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GENETIC TRANSFORMATION OF RASPBERRIES BY MEANS OF *AGROBACTERIUMTUMEFACIENS*

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KEY WORDS: raspberry (Rubus idaeus); transformation; regeneration; npt II; Agrobacterium tumefaciens

ABSTRACT

Genetic transformation of red raspberry (Rubus idaeus) was achieved using Agrobacterium tumefaciens. Leaf petioles and leaf segments of cv. Samodiva and cv. Elit-1 were infected with Agrobacterium strains EHA 101, LBA 4404 and LBA 4404 carrying 35 S and 2x35 S promoters. The regenerants were obtained on MS nutrient medium enriched with 0.3 mg/l IBA, 0.01 mg/l 2,4-D and 2 mg/l TDZ. The Agrobacterium growth was inhibited with cefotaxime. Kanamycin was used as a selective agent for the transformants. The transformation efficiency was within the range of 0.54-2.08 % for Elit-1 transformed with Sac B and cod A gene. The integration of the marker genes npt and Hygromycin in putative transgenic plants was confirmed by PCR analysis using primers, the nucleotide sequence of which was complementary to these genes.

INTRODUCTION

The "green revolution" of the past century contributed to conserve the population diversity. In contrast to it, the rise of "gene revolution" gave the biological science new dimensions and technology for increasing the yields and quality of plant population. The development of the technology for obtaining genetically improved plants progresses at quick rates in the world and the areas sown with them increase every year (Datta, 2000).

Raspberry is a widespread fruit crop, not only in our country, but also in the world, owing to the multipurpose use of its fruits (Georgiev et al., 2004; Ludneva et al., 2008). The raspberry fruits are richer than the strawberry ones with regard to their biochemical composition. The main problems confronting the breeders are related to development of resistant cultivars to abiotic stress factors, economically important phytopathogens and insect pests (Hristov et al., 1981; Boycheva et al., 1994).

The improvement of the cultivars possessing a high genetic potential is based on combination of the traditional methods with the contemporary biotechnological approaches (Goranova et al., 2005). With the development of the molecular biology, genetics and genetic engineering, it became possible to integrate genes responsible for the expression of economically useful agricultural qualities into raspberry genomes (Klee et al., 1987; Moerbe et al. 1988).

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MATERIAL AND METHODS

Plant regeneration

Ten regeneration capacity of raspberry genotypes were studied and tested it by different media for inducing adventitious shoots. The regeneration medium containing MS basic salts and enriched with 0.3 mg/l IBA, 0.01 mg/l, 2,4-D and 2 mg/l TDZ pH 5.7 was the best for the studied. Leaves of the middle storey of 21-day plants were used as explants. From each leaf a square leaf segment , containing the main nerve, was cut off and placed with its adaxial surface towards the nutrient medium and the leaf petioles were injured with two perpendicular sections. Ten explants were laid out in each of Petri dishes. The cultures were grown for eight weeks, at a photoperiod of 16/8 hours and temperature of 23-25 ˚C. The leaf explants were examined on days 21, 28, 35, 42, 49 and 56 for regeneration capacity and average number of adventitious shoots per explant.

Bacterial strains and plasmids

The following bacterial strains and plasmids were used for the transformation:

Escherichia coli strain DH5α (Hanahan, 1983), subsidiary strain HB101carrying plasmid pRK2013 (Lam et al. 1985) and *Agrobacterium tumefaciens* strain LBA4404 (Hoekema et al. 1983). kDNA for *AtP5CS* (Yoshiba et al. 1997) or *SacB* gene (in pKP construct) (Ebskamp et al. 1994) were put under the control of 35S promoter of cauliflower mosaic virus (CaMV) in binary vector pNFHK1 containing selective marker genes coding neomycin – and hygromycin-phosphotransferase (Table 1). The bacterium development and plasmid DNA isolation were performed according to Sambrook et al., 1989.

The binary vector pGAH carrying *codA* gene was introduced into *Agrobacterium tumefaciens* strain EHA101 (Hayashi et al. 1997) and kindly provided to the AgroBioInstitute by Prof. Norio Murata, National Institute for Basic Biology, Japan.

Table 1

Used strains of *Agrobacterium tumefaciens* and binary vectors

Agrobacterium tumefaciens	Binary vector	Marker genes	Promoter	Transgene
LBA4404	pNFHK1	Km, Hyg	35S	*AtP5Cs**
LBA4404	pNFHK1	Km, Hyg	2 X 35S	*SacB**
EHA101	pGAH	Km, Hyg	35S	*codA*

- The genes were involved in pNFHK1 vector by prof. Oleg Georgiev, Institute fur Molecularbiologie, Universitat Zurich-Irhel, Zurich, Switzerland, for the needs of ABI.

The used genes code key enzymes of byosynthetic pathways of proline, fructan or glycine-betaine, the latter being nonspecific compounds for raspberry (Table 2). *SacB* gene is fused with the sequence of transit peptide for localization in the vacuole (*cpy*, Valls et al. 1987), and *codA* – for localization in the chloroplasts (*rbcS tr*, Pinck et al. 1984).

Table 2

Genes used for transformation

Gene	Origin	Enzyme	Function
AtP5Cs	*Arabidopis thaliana*	Δ^1-pyroline-5-carboxylate synthetase	Proline biosynthesis
VacP5Cs	*Vigna aconitifolia*	Δ^1-pyroline-5-carboxylate synthetase	Proline biosynthesis
SacB	*Bacillus subtilis*	Levansucrase	Fructan biosynthesis
codA	*Artrobacter globiformis*	Choline oxidase	Glycine-betaine biosynthesis

Procedure for inoculation of the explants and cocultivation

The isolated and injured explants were inoculated in a Petri dish by pouring on a 5 ml bacterial suspension for 20 minutes. Then they were dried up carefully with sterile filter paper and put on the solid regeneration medium. The cocultivation took place for 48 hours in the dark at a temperature of 28 ºC (Mezzetti et al., 2002).

After expiration of the period of cocultivation, the bacterium was removed by fourfold successive washing in liquid regeneration medium and simultaneously with the bacterium elimination supplemental nutrition of the plant explants was also conducted.

The transformed material was well dried up on sterile filter paper and then transferred on solid regeneration medium containing 500 mg/l cefotaxime sodium for elimination of bacterial development. A system of late selection was applied, the selective dose of 50 mg/l kanamycin sulfate being added at second passage, for regeneration only of the transformed tissues.

Every two weeks explants were transferred on fresh medium containing 500 mg/l cefotaxime sodium and 50 mg/l kanamycin sulfate. After two-month culture on selective medium the plant material was released of the antibiotic and subcultured on a medium to finish its growth.

Determination of the dose curve of kanamycin sulfate

Cultivar sensitivity to the selective agent was studied with intact *in vitro* plants. A number of ten clone microplants were put in each of tubes with MS medium enriched with 0.3 mg/l IBA and varying concentration of kanamycin sulfate (0; 50; 100; 150; 200 mg/l). The results were recorded after 8 weeks.

Detection of gene transfer

The following modification of the method of Dellaporte et al., 1983 allowing efficient isolation of high-quality DNA, was used in this work:

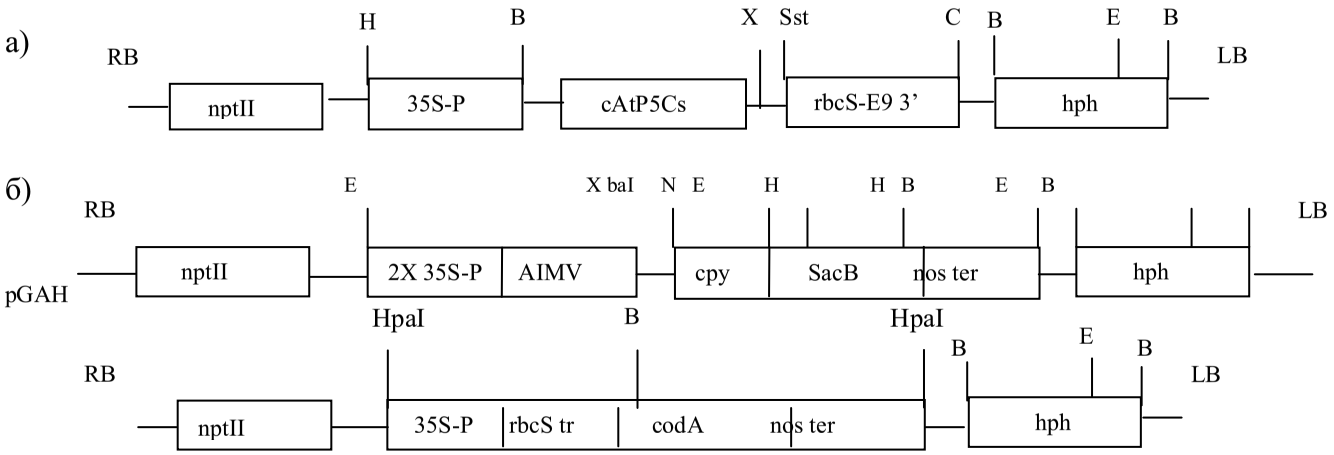
Leaf material (0.2 g) was put in an Ependorf tube, frozen in liquid nitrogen and ground. 500 µl extraction buffer (10 mM Tris–HCl, pH 8.0; 50 mM EDTA, pH 8.0; 100 mM NaCl; 1 % SDS) preheated at 65 °C was added. The samples were incubated for 15 minutes (up to 30 minutes) in water bath at 65 °C to lyse the cells. They were cooled at ambient temperature for 5 minutes. 165 µl 5 M potassium acetate of pH 7-7.5 were added and left on ice for 30 minutes for precipitation of proteins. The latter were extracted with 600 µl chloroform:isoamyl alcohol (24:1). The obtained precipitate of ammonium acetate – SDS was settled by centrifugation at high speed (13 500 g).

The upper phase was pipetted and transferred into a new Ependorf tube. 8 µl PH–ase of 10 mg/ml concentration were added and incubated at 37 °C for 30-60 minutes. 500 µl chloroform:isoamyl alcohol (24:1) were added. Centrifugation for 5 minutes at 13 500 g and transfer of the supernatant into an Ependorf tube. 700 µl isopropanol were added to precipitate the high-molecule DNA. After centrifugation for 10 minutes at maximum rotations, the obtained sediment was washed with 1 ml 75 % cold ethanol and then dried and resuspended in 50 µl TE (10mM Tris–HCl, pH 8.0; 1mM EDTA, pH 8.0). The concentration and quality of the so isolated DNA were determined spectrophotometrically at 260 and 280 nm and by electrophoresis in agarose gel using lambda (λ) DNA standards of a strictly fixed concentration.

The constructs used in our experiments are presented in Figure 1.

Figure 1. Constructs used in the transformation experiments

pNFHK1



For PCR amplification of sites of the coding regions of the *AtP5Cs* and *codA* genes the following specific oligonucleotide primers were used:

Hygromycin primer:

Forward primer: (5'–3'): AAAAGTTCGACAGCGTCTCCGACC

Reverse primer: (5'–3'): TTGGCGACCTCGTATTGGGAATCC, amplify a site of 643 bp.

NFT II primer:

Forward primer: (5'–3'): AGAACTCGTCAAGAAGGCGA

Reverse primer: (5'–3'): ATTGCACGCAGGTTCTCCGG, amplify a site of 772 bp.

The apparatus GeneAmp PCR System 9700 (Perkin Elmer) was used for the PCR analysis. The results of the PCR amplification were analyzed by electrophoresis in 1 % agarose gel, in 1xTAE buffer (0.04M Tris–acetate, 0.001M EDTA, pH 8.0) and DNA staining with ethide bromide.

The PCR conditions for amplification of *npt II* gene were 94 for 4 min, followed by 35 cycles of 94 ˚C for 30 sec, 57 ˚C for 1.40 min, 72 ˚C for 1.4 min and 72 ˚C for 7 min.

RESULTS AND DISCUSSION

Effect of kanamycin on the rooting of *in vitro* plants

The minimum concentration inhibiting the rooting of *in vitro* plants was 50 mg/l (Figure 2).

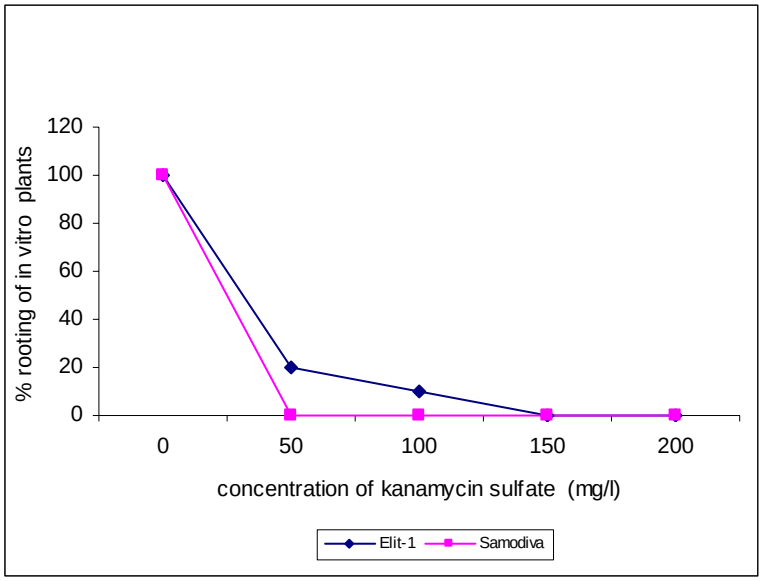


Figure 2. Dose curve of resistance to kanamycin sulfate of adventitious shoots of the Elit-1 and Samodiva genotypes

The antibiotic effect resulted in strong chlorosis and necrosis of plant tissues and gradual dying of microplants. The concentration of 50 mg/l was chosen as an upper limit for selection in our experiments of genetic transformation. The same concentration was also used in the experiments with fig (Yancheva et al. 1994, 2005). No root formation was observed for the other studied combinations, the plants had suppressed growth, bleached apical part and young leaves, as well as necrotized old leaves.

Kanamycin effect on the morphogenetic capacity of leaf explants

Gradual bleaching and necrotization of leaf blades and strong thickening of petioles were observed on selective medium containing 50 mg/l kanamycin.

The analysis of the data in Figure 2 shows that the raspberry plants cultured under *in vitro* conditions were sensitive to concentrations of 50 mg/l kanamycin sulfate, which corresponded to the studies of Graham et al. (1990) where the concentration of 50 mg/l antibiotic proved to be lethal (critical) to the clonal raspberry plants.

The raspberry regenerants were very sensitive to kanamycin that compromised the regeneration of the transformed plants. The use of geneticin 7, instead of kanamycin, in *npt II* transformation system and the use of hygromycin in *hpt* transformation system is a promising component for successful gene transfer (Swartz et al., 1996). The observed results were similar to the communication of Faria et al. (1997) reporting for oversensitivity of raspberry tissues to kanamycin sulfate at 40 mg/l concentration. With regard to the tolerance to the mentioned antibiotic, an exception is the arctic raspberry (*Rubus arcticus L.*), for which Kokko et al. (1998) found that it is not sensitive to kanamycin sulfate as

other members of *Rubus* genus. The lack of root formation of the studied genotypes at 100, 150 and 200 mg/l kanamycin sulfate was probably due to the strong toxic effect of the antibiotic on the plant tissues of the *in vitro* plants.

Genetic transformation of leaf explants from raspberry

Approximately twenty microplants regenerated from a total of 3600 leaf explants after incubation of different explants from the studied raspberry cultivars and elites with the used *Agrobacterium tumefaciens* strains. No regeneration was achieved after the applied scheme in cv." Samodiva". The *CodA* gene isolated from Artrobacter globiformis produced a good percentage - 2.08 % transformed plants. When comparing the strain virulence to the raspberry genotypes, the *SacB* gene proved to be the lowest efficient for which only one regenerant was registered (Table 3).

Table 3

Transformation frequency of leaf explants from Elit-1 infected with *Agrobacterium* on medium containing 50 mg/l kanamycin sulfate and 500 mg/l cefotaxime sodium

Explant type	Bacterial plasmid	Total explant number	Genetic transforma-ion, explant number	Regene-rated shoots, Number	Rooted plants, number	Transfor-mation efficiency %
Leaf	*AtP5Cs* – LB4404	300	154	0	0	0
	CodA – EHA101	300	84	0	0	0
	SacB – LB4404	300	136	0	0	0
Petiole	*AtP5Cs* – LB4404	300	91	13	0	0
	CodA – EHA101	300	144	6	3	2,08
	SacB – LB4404	300	184	1	1	0,54

The following was successfully obtained in our experiments of gene transfer:

- 13 plants from petioles of Elit-1 transformed with construct *AtP5Cs*;
- 6 plants from petioles of Elit-1 transformed with construct *codA*;
- 1 plant from petioles of Elit-1 transformed with construct *SacB*.

Table 4

Control explants laid out simultaneously with the transformation experiment

Cultivar	Laid out explant number	Regenerated explant number	% regeneration	Total regenerant number per explant	Average regenerant number per explant
Leaf					
Elit-1	116	51	44	99	1,9
Samodiva	116	51	44	121	2,4
Petiole					
Elit-1	232	100	35,5	278	2,8
Samodiva	148	34	23	61	1,8

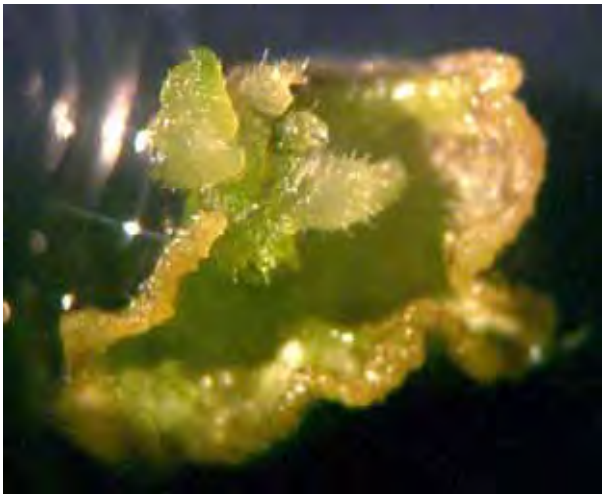


Figure 3. Regeneration from transformed leaf segments with *codA*gene on A-1 medium after 60 days of culture

Better results of the study of the gene transfer technology were obtained from the petioles of Elit-1 (controls - 35.5 %, transformed – 0.14 % on A-1 medium), as compared to the leaf segments (from 44 % to 0 %), respectively, which could be due to their richer supply of reserve assimilates and sugars (higher content of anuline near to main nerve and petiole), as well as to differences in anatomical texture of both plant organs (Table 5).

No regeneration was achieved from leaf explants of cv.” Samodiva”.

Table 5

Effect of kanamycin sulfate and infection on the plant regeneration of adventitious shoots

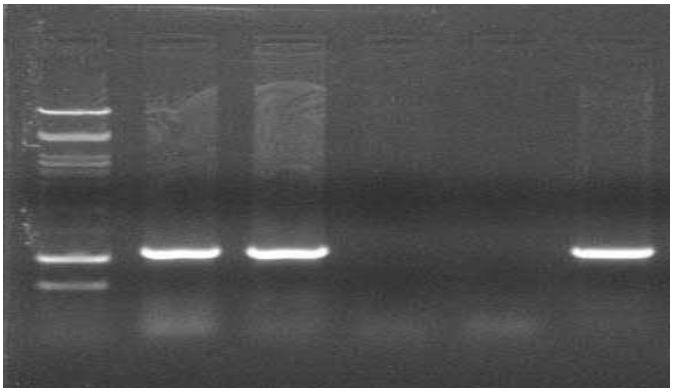
Genotype	Explant type	Regenerating explant percentage		
		(-) *Agrobacterium*	(-) *Agrobacterium*	(+) *Agrobacterium* strain *ATP5Cs*
		(-) Kanamycin sulfate 50 mg/l	(+) Kanamycin sulfate 50 mg/l	(+) Kanamycin sulfate 50 mg/l
Samodiva	Leaves	44	0	0
	Petioles	23	0	0
Elit-1	Leaves	44	0	0
	Petioles	35,5	0	0,14

PCR – analyses

The PCR analysis of probable transformants proved the incorporation of *npt II* Hygromycin into the genome of Elit-1.

DNA samples of putative transgenic plants obtained after transformation with *codA* gene were analyzed with the appropriate primers for presence of *npt* II or Hygromycin marker genes. DNA samples isolated from the working constructs were used as positive controls. In the transformed plants there was an amplification product corresponding to that obtained under amplification of plasmid DNA containing the respective gene (Figure 5, Figure 6). The obtained amplification mixture from the polymerase chain reaction had a size of 772 kb in the transformed plants carrying *npt*

(Figure 6) and 643 kb which confirmed the presence of Hygromycin gene. No positive signal was observed in the DNA samples from nontransformed plants.

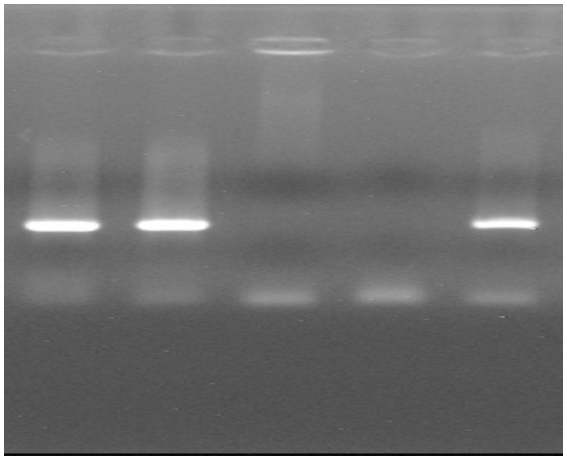


1 2 3 4 5 6

Figure 4. Detection of the integration of Hygromycin marker gene into the genome of Elit-1 through PCR

Legend:

1 – λ DNA/PstI Marker, 24; Clone 2,3 - transformants carrying Hygromycin gene; 4 - nontransformed control; 5 - water; 6 – positive control (plasmid DNA carrying Hygromycin gene).



1 2 3 4 5

Figure 5. PCR analysis confirming the presence of *npt* II in the genome of Elit-1

Legend:

Clone 1 – 2 transformants carrying *npt* II gene; Clone 3 – nontransformed control; 4 – water; 5 – plasmid

CONCLUSION

The trials of genetic transformation through *Agrobacterium tumefaciens* in raspberry gave a positive signal on the grounds of which we can continue the experiments.

Two different selective marker genes were used: hygromycin phosphotransferase (*hpt*), which gives resistance to hygromycin B and neomycin phosphotransferase (*npt II*), which provides resistance to kanamycin (Graham et al., 1990; Hassan et al., 1993; Mathews et al., 1995; Faria et al., 1997; Kokko et al., 1998). The explants isolated from raspberry showed oversensitivity to kanamycin, therefore we have good reasons to apply a later selection scheme. The use of *geneticin* 7 instead of kanamycin in *npt II* transformation system and the use of hygromycin in hpt transformation system is a promising component (Swartz et al., 1996). The recorded percentage of transformed raspberry shoots was low and many of the obtained plants are chimeras, because they have multicellular origin.

The genetic transformation of raspberry varieties through *Agrobacterium tumefaciens* realized a low regeneration frequency and low transformation frequency that impeded to bring it to an end. On the grounds of the primary results obtained from the genetic transformation of raspberry with *AtP5Cs, codA* and *SacB* genes we can assume that their application to improve the selection quality of raspberry is possible and is a reliable alternative in the conventional selection.

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LICHEN MYCOTA ALONG ULUDAĞ FIR (*ABIES BORNMUELLERIANA* MATTF.)

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KEY WORDS: lichen mycota of tree, epiphytic, corticolous, fir, research forests of Turkey

ABSTRACT

For the purpose of investigating distribution of epiphytic lichen taxa along a tree, mycota of 5 cut-fir trees was studied in Şerif Yüksel Research Forest in the province of Bolu, for the first time in Turkey.

INTRODUCTION

The present contribution is about lichen mycota along a tree, in order to determine if lichen species have any selective behavior about where to colonize on a tree, from the ground to its peak. Can the all lichen mycota be detected enough collecting on reachable lower parts of trunk or are some species missing? No other similar papers present in Turkish lichen literature.

The presented survey was performed with epiphytic lichens on fir trees in Şerif Yüksel Research Forest situated in the province of Bolu in western black sea region of Turkey. The forest is differentiated from Aladağ Forestry Management. It is located between 40° 35' 00" – 40° 39' 00" northern latitudes and 25° 33' 00" – 25° 38' 00" eastern longitudes. It has totally 1544 ha covering area with the highest point of 1640 m and the lowest point of 1330 m and moderately rough (Tosun 2003). According to Şerif Yüksel Research Forest Meteorological Station, the average annual mean temperature is 5.7 °C (1975–1995) and the annual precipitation is about 882.6 mm The climate type is symbolized as $B_4C_2'rb_2'$ according to Thornthwait, that is humid, micro thermal, not or very few lack of water and partly under sea impact (Serin 1998). Dominated tree species in the study area are Uludağ fir (*Abies bornmuelleriana* Mattf.) and Scotch pine (*Pinus sylvestris* L.) (Bozakman 1976).

MATERIALS AND METHODS

The corticolous lichen samples were collected on 5 newly cut Uludağ firs (*Abies bornmuelleriana* Mattf.) in Bolu Şerif Yüksel Research Forest in July 2005 during another lichenological study. The browsed trees (5) have heights of 27.6, 33.5, 35.1, 37.2 and 37.6

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m with respectively the following ages; 192, 212, 256, 263 and 208. Each tree trunk was vertically divided into 2-meter-parts (0-2, 2-4, 4-6, … m), and encircled lichen samples of all morphological types (foliose, fructicose and crustose) were collected on every division.

The collected lichens were let air dried and then identified with the aid of several publications (Clauzade & Roux 1985, Purvis *et al.* 1992, Wirth 1995). The nomenclature follows recent literature (Blanco *et al.* 2004). The specimens are kept in the herbarium of the Faculty of Science and Arts, Marmara University (MUFE), and some duplicates in the herbarium of Faculty of Forestry, Istanbul University (ISTO).

RESULTS AND DISCUSSION

The total list includes 72 lichen species detected along 5 sampled Uludağ firs (*Abies bornmuelleriana* Mattf.) on every 2-m divisions. The list of species was given in alphabetical order at the Table 1, and each of the lichen species along tree mycota is enough to be marked even if present only on any of 5 fir trees.

The abbreviations are as follows: for lichens collected only on trunk "T", only on branches "B", and on both trunk and branches "A".

Table 1

Distribution of lichen species along five sampled fir trees.

LICHEN SPECIES	DIVISIONS ON FIVE SAMPLED FIR TREES (m)																			
	0--2	2--4	4--6	6--8	8--10	10--12	12--14	14--16	16--18	18--20	20--22	22--24	24--26	26—28	28--30	30--32	32--34	34--36	36--38	Frequency of taxa (m)
Alectoria sarmentosa	T	T	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B		18 (0-36)*
Anaptychia ciliaris								B				B	A	A	A	A	A	T		8 (14-36)
Arthonia radiata						T					B	A								3 (10-24)
Bacidia laurocerasi			B			B	B		B	T		T		A						7 (4-28)
Bacidia rosella												T	T							2 (22-26)
Bryoria capillaris	T	T	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	19 (0-38)*
Bryoria fuscescens		T	T	T	A	A	A	A	A	A	A	A	A	A	A	A	T			16 (2-36)*
Bryoria implexa							T	T												2 (12-16)
Buellia disciformis								T	T			T		T	T	T				6 (14-32)
Buellia griseovirens	T	T	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B		18 (0-36)*
Calicium salicinum	T	T	T	T	T	T	T	T												8 (0-18)
Calicium viride	T	T	T	T	T	T	T	T	T											9 (0-20)
Caloplaca herbidella		T	B		B	T	B	B	B	B	B	B	T	A	A	B	B			15 (2-34)*
Candelariella reflexa												T		T		B	B			4 (22-34)
Candelariella xanthostigma												T						B		2 (22-36)
Cetrelia olivetorum					B				B											2 (8-18)
Chaenotheca brunneola				T																1 (6-8)

Chaenotheca chrysocephala	T	T	T	T	T	T	T												7 (0-14)
Chaenotheca trichialis	T	T	T		T	T													5 (0-12)
Chrysotrix candelaris	T	T	T	T	T	T	T	T											8 (0-16)
Cladonia coniocraea	T		T										T						3 (0-28)
Cladonia fimbriata	T	T		T	T			T	T			T		T					8 (0-28)
Cyphelium inquinans		T	T		T		B			T									5 (2-20)
Evernia divaricata	T	T	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	18 (0-36)*
Evernia prunastri					B		B										B		3 (8-34)
Graphina ruiziana	T	T	T	T	T	T													6 (0-12)
Hypogymnia physodes	T	T	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	18 (0-36)*
Hypogymnia tubulosa	T		B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	17 (0-36)*
Lecanora albella	T	T	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	18 (0-36)*
Lecanora argentata		T	T	T	A	B	T		A	B	B		B		B		B		12 (2-34)
Lecanora carpinea			B	B	T	A	T	A	A	A			T	T					10 (4-28)
Lecanora chlarotera			B	T	A	B	B	T	A	A	A	A	A	A	A	A	A	T	16 (4-36)*
Lecanora expallens											B	B							2 (20-24)
Lecanora intumescens								T											1 (14-16)
Lecanora symmicta	T	T	B							B			B						5 (0-26)
Lecanora varia						A		A		A		T			T	T	T		7 (10-34)
Lecidella elaeochroma	T	T	A	A	B	A	A	T	A	A	T	A	A	A	A	A	A	B	18 (0-36)*
Lobaria pulmonaria							A	A	T	A	B	T	T	T					8 (18-28)
Melanelixia fuliginosa			B	B	B	B		B	A	A		B	B	B	B	B	B		13 (4-34)
Melanohalea exasperatula			B		B	B	B	A		A	T	A	A	A	A	A	A	A	14 (4-36)
Opegrapha atra	T	T	T	T	T														5 (0-10)
Ochrolechia androgyna		T	T	T	T		A	A	B	B	B								9 (2-22)
Ochrolechia tartarea						T	T	T	T										4 (10-18)
Ochrolechia turneri	T	T	T	T	A	T	T	T	A		T	A	A	B					13 (0-28)
Parmelia saxatilis	T	T	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	T	18 (0-36)*
Parmelia submontana					B		B	A	A	B	B		B		B	A			9 (8-32)
Parmelia sulcata	T		B	B	A	A	A	A	A	A	A	A	A	A	B	T	B	B	17(0-36)*
Parmeliopsis ambigua	T	T	T	T					B										5(0-18)
Pertusaria albescens	T	T	A	A	A	A	A	A	B	A	A	A	A	B	A	T			16(0-32)*
Pertusaria coronata	T		T													B			3(0-32)
Pertusaria hemisphaerica	T	T	T	T	A	T		T											7(0-16)
Phlyctis argena			B					B		B		B		B	B	B			7(4-32)
Physcia adscendens														B					1(26-28)

Physcia leptalea									B				B	B		B	A	A		6(16-36)
Physcia tenella						B							B			B	A	A		5(10-36)
Physconia distorta			B	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A		16(4-36)*
Platismatia glauca	T	T	A	A	B	B	A	A	B	B	B	B	T	B						14(0-28)
Pleurosticta acetabulum																	B			1(32-34)
Pseudevernia furfuracea		T	B	A	A	A	A	B	B	A	A	B	B	A	B	B	A	A		17(2-36)*
Ramalina calicaris			T	T	T		B	T	T	T	T	T								9(4-24)
Ramalina farinacea	T	T	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A			17(0-34)*
Ramalina fastigiata					A		T	T	A	T	T	A	T	A	A		B			11(8-34)
Ramalina thrausta		T	T	T	A	A	B	A	A		T	T	T	B						12(2-28)
Rinodina exigua				B		B			B	A	B	B	B	A	A	A	A	A		12(6-36)
Schismatomma graphidioides	T	T	T	T	T	T	A	T	T											9(0-18)
Tuckermannopsis chlorophylla	T	T	A	A	A	A	A	A	A	A	A	A		B	B	B				14(0-30)
Usnea cavernosa				T			T		T	T	T		B	T						7(6-28)
Usnea filipendula			T	T	A	B	B	A	T	B	B		B		B	B	B			13(4-34)
Usnea florida	T	T	T	A	A	A	A	A	A	A	A	A	A	A	T	T				16(0-32)
Usnea fulvoreagens							T	T	T		T		B							5(12-26)
Usnea hirta						B		T					T			T				4(10-32)
Usnea subfloridana	T	T	T	A	A	A	A	A	A	A	T	A	A	A	A	T				16(0-32)*
TOTAL NUMBER OF SPECIES 72	31	34	46	41	45	43	44	47	44	38	36	39	40	39	31	33	29	19	1	

The data obtained from 5 sample fir trees indicated that the lichen-intensive heights of tree trunks are 4-18 m in general. The trees have no branches up to 4 m. A total of 31 species are detected from the ground level to 2 m (0-2 m). Some lichen species begin to occur above 2 m from the ground, such as *Bryoria fuscescens, Caloplaca herbidella, Cyphelium inquinans, Lecanora argentata, Ochrolechia androgyna, Pseudevernia furfuracea* and *Ramalina thrausta.*

Number of lichen species on trunk (T) is the highest on 14-16 m, and on branches (B) are the highest at divisions of 4-6, 24-26 and 32-34 m. Of the 72 species, 6 species are collected only on branches (4 to 36 m), 15 species only on trunk (0 to 32 m), and the remaining 51 species on both (4 to 38 m) (Tab. 1). Eleven percent of the total number of species is collected only above 6 m that is higher than eye-level *(Bacidia rosella, Bryoria implexa, Candelariella reflexa, Canderiella xanthostigma, Cetrelia olivetorum, Chaenotheca brunneola, Lecanora expallens, Ochrolechia tartarea, Physcia adscendens, Physcia leptalea, Pleurosticta acetabulum*). This means during common sample collection, some part of lichen taxa may probably remain uncollected. That is the lichen mycota of a forest should be completed with a detailed collecting without lacking those species on unreachable parts of trees.

The most frequent species, collected on at least 15 divisions along the tree (average of five fir trees), are *Alectoria sarmentosa, Bryoria capillaris, Bryoria fuscescens, Buellia griseovirens, Caloplaca herbidella, Evernia divaricata, Hypogymnia physodes, Hypogymnia tubulosa, Lecanora albella, Lecanora chlarotera, Lecidella elaeochroma, Parmelia saxatilis, Parmelia sulcata, Pertusaria albescens, Physconia distorta, Pseudevernia furfuracea, Ramalina farinacea, Usnea subfloridana.* Comprehensively the least frequent species, collected only on 1 division along the tree, are *Chaenotheca brunneola* (T/6-8 m)*, Lecanora intumescens* (T/14-16 m)*, Physcia adscendens* (B/26-28 m)*, Pleurosticta acetabulum* (B/32-34 m) (Tab. 1). This does not mean those species are never present on other parts of trees in general, but this data is valid only for these 5 sample trees. Can the all mycota be present on lower trunk or are some species missing on higher trunk and/or branches? To document lichen mycota of a forest, were the samples enough to be collected only on eye-level of trunks? The total lichen mycota of the same forest has been represented with 102 species (Cobanoglu *et al.* 2008).

CONCLUSIONS

As the results show that species frequency on tree-trunk below 4 m with 38 species becomes only about 53 % of the whole lichen mycota of a tree, the lichen mycota can not be completed by collecting only on reachable levels of tree trunks. In other words, the number of species present above 4 m, which is the beginning of branching, is 34 that is 47 %. So, if collecting does not occur all along a tree, some big amount of species has probably not been noticed.

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POLLUTION INDICATORS SPECIES. THE QUANTITATIVE DETERMINATION OF Hg AND Pb BY AAS SPECTROMETRY FROM *Achillea millefolium L.,Centaurea cyanus L.* AND *Plantago major L.*

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KEY WORDS: atomic absorption spectrometry, toxic metals, polluted zone

ABSTRACT

An atomic absorption spectrometric method is described for the determination of lead and mercury in plant tissues.

The procedure described in this paper, highlight the degree of exposure of plants to certain toxic metals : Hg and Pb, the plants being harvested from a polluted industrial zone (Sinteza Oradea) and from a non polluted zone (Beius - Bihor).

Samples used are formed from vegetal tissues, obtained from the following species: Achillea millefolium, Centaurea cyanus L. and Plantago major L..

INTRODUCTION

The pollution indicators are sensible vegetal species which indicates the presence of a certain pollute by the appearence of some lesions or malformations and give indications, by the tissue analysis, linked by few amounts of toxic substances from the environment

Achillea millefolium L. is part of the Compositae family.

Centaurea cyanus L. is part of the Apiaceae family.

Plantago major L. is part of the Plantaginaceae family.

The speciality works indicates for the analysing of the toxic metals from the plant tissues, the use of solvent mixture such as :nitric acid, sulphuric acid and perchloric acid.

In the present paper, we choose to using of nitric acid, because metalic nitrates does not interfere in the atomic absorption spectrometry. Traces of toxic metals present in the studied vegetal tissues, are transformed after treatment in nitric acid in Pb and Hg nitrates. In the year of 2006, according the dates CORINVENT, it results the following amounts of heavy metals:

Hg – 21.97 kg

Cd – 2.91 kg

Pb – 24 kg.

The total amount of emission of heavy metals during the year 2005 in Bihor County is 924.750kg , as can be seen in table 1.

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Table 1

The total amount of emission of heavy metals (kg).

As	Cr	Cu	Ni	Zn	Cd	Hg	Pb
93.3	91.2	1.28	95.5	256.07	9.18	252.42	20.58

MATHERIAL AND METHODS

Atomic absorption spectrometry is widely used in biological analysis. Nitric acid alone, hydrofluoric acid along with nitric, perchloric acid, sulphuric acid and hydrochloric acid, all have been used for this purpose. The proposed method makes use of HNO3 -HCl digestion and subsequent disolving of the residue from the digestion in 10% (v/v) HCl. The lead is separated from the acidic solution in methyl-iso butyl-ketone MIBK by the extraction procedure, because lead is susceptible to inter element interferences. The mercury compounds are dissolving in sulphuric acid – nitric acid.

A Carl Zeiss Jena atomic absorption spectrophotometer was used for all determinations with the instrument settings shown in table 2.

Table 2

AAS 30 settings for determination of Pb and Hg

Metal	Lamp current (mA)	Wave length (nm)	Slit (nm)
Pb	5.0	217.0	1.0
Hg	5.0	229.0	1.0

The device use an air /acetylene flame.

Reagents and standards

Prepare individual 1000 mcg /mL stock solution by dissolving of 1.077g PbO in 100 mL of concentrated hydrochloric acid and diluting to 1 L with water. Then, prepare an aqueous lead solution (10 mcg/mL) by dilution with 10 % HCl. Add 10 mL iodide reagent and 2g of ascorbic acid, and dilute to 30 mL with 10%HCl. Then, add 10 mL of methyl iso-butyl-ketone MIBK solution and shake for 30s.

HgO is dissolved in sulphuric acid – nitric acid =2:1 v/v.

From the prepared solutions, there were obtained a concentration of 10mcg/mL for each ion, using succesive dilutions.

Weigh 1-2 g of biological material into a 100mL glass beaker. The samples were treated three times with a combination of 10mL of hydrochloric acid and 5mL nitric acid. For the determination of lead, transfer a 20mL aliquot of the sample solution to a marked 25x100mm screw- cap tube. Add 2g of ascorbic acid and 10mL of iodide reagent. Shake to dissolve the ascorbic acid. Add 10 mL of MIBK solution and shake for 30s. Allow the organic and aqueous phases to separate, and aspirate the upper organic layer into the flame.

For analysing of mercury, 1-2g of dry weigh , add 15 mL 2:1 18Msulfuric acid-2M nitric acid. Place the volumetric flasks in a shaking water bath set for 50-60°C and digest for 2h. The added volume is 25mL of mixture. The mixture is warmed up to 100°C, it is centrifugated, and the supernatant liquid is diluted with distilated water at a volume of 50mL. The calibration curves for each analysed metal are liniar in shape, thus obtaining the interval of valability of the Lambert-Beer low.

RESULTS AND DISCUSSION

The experimental results are presented in table 3.

Table 3

The experimental results

Metalic ion (µg/g)	**Polluted zone SINTEZA**			**Non polluted zone BEIUS**		
	Achillea millefolium	*Centaurea cyanus*	*Plantago major*	*Achillea millefolium*	*Centaurea cyanus*	*Plantago major*
Hg	245	210	100	5	2.4	2.0
Pb	359	260	90	2.3	1.5	1.2

Accordingly to the dates presented in table 3, it is evident that the plants from the polluted zone situated in Sinteza, were more affected by the pollution, the metal ions have recorded levels that at much higher than from the non polluted zone. Prelevation of the experimentead vegetal material was made in august.

Table 4

Measurements effected for *Achillea millefollium*

Bioindicators	Root		Stem		Inflorescence	
	Sinteza	**Beius**	**Sinteza**	**Beius**	**Sinteza**	**Beius**
Average	4.93	5.3	32.06	42.52	4.45	6.26
Standard deviation	1.7983	1.9898	11.8737	11.1634	1.9171	2.0683
Coefficient of variation	36.4461	37.5428	37.0360	26.2545	43.0246	33.0403

The measurements made at bioindicator species from the polluted zone and from the non polluted zone, are presented in tables 4, 5 and 6.

The species of bioindicator plants, analised in the experiment, in the polluted zone Sinteza, proved to be more sensible at chemical pollutes, from the water and soil, fact revealed by noticing of some vegetal tissue damages.

The species of bioindicators plants analised from the experiment that has been made in Sinteza area, proved to be more sensible at chemical pollutes from air and soil, fact revealed by the observation of some lesions of the vegetal tissues, small leaves, weak inflorescence, pale color and a small developing in high of the plants.

Table 5

Measurements effected for *Centaurea cyanus L.*

Bioindicators	Root		Stem		Inflorescence	
	Sinteza	**Beius**	**Sinteza**	**Beius**	**Sinteza**	**Beius**
Average	45.18	54.85	7.33	8.56	1.03	1.23
Standard deviation	9.1327	11.3433	2.6146	2.9364	0.1620	0.1832
Coefficient of variation	20.2125	20.6807	35.6537	34.2773	15.7100	14.0028

Table 6

Measurements effected for *Plantago majorL.*

Bioindicators	Root		Stem		Inflorescence	
	Sinteza	**Beius**	**Sinteza**	**Beius**	**Sinteza**	**Beius**
Average	3.38	13.26	10.24	42.58	23.08	48.68
Standard deviation	1.4274	3.2876	2.4707	8.2662	6.0028	15.5148
Coefficient of variation	41.7592	24.7936	24.1281	19.4133	26.0089	31.8711

There have been identified some mechanism at cellular level by which the toxic metals can produce damages, such as:

- blockage of the functional groups of important biological molecules: enzymes, polynucleotydes, the transport system for nutritive ions.
- removing or/and replacing of the essential metalic ions from the biomolecules and the functional cellular units.
- enzyme denaturation and inactivation.
- destruction of the integrity of cell membranes and of the organites membranes.

The toxic symptomes are the results of some damaging effects of the metals up on some physiological process that include: inhibition of the breathing and photosynthesis, damage of the relation plant-water that determines water stress, lowering the permeability of the plasmatic membranes of the cells from the root, reverse effects upon the metabolic enzymes activity.

There have been suggested some criteria symptomes for determination of the toxicity of a metal upon the plants. These are:

1. plants presents persistent damages.
2. plant tissue accumulation of the potential toxic metal.
3. biochemical modifications.

This fact was confirmed also by the atomic absorption spectrometry of the heavy metals. The vegetal samples were taken from the environment of Sinteza. The most important toxic metals from the nature are Pb, Hg, Cr and Mn. The amount of heavy metals provided by human activities is 29 times greater than provided from natural process. These are five groups of human activities responsible for the contamination with heavy metals.

CONCLUSIONS

These pollutes are coming from the departments of Sinteza, special from the pigment department of Cr, Pb and Cd, organophosphorics and drugs. Although in the present paper, we did not followed the atmospherical pollution, we can notice that the Sinteza factory determines a great degree of air and soil, observed by the concentrations of some gases.

Finally, the experimental dates showed us that the most sensible pollute bioindicator was *Achillea millefolium,* showing great values due to metalic ions, the variability coefficient being greater in comparation with that of the other species. The variability coefficients have the following values: the length of the roots is 36.44 in the polluted zone and 26.25 in the non polluted zone; the diameter of the inflorescence is 43.20 in the polluted zone and 33.04 in the non polluted zone. The main factors of pollution in Sinteza area are: the presence of acids rain, air speed, soil pollution and residual waters.

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XX INTERNATIONAL CONGRESS OF GENETICS *GENETICS – UNDERSTANDING LIVING SYSTEMS* BERLIN, GERMANY, JULY 12-17, 2008

Corneanu C.G[1]., Corneanu Mihaela[2]

KEY WORDS: XX International Congress of Genetics, Berlin

ABSTRACT

*The **XX International Congress of Genetics**, with device **Genetics-Understanding Living Systems**, took place in Berlin in the period 12-17 July 2008. At this remarkable scientific event, attended over 2500 scientist from all over the world, 6 Nobel laureate, and over 280 of the world's most prominent genetics. In one Keynote Symposium, 9 Plenary Lecture, 54 Concurrent Symposia, 2 Poster Sessions and 2 Workshops, were presented over 2000 papers, which illustrate the genetics development at the level of 2008 year. From Romania, attended 10 specialists with 26 papers.*

The capital Berlin, harbor in the period 12-17 July 2008, a remarkable event, The **XX International Congress of Genetics** with device ***Genetics – Understanding Living Systems***. This Congress took place at five years, under auspices of **International Genetics federation** (***IGF***). This Congress dated from 1899 year, first edition being entitled ***International Conference on Hybridisation and Cross-Breeding of Varieties*** (London), and the second (New York, USA), ***International Conference on Plant Breeding and Hybridization*** (New York, 1902), the name of ***International Conference of Genetics*** (***Congress*** from 1927, Berlin), being received at the third edition (London, 1906), at the proposal of William Bateson.

The XX ICG was organized by ***German Society of Genetics*** (***GfG***) in partnership with editorial group ***Nature / Genetics***. ***Congress President*** was Prof. dr. **Rudi Balling**, (Braunschweig, Germany, President of the VBIO Society), and ***Honorary Presidents***, **Chrystiane Nüsslein-Volhard** (Nobel Laureate, Tübingen, Germany), **Tomoko Ohta** (Mishima, Japan) and **Rudolf Jaenisch** (Cambridge, USA).

International Program Committee (**IPC**), which selected the presented papers, was formed from 64 scientific personalities from 17 countries (Australia, Brazil, Canada, China, France, Germany, Great Britain, Holland, Ireland, Japan, Mexico, Singapore, South Korea, Sweden, Switzerland, Taiwan, USA), being co-ordinate by two **Co-Chairs**, respectively, **Rudi Balling** (Braunschweig, Germany) and **Charles H. Langley** (Davis, USA).

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[2] University of Agricultural Sciences and Veterinary Medicine *Banatul* Timisoara, Genetic Engineering Dept., Faculty of Horticulture and Forestry

Sponsors, depending of their contribution, were distributed in five groups: ***Gold sponsor***, Roche society; ***Silver sponsors***: Applied Biosystems and Illumina; ***General sponsors***: Affymetrix, Agilent Technologies, Boehringer Ingelheim, Elsevier, Helicos, hp Invent, Sequenom; ***Special sponsor***: Science AAAS, and ***non-corporate sponsors***.

The XX International Genetic Congress took place in the International Congress Center (ICC) from Berlin. "The ICC Berlin ranks among the biggest, most advanced and most successful congress centers in the world. With its 80 halls and rooms seating between 20 and 9.100, its versatile facilities, superb technical installations and comprehensive range of services, the ICC Berlin" was perfect choice for the XX ICG. At this Congress attended over 2500 specialists from all over the world. From Romania, attended 10 genetists from some Universities and research institutes from Bucuresti, Craiova, Iasi, Timisoara and Otopeni.

Photo 1. International Congress Center (ICC) from Berlin.

The opening ceremony of XX ICG, took place in the afternoon of July 12 2008, the welcome speeches being addressed by Prof. **Rudi Balling** (Congress President), Prof. **John W. Drake** (North Carolina, USA; President of the International Genetics Federation) and Prof. **Alfred Nordheim** (Tübingen, Germany; President of the German Society Genetics). **Opening Lecture**, ***Decisive Steps to a Global Research Area***, was presented by Prof. Dr. **E.-L.Winnacker (ERC)**.

In the ***Welcoming Message***, Prof. dr. **Rudi Balling** and Prof. dr. **Alfred Nordheim**, underlined that "The ICG in Berlin will present the latest genetic and genomic insights in one Keynote Symposium, 9 Plenary Lectures and 54 Concurrent Symposia, 280 of the world's most prominent genetics researches will speak", as well as 6 Nobel Laureate. Also, **Klaus Wowereit**, Governing Mayor of Berlin, address to all participants a ***Welcoming Message***, underlined that the latest Congress has taken place in Berlin before, in 1927 year.

Photo 2. Prof. Rudi Balling - Congress President

Photo 3. Prof. Alfred Nordheim - President of German Genetics Society

Photo 4. Prof. Nüsslein-Volhard Christiane

Photo 5. Prof. Smithies Oliver.

In the inaugural symposium, **Keynote Symposium** ***Mutating Genomes***, three Nobel laureates presented personal contributions to genetics development and research direction for which they were awarded with the Nobel Prize:
- **Nüsslein-Volhard Christiane** (Nobel Laureate, Max-Planck-Institut für Entwicklungs-Biologie, Tübingen, Germany), ***Gene Mutations: A historical perspective;***
- **Smithies Oliver** (Nobel Laureate, University of North Carolina at Chapel Hill, USA), ***From Gels to Genes***;
- **Capecchi Mario** (Nobel Laureate, University of Utah, Howard Hughes Medical Institute, Salt Lake City, USA), ***Modeling Human Disease in the Mouse: from cancer to neuropsychiatric disease***.

Photo 6. Prof. Capecchi Mario.

In 2007, **Smithies Oliver, Capecchi Mario and Evans Martin** were awarded with Nobel Prize, for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells.

Photo 7. The Conference Hall

In the next days, were presented **9 Plenary Lecture**, took place **54 Concurent Symposia** (every with 8-10 oral papers oral), **two Poster Sessions** in which were presented and discussed 1368 papers, and **two Workshops**.

The authors were divided in three groups: (a) *Invited Speakers* – 440; (b) *Workshops Authors* – 30; (c) *Authors for oral and poster presentations* – over 2000 specialists.

Photo 8. Prof. Jaenisch B.

Photo 9. Prof. Blackburn E.H.

In the each of the **9 Plenary Lectures** (five days, one or two per day, time of one hour each), the well known specialists presented the latest novelty, research directions and perspectives in different branches of genetics. These were:

- Jaenisch B. (Gruber Laureate, Whitehead Institute for Biomedical Research, Department of Biology, M.I.T. Cambridge, USA), ***Stem cells, pluripotency and nuclear reprogramming.***

- Spradling C. Allan (Carnegie Institution of Washington, USA), ***Regulation of Drosophila stem cells by tissue niches*** (the **Gruber Genetics Prize**, in value of $ 500,000, offered annual by Peter and Patricia Gruber Foundation, represent a correspondent of the Nobel Prize for Genetics).

- Blackburn H. Elisabeth (Gruber Laureate, University of California, San Francisco, USA), ***Telomeres and telomerase: their implication in human health and disease.***

- Lander E.S. (Broad Institute of Harvard and MIT, Cambridge, USA), ***Reading to human genome: implications for biology and medicine***.

- Sharp A. Phillips (Nobel Laureate, MIT, Koch Institute, Cambridge, USA), ***Gene regulation by small RNAs***.

- Pääbo Svante (Max Planck Institute for Evolutionary Anthropology Leipzig, Germany) and his co-workers (Green R.E., Maričić T., Krause J., Briggs A., Kelso J., Pruefer K., Stenzu U., Visagie J., Affourtit J., Simons J.F., Du L., Knight J., Egholm M., Rothberg J., Brajković D., Gušić I., Rudan P., Kučan Z., Reich D., Patterson N., Mullikin J., Mallaspinas S.-A., Johnson P., Slatkin M., ***Neanderthal Genomics***.

- Axel Richard (Nobel Laureate, Columbia University, Howard Hughes Medical Institute, USA), ***A molecular logic of olfactory perception***.

- Ohsumi Y. (National Institute for Basic Biology, Okazaki, Japan), ***Autophagy in yeast – Molecular dissection of autophagy in yeast***.

- Kremer A., (INRA, Cestas, France), ***Ecological, genetics and environmental changes: are forest tree III-equipped to face climatic trends?***

Photo 10. Prof. Sharp A. Phillips

Photo 11. Prof. Pääbo Svante

The **54 Concurrent Symposia** (with 5-8 papers each, in total 269 papers), covered the all branches and researches direction from contemporary genetics, being moderates by valuable personalities from genetics, inclusive by Nobel Laureate (**Prof. Eric F. Wieschaus** (Nobel Laureate, Princeton University, USA). Some of these topics were: ***Epigenetics, Mechanisms & Chromatin; Agricultural Applications; Quantitative and Statistical Genetics; Genetic Model Organisms: Discovery to Translation; Epigenetics, Development; Biotechnology; Microbial Genetics; Comparative Genomics; Populations Genetic; Biodiversity and Adaptation; Pharmacogenomics; Genetics of Sex; Plant Genetics; Cancer Genetics; Evolution / Speciation; Genome-Environment Interactions / Ecology; Societal, Ethical, Legal Issues of Genetics (SELIG); Human genetics; Veterinary Genetics; Stem Cells; Evolution of Development (Evo-Devo); Evolution of Humans; Neurogenetics; Immunogenetics; Genetics of Symbiosis; Evolutionary Genomics; Genetics of Fungi; Omics: Genomics, Transcriptomics, Proteomics, Metabolomics; Genetics of Cell Biology; Metagenomics; Mutation / DNA Repair / Recombination; Genetics of Parasitism; Mammalian Genetics***, a.o.

In **two Poster Sessions**, time of four days, were presented 1368 papers, grouped on the 54 topics of the Concurrent Symposia.

The specialists group from Romania, belonging to Universities and Research Institutes, presented an oral paper at **Concurrent Symposia:** ***Genome-Environment Interaction/Ecology*** as well as 25 poster papers, in 13 sections of the **XX ICG**: ***Agricultural Applications***, ***Biodiversity & Adaptation***, ***Cancer Genetics***, ***Epigenetic Mechanisms & Chromatin***, ***Epigenetics & Development***, ***Human Genetics***, ***Plant***

Genetics, ***Teaching Genetics***, ***Evolution of Humans***; ***Mutation / DNA Repair / Recombination***, ***Complex Traits***, ***Comparative Genomics*** and ***Human Genetics***. After affiliation of the first authors, these papers belonging to: Bucuresti (15 papers: UMF, Genetics Institute from University of Bucuresti and a Research Institute), Timisoara (6 papers: USAMVB and UMF), Iasi (2 papers: *A.I.Cuza* University), Craiova (1 paper, University of Craiova) and Otopeni (1 paper, Biotechnos S.A.).

The **two Workshops**, presented two different topics: **Workshop I** (5 papers), ***Functional Genomics: From Disease Genes to Protein Networks*** (5 papers) and **Workshop II** (4 papers), ***Plant Artificial Minichromosomes – Potential Vectors for Gene Transfer***.

Papers which shocks all assistance through their content and investigation perspectives, were presented by **Phillip A. Sharp** (*The small RNAs,* implication in the gene regulation), **Svante Pääbo** (*Neanderthal Genomics*, which reveal a high similarity between genome of Neanderthal Homo and actual Homo), **A. Kremer**, with an *Ecological Genetics* topic, or **Richard Axel**, which presented the molecular mechanisms of the olfactory perception in evolution, a.o. An **Abstract Book**, of 408 pages, reunites the abstract of the all papers presented at the XX International Congress of Genetics.

Photo 12. Debates with Prof. Paul Sharp

The next ICG will be take place in Singapore in the 2013 year.

Numerous companies, publishers, as well as none profit organizations were present at the XX ICG as exhibitors. From these, can be mentioned: *Agilent Technologies*, *Applied Biosystems*, *Bio-Rad Laboratories GmbH*, *BioGenes GmbH*, *Cambridge University Press*, *CLC Bio*, *Delphi Genetics*, *European Molecular Biology Organization*, *Genetics Society of Japan*, *Illumina Inc.*, *Invitrogen*, *Karger*, *Max Delbrük Center for Molecular Biology*, *MetaSystems GmbH*, *Promega*, *Texas Institute for Genomic Medicine*, *Wiley-Blackwell*, a.o. Also, was organized industry exhibition for different genetics branches.

The editorial house presented at XX ICG, put to specialists disposal scientific books with a substantial discount (*Blackwell*, *Cambridge Univ. Press*, *CSHL*, *Elsevier*, *Karger*, *Oxford Univ. Press*, *Springer*, a.o.), as well as numerous journals free of charge. Can be mentioned the following editorial house and journals: **AAAS** (*Science*): **Blackwell Pub.** (*Aeging Cell*, *Biological Journal*, *Botanical Journal*, *Cell Proliferation*, *International Journal of Immunogenetics*, *Journal of Evolutionary Biology*); **Cambridge Univ. Press** (*Plant Genetic Resources*); **Cell Press** (*Trends in Genetics*); **Karger** (*Cytogenetic and Genome Research*): **Nature Pub. Group** (*Nature*, *Nature Reviews – microRNA collection*, *Nature Genetics*, *Cancer Gene Therapy*); **Oxford Open** (*Behavioral Ecology*, *Molecular Biology and Evolution*), **Springer** (*Genetica*, *Molecular Genetics and Genomics*, *Theoretical and Applied Genetics*); **Royal Society Pub.** (*Biological Letters*, *Proceedings of the Royal Society*); **Whiley-VCH** (*American Journal of Medical Genetics*, *Proteomics*), a.o., as well as scientific synthesis offered by different societies as **Max Delbrück Center for Molecular Biology** (*Geneticists in Berlin-Buch*, *Research Rapport 2008*), **European Molecular Biology Organization** (*Stem Cell Research*), a.o.

Photo 13. Kaiser-Wilhelm-Gedächtnis-Kirche

Photo 14. Reichstag.

The social program included many actions, which refer to tours of about 3-7 hours each, with a different destination (*Berlin Underground*, *Jewish Life in Berlin*, *The New Berlin*, *Art and Architecture in Berlin*, *The Third Reich in Berlin*, *Potsdam*, *History around the Wall*, a.o.), or visit some from over 70 Museums from Berlin (*Ägiptisches Museum and Papyrussamlungen*, *Bodenmuseum*, *Deutscher Dom*, *Jüdisches Museum Berlin*, *Museum of Natural History*, *New National Gallery*, *Old National Gallery*, *Pergamonmuseum*, a.o.), as well as the famous *ZooGarten* (the oldest from Germany), *Reichstag*, *Brandenburger Tor*, *Unter den Linden*, *Alexanderplatz*, *Potsdamer Platz*, *Kaiser-Wilhelm-Gedächtnis-Kirche*, *Schloss Charlottenburg*, *Kurfürstendamm*, a.o.

Revived from the proper ruins from the Second World War similar to *Phoenix Bird*, the Berlin city remain in our memory through fabulous **XX International Congress of Genetics**, numerous hotels, restaurants, with varied menu from all over Germany and from all over the World (the Rogaki area is the better location), a remarkable system of urban transport, the amiability and hospitality of its inhabitants.

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RESEARCHES CONCERNING SOME FACTORS INFLUENCE ON CALLUS INDUCTION OF BLUEBERRY BUSH (*Vaccinium myrthilus*)

Danci M., Botău Dorica, Danci Oana[1]

KEY WORDS: callus, phitohormons, culture medium, explants

SUMMARY

Results regarding callus proliferation potential derived from two types of blueberry bush explants, using two culture media and two hormonal balances are presented in the following paper. The two culture media used in this study were Anderson and WPM, each type of culture having two hormonal balances variants. Foliar tissue fragments and stalk tissue fragments constituted the explants used in this experiment.

This study results proved that a great variability exists between the two types of explants regarding callus genesis depending on both the culture media and hormonal variants. The highest callus percentage was obtained on the hormonal variant containing 2 mg/l 2,4D and 1,5 mg/l KIN.

INTRODUCTION

Unlimited proliferation potential of plant somatic cell and its high information plasticity, that represent the base of *in vitro* totipotency express, permit tackling some researches themes for different plants in order to obtain cellular mass (callus). Callus can be used as source of secondary metabolites production and induction of some morphogenetic specific processes.

The callus represents a particular system constituted of a cells mass with a uniform histological structure. Callus cells genetic instability and the easy polyploids generation, in some culture conditions, make the callus culture a valuable biological material that permits obtaining plants lines with special qualities.

This paper presents results regarding callus proliferation potential generated of two blueberry bush explants on two basal media and two different hormonal balances.

MATERIAL AND METHOD

The biological material used in this experiment was constituted of different types of explants as stalk fragments of about 1 cm long and leaf fragments of about 1cm^2, excised of blueberry bush plants grown in the field. Sterilization of the biological material was realized in $HgCl_2$ 0,1% for 2 minutes followed by successive (5 times) washes in sterile distilled water.

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Two basal culture media have been used, as Anderson and WMP, and two hormonal balances (table 1).

Culture vessels were incubated in growth room at 23-25°C temperature, 70-80% humidity and 16/8 hours photoperiod.

Table 1

Culture media hormonal composition used to induce callus formation from different blueberry bush explants

Experimental step	Culture media	Phytohormons used mg/l			
		V_1		V_2	
		ANA	BAP	2,4D	KIN
Callus induction	Anderson	1,5	1	2	1,5
	WPM	1,5	1	2	1,5

RESULTS AND DISCUTIONS

Observations have been made at 14, 30 and 45 days post-inoculation. Initiation of callus formation was observed even at 14 days after explants inoculation. Both types of explants generated callus on both culture media and on both hormonal balances, statistical differences between all the experimental variants were not significant.

Differences between the experimental variants were observed only after 30 days of culture. Thus, callus formation capacity was proved to depend on the type of the explant and also on the basal culture media and hormonal balance (table 2).

Table 2

Blueberry bush callus induction frequency depending on the explants type, basal culture medium and hormonal balance at 30 and 45 days

Explants type	Basal culture media	Callus formation frequency (%)			
		30 days		45 days	
		V_1	V_2	V_1	V_2
Stalk fragments	Anderson	37,5	38,7	56,9	64,8
	WPM	32,2	35,0	47,7	52,1
Leaf fragments	Anderson	43,6	53,1	70,3	71,4
	WPM	34,5	41,7	51,0	59,3

In order to emphasize phytohormons nature and combination role in callus formation, different types of explants were used. Leaf fragments were taken from young and old leaves, from the base or the vertex of the leaf and also stalk fragments were taken from different parts of the plant. No significant differences were observed between the same types of explants indifferent of the part of the plant they were taken of. Significant

differences were registered between the same types of explants depending on the culture media and on the hormonal balance.

Data from figure 1 show that significant differences regarding callus generation capacity were registered between stalk fragments inoculated on the same culture medium, namely Anderson, especially after 45 days of inoculation.

Comparing the two basal media it was observed that culture medium Anderson assures a higher callus formation frequency on both hormonal variants indifferent of the period that culture was done (Fig.1). These remarks proved that basal medium Anderson can be used as optimum for blueberry bush stalk tissue culture.

Explants constituted of leaf fragments assured a higher callus induction percentage on the same basal culture media, namely Anderson, and on the hormonal variant containing 2 mg/l 2,4D and 1,5 mg/l KIN.

Highest callus formation frequency differences were registered between all the experimental variants after 30 days. 45 days old cultures showed lower differences regarding callus generation capacity of leaf fragments on both culture media and indifferent of the hormonal variant (Fig.2).

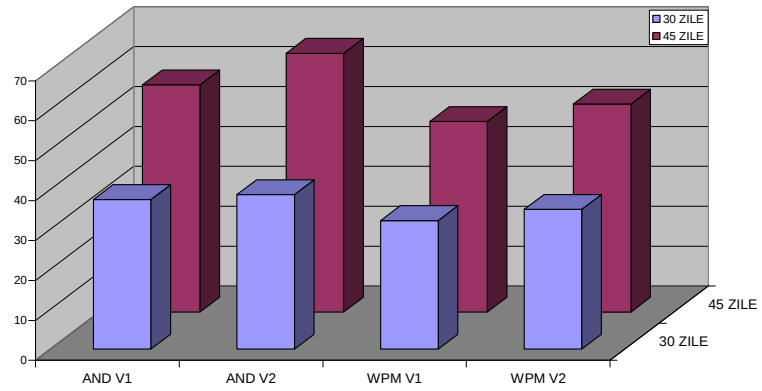

Fig. 1. Callus generation frequency on both culture media and both hormonal variants using explants constituted of stalk fragments.

Comparing the two types of explants taken into experimentation it was observed that a higher callus generation capacity was given by the explants constituted of leaf fragments on both culture media and both hormonal variants.

These observations leaded us to the conclusion that in callus generation capacity the most important role is played by the tissue type, foliar tissues are more suitable to *in vitro* culture and regeneration comparing with stalk tissues.

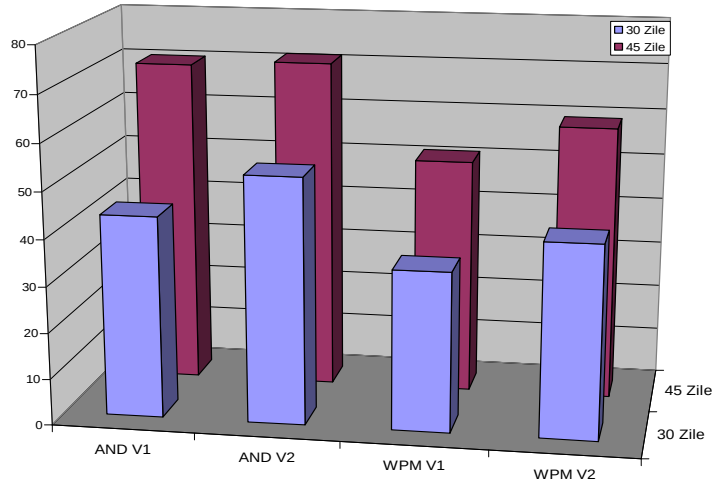

Fig. 2. Callus generation frequency on both culture media and both hormonal variants using explants constituted of leaf fragments

CONCLUSIONS

1. Callus generation capacity differs in a high manner in accordance with the explants types, basal culture media chemical composition and on the hormonal balance used.

2. A high influence in callus formation is given by the hormonal balancer used and regarding blueberry bush tissues the best callus generation frequency was obtained on media containing 2 mg/l 2,4D and 1,5 mg/l KIN.

3. In callus generation capacity the most important role is played by the tissue type, foliar tissues are more suitable to *in vitro* culture and regeneration comparing with stalk tissues, on both basal culture media and both hormonal variants used.

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THE LEAF BLADE TRICHOMES COMPARATIVE STUDY OF SOME *DATURA* AND *NICOTIANA* SPECIES (SOLANACEAE)

Rodica Bercu[1]

KEY WORDS: Datura, glandular hairs, histology, protective hairs, Nicotiana

ABSTRACT

The article comprises histological aspects of the protective and glandular hairs from the leaf blade of two species belonging to Datura and Nicotiana genus (Solanaceae) - Datura stramonium L. Datura suaveolens Humb. & Bonpl. ex Willd., Nicotiana alata Link. et Otto. and Nicotiana tabacum L. Some cytological elements are disscused. The paper reveals the main histological trichomes patterns concerning their type, structure, shape, size, the cells arrangement on the blade surface etc. as well. In literature an exclusively study concerning the blade hairs mostly of the ornamental plants almost lack, that is why we consider that any study of the foliar hairs is in need.

INTRODUCTION

One of the most important taxonomical criteria is the glandular or protective leaf's hairs. *Datura stramonium* is an erect annual herb, on average 30 to 150 cm tall with erect, forking and purple stems. The leaves are large. The white flower's corolla opens and closes at irregular intervals during the evening, earning the plant the nickname moonflower. *Datura suaveolens* Humb. & Bonpl. ex Willd. syn. *Brugmansia suaveolens* Bercht & K. Presl (angel's trumpet) is a large woody perennial shrub (mature high 5.00m).

The plant is cultivated for its very large attractive nocturnally fragrant with large trumpet-shaped white or red-pink flowers. It is also known as Night Queen (Bavaru 2005, Preda 1979).

The plant, such as the most *Datura* species contain alkaloids mostly hyoscyamine, atropine, scopolamine used in medicinal purpose (Weiner, 1980). *Nicotiana alata* is a species of tobacco called Winged Tobacco or Jasmine Tobacco. It is a perennial species mainly growing as an ornamental plant up to 0.6m by 0.3m. The cannular flowers are white enclosing during the day light (Chittendon 1951, Diaconescu 1970, Huxley 1992). *Nicotiana tabacum* is a perennial herbaceous plant growing to heights between 1 to 2m. The cannular flowers are usually pink. The histological study of the studied species epidermal structures - trichomes – contributes to the knowledge of their variety and implications in the plant life, in accordance to their role in the plants defence and metabolism.

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MATERIAL AND METHODS

The leaves were collected from mature plants of S. C. Iris International S.R.L. greenhouse, Constantza. The leaves trichomes used for microscopy were taken from the upper and lower blade surface using the classical methods used in vegetal histology (Bercu & Jianu 2003). The samples were colored with saphranin 1%. Observations were made with a BIOROM-T bright field microscope, equipped with a TOPICA-6001A video camera. The micrographs were obtained from the video camera through a computer. The drawings were obtained with a camera lucida attached to the same microscope.

RESULTS AND DISCUSSIONS

Datura stramonium leaves are large, 7 to 20cm long and have irregular teeth with a slightly sharp tip. The plant leaves are dark green on the upper surface and light green on the lower one.

Functionally, the trichomes of the both blade surfaces are protective and glandular. Few glandular hairs on the epidermal surface occur. Remarkable is the abundance of the protective trichomes. Structurally, the simple multicellular protective trichomes are taller than the glandular trichomes. The stalk possesses 4 or 5 cells placed in between the epidermal cells or stomata. The size of the rest stalk cells is reduced from the base to the tip. Remarkable is the last cell possessing a sharp tip (Fig. 1, A). The glandular trichomes are small and represented by one or two small cells (Fig. 1, B).

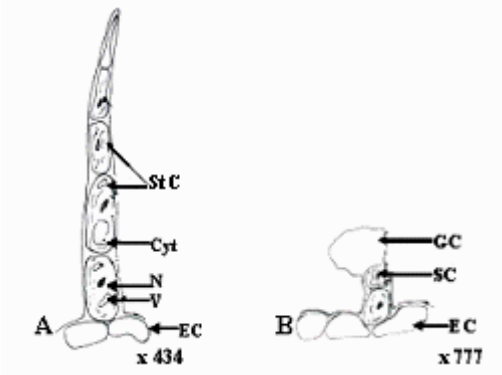

Fig. 1. Epidermal trichomes of the upper and lower epidermis of *Datura stramonium* L. blade. Protective trichome (A). Secretive trichome (B): Cyt- cytoplasm, EC- epidermal cell, GC- glandular cell, N- nucleus, SC- stalk's cells, V- vacuole (orig.).

D. suaveolens leaves are large. They are petiolate leaves, ovate-elliptical in shape with entire margin, slightly cuspidate and a sharp tip. In our country it is known as an ornamental plant. On both blade epidermis the mature simple protective hairs are silver-like, dense, with a perpendicular arrangement on the blade surface.

On both blade surface, the same functional type of trichomes such as *D. stramonium* Humb. & Bonpl. ex Willd possesses *D. suaveolens*. Few protective trichomes on the epidermal surface occur. Structurally, the protective trichomes are represented by three elongated small cells. The stalk size cells are reduced from the base ending in a sharp tip cell (Fig. 2, A). The simple multicellular glandular trichomes are taller than the protective hairs.

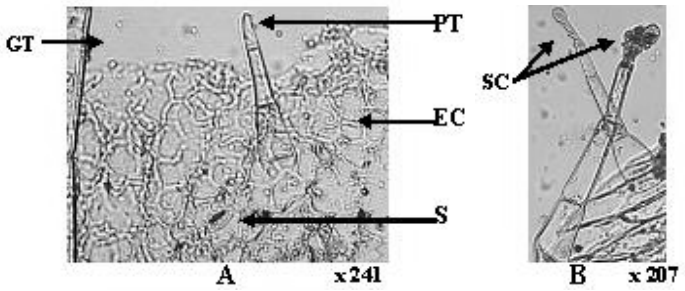

Fig. 2. Upper epidermis trichomes of *Datura suaveolens* Humb. & Bonpl. ex Willd blade. Protective hair (A). Secretive hair (B): EC- epidermal cell, GT- glandular trichome, PT- protective trichome, S- stoma, SC- secretive cells (orig.).

The stalk possesses 4 or 5 long cells. Remarkable is the voluminous basal cells placed in between the epidermal cells (Fig. 2, B; Fig. 3, A). On the lower epidermis, here and there, few glandular trichomes with a thin middle-stalked body are present (Fig. 3, B). Each cell contains a dense cytoplasm, nucleus and rare vacuoles. The glandular part of the trichomes is represented by secretive cells.

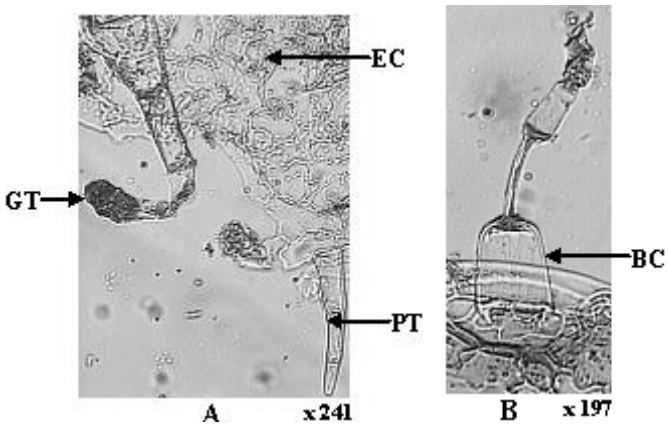

Fig. 3. Epidermal trichomes of the lower epidermis of *Datura suaveolens* Humb. & Bonpl. ex Willd blade. Protective hair (A). Secretive hair (B): BC- basal cell, EC- epidermal cells, GT- glandular trichome, PT- protective trichome (orig.).

N. alata obovate sessile green asymmetrical leaves are rough possessing undulate or slightly dentate margins and an acute apex. The leaves extend down and adnates to the stem – decurrent leaves.

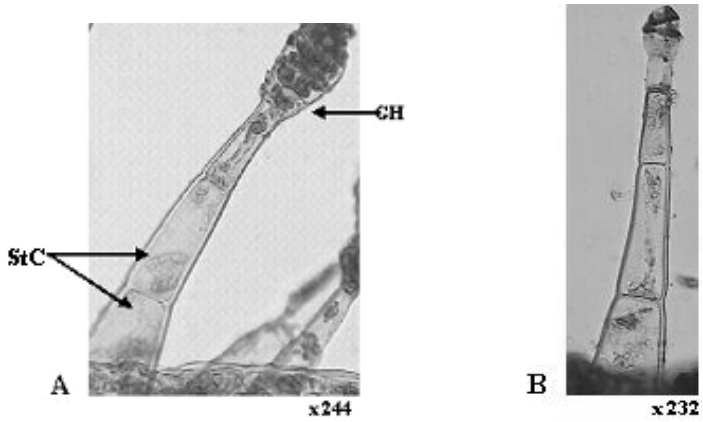

Fig. 4. Glandular trichomes of the upper epidermis of *Nicotiana alata* Link. et Otto balde (A, B): GH- glandular head, StC- stalk cells (orig.).

Structurally, the trichomes of both surfaces of the leaf blade are multicellular glandular hairs. However, the upper epidermis glandular cells are numerous then the lower one. The stalk consists of 3-4 cells. The basal cell is large almost equable in thickness followed by a thinner second cell whereas the third cell is smaller then the other. The tip is represented by a multicellular (Fig. 4, A; Fig. 5, A) or unicellular head (Fig. 4, B; Fig. 5, B) representing the glandular part of the trichome.

The glandular trichomes exhibit characteristics common to gland cells - a dense cytoplasm, the nucleus and little vacuolation. A dense secretory product is observed between the protoplast and the cell wall and within the intercellular spaces. In between the normal glandular cells may occur some glandular hairs with different configuration (Fig. 5, A, B).

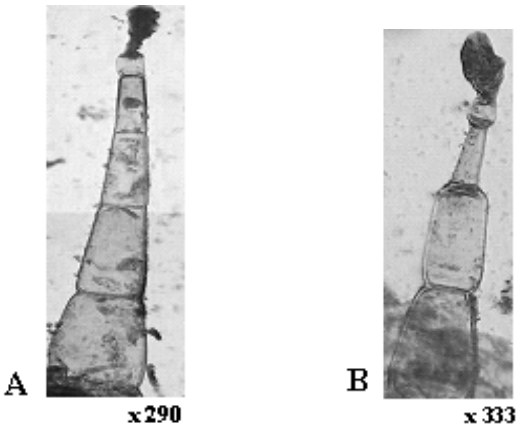

Fig. 5. Glandular trichomes of the lower epidermis of *Nicotiana alata* Link. et Otto blade (A, B) (orig.).

N. tabacum winged leaves are ovate or oblong-lanceolate shaped and most usually borne directly (sessile) on the main stem. The leaf surface has a matte appearance. The

leaves are antispasmodic, diuretic, emetic, expectorant, irritant, narcotic and sedative (Bavaru & Bercu 2002, Emboden 1979; Grieve 1984; Weiner 1980).

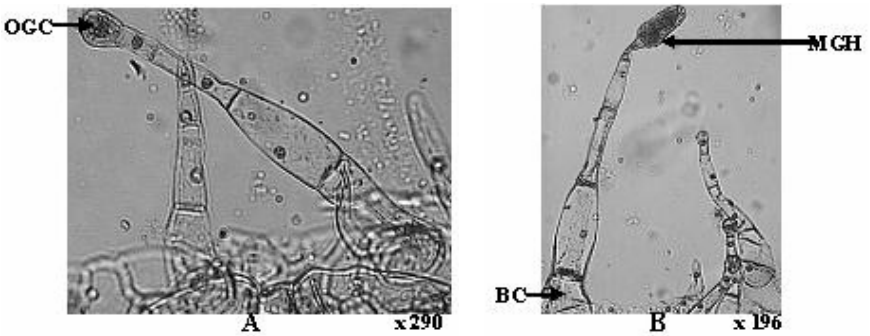

Fig. 6. Glandular trichomes of the upper epidermis of *Nicotiana tabacum* L. blade (A, B): BC- basal cell, OGC- one glandular head, MGH- multicellular glandular head (orig.).

Structurally, the tobacco leaf trichomes of the upper and lower epidermis are glandular (such as Akers et al., 1978 reported) and they are two different type tall trichomes and short trichomes.

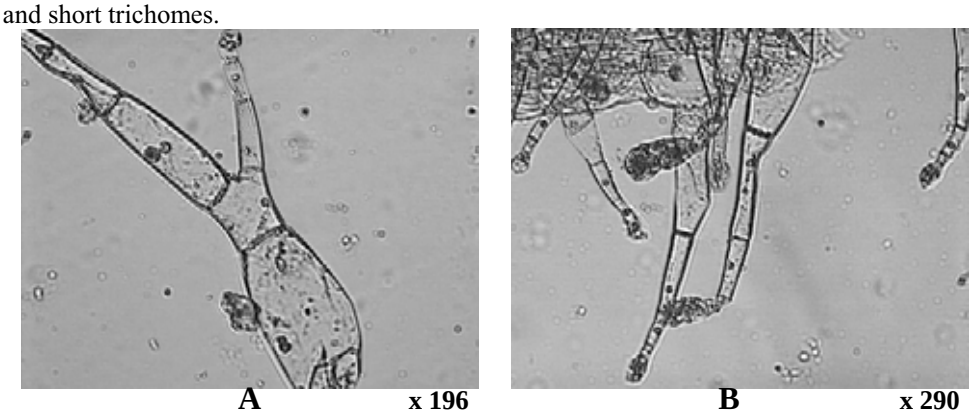

Fig. 7. *Nicotiana tabacum* L. Branched glandular trichomes of the upper epidermis (A). Glandular trichomes of the lower epidermis (B) (orig.).

The tall trichomes have a multicellular stalk represented by 4-5 cells different in size, with a multicellular glandular head, whereas the short trichomes stalk possesses 3-4 cells with a single glandular cell in the tip (Fig. 6, A, B; Fig. 7, B). The basal cell such as the following one is large and voluminous whereas the two cells above are elongated ending with a small one. The glandular head is represented by one or more secretive cells such as *N. alata*.. Here and there may be observed multicellular branched glandular trichomes (Fig. 7, A).

Both types of hairs consists a nucleus, a dense cytoplasm, little vacuolation. Akers et al. (1978) reported that the electron microscopy reveals that the tall trichome contains structurally well developed chloroplasts, an elaborate network of endoplasmic reticulum and numerous mitochondria. The short trichomes contain undifferentiated plastids. The same secretory product observed in *N. alata* glandular hairs may be seen between the protoplast and the cell wall and within the intercellular spaces.

CONCLUSIONS

The two *Datura* species possesses on both epidermis (upper and lower) protective and glandular trichomes. Structurally the protective hairs of *Datura stramonium*'s blade surface are multicellular and simple with a sharp tip whereas the glandular trichomes are very short with a large secretive cell in the tip. Comparatively, *Datura suaveolens* blade glandular trichomes are taller then the protective hairs.

Nicotiana alata and *N. tabacum* blade such as other Solanaceae species (*Hyoscianum niger*, *Atropa belladona* etc.) (Batanouny 1992, Palade 1998, Serbanescu-Jitariu 1975) possesses on upper and lower epidermis glandular trichomes of two types (tall and short hairs), different in size and shape cells. All secretory trichomes exhibit characteristics common to gland cells. The secretory product may be seen between the protoplast and the cell wall and within the intercellular spaces.

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ANATOMY OF THE AQUARIUM PLANT *APONOGETUM CRISPUS* THUNB. (APONOGETONACEAE)

Rodica Bercu[1]

KEY WORDS: anatomy, Aponogeton crispus, leaf, root

ABSTRACT

The article comprises investigation of the vegetative organs anatomy of an aquatic plant Aponogeton crispus *Thunb. This species belongs to Aponogetonaceae family, living submerged. In our country it is frequent known and cultivated as an aquarium plant Our purposes were to show some features of anatomical interest concerning the vegetative organs of this submerged plant, in accordance with it hydrophytic habit. The anatomical aspects of* Aponogeton crispus *'s adventitious root and leaf has been described and discussed.*

INTRODUCTION

Aponogeton crispus Thunb. (fam. Aponogetonaceae) known as Crinkled Aponogeton is a submerged aquatic plant and grows up to 25cm in size. The plant has a fibrous, cylindrical rhizome (2–3cm in diameter). The plant forms long and narrow lance shaped green to olive green-brown, (20-35cm) leaves. The leaves have a wavy margin and a petiole up to 45cm long. *Aponogeton crispus* will not grow any floating leaves. The flowers are produced on an erect stem up to 80cm tall with an apical white spike-like raceme up to 18 cm long (Allgayer & Teton 1987, Benl 1971, Long, 1976). The plant is native to Sri Lanka in southeastern Asia and is found naturally in still and running waters (Muhleberg 1982, Stodola 1967). *Aponogeton crispus* is one of the most robust Aponogetons and is often cultivated as an aquarium plant (Scheurmann & Stevenson 2000). In the literature a study into the anatomy of this species almost lack, excepting some taxonomic studies.

MATERIAL AND METHODS

The plant was collected form the faculty's laboratory aquarium. Small pieces of the adventitious root and leaf were fixed in F.A.A. (formalin:glaciar acetic acid:alcohol 5:5:90). Cross sections of the vegetative organs were performed using the classical technique used in vegetal anatomy studies (Bercu & Jianu 2003). The samples were stained with alum-carmine and iodine green. Histological observations and micrographs were performed with a BIOROM –T bright field microscope, equipped with a TOPICA 6001A video camera. The microphotographs were obtained from the video camera through a computer.

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RESULTS AND DISSCUSSION

Cross section of the root reveals that the epidermis, the outermost layer is composed of one layered cells. Hairs are absent. The cortex is well developed and rough differentiated into two zones. The outer cortex – exodermis - consists of one compactly arranged parenchymatous cell. The inner cortex is well developed and covers the major portion of the root. It consists of 7-8 layers of parenchymatous cells, enclosing large intercellular spaces. The cells are nearly spherical in shape and regularly arranged in concentric layers (Fig. 1 A). Characteristically, the endodermis is one-layered, possessing Casparian strips alternating, at places, with the pericycle cells.

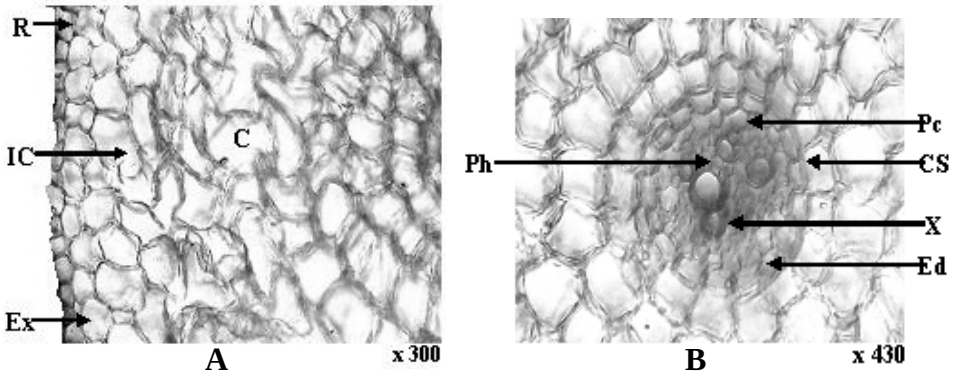
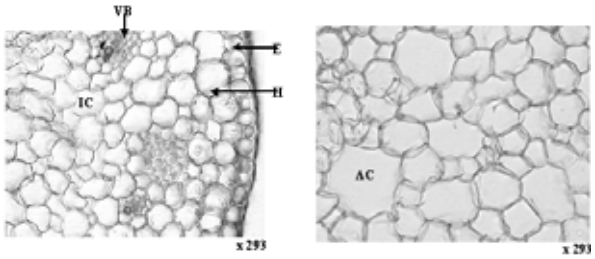


Fig. 1. Cross section of the root. Portion with epidermis and cortex (A). The stele (B): Ed- endodermis, Ex- exodermis, C- cortex, CS- casparian strip, IC- inner cortex, Pc- pericycle, Ph- phloem, R- rhizodermis, X- xylem (orig.).

The stele is enclosed by a single layered pericycle. The vascular system is radial type and contains xylem and phloem bundles. The xylem shows exarch condition, metaxylem towards the centre and protoxylem facing the periphery. Phloem is well developed and present among the xylem groups (Fig. 1 B).

Cross section of the petiole reveals a slightly undulated outline. Epidermis is one-layered with thin-walled cells. The cortex lies beneath the epidermis and is differentiated in two distinct zones. As other authors reported for the hydrophytic plants (Bavaru & Bercu 2002, Raven et al. 1992), the sub-epidermal region (hypodermis) is composed of thin walled compactly arranged parenchymatous cells, whereas the inner region is made up of symmetrically cortical arranged air spaces separated by thin multicellular partitions made up of a single layer of thin-walled cells (Fig. 2, A, B). Epidermis and hypodermis contain chloroplasts.

The stele, present in the basic tissue, is represented by 25 small collateral bundles with peripheral arrangement and centrally a larger one occurs. Each vascular bundle is formed by few xylem and phloem elements. The large central vascular bundle consists of few metaxylem vessels and a protoxylem lacuna. Phloem is well developed (sieve vessels, companion cells and few phloem parenchyma cells). The endodermis and pericycle are absent and the protection of the vascular tissue is afforded by parenchymatous cells surrounding them (Fig. 3).



A B

Fig. 2. Cross sections of the petiole. Portion with epidermis, cortex and aerenchyma (A). Middle portion with air chambers (B): AC- air chamber, E- epidermis, H- hypodermis, IC- inner cortex (orig.).

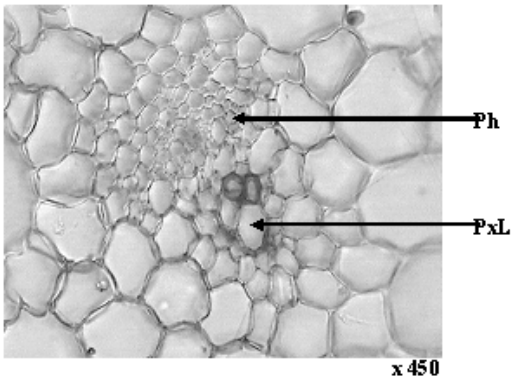


Fig. 3. Cross sections of the petiole. The large vascular bundle (C): Ph- phloem, PxL- protoxylem lacuna (orig.).

A transversal section through the blade exhibits the usually succession of tissues. The upper epidermis such as the lower one contains a single layer of thin-walled cells. The lower epidermis forms an arch below the large veins. As other underwater plants species blade (Arber 1963, Batanouny 1992, Bercu, 2007), the mesophyll is undifferentiated consisting large thin walled-cells interrupted by two large air chambers placed both sides of each large vein and homogenous to the margins (Fig. 4, A). The mesophyll cells, placed just below the upper epidermis, consists abundant chloroplasts whereas the rest layers of cells few.

The vascular system of a large vein is represented by few xylem and few phloem elements. Xylem is placed to the upper epidermis and phloem to the lower one. The vascular bundle is unprotected by a parenchymatous sheath (Fig. 4 B).

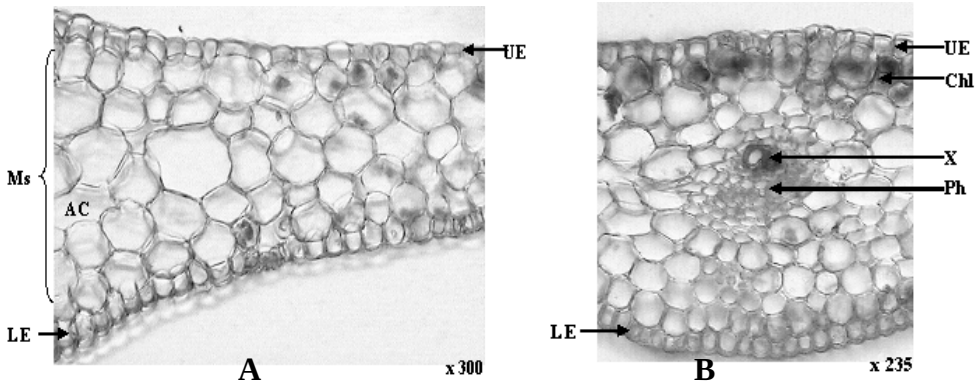


Fig. 4. Cross section of the blade. Portion with mesophyll (A). A vein vascular bundle (B): AC- air chamber, Chl- chloroplasts, LE- lower epidermis, Ms- mesophyll, Ph- phloem, UE- upper epidermis (orig).

CONCLUSIONS

Results indicate that the root of *Aponogeton crispus* possesses a typical dicots primary structure. However, the cortex is well-developed, containing a large number of parenchymatous cells with large intercellular spaces. The leaf petiole epidermis is thin-walled, lacking cuticle. The cortex possesses air chambers and a number of small close collateral vascular bundles embedded in the aquatic parenchyma tissue. Phloem is normal, located in the outer region of the bundle, whereas xylem frequently consist a large protoxylem lacuna. Remarkable are the mesophyll two air chambers placed near by the veins. Among the veins and to the margins, the mesophyll is homogenous. Mechanical tissues are absent. The histo-anatomical features of the plant organs are in accordance with it hydrophytic nature.

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ABOUT SOME LOW ALTITUDE POPULATIONS OF *SALAMANDRA SALAMANDRA* AND *RANA TEMPORARIA* FROM THE PRUNISOR-IGNESTI AREA, ARAD COUNTY, ROMANIA

Covaciu-Marcov Severus-Daniel[1], Cicort-Lucaciu Alfred-Ştefan[2], Balint (Szeibel) Noemi[1], Szabo Attila-Levente[1], Lazăr Olivia[1]

KEY WORDS: Salamandra salamandra, Rana temporaria, low altitude

ABSTRACT

Salamandra salamandra and Rana temporaria are two amphibian species found in Romania in hilly and mountain regions, at altitudes of more than 200 m asl. We have identified populations of these two species to as little as 135 m asl. in the Prunisor-Ignesti area from Arad County. The two species occur in wet habitats, being present near the streams that cross the forests in the region. These results increase the particularities of the herpetofauna from this area, where many species come down to lower altitudes than in other areas from the country.

INTRODUCTION

In Romania, *Salamandra salamandra* and *Rana temporaria* are two species characteristic to the hills and mountains, being generally found at altitudes over 200 m (Fuhn 1960, Cogălniceanu et al 2000, Iftime 2005). The situation also applies to the western part of the country where the two species are usually found together, at the same altitudes and in the same humid and afforested habitats (Covaciu-Marcov et al 2000, 2002, 2003 a, b, c, 2004, 2005 a, b, 2006 a, b, 2007 a).

These correspond with the ecological needs of the two species (Fuhn 1960, Cogălniceanu et al 2000, Iftime 2005, Veith 1997), but also with their history because *Salamandra salamandra* is a species that entered Romania in the postglacial, together with the forests while *Rana temporaria* is considered a glacial resident here (Stugren 1957). However, recent work has indicated both species at more reduced altitudes in western Romania, having been pointed at 150 m asl. in Livada, Satu-Mare County, a fact that was linked with the cold and humid climate of the region (Covaciu-Marcov et al 2007 b, 2008). The present study signals new populations of these two species at lower altitudes than previously known, but in a area with a warmer climate, meaning Arad County.

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[2] PhD candidate in Biology, Babeş – Bolyai University, Faculty of Biology and Geology

MATERIAL AND METHODS

Our study took place in spring 2007, between the months of March and May.

We've made different investigations on the herpetofauna in the region, mainly regarding the biology and ecology of some newt species, the results about the occurrence of the two species at low altitudes having not been planned initially.

Afterwards, after finding the firsts individuals at these low altitudes, we've undergone explicit researches for this matter. We've used the transects method (Cogălniceanu 1997), making numerous transects in and around the forests from near Prunisor and Ignesti. We've observed and counted both adults and larvae in the case of the salamanders, using the direct observation method (Brown 1997). Some of the samples were captured and photographed, being afterwards set free in their habitat of origin.

Our research area is situated in the central-northern part of Arad County, near Sebis, close to the Teuz' River superior course (Tufescu 1986). The region is located at the limit between the Sebis Depression and the Teuz Hills, the latter ones being found to the south-west of the Codru Moma Massive (Posea & Badea 1984). The Sebis Depression represents an appendix of flat plain that broke through alongside the Teuz and the Crisul Alb Rivers.

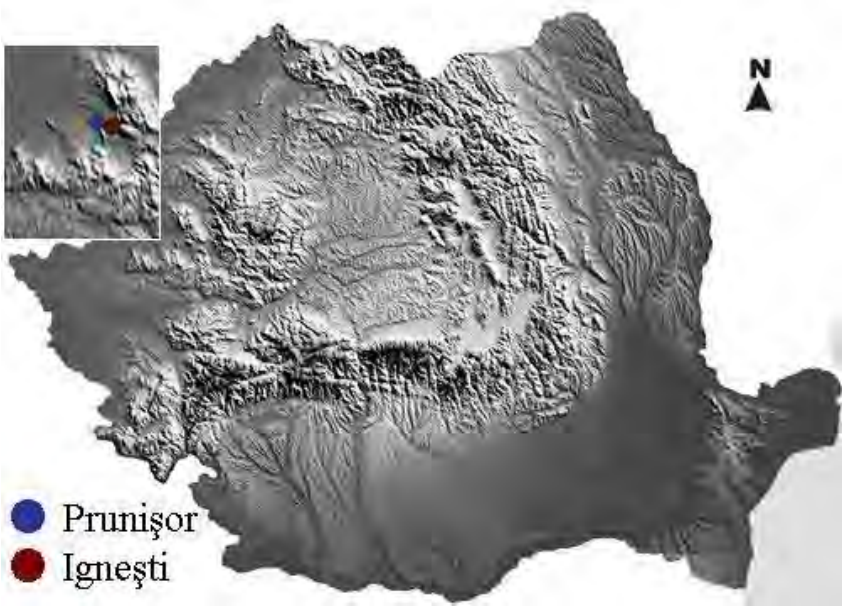

Photo no. 1. Geographical position of the studied localities

RESULTS

In the area of the Prunisor and Ignesti localities from Arad County, *Salamandra salamandra* and *Rana temporaria* populations are present at lower altitudes than previously indicated for Romania. This is how *Salamandra salamandra* was encountered at 140 m asl.,

while *Rana temporaria* at 135 m asl. These are, until now, the lowest altitudes at which the two species have been observed, a least for western Romania.

The two species occur together, populating the same habitats. These are represented by humid areas from inside the forests of the region, especially near some streams that cross the woods. The forests here are vast and linked together, consisting mainly of Quercus species, and more accurately *Quercus sesilliflora*. (Stoenescu et al 1966). In these biotopes, alongside these two species, one can also frequently find the agile from (*Rana dalmatina*), *Bombina variagata* or *Bufo bufo*, expectable elements in this area.

The larvae of the salamanders are mainly present in the streams that cross the forest, near the habitats populated by the adults, a fact that corresponds with the needs of the species (Veith 1997). With all that, we've observed larvae in temporary puddles located in open areas but close to the forest, including some puddles used by the local pigs as drinking water.

Rana temporaria is mating in a system of ponds situated at only 135 m asl., at about 20 meters from the forest, in teh side facing the plain. The habitat consists of a system of larger ponds that continues with a marshland. The two puddles located right near the forests are permanent, having their own stream, while the other ones dry out during the summer. The depth of the water reaches over 80 cm and the substratum is made of mud. The puddles closer to the forest have an abundant aquatic vegetation, represented both by algae and cormophitas, which sometimes cover entirely the surface of the water. Between the ponds and the forest, there is a road. In two of the system's ponds, the ones closest to the forest, we noticed *Rana temporaria* spawn on the 10th of March 2007. One of the ponds contained a large gathering of about 27 spawn, while the other one a smaller one, consisting of about 8 independent spawn. Simultaneously, in the water there were more than 100 *Rana dalmatina* spawn. During that time, we also found two *Rana temporaria* males in the water, indicated the end of the breeding period.

DISCUSSIONS

The finding of the two species in the area at such low altitudes raises some questions. This is especially because in this region, some other species like *Messotriton alpestris* and *Vipera berus* bond in Romania with much higher altitudes come down here to very low ones (Covaciu-Marcov et al 2006 a). Further more, the fact that these species are found at these altitudes here, in Arad County – their southern limit of distribution for western Romania – modifies the previous points of view regarding the descent of the mountain species in depressionary wet and cold areas (Micluță 1970, Covaciu-Marcov et al 2003 a, 2004, 2007 a, b). Thus, the Prunisor-Ignesti region from Arad Couty benefits from warmer temperatures (Stoenescu et al 1966, Mândruț 2006) than the regions from the Western Plain in which the species were pointed out at low altitudes before. In this area, Rana temporaria comes down to 135 m asl., but in Transylvania, a region also with lower average temperatures, it's only present at 400 m asl. or more (Ghira et al 2002). This is how the present result are joined with the other particularities of the herpetofauna from the superior course of the Teuz River hydrograph basin (Covaciu-Marcov et al 2006 a).

The explanation behind the presence of these species at such low altitudes can not be given by the actual climate but by the history and evolution of this fauna group in this region, a group found here under special conditions. Thus, these results put together with our previous ones from the area, suggest that this place was a refuge in the last glacial period for these species tied to a colder and more humid climate. Such a possibility was also indicated for the low altitude populations from the forest from Livada (Covaciu-

Marcov et al 2007 b). The Prunisor-Ignesti area is positioned to the south, being sheltered to the north-east by the Codru-Moma Mountains, with peaks exceeding 1000 m (Tufescu 1986). These points of view expressed here are sustained by recent studies that demonstrate that the Pannonian Basin contained the most nordic forest in the last glacial maximum (Ravazzi 2002). The existence of a northern refuge in the Carpathian Basin was recently indicated for several species (Wallis & Arntzen 1989, Ursenbacher et al 2006). Further more, it was argued that the glacial refuges were not uniform but in fact devised in more distinct sub-refuges or even refuges inside refuges (Gomez & Lunt 2006). As a conclusion, we can accept that in the last glacial peak, in the forests from the nowadays western Romania, several independent regions were present, in which due to some microclimate differences these species have survived. Thus, the low altitude populations are relicts, a fact suggested by the differences of the inferior limits of their areal in different regions of Romania.

Despite their location at the southern limit of their areal, the populations of the two species appear to be rather large. Thus, in the end of April 2007, on a 500 m transect alongside a stream from the forest, we managed to count 25 *Salamandra salamandra* and 33 de *Rana temporaria* adults. In the case of the salamanders, the results are similar with the ones recorded for the low altitude population from Livada (Covaciu-Marcov et al 2007 b), but the populations are smaller than the ones indicated at altitudes of about 400 m asl. in Hungary, where on favorable transects about 90 specimens could be counted on a stretch of 1 km (Puky et al 2005).

The low altitude populations of the two species from the Prunisor - Ignesti area are not under major threat. The main dangers for these populations are represented by clearings of the forests, grazing and traffic, the first two affecting both species while the latter only the salamanders. Right near or around the habitat there aren't any massive clearings, but at some kilometers away, organized exploitations take place. Grazing affects all the areas around the forest, large herds of pigs living near Ignesti. Their impact is not so much on the adults, but on their larvae, big mammals consuming the small ones while drinking the water or pigs killing them while bathing in the puddles. We also found dead salamander bodies on the sector of the roads that passes through the forest at Ignesti, on rainy days from April and May. Luckily, the road has rather little traffic, also limited by speed because of the bad status of the asphalt and so we only found 3 salamander bodies.

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NEW CONTRIBUTIONS TO THE STUDY OF THE GEOGRAPHIC DISTRIBUTION OF THE HERPETOFAUNA OF THE SOUTH-WEST DOBRUDJA, ROMANIA

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KEY WORDS: Herpetofauna, geographic distribution, Dobrudja

ABSTRACT

This present paper comes as an addition to the paper we published by us in 2006 (Covaciu-Marcov et al 2006) and intends to indicate new localities for the geographic distribution of the herpetofauna from the south-west Dobrudja. The research was carried out during 2007 and 2008 and this paper contains only the new data from that period. Thus, we amalyzed 14 localities in which we found 6 amphibian species: Bombina bombina, Hyla arborea, Pelobates fuscus, Bufo viridis, Rana ridibunda, Rana dalmatina and 11 reptile species: : Emys orbicularis, Testudo graeca, Ablepharus kitaibelli, Lacerta viridis, Podarcis taurica, Podarcis muralis, Darevskia praticola, Natrix natrix, Natrix tessellata, Dolichophis caspius, Vipera ammodytes. The most important signalation is that of the new locality for Darevskia praticola, the rarest lizard species from the south-west Dobrudja.

INTRODUCTION

Dobrudja is a region of Romania that contains a rich and special herpetofauna, from all the following points of view: number of species, as ecological needs and as history, in comparison to other regions of the country (Fuhn & Vancea 1961, Iftime 2005, Covaciu-Marcov et al 2006). This fact is a consequence of the higher thermal regime than others from Romania (Stoenescu et al 1966), but also of the location of the Danube, right to the north of this region, so that it would not consist in a natural barrier for the southern species. This is how there were a lot of studies referring to the herpetofauna made for this particular region of our country (Andrei 2002, Băcescu 1934, Fuhn 1952, Fuhn & Hârsu 1962, Iana 1970, Kotenko 1993 a, b, 2001, Oțel 1998, Popescu 1977, Torok 1997, 1998 1999).

However, nowadays most of Dobrudja is degraded and affected by human activities and this is why we managed to identify only two relatively natural areas in this entire region: the northern part of Dobrudja, with the Babadag Plateau and Macin Hills and the southern part, comprising the Oltina Plateau – the area with the most special herpetofauna (Covaciu-Marcov et al 2006). This fact is also indicated by explicit studies for this area (Andrei 2002, Iftime 2002, Sos et al 2008).

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We've recently made a vast study regarding Dobrudja, as well (Covaciu-Marcov et al 2006). However, it only ofers a general view, due to the vast territory of Dobrudja and to the diversity of its herpetofauna. As such, in the last 2 years, the investigations in the south-western part of the region have brought new data that complete the former ones. Therefore, this article aims to present the obtained results in this time frame, contributing to the forming of a clearer image upon Dobrudja's herpetofauna.

MATERIAL AND METHODS

The study took place in 2007 and 2008. In each year we made a field trip in spring, in April, and one in the summer, in August, each trip comprising 2-3 days of field work. Almost all localities from the south-western Dobrudja were investigated, but this present paper will only present the new data – the one not found in the 2006 paper – and therefore it will consist of only 14 localities. The analyzed are comprises the triangle formed between the border with Bulgaria to the south, the Danube to the north and an imaginary line to the east, somewhere between the town of Cernavoda and Negru Voda. From the geographic point of view, the area overlaps the Oltina Plateau(Mândruț 2006).

We used the transects method (Cogalniceanu 1997), making numerous surveys in each investigated locality. The animals were determined mostly directly, without the necessity of capturing them. When the capture of some specimens was compulsory, it was usually made by hand. After determining the captured species, they were set free in their habitats of origin. An important role in the charting of the herpetofauna of the investigated region was played by the dead animals that we found, killed either by cars or by local people.

RESULTS AND DISCUSSIONS

In the researched are, we identified a total of 17 species for the herpetofauna. Among these, 6 belong to the amphibians (*Bombina bombina, Hyla arborea, Pelobates fuscus, Bufo viridis, Rana ridibunda, Rana dalmatina*) and 11 to the reptiles (*Emys orbicularis, Testudo graeca, Ablepharus kitaibelli, Lacerta viridis, Podarcis taurica, Podarcis muralis, Darevskia praticola, Natrix natrix, Natrix tessellata, Dolichophis caspius, Vipera ammodytes*). We analyzed the distribution of these 17 species of the herpetofauna in all 14 localities from the studied region. Consequently, we managed to identify 41 localities for the encountered species in the 14 field localities (Appendix 1).

Bombina bombina is relatively rare in the studied region being indicated in the present paper in only two locations, new compared to the previous study. The species is present in both large brackish water, like Iortmac Lake, and in streams with a relatively fast flow and low water debit, in which it was observed near Peştera Sfântului Apostol Andrei Monastery.

Hyla arborea is distributed only in the afforested areas, according to its ecological needs (Fuhn 1960). Aside for our previous data, we managed to indicate this species for the forests near Iortmac Lake.

Pelobates fuscus was identified in a new locality, aside the ones from our previous study Covaciu-Marcov et al 2006), also at Iortmac Lake. This signalation is very important because here the species is found inside the Oltina Plateau, while all the previous points were right against the Danube. However, although Iortmac Lake is located at about 10 km inside the Dobrudjan platform, it is directly tied to the Danube. The areas around the lake

have a favorable soil for the spadefoot toads, the species being present around it and probably around the canals that link it to the Danube. On the roads near the lake we unfortunately found numerous specimens killed by the traffic.

Bufo viridis is the most common amphibian species in Dobrudja (Covaciu-Marcov et al 2006), a statement valid for this area, too, where we saw the species in many lew locations.

Rana ridibunda is rarer due to the scarce supply of permanent waters. Presently, we are signaling the species in two new localities, occurring alongside some quasi-permanent streams of Iortmac Lake, where numerous samples were seen.

Rana dalmatina is a species bond with forests and therefore rare in Dobrogea, only recently indicated for the south-western part (Andrei 2002). The two now points for this species are found in forests, too, but the agile frog isn't well represented here.

Emys orbicularis is signaled as a premiere in comparison to our previous data (Covaciu-Marcov et al 2006). As expected, the largest populations are present near the Danube, where the species has been previously mentioned (Iiftime 2005). However, the water terrapin reaches inside the Oltina Plateau, being observed in large and permanent water accumulations from Iortmac Lake. Further more, the species even reaches the farther inside the Dobrudjan platform, all the way to Dobromir, near the border with Bulgaria. Here, *Emys orbicularis* occurs in a permanent stream but with little water flow. The riverbed of the stream is about 0.5 m wide, but forms several larger permanent ponds (tens of meters wide) between Dobromir and Dobromiru de Deal. These represent favorable habitats for the terrapin, but also for macrozoobentic invertebrate communities which are well represented here, indicating well oxygenated waters (Hercuț et al 2008). In spring, we identified several *Emys orbicularis* bodies, mostly juveniles, including at Dobromir, a fact that indicates large populations but also the massive anthropogenic stress of the species.

Testudo graeca is a typical species for Dobrudja, being pointed out for many southern localities in the past, too (Covaciu-Marcov et al 2006). In 2007 and 2008 we've identified the species in other 4 locations (Table 1.).

Ablepharus kitaibelli is also a southern element, well represented in Dobrudja. Presently, we mention the species in 3 extra localities. The new populations appear to be big, occupying in all three cases forest habitats, generally inside the woods and no so much at skirts of forests and only exceptionally in the grasslands from right near the forests.

Lacerta viridis is a common species for the south-west Dobrudja.

Podarcis taurica is the most common lizard in the region, inhabiting habitats affected by human activities, too.

Podarcis muralis is a much rarer species, all across Dobrudja occurring only in the south-west (Iftime 2005). As opposed to our previous study, we are mentioning the species in two localities. At Tufani, the lizard is present on a rock and bushy hill side, situated near a forest. Near the Iortmac Lake it occupies ruins and concrete foundations, but also the base of the bridge that crosses the end of the Lake.

Darevskia praticola is an extremely rare species in the region, its presence not being noticed by us in our previous study, although it was pointed out before in the past (Fuhn & Hârsu 1962, Andrei 2002). We've observed the species in a new locality, at Goruni, where it inhabits the local forest. The species is rare here, too, being the least represented lizard in the forest. It occurs in the more opened and grassy sectors of the woods, living among the fallen leaves, the grassy vegetation but also on the sides of the road that crosses the forest near the Goruni and Carvan villages.

Natrix natrix is signaled for only on new locality, but is a common presence in the area. We've confirmed the species in many of the locations we've seen it in our previous study.

Natrix tessellata was not indicated for this region in our preceding study anterior (Covaciu-Marcov et al 2005), although it was previously cited in the Danube's Meadow (Iftime 2005). The speceis was observed in 4 new localitites, 3 of these being situated near the Danube and the forth one neat Iortmac Lake, inside the Dobrudjan platform. The populations seem large, but we also managed to identify 4 samples killed on the road parallel to the Danube, in April 2008.

Dolichophis caspius is another typica species for Dobrudja, being previously pointed in many localities in the area (Covaciu-Marcov et al 2006).

Vipera ammodytes is a very rare species, signaled in only one location, at Tufani. It inhabits the same habitat with *Podarcis muralis*; we observed only one specimen in April 2007.

Table 1

Geographical distribution of amphibians and reptiles in the investigated region.

Localitatea	**Bb**	**Ha**	**Pf**	**Buv**	**Rr**	**Rd**	**Eo**	**Tg**	**Ak**	**Lv**	**Pt**	**Pm**	**Dp**	**Nn**	**Nt**	**Dc**	**Va**
Aliman	-	-	-	-	-	-	X	-	-	-	-	-	-	-	X	-	-
Carvăn	-	-	-	X	-	X	-	X	X	X	X	-	-	-	-	-	-
Cochirleni	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-
Dobromir	-	-	-	-	-	-	X	X	-	-	-	-	-	-	-	-	-
Dobromiru de Deal	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Dumbrăveni	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-
Esechioi	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-
Goruni	-	-	-	-	-	X	-	X	X	-	X	-	X	-	-	-	-
Lacul Iortmac	X	X	X	X	X	-	X	X	-	X	X	X	-	X	X	-	-
Lespezi	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	X	-
Mănăstirea Peştera Sfântului Apostol Andrei	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-
Rasova	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-
Tufani	-	-	-	X	-	-	-	-	X	-	-	X	-	-	-	-	X
Vlahii	-	-	-	-	-	-	X	-	-	-	-	-	-	-	X	-	-
Σ X	2	1	1	4	2	2	4	5	3	3	3	1	1	1	4	3	1

Bb=*Bombina bombina,* **Ha**=*Hyla arborea,* **Pf**=*Pelobates fuscus,* **Buv**=*Bufo viridis,* **Rr**=*Rana ridibunda,* **Rd**=*Rana dalmatina,* **Eo**=*Emys orbicularis,* **Tg**=*Testudo graeca,* **Ak**=*Ablepharus kitaibelli,* **Lv**=*Lacerta viridis,* **Pt**=*Podarcis taurica,* **Pm**=*Podarcis muralis,* **Dp**=*Darevskia praticola,* **Nn**=*Natrix natrix,* **Nt**=*Natrix tessellata,* **Dc**=*Dolichophis caspius,* **Va**=*Vipera ammodytes.*

CONCLUSIONS

Compared to our previous paper, we managed to signal more species for the south-west region of Dobrudja (*Emys orbicualris*, *Darevskia praticola, Natrix tessellata*). The most important areas for the herpetofauna are the"islands" of forests that are nowadays reduced in surface and isolated one to another.

These shelter almost all the species in the region and therefore they need to be strictly protected. Someone also needs to put a stop to the clearings and to the re-plantation of the Acacia trees. Also, another set of important areas for the herpetofauna are the sectors near the Danube, near Aliman, Rasova, Vlahii and the ones near Iortmac Lake, where we have a concentration of the species linked with a higher degree of humidity.

The human impact on the herpetofauna is presently relatively reduced, but it was massive in the past, with massive clearings of the forests, increasing the agricultural fields, or with terracing the hill sides for vine cultures. Most of the latter areas are presently abandoned. The strongest impact in the present days is represented by the traffic, which affects most of the speceis from the region. Further more, over-grazing has both direct and indirect negative impact, affecting many habitats.

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CONTRIBUTIONS TO THE STUDY OF THE MACROZOOBENTHIC INVERTEBRATE COMMUNITY IN PRUNIŞOR AREA (ARAD COUNTY, ROMANIA)

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KEY WORDS: macrozoobenthos, semi temporary pond, aquatic invertebrate community, anthropic area

ABSTRACT

This paper deals with the study of the structure and dynamics of the macrozoobenthic invertebrate community from a semi temporary pond from Prunişor village area (Arad County). The study was made in the spring period (March-May), period in which the pond persists. The study consists from the determination of the structure and dynamics of the macrozoobenthic invertebrate community depending on the variations of the environmental conditions and the characteristics of the life cycle of each species. The pond is important for the conservation of biodiversity because it is situated in an anthropic area with agricultural fields around it. Because of this reason it is a refuge for a number of aquatic invertebrate species and also amphibians which lays their eggs here. Maintaining these kinds of ponds ensure the survival of different species in anthropic areas, where they have an insular distribution and their habitat is endangered.

INTRODUCTION

Prunisor area is situated at 82 km from Arad and it is localized in the Crisul Alb Valley in the western part of the country (Fig. 1). Here we have studied a temporary pond with a relatively big surface of appreciatively 75 m^2. The pond is situated near a road and has a dam around it. The depth of the pond is relatively high around 1 m, at the deepest point (Fig. 2). The substrate is covered by muddy soil land the vegetation of the pond is scarce formed from especially terrestrial plant species. The nature of the substrate is an essential factor for the structure of the benthic community (Silveira, et al., 2006).

In this pond during the spring different amphibian species come to feed and to lay their eggs. Also here different insect species with aquatic larvae find refuge and reproduction places in this highly anthopised area, where are a lot of agricultural fields. So this pond it is an island where the biodiversity can be maintained and a „green corridor" for the spreading of different insect species (Bănărescu 1995, Cogălniceanu & Venczel, 1993).

We have studied in this pond the structure and dynamics of the benthic macroinvertebrates in the spring period. At the end of May the pond dry out and the animal

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species are looking for other refuges, they pass through anabiosis or dry out together with the water.

Similar studies upon the benthic communities in temporary waters was made recently in the western part of our country and abroad (Cupşa et al. 2002, Cupşa et al. 2007 a and b, Williams 1997)

MATHERIAL AND METHODS

The samples where taken from the pond from March to May, after that the pond dried out. The samples where preserved in 4% formalin in the field and transported in the lab. In the lab the samples where sorted under a 40X magnifying glass and the organisms where separated by taxonomic groups and transferred in 80% ethylic alcohol. The species where determined where it was possible at the species level using specialty literature (Chiriac & Udrescu 1965, Godeanu 2002, Bouchard 2004).

We have calculated statistical indexes as abundance, frequency, constancy and density for each sampling period (Sîrbu & Benedek 2004).

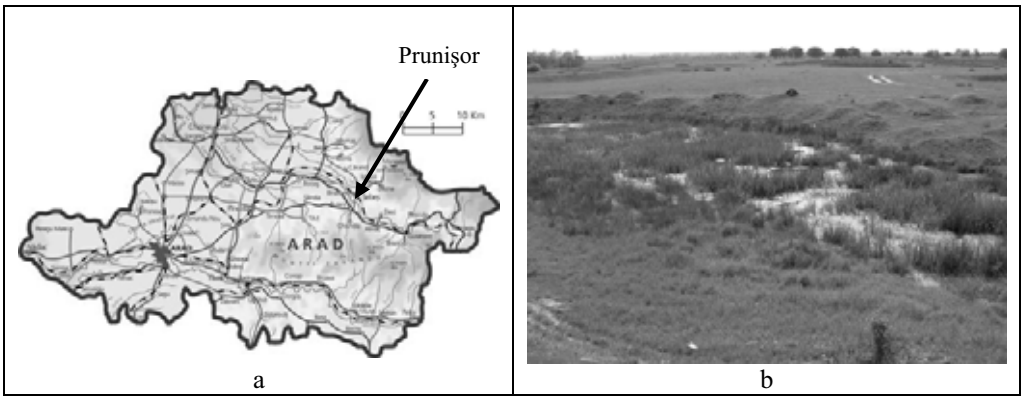

Figure 1. Prunisor localization in the county of Arad (a) and the aspect of the researched pond (b).

RESULTS AND DISCUSSIONS

During our research we have found a number of 10 invertebrate groups from which 6 where insects. One group of insects (Coleoptera) was represented by adults and the other by larvae. From the identified groups some (Isopoda, Heteroptera larvae, Coleoptera) are not characteristic for the benthic habitat, but considering the small depth of the water the can often found on the substrate. Those groups which can live exclusively in the water in all life stages (Oligochaeta. Gastropoda, Acarina) were found in small density because they dry out with the water in the summer period. Similar benthic community structure we can find in some sand-pit lakes unaffected by pollution (Celik, 2002). The Isopoda which are also exclusively aquatic species were found with big densities in two of the sampling periods. This is due to the fact that they probably reproduced in the spring, because we

found individuals of different sizes and also they have resistant eggs which can survive through the summer period without water.

The insect larvae were found with a relatively high density especially Ephemerella ignita. The Heteroptera and Coleoptera have lower densities, but the Coleoptera are represented by a great number of species (10) (Table 1). This lower density can be explained by the fact that the pond is not very large and deep, so the species do not have enough space to develop big populations and also the food resources are limited especially if we consider that it is a temporary pond.

The species composition shows that the water quality is relatively good; the organic content is not very high, because the Chironomida larvae are not very dense. We won't find very exacting species because the shallow water of the pond suffers great temperature variations during the day and can also have and oxygen deficit in the substrate when the water is warm.

Table 1

The macrozoobenthic invertebrate groups density during the study period

Nr crt	Invertebrate groups	Species	10.03. 2007	25.03. 2007	5.04. 2007	21.04. 2007	5.05. 2007
1.	**Oligochaeta**	-	-	5	-	7	-
2.	**Gastropoda**	-	-	-	-	1	-
3.	**Acarina**	*Fam. Limnebiidae*	-	1	-	1	-
4.	**Isopoda**	*Assellus aquaticus*	1	170	-	29	195
5.	**Ephemeroptera larvae**	*Cloeon dipterum*	-	12	-	-	-
		Ephemerella ignita	-	228	-	260	39
6.	**Odonata larvae**	*Coenagrion hastulatum*	-	8	-	8	15
		Ichnura elegans	-	8	-	-	-
		Libellula depressa	-	12	-	6	16
7.	**Heteroptera larvae**	*Notonecta viridis*	-	3	-	4	1
		Notonecta glauca	-	2	-	-	-
		Corixa punctata	-	-	-	1	-
8.	**Lepidoptera larvae**		1	3	-	-	-
9.	**Coleoptera**	*Fam. Haplididae*	-	-	-	-	8
		Fam. Haliplidae	-	-	-	17	-
		Haliplus flavicolis	-	-	-	-	7
		Haliplus obliquus	-	6	-	9	-
		Fam. Hydraenidae	-	-	-	-	2
		Hydrobius fuscipes	-	-	1	5	15
		Colymbetes fuscus	-	2	-	-	-
		Dytiscide larvae	-	4	-	2	1
		Dytiscus sp.	-	-	-	-	1
		Dytiscus marginalis	-	2	-	-	-
10.	**Diptera larvae**	*Fam. Chironomida*	1	123	-	71	15
	Total density		3	589	1	421	315
	Shannon-Wiener diversity index		1.09	1.37	0	1.17	1.27

In two of the study periods (10.03.2007, 05.04.2007) the benthic community was almost absent being represented by 3 species in the first case and 1 in the other. This fact is due probably to the low temperature of the water in these periods which made the fauna to be inactive. Also some of the insect larvae may become adults and leave the water.

If we analyze the abundances we can see (Fig. 2) that three species have great abundances: *Assellus aquaticus* in two of the studied periods, *Ephemerella ignita* and the Chironomida larvae. The rest of the species have small abundances in all the study period.

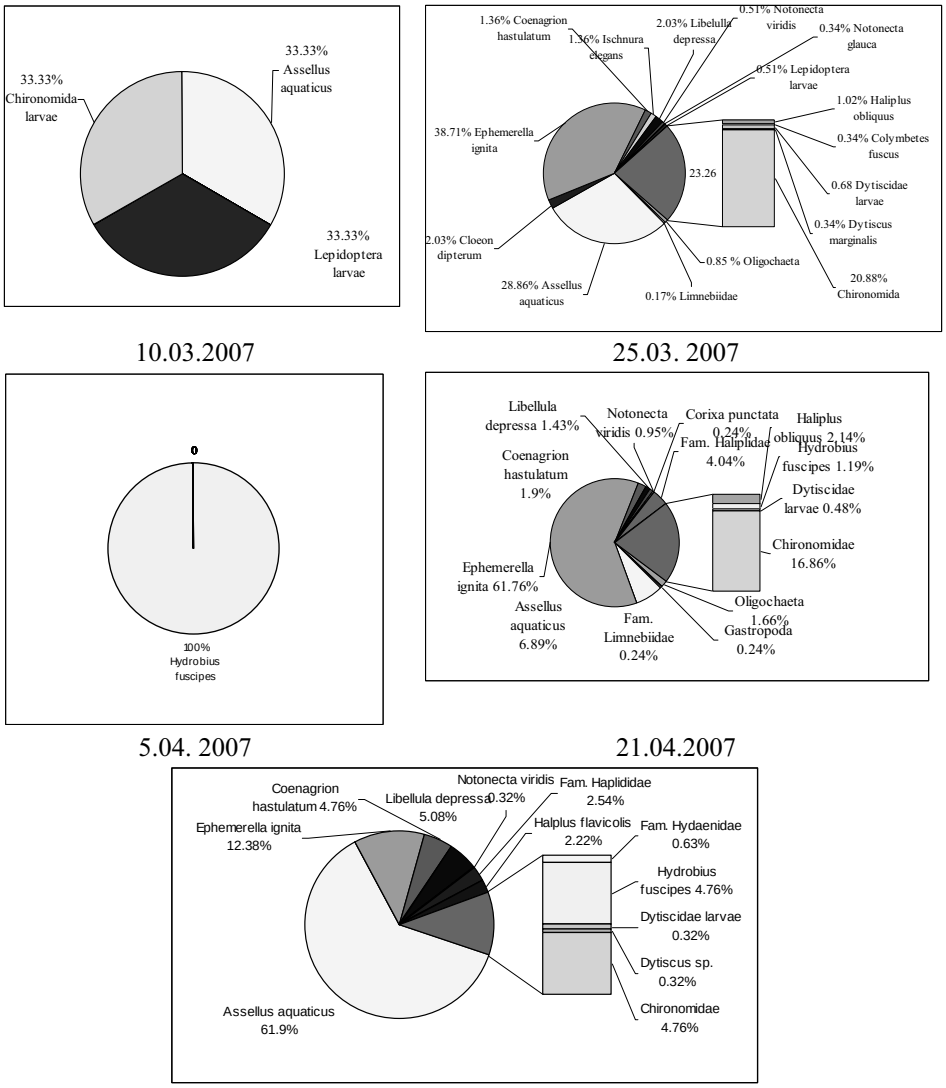

Figure 2. The relative abundances of the macrozoobenthic invertebrates in each sample.

The constancy of the species is not very high except the Chironomida larvae and the Isopoda which were found in almost all samples. The other species are less frequent which means that the analyzed pond it's not their permanent living environment. The community is made up from 33.33% euconstant and constant species (Fig. 3), 50 accidentally ones and 17% accessory. This fact means that is not a very stable community and this fact is due exactly to the temporary character of the pond.

The diversity is not very high in the all study period and in 05.04.2007 it is 0, because we found only one species. The small diversity is characteristic for a temporary pond in which only a small number of species can survive.

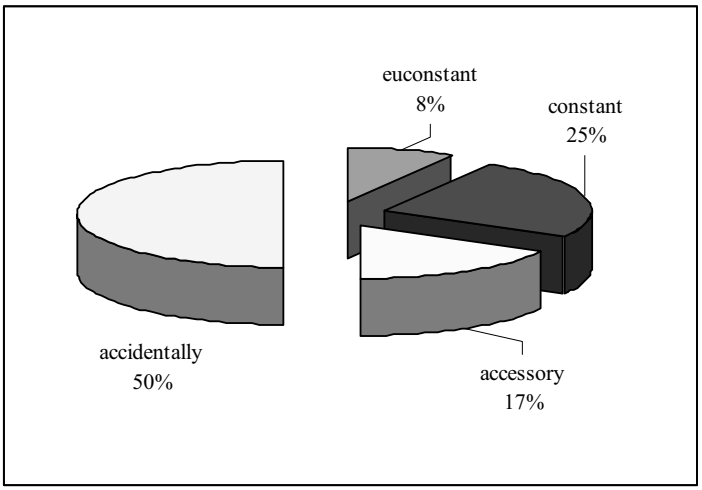

Figure 3. The constancy of the species during the research period

CONCLUSIONS

During our study we have found a number of 24 species and 1329 individuals. The dominant species were *Assellus aquaticus*, *Ephemerella ignita* and Chironomida larvae. The density of the individuals is generally low because of the variable life conditions in the pond and the summer dry out.

The most constant species were the Isopoda and Chironomida larvae. The community is not very stable this fact being shown by the small number of constant and euconstant species.

The diversity is not very high due to the specific environmental conditions of the pond.

The studied pond shows that can offer refuge and reproductive conditions to a relatively high number of species so it is an important environment for the biodiversity conservation in this region where the landscape is very antropised and the most of the fields are in agricultural use.

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THE TROPHIC SPECTRUM OF A *BUFO VIRIDIS* POPULATION FROM VODIȚA VALLEY, MEHEDINȚI COUNTY, ROMANIA

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Toth Gabriela[1], Aszalós Ágnes[1]

KEY WORDS: Bufo viridis, Mehedinți, trophic spectrum

ABSTRACT

We analyzed the diet of 98 individuals of Bufo viridis, captured on the 12th and 14th July 2006 and 12th,14th and 15th September 2007, in order to observe the evolution of the feeding spectrum. The most important categories of prey taxa were consumed in the highest quantities and have the highest frequency are represented by Hymenoptera- Formicida and Crustacean-Izopoda. During our study, the examined individuals presented a high feeding intensity, which reveals good feeding conditions.

INTRODUCTION

The feeding data are crucial for the understanding of the anuras' lifecycle and the impact that the modification of the habitats have on them (Anderson et al.1999). In our country, *Bufo viridis* is a common species, covering a wide area except the alpine zones. Despite all of this, a decline of this species has been observed in some countries from Europe (Carrier & Beebee 2003), so studies like this one, which deal with different aspects of their ecology, are of great importance in stopping the disappearance of the scabby frog populations. There are few studies that focus on the analysis of the food composition of the green scabby frog (Covaciu-Marcov et al.,2005, Tesio & Teodorescu 1999, Nicoară et al 2005), in the studied area this is the first one. Thus, through this study we observed the seasonal variation of the trophic spectrum of a population of *Bufo viridis* from Mehedinți county.

MATERIALS AND METHODS

The study took place in July 2006 and August and September 2007, a number of 98 stomach contents being drawn. The green scabby frogs were captured by hand, during the night, between 22^{oo}-2^{oo} around Vodița monastery or near Vodița valley. They were found in artificially lighted zones or with the help of flash lights. Stomach contents were collected using the stomach-flushing method (Cogălniceanu 1997). In this way, we can carry out feeding studies without killing the investigated individuals. The samples were

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preserved in separate airtight test tubes, which contain a 4% solution of formaldehyde. Afterwards, the frogs were released closed to the place where they were caught, in an attempt to diminish the impact that our activity could have on them. The processing of the samples was made in the laboratory, with the help of a binocular microscope.

RESULTS AND DISCUSSIONS

In the stomach contents of the 98 studied individuals we identified 3395 preys, which were grouped in 32 taxonomic categories. In the stomach contents we determined plant remains and minerals, both registering high amounts during the analyses. The plants are accidentally swallowed, together with the animal prey, or they are mistaken with these (Covaciu-Marcov et al. 2000), at least the ones represented by different seeds and capsules that can be carried by the wind, the frogs being drawn by their movement. Amphibian adults generally consume animal organisms, being carnivore-insectivore, only the larvae feed on plant material, especially algae (Jenssen 1967). Other authors consider that the plant remains are consumed in order to help crush the exoskeleton of the insects, eliminate intestinal parasites (Evans and Lampo1996), or they consist an additional water supply (Anderson et al. 1999).

Table 1

Number of analyzed individuals; plant and mineral frequency; the total, maximum and average number of prey/individual; the amount of aquatic and terrestrial prey

	12.07. 2006	14.07. 2006	12.08. 2007	15.09 2007	14.09. 2007
No. of analyzed individuals	21	17	39	9	12
Plant fragments	71,43	5,13	88,46	66,67	87,50
Minerals	42,86	3,42	61,54	66,67	37,50
Total no. of prey	957	326	1713	174	225
Max. no. of prey/individual	93	55	462	39	55
Aver. no. of prey /individual	45,57	19,73	43,92	19,33	18,75

Minerals, represented especially by pebbles, sometimes soil, have also been determined in a smaller amount, in comparison to the plants (Table no. 1). These are also accidentally swallowed together with the prey or as a consequence of an unsuccessful attack. The maximum number of prey eaten by an individual was registered in August (462), 450 of which being Formicida.

Due to their small size, ants have to be consumed in bigger quantities, in order to provide the necessary energy. During the study we didn't find any individual that had an empty stomach, the feeding conditions being optimum.

In September the average and maximum number of prey captured by the frogs is smaller than in the other periods, the diminution of the temperature having a direct effect on the number of potential prey.

The highest feeding intensity is signaled in the summer months, when the environmental conditions can provide the development of a great number of invertebrates.

Table 2

The weight of the prey taxa in the analyzed stomach contents

	12.07. 2006	14.07. 2006	12.08. 2007	15.09 2007	14.09. 2007
Anelide-Oligochete	-	-	0,35	-	0,67
Anelide-Polichete	-	-	0,35	-	2,67
Gasteropode-snails	0,16	-	0,18	-	-
Crustacee-Isopoda(t)	31,50	4,15	31,35	-	24,00
Arahnide-Araneide	1,72	2,30	3,33	24,14	18,67
Arahnide-Acarieni	0,94	0,92	-	-	-
Pseudoscorpionide	-	-	0,35	-	0,67
Scorpionide	-	-	0,17		0,44
Arahnide-Opilionidae	-	-	-	0,86	-
Myriapoda-Chilopoda	2,98	2,30	0,35	3,45	-
Myriapoda-Diplopoda	0,16	0,46	1,05	5,17	4,00
Collembola	1,72	-	-	-	2,00
Ortoptera	2,19	1,84	0,79	1,72	0,67
Blatodiae	-	-	-	2,59	-
Dermaptera	-	-	0,18	3,45	1,33
Heteroptera (t)	0,94	1,38	-	-	-
Heteroptera(a)	-	-	-	-	0,67
Lepidoptera [L,]	0,16	1,38	-	-	-
Lepidoptera	0,47	-	0,18	1,72	-
Coleoptera-undet,	1,10	2,76	1,40	-	2,00
Coleoptera-Carabida	0,31	0,46	5,78	6,90	2,67
Coleoptera-Curculionida	0,31	-	0,44	5,17	0,67
Coleoptera-Cryzomelida	-	0,46	0,79	-	-
Coleoptera-Scarabeida	0,31	-	-	-	0,67
Coleoptera-Elaterida	0,78	0,46	-	-	-
Coleoptera-Stafilinida	-	0,46	-	-	-
Diptera-Brahicera (L)	-	-	0,26	-	0,67
Diptera-Brahicera	0,16	-	-	-	-
Diptera-Nematocera	-	0,46	-	0,86	0,67
Hymenoptera-undet,	0,16	-	-	0,86	2,00
Hymenoptera-Formicida	50,78	74,65	52,89	43,10	35,33
Hymenoptera-Apida	2,98	5,53	-	-	-
Hymenoptera-Vespida	0,16	-	-	-	-

Regarding the amount of prey taxa, the highest value is owned by the Hymenoptera-Formicida, which were consumed throughout the entire period of the study (Table no. 2).The highest value was registered on 14th July 2006, 74.65%, when the ants were abundantly present in this habitat, living in big-sized, easily captured colonies.

Table 3

The frequency of the prey taxa in the analyzed stomach contents

	12.07 2006	14.07. 2006	12.08. 2007	15.09 2007	14.09. 2007
Anelide-Oligochete	-	-	7,69	-	12,50
Anelide-Polichete	-	-	11,54	-	37,50
Gasteropode-melci	7,14	-	7,69	-	-
Crustacee-Izopode(t)	71,43	3,42	76,92	-	90,90
Arahnide-Araneide	42,86	2,56	46,15	66,67	75,00
Arahnide-Acarieni	28,57	1,71	-	-	-
Pseudoscorpionide	-	-	11,53	-	12,50
Scorpionide	-	-	7,69		9,09
Arahnide-Opilionidae	-	-	-	16,67	-
Myriapoda-Chilopoda	57,14	2,56	15,38	33,33	-
Myriapoda-Diplopoda	7,14	0,85	30,77	66,67	50,00
Collembola	7,14	-	-	-	12,50
Ortoptera	42,86	3,42	26,92	33,33	12,50
Blatodiae	-	-	-	16,67	-
Dermaptera	-	-	7,69	50,00	25,00
Heteroptera (t)	35,71	1,71	-	-	-
Heteroptera(a)	-	-	-	-	12,50
Lepidoptera [L,]	7,14	2,56	-	-	-
Lepidoptera	21,43	-	7,69	33,33	-
Coleoptera-undet,	28,57	2,56	19,23	-	25,00
Coleoptera-Carabida	14,29	0,85	80,77	83,33	25,00
Coleoptera-Curculionida	14,29	-	11,54	50,00	12,50
Coleoptera-Cryzomelida	-	0,85	19,23	-	-
Coleoptera-Scarabeida	14,29	-	-	-	12,50
Coleoptera-Elaterida	21,43	0,85	-	-	-
Coleoptera-Stafilinida	-	0,85	-	-	-
Diptera-Brahicera (L)	-	-	11,54	-	12,50
Diptera-Brahicera	7,14	-	-	-	-
Diptera-Nematocera	-	0,85	-	16,67	12,50
Hymenoptera-undet,	7,14	-	-	16,67	25,00
Hymenoptera-Formicida	100	7,69	69,23	66,67	75,00
Hymenoptera-Apide	50,00	5,13	-	-	-
Hymenoptera-Vespida	7,14	-	-	-	-

The frequency of the Formicida is also high during the study, for example in the first period of the research they were identified in all of the stomach contents (Table no. 3). This type of food is the most important one for the analyzed population; all of the individuals can feed on this category. Some papers reveal that alkaloids from the scabby

frog's skin are due to the specialized feeding on ants (Daly et al 2000; Bonansea & Vaira, 2007).

Another category which has significance in the feeding of the studied frogs is represented by the Crustacean-Isopoda, which are easily captured by the scabby frogs (Yüyüt et al 1997) and because of their close relation to humidity, they can be very abundant at night when the individuals of the studied species display their activity. The Isopoda are consumed mainly in the first, 12 august 2007, and last period of the study, in the other parts the weight and the frequency have smaller values. This fact can be caused by the unfavorable conditions for their development, a certain level of humidity being needed in order to be active, the higher temperatures in those periods having a direct influence on the lack of Isopoda in the habitats. Thus we can observe that the diet of the studied individuals depends on the variations of the environmental factors which influence the life cycle of the preys, these being available as food for the frogs only in certain conditions.

Arachnids-Araneidas are present in the diet of the frogs throughout the entire study, but a bigger number in the stomach contents was registered on 15th September , the weight of these being 24.14% (Table no. 2.).The highest frequency was registered on 14th September 2007, this type of prey being identified in the stomach of 75% of the studied individuals. The rising rate of the consumption of the spiders in this period is probably due to the lack of Crustacean caused by some unfavorable conditions, which don't have an effect on the spiders. Arachnids represent an important element in the food of other green scabby frog populations (Covaciu-Marcov et al., 2005, Tesio & Teodorescu 1999).

Coleoptera-Carabidae have also been identified in the stomach contents of the individuals, but their amount is low. On 12th August and 15th September the frequency with which they were consumed surpassed 80% (Table no. 3). The considerable difference between the amount and the frequency indicates that a large number of individuals consume a small number of Carabidae, due to the fact that this type of prey is homogeneously spread on the entire habitat, but the number of individuals is reduced. Other prey taxa which have a higher frequency value are represented by prey such as Myriapodes.

An interesting fact is the appearance of the Vespide in the stomach contents of the frogs, represented by species such as *Euscorpius carpaticus* or *Scolopendra,* although they have a small weight. The fact that the prey which have methods of protection through the emission or inoculation of vesicant substances aren't avoided, shows that the feeding isn't made selectively, the individuals capturing everything that comes in sight and has the appropriate measures to be swallowed. It is possible that because of the speed with which they are captured, they can't defend themselves.

Regarding the origin of the prey's environment we can observe that all the prey identified in the stomach contents live in a terrestrial environment, which is predictable due to the fact that this species is seen in the water only when it lays its eggs, our study being carried out in the postreproductive period.

CONCLUSIONS

The most important prey categories identified are represented by Hymenoptera-Formicidae and Crustacean-Isopoda. The environment has an influence on the food composition (temperature, precipitation, humidity), which can have a negative impact on the potential prey, thus determining a diminution in the food offer. The highest feeding intensity was registered in August 2007 and in the summer months, when there were optimum conditions for the development of the prey. As fall came closer, the environment

changed and as the hibernating period approached the number of the identified prey in the analyzed samples decreased. As a conclusion we can establish that the *Bufo viridis* population from this habitat has optimum feeding conditions, in different parts of the year the frogs consume the preys which are the most abundant in the habitat in that moment.The environmental factors and the life cycle of the prey taxa determine the elements of the feeding spectrum of the analyzed individuals.

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FEEDING OF SOME HYBRID POPULATIONS BETWEEN *BOMBINA BOMBINA* AND *BOMBINA VARIEGATA* FROM ARAD COUNTY, ROMANIA

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KEY WORDS: feeding, Bombina bombina, Bombina variegata, hybrids

ABSTRACT

This study deals with the analysis of the trophic spectrum of three hybrid populations between Bombina bombina and Bombina variegata, totalizing 92 individuals. We identified four categories of stomach contents, animal prey, plant remains, shed-skin fragments and in an extremely low proportion minerals. The most substantial values in the food composition are held by Coleoptera, Lepidoptera larvae, aquatic Isopoda and Araneida. In connection with this situation, almost all of the prey originates from terrestrial environment. As well as presenting quantitative and qualitative facts, this paper underlines the differences that the sexes and habitats have on the feeding process.

INTRODUCTION

The main connections between an organism and the environment are established through the process of feeding (Pop, 1977). Thus, knowing the trophic spectrum represents a key factor in understanding any animal's ecology (Perry et al. 1990). At the same time, the analysis of this element can provide valuable information regarding the quality of that animal's habitat.

This study analyses the trophic spectrum of some hybrid populations between two species of the gender *Bombina, Bombina bombina* and *Bombina variegata.* The two species present an interesting characteristic, diverting from the classical definition of the species, based on reproductive isolation (Mayr, 1942). Thus, the toads aren't reproductively isolated, hybrid populations being created in the joining areas between their habitats (Szymura, 1993).

Different studies related to this subject have been carried out in Romania (Ghira & Mara 2000, Covaciu-Marcov et al. 2002, 2004), research that underlines the morphological, chromatic, genetic and ecological features of the individuals. However, the data concerning the diet of the hybrids are fewer than those of the parental species.

The purpose of this paper is to improve the knowledge in this area, or to strengthen certain conclusions regarding the feeding of the hybrids.

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MATERIALS AND METHODS

Our study took place in May 2007 in Teuzului Valley, situated in the north-eastern part of Arad County. During our activity, we analyzed the stomach contents of 92 hybrids, collected from two different habitats. The first one lies in Igneşti commune, which is situated at the southern foot of Codru Moma Mountain. It comprises three puddles of different sizes, set near the road. The toads from the second habitat were captured on two different dates, 5th May (Prunişor 1) and 20 th May (Prunişor 2). The toads were captured either by hand, or with the help of a net, the individuals being released afterwards. Stomach contents were collected using the stomach-flushing method (Opatriny, 1980), which has the advantage of not harming the animal. The samples were stored in airtight test tubes, preserved with a 4% solution of formaldehyde, accompanied by labels with the toad's sex. The preys were identified in the laboratory with the help of a magnifying glass and scientific literature. The study has several objectives: 1. Determining the taxonomic affiliation of the identified preys and the origin of their habitat (terrestrial/aquatic) 2. Analyzing the maximum and average number of prey /individual 3. Highlighting certain differences between the female's and male's food 4**.** Discussing the weight and frequency of the most important prey items

RESULTS AND DISCUSSIONS

We determined a number of 839 animal preys, arranged in 29 taxonomic categories (grid number 2). Besides animal prey, we also found plant fragments, shed-skin and minerals (grid number 1). From a total of 92 individuals, 8 toads didn't present stomach contents, thus the feeding activity ratio is quite high (91.30%). This suggests the presence of good feeding conditions, although one can observe human influence through the building of the road. The maximum prey/ individual was determined at a female from Prunişor 1, which ate 32 preys. In the case of these populations we can conclude that the females have a higher value of the maximum and average number of preys than the males. The females feed on smaller prey, in comparison to the males, so they have to eat in larger quantities in order to sustain their energetic needs.

Amphibians often consume plant fragments, although they are carnivores. Scientists have different opinions regarding this phenomenon. Some claim that they are accidentally swallowed together with the animal prey (Whitaker et al 1977), while others suggest that plants help crush the exoskeleton of the Gasteropodes, or eliminate intestinal parasites (Evans and Lampo 1996). In this study, plant materials have a high frequency, the maximum (69.23%) being recorded by a female from Prunişor 1. This habitat also registered the maximum for the males (60%) and, on the opposite side, the minimum for both females (29.62%) and males (33.33%). The presence of vegetal fragments in the diet of the amphibians has been noticed in most of the studies dedicated to this subject, regarding the parental species, *Bombina variegata* (Toth et al 2007- 66.66%) and *Bombina bombina (*Radu et al 2007- 60%). In this case, the direct correlation between the weight of terrestrial prey and the frequency of plant remains, suggests the involuntary swallowing of the parts.

The second type of food category is represented by shed skin consumption, which is believed to be a good epidermal protein source (Weldon et al 1993). Although this element has a lower value in comparison to that of the plant remains, it is thought to be ingested deliberately. Analyzing the facts, we noticed a higher frequency in the population

from Prunişor 2 (26.19%), were the males registered the maximum value (26.66%). The minimum value was determined at Igneşti (4.76%), were the females reached the highest value from all the populations (33.33%). Based on the results we can establish a link between the diversity of the food and the low rate in which shed-skins were consumed. Thus, due to the good feeding conditions, the toads didn't have to recycle their epidermal proteins.

Minerals have the lowest frequency in the hybrids' diet, this fact being observed in other populations as well (Ferenți et al 2007- 1.05%), in some cases the value is null. In this study, the percentage of inorganic elements is almost imperceptible, being determined at only one female from Prunişor (6.66%).

Table 1

The frequency of stomachs containing plant remains, shed-skin and minerals
The origin of the prey taxa (M-male, F-female, T-total)

	Igneşti			Prunişor 1			Prunişor 2		
	M	F	T	M	F	T	M	F	T
Plant fragments	44.82	33.33	43.75	60	69.23	66.66	33.33	29.62	30.95
Shed-skin	3.44	33.33	4.76	20	7.69	11.11	26.66	25.92	26.19
Minerals	-	-	-	-	-	-	-	6.66	2.38
Aquatic prey	-	4	0.55	42.6	28.36	32.3	-	1.37	1.07
Terrestrial prey	100	96	99.45	57.4	71.64	67.7	100	98.63	98.93

The last and most important food category is represented by animal prey. We identified 839 preys, which we classified in 29 categories. During the study, we separated the Lepidoptera and Nematocera larvae from the adults, as well as the terrestrial Isopoda from the aquatic ones. Some specialists assert that there is a difference between the adult insects and their larvae forms, regarding their nutritive contents, the latter having a higher lipid value (Redford & Dorea 1984). The females prefer the larvae forms of certain insects, like those of the Lepitoptera, due to their accessibility.

Most of the 29 prey taxa, (21) belong to the Insects class. The most important weight is held by Coleoptera, which occupies first place in Igneşti (62,43%) and Prunişor 2 (62.99%). In Prunişor 1, the majority is owned by the aquatic Isopoda, the population registering a value of 32.31% and the males, 42.59%. The other prey taxa have low values in comparison to the above-mentioned orders. An interesting aspect in the analysis of the trophic spectrum is the exclusive presence of certain prey taxa. Thus, in the stomach contents of the toads from Prunişor 2 we identified Gasteropoda (snails and Limax), Acarieni, Miriapoda (Chilopoda) and Trichoptera. The variety is much smaller in the case of the other populations. In Prunişor, we found aquatic Isopoda and Scarabeidae, while in Igneşti we determined the presence of Cantharidae. This differentiation is related to the influence of the habitat.

Comparing the weight and the frequency of the prey taxa, we can observe a certain similarity between them. This can indicate a homogeneous trophic spectrum. However, due to their small sizes, the toads could be constrained to eat only certain types of animals. The Coleopteras have the highest frequency in all the analysed populations, the maximum being registered at Prunişor 2 (90.47%). Here, all the females ate this taxon. The Lepidoptera larvae occupy an important place, 80% of the males from Prunişor 1 having consumed this

prey. On the other hand, in some cases, we can notice discrepancies between the weight and frequency of the prey. Some animals don't have to be consumed in large quantities, because of their size, or high nutritive contents.

Table 2

The amount of the prey taxa
(M-male, F-female, T-total, t.-terrestrial, a.-aquatic, L.-larvae)

	Igneşti			Prunişor 1			*Prunişor 2*		
	M	F	T	M	F	T	M	F	*T*
Lumbricidae	2.56	8	3.31	1.85	1.42	1.54	1	-	*0.22*
Gasteropoda-	-	-	-	-	-	-	6	0.28	*1.52*
Gasteropoda-Limax	-	-	-	-	-	-	-	0.28	*0.22*
Araneidae	5.13	4	4.97	1.85	2.84	2.56	7	2.21	*3.25*
Acarieni	-	-	-	-	-	-	2	0.55	*0.87*
Izopoda (t.)	-	-	-	-	0.71	0.51	1	1.66	*1.52*
Izopoda (a.)	-	-	-	42.59	28.37	32.31	-	-	-
Miriapoda-Chilopoda	-	-	-	-	-	-	-	0.28	*0.22*
Collembola	-	-	-	-	1.42	1.03	-	1.66	*1.3*
Blatoideae	0.64	-	0.55	-	-	-	4	0.55	*1.3*
Heteroptera	1.92	4	2.21	-	-	-	-	0.28	*0.22*
Homoptera-Afidine	3.85	-	3.31	-	7.09	5.13	9	14.92	*13.64*
Homoptera -Cicadine	5.13	-	4.42	1.85	-	0.51	1	0.55	*0.65*
Coleoptera -nedet.	66.03	40	62.43	18.52	36.88	31.79	54	65.47	*62.99*
Coleoptera - Elateridae	0.64	-	0.55	-	-	-	1	-	*0.22*
Coleoptera - Carabidae	1.92	-	1.66	-	-	-	1	0.55	*0.65*
Coleopt - Chrysomelidae	0.64	-	0.55	5.56	-	1.54	-	0.28	*0.22*
Coleoptera - Cantharidae	0.64	-	0.55	-	-	-	-	-	-
Coleopt. - Staphylinidae	-	-	-	-	2.84	2.05	-	0.83	*0.65*
Coleopt. - Curculinoidae	-	-	-	3.7	4.96	4.62	-	0.55	*0.43*
Coleoptera - Scarabeidae	-	-	-	1.85	2.13	2.05	-	-	-
Lepidoptera (L.)	3.85	12	4.97	11.11	3.55	5.64	2	1.1	*1.3*
Trichoptera	-	-	-	-	-	-	1	0.28	*0.22*
Diptera – Nemat. (L.)	-	4	0.55	-	-	-	-	1.38	*1.08*
Diptera Nemat.Culicidae	2.56	-	2.21	1.85	0.71	1.03	1	0.55	*0.65*
Dipter Nemat Typulidae	1.28	-	1.1	1.85	1.42	1.54	-	-	-
Diptera - Brahicera	0.64	-	0.55	1.85	2.13	2.05	2	1.1	*1.3*
Hymenoptera- nedet.	0.64	-	0.55	1.85	0.71	1.03	-	0.28	*0.22*
Hymenopt. Formicidae	*1.92*	*28*	*5.52*	*3.7*	*2.84*	*3.08*	*7*	*4.7*	*5.19*

An important aspect regarding the hybrids' diet is the origin of the prey's environment. Amphibians live and eat in both terrestrial and aquatic habitats. Thus, the majority of the prey has terrestrial origin, the males from Prunişor 2 and Igneşti having consumed only this type of prey. In these populations, the females ate in very small quantities aquatic prey, represented by Nematocera larvae. Analyzing the data from

Prunişor 1, we can observe that the differences between the terrestrial prey and the aquatic one is smaller, due to the presence of the aquatic Isopoda.

Table 3

Frequency of occurrence the prey taxa
(M-male, F-female, T-total, L.-larvae, a.-aquatic, t.-terrestrial)

	Igneşti			Prunişor 1			*Prunişor 2*		
	M	F	T	M	F	T	M	F	T
Lumbricidae	10.34	66.66	15.62	20	7.69	11.11	6.66	-	2.38
Gasteropoda-	-	-	-	-	-	-	26.66	3.7	11.9
Gasteropoda-Limax	-	-	-	-	-	-	-	3.7	2.38
Araneidae	24.13	33.33	25	20	23.07	22.22	40	22.22	28.57
Acarieni	-	-	-	-	-	-	13.33	7.4	9.52
Izopoda (t.)	-	-	-	-	7.69	5.55	6.66	3.7	4.76
Izopoda (a.)	-	-	-	60	38.46	44.44	-	-	-
Miriapoda-Chilopoda	-	-	-	-	-	-	-	3.7	2.38
Collembola	-	-	-	-	7.69	5.55	-	7.4	4.76
Blatoideae	3.44	-	3.12	-	-	-	20	7.4	11.9
Heteroptera	10.33	33.33	12.5	-	-	-	-	3.7	2.38
Homoptera-Afidine	6.89	-	6.25	-	30.76	22.22	26.66	44.44	38.09
Homoptera -Cicadine	13.79	-	12.5	20	-	5.55	6.66	7.4	7.14
Coleoptera -nedet.	68.96	66.66	68.74	60	92.3	83.33	73.33	100	90.47
Coleoptera - Elateridae	3.44	-	3.12	-	-	-	6.66	-	2.38
Coleoptera - Carabidae	6.89	-	6.25	-	-	-	6.66	7.4	7.14
Coleopt -Chrysomelidae	3.44	-	3.12	40	-	11.11	-	3.7	2.38
Coleoptera -Cantharidae	3.44	-	3.12	-	-	-	-	-	-
Coleopt. - Staphylinidae	-	-	-	-	30.76	22.22	-	11.11	7.14
Coleopt. -Curculinoidae	-	-	-	40	7.69	27.77	-	7.4	4.76
Coleoptera -Scarabeidae	-	-	-	20	23.07	22.22	-	-	-
Lepidoptera (L.)	17.24	33.33	18.75	80	23.07	38.88	13.33	14.81	14.28
Trichoptera	-	-	-	-	-	-	6.66	3.7	4.76
Diptera – Nemat. (L.)	-	33.33	3.12	-	-	-	-	11.11	7.14
Dipt. Nemat.Culicidae	13.79	-	12.5	20	7.69	11.11	6.66	7.4	7.14
Dipter Nemat Typulidae	3.44	-	3.12	20	7.69	11.11	-	-	-
Diptera - Brahicera	3.44	-	3.12	20	23.07	16.66	6.66	14.81	11.9
Hymenoptera- nedet.	3.44	-	3.12	20	7.69	11.11	-	3.7	2.38
Hymenopt. Formicidae	6.89	33.33	9.37	20	30.76	27.77	26.66	22.22	3.8

CONCLUSIONS

Amphibians occupy a special place in the ecosystem, due to the fact that they live in two different habitats, thus they consume both terrestrial and aquatic prey. This aspect is also visible in our paper, which studies the trophic spectrum of three hybrid populations. As a result of our analysis, we identified four types of food in the stomach contents of the

toads, the most important one being the animal prey. The Coleopteras hold the majority at Prunişor 2 and Igneşti, while the aquatic Isopoda occupy first place at Prunişor 1. The same connection is established regarding the origin of the prey. There are certain differences between the habitats, regarding the populations. The females eat more small-sized prey in comparison to the males, as a result, the maximum and average number will be higher in the case of the females.

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NOTE ON THE TROPHIC SPECTRUM OF A *TRITURUS CRISTATUS* POPULATION FROM LIVADA FOREST, SATU MARE DISTRICT, ROMANIA

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KEY WORDS: Triturus cristatus, trophic spectrum, sexes, ontogenetic development

ABSTRACT

We have analyzed the trophic spectrum of 31 species of crested newts, caught in May 2007 in Livada forest. In order to get the samples, we have used the method called stomach lavage. In the stomach contents we have identified plants, skin remainders, eggs and minerals, and animal preys, among which the most important are the Cladoceras, the Ephemeroptera larvae, Nematocera larvae and aquatic Isopodas. We have also identified one Triturus vulgaris type in the newts' stomach. Most of the preys live in water.

INTRODUCTION

The most important conditions for the newts to survive are winter and summer unpolluted habitats, proper humidity and food supply values. In addition, the protection against predators and extreme weather seem to play a key role (Kinne 2006).

The decline in most of the amphibian populations is due to the anthropic factors (Semlitsch 2003). That is why these habitats need to be protected, which can be done only if we properly know the amphibians' ecology and biology. Different studies suggest food plays an important role concerning the amphibians relation with the environment (Duellman 1967), the trophic spectrum providing thus information upon the respective species' ecology and biology.

The crested newt prefers larger ponds, rich in vegetation, but we can spot it also in concrete basins (Cogalniceanu et al 2000), or in ponds formed in abandoned stone pits, which is in fact our case. We can see it in Romania towards the northern part, in the leafy forests (Stugren 1957).

Today we can see it starting with 100 m altitude up to 1000 m (Fuhn 1960). Research on the Romanian trophic spectrum took place especially over tha last few years (Cicort Lucaciu et al 2005a, b, 2006, 2007). Our study's goal is to analyze the Livada forest trophic spectrum, taking into account both the sex variations and the ontogenetic development stage.

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MATERIAL AND METHODS

In May 2007 we have studied the feeding process of a crested newt population from Livada forest. The habitat is on the northern slope of Mujdeeni hill, a slope through which the hill ends towards the Talna field.

The 31 *Triturus cristatus* samples under study have been taken from a habitat made of several ponds in a stone pit, which strongly affects the former anthropically, as the vegetation is made of reed and pewter. These ponds are relatively large, and never drain.

The newts were caught using a net having a long metal stick. In order to get the stomach samples we have used the stomach lavage method, using also a syringe and a perfusion on top of it. This is a highly used and recommended method, as it allows studying the amphibians without killing them (Fraser 1976, Legler & Sullivan 1979). As the Amphibians' digestion is quite a short one, we have tried to reduce the time between catching the *Triturus cristatus* and getting the stomach samples (Caldwell 1996). After getting the samples the newts were released in their environment. The stomach contents were then kept in test tubes with 4% formol. The test tubes were provided with labels containing information about the types' sex, as 11 individuals were males, 10 females and 10 juveniles. The samples' analysis was later undertaken in laboratory conditions, using a binocular magnifying glass and also the reference literature (Crisan & Muresan 1999, Moczar 1990, Radu & Radu 1967).

The results were included in statistics, using the following parameters: the feeding rate, the feeding intensity (we have taken into account the average number of preys / individual and the maximum number of preys / individual), the weight and the frequency of the prey taxons and their origin. The results were analyzed by comparison, in point of sexes and the ontogenetic development.

RESULTS AND DISCUSSIONS

Our study took place in May 2007, in Livada forest, analyzing the trophic spectrum of 11 *Triturus cristatus* males, 10 females and 10 juveniles. After analyzing the stomach contents we have identified several elements: plant fragments, skin remainders, minerals and eggs. The most important element in these frogs' trophic spectrum is represented by animal preys, including here both Invertebrates and Vertebrates.

Several authors stated that climate changes had a direct effect on the frogs' feeding process (Tyler & Hoestenbach 1979). All the individuals we studied had stomach content, thus the feeding rate was 100%, which indicates the temperature and humidity of the environment were favourable. This connection between the newts' feeding and the environment conditions was also noticed in other *Triturus cristatus* populations (Cicort Lucaciu et al 2005 a,b, 2006, 2007a).

As for the feeding intensity we can observe this is influenced by both the environment conditions and the individuals' energy needs. The average number of preys/individual is 9,03, this being the largest in juveniles, followed by males and females. This order can be noticed as far as the maximum number of preys/individual is concerned. Some authors believe females eat more (Cicort Lucaciu et al 2005 b). In our case the juveniles and the males have eaten very small preys, while the females' trophic spectrum is made of bigger preys, which they had to consume in larger quantities to get the same energy amount.

The plants diet is wide spread in Amphibians, but also in Urodeles (Cicort Lucaciu et al 2005a, 2006, 2007 a), and Anurans (Peter et al 2007, Dobre et al 2007, Sas et al 2005).

Some believe that plants are swallowed by accident together with mobile preys (Whitaker et al 1977), the plant fragments being taken for animal preys (Turner 1959). In our case the stomachs containing plants have a frequency of 42%, which is higher in females. We have not found any type having a stomach only with plants in it, which leads to the fact the plants were not ingested voluntarily.

Table 1

The prey taxons' weight (W) and frequency (F)

	Males		Females		Juveniles		Total	
	W	F	W	F	W	F	W	F
Plants	-	54,54	-	60	-	40	-	51,61
Skin remainder	-	27,27	-	30	-	30	-	29,03
Minerals	-	0	-	0	-	10	-	3,22
Eggs	-	18,18	-	30	-	30	-	25,80
Arachnidas-Araneidas	0	0	0	0	0,85	10	0,35	3,22
Coleopteras - undet.	1,13	9,09	2,66	20	0,85	10	1,42	12,90
Cladocera	42,04	18,18	49,33	20	55,55	20	49,64	19,35
Dipt.-Nematoceras L.	37,50	81,81	22,66	50	28,20	80	29,64	70,96
Dipteras - Brahiceras ad.	0	0	1,33	10	0	0	0,35	3,22
Ephemeroptera L. acq.	10,22	36,36	10,66	70	7,69	50	9,28	51,61
Heteropteras acq.	0	0	0	0	0,85	10	0,35	3,22
Hymenopteras-Formicidae	1,13	9,09	0	0	0	0	0,35	3,22
Isopodas acq.	7,95	18,18	8	40	4,27	20	6,42	25,80
Odonata L.	0	0	4	20	1,70	20	1,78	12,90
Triturus vulgaris	0	0	1,33	10	0	0	0,35	3,22

Many authors believe that eating skin remainders together with mobile preys is also accidental (Sas et al 2005), and according to Weldon et al (1993), this is related to the recycling of the epidermal proteins. In our case the values are not very different in point of sexes and the ontogenetic development either – they are low in each situation. Other *Triturus cristatus* populations have also skin remainders in their trophic spectrum (Cicort Lucaciu et al 2005 a, 2006).

The minerals were ingested only by one *Triturus cristatus* juvenile sample, which we believe they were eaten by accident, as the newt was found in a pond in a former stone pit.

We have also noticed eggs in the newts´ diet, which were higher in the females' case. As the females lack the back ridge, they cannot hunt quicker preys. Thus, eggs are an important energy source for them, and also easy to catch. Females need this energy in order

to reproduce. It is thus obvious that eggs are a common food source for newts (Covaciu Marcov et al 2002, Cicort Lucaciu et al 2005a, 2006).

On the whole we have identified 280 animal preys, belonging both to Invertebrates and Vertebrates.

In one female's stomach content we have found a *Triturus vulgaris* type, which shows that crested newts have an opportunistic way of feeding, eating everything around them that has a proper size. Other *Triturus cristatus* populations include also in their diet Vertebrates (Dobre et al 2007)

The Invertebrates preys are the most important part of the crested newts' trophic spectrum. The most important taxon is represented by the Cladoceras, which is high in both sexes. The differences in point of sexes and ontogenetic development are noticeable in the case of the next taxons, such as Nematocera larvae, Ephemeroptera larvae, aquatic Isopodas and Odonata larvae.

As for the terrestrial preys, we can notice certain differences in point of sexes and also the ontogenetic development stage.

The bigger preys (Odonata larvae, Isopodas, Coleopteras, Triturus vulgaris) are mostly to be found in females, which suggests they use the 'sit-and-wait' method (Duellmann & Trueb 1994). Thus the females tend to catch their prey when they actually spot it. As for the preys' size in males, the smaller taxons seem to prevail (Nematocera larvae), which suggest they use the active foraging method (Huey and Pianka 1981). As for the juveniles, the trophic spectrum is made of both big and small preys, so they use both the hunting methods – they have higher energy needs as they need to grow up. On the other hand, this fact suggests that the juveniles (but also the adults) do not feed in a selective way, but simply catch the easiest prey around them.

Several authors believe larvae are richer in lipids, and thus a richer nutritive content (Brooks et al 1999). The different insects' larvae are to be found in both the sexes (Nematoceras, Ephemeropteras), whereas libellula larvae are to be found only in females and juveniles. This aspect of the trophic spectrum can be considered as an expression of energy saving. The libellula larvae are an easy to catch energy source – that is why females and juveniles do not need to eat too much, they prefer to save energy in order to mate and to grow (in the case of the juvenile ones).

The frequency of the prey taxons varies according to sex. Thus, we can notice males eat more mosquito larvae, whereas females prefer Ephemeroptera larvae – Isopoda and Odonata larvae are also to be found in their trophic spectrum. This is due to the males' back ridge, which allows them to catch quicker preys, while females tend to catch slower preys.

The Cladoceras have a high frequency, whereas their frequency value was relatively a low one. This is due to the fact that very few individuals eat them (in great amounts), but accidentally. Thus, although the Cladoceras are high in number in the environment, we can say their frequency is quite a low one, as only few newts stomachs contain Cladoceras.

As for the prey taxons' origin, the aquatic preys come first, as our study was made in the crested newts' aquatic stage. Also, the same was noticed in the case of a crested newt population from Turt (Cicort Lucaciu et al 2007).

CONCLUSIONS

To sum up, we can say the frogs' feeding is very much related to the environment conditions; in our case the best feeding conditions are reflected in the maximum value of the feeding activity rate. The crested newts' feeding is not selective, as both big and small preys are to be found in their trophic spectrum. Thus the newts catch the preys that are easy to catch and also those having the proper size from their environment. So we can say newts have an opportunistic way of feeding.

Females tend to save energy when eating. Thus they mostly eat bigger preys, using the 'sit and wait' hunting method. Respectively, they prefer the preys that have a higher nutritive value, such as Odonata or Ephemeroptera larvae. The males' trophic spectrum is made of quicker preys (mosquito larvae), because of the former's back ridge, as opposed to females, which prefer slower preys. The young newts have a higher need of energy, that is why they eat more, having an opportunistic way of feeding. We reached this conclusion because the maximum number of preys/young individual is high, and also we noticed both big and small preys.

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POLLUTION INDICATORS SPECIES. THE QUANTITATIVE DETERMINATION OF Cu, Cd AND Zn BY AAS SPECTROMETRY FROM *Achilea millefolium L., Pastinaca sativa ssp. pratensis L.* AND *Matricaria inodora L.*

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KEY WORDS: atomic absorption spectrometry, toxic metals, polluted zone.

ABSTRACT

The procedure described in this paper, highlight the degree of exposure of plants to certain toxic metals: Cu, Cd, and Zn, the plants being harvested from a polluted industrial zone (Sinteza Oradea) and from a non pollute zone (Suncuius Bihor). An atomic absorption spectrometric method is described for the determination of copper, zinc and cadmium in biological materials. Copper, zinc and cadmium are determined directly by aspirating the solution into an air- acetylene flame.

Samples used are formed from vegetal tissues, obtained from the following species: Achilea millefolium, Pastinaca sativa ssp. Pratensis and Matricaria inodora L.

INTRODUCTION

Atomic absorption spectrometry is widely used in biological analysis. The speciality works indicates for the analysing of the toxic metals from the plant tissues, the use of solvent mixture such as :nitric acid, sulphuric acid and perchloric acid.

In the present paper, we chose the using of nitric acid, because metalic nitrates does not interfere in the atomic absorption spectrometry. Traces of toxic metals present in the studied vegetal tissues, are transformed after treatment in nitric acid in Cu, Cd, and Zn nitrates. The proposed method makes use of nitric acid – hydrochloric acid digestion and subsequent dissolution of the residue from the digestion in 10 % (v/v) HCl.

MATHERIAL AND METHODS

The determinations have been done with an atomic absorption spectrometer Carl Zeiss AAS 30, using for each metal a uni cathod lamp which is specific for each analysed element. The device use an air /acetylene flame.

A Carl Zeiss Jena atomic absorption spectrophotometer was used for all determinations with the instrument settings shown in table I.

For each studied metal, there were prepared four solutions with a concentration of 1000 mcg/mL, obtained from solving the following substances:

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1. Cd (II) – CdO is solved in 100mL of concentrated hydrochloric acid and diluting to 1 L with water.
2. Cu (II) – CuO is solved in 100mL of concentrated hydrochloric acid and diluting to 1 L with water.
3. Zn (II) – ZnO is solved in 100mL of concentrated hydrochloric acid and diluting to 1 L with water.

From the prepared solutions, there were obtained a concentration of 100mcg/mL for each ion, using succesive dilutions. There have been mixed different volumes from each standard solution, obtaining the following standard combination:

1.Cd (0.5mcg/mL); Cu (2.0mcg/mL); Zn (10.0mcg/mL).
2.Cd (1.0mcg/mL); Cu (4.0mcg/mL); Zn (20.0mcg/mL).

From Cu (II) and Cd (II), the spectrometric determination was made at λ=228.8nm and 324.7 nm. For Zn (II), the spectrometric determination was made at λ=213.9nm. The calibration curves for each analysed metal are liniar in shape, thus obtaining the interval of valability of the Lambert-Beer low.

All the samples from the vegetal tissue were kept in cold. Each sample of vegetal tissue has been treated identicaly.

2 g of vegetal tissue was treated with a mixture of HNO_3 16M -HCl12M = 1:1 v/v. The added volume is 25mL of mixture. The mixture is warmed up to 100°C, it is centrifugated, and the supernatant liquid is diluted with distilated water at a volume of 50mL.

Table 1

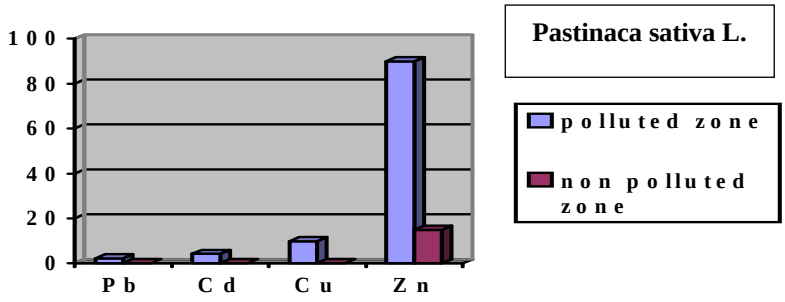
AAS Carl Zeiss Jena settings for determination of Cu, Cd and Zn

Metal	**Lamp current (mA)**	**Wave length (nm)**	**Slit**
Cu	2.5	324.7	0.5
Cd	2.5	228.8	0.5
Zn	5.0	213.9	0.5

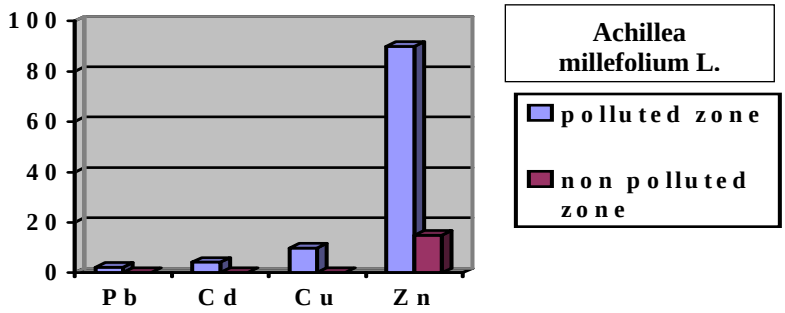
RESULTS AND DISCUSSION

The experimental results are presented in table II.

Accordingly to the dates presented in table II, it is evident that the plants from the polluted zone situated in Sinteza, were more affected by the pollution, the metal ions have recorded levels that at much higher than from the non polluted zone.



A



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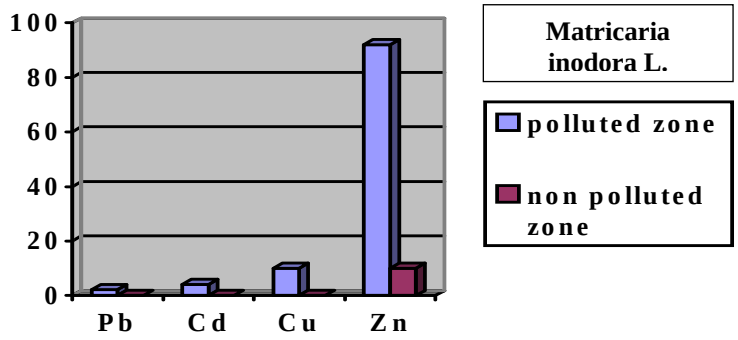

C

Figure1. Graphic aspect of the experimental samples

Table 2

The experimental results obtaining by AAS spectrometry,
from polluted zone and non polluted zone.

Metalic ion mg/g	Polluted zone Sinteza			Non polluted zone Suncuius		
	Pastinaca sativa	***Achilea millefolium***	***Matricaria inodora***	***Pastinaca sativa***	***Achilea millefolium***	***Matricaria inodora***
Cd(II)	4.10	4.20	4.05	0.05	0.06	0.04
Cu(II)	8.70	9.00	10.12	0.10	0.13	0.07
Zn(II)	85	90	87	15	20	10

The figure1 shows that the pollution with Cd was much higher at *Achilea millefolium* from the polluted zone (4.20mg/g) and in non polluted zone, 0.06mg/g.

The pollution with Cu presented high values (10.12mg/g) in the polluted zone at *Matricaria inodora* and 0.13mg/g at *Achilea millefolium.*

The Zn pollution presented very high levels in the polluted zone at all studied species, the *Achilea millefolium* presenting a maximum of 90 mg/g. Concerning the non polluted zone, the levels at the toxics is little, 10-20mg/g, maximal values are 20mg/g, at *Achilea millefolium.*

The spectrometric determinations has been performed at λ=200-400nm. The mixture of HNO_3 16M and HCl 12M has been used for:

- destruction of the organic substances until mineralization.
- transform and dissolved of the heavy metals from nitrates into salts.
- heating them for transforming into solution
- centrifugation, to remove the vegetal rest

The prelevation of the vegetal material has been performed in the summer (august). The measurements that have been dove at the bioindicator species from the polluted and non polluted zone, are present in tables 2, 3,and 4.

The species of bioindicator plants , analised in the experiment, in the polluted zone Sinteza, proved to be more sensible at chemical pollutes, from the water and soil, fact revealed by noticing of some vegetal tissue damages.

Table 3

Measurements effected for *Pastinaca sativa*

Bioindicators	Root		Stem		Inflorescence	
	Sinteza	**Suncuius**	**Sinteza**	**Suncuius**	**Sinteza**	**Suncuius**
Average	3.38	5.33	9.71	29.4	42.36	16.16
Standard deviation	1.4274	1.6143	4.4493	8.9988	14.4444	5.5193
Coefficient of variation	41.7592	30.5953	45.8224	30.6083	34.0993	34.1546

Table 4

Measurements effected for *Achilea millefolium*

Bioindicators	Root		Stem		Inflorescence	
	Sinteza	**Suncuius**	**Sinteza**	**Suncuius**	**Sinteza**	**Suncuius**
Average	4.93	5.30	32.06	45.52	4.45	6.26
Standard deviation	1.7983	1.9898	11.8737	11.1634	1.9171	2.0683
Coefficient of variation	36.4461	37.5428	37.0360	26.2545	43.0246	33.0403

Table 5

Measurements effected for *Matricaria inodora*

Bioindicators	Root		Stem		Inflorescence	
	Sinteza	**Suncuius**	**Sinteza**	**Suncuius**	**Sinteza**	**Suncuius**
Average	5.40	6.12	29.26	24.74	17.14	25.06
Standard deviation	1.5844	2.1819	9.0120	8.3977	5.7711	6.3804
Coefficient of variation	29.3400	35.6524	30.7998	33.9441	33.6708	25.4608

CONCLUSIONS

These pollutes are coming from the departments of Sinteza, special from the pigment department of Cr, Pb, and Cd, organophosphorics and drugs. Although in the present paper, we did not follow the atmospherical pollution, we can notice that the Sinteza factory determines a great degree of air and soil, observed by the concentrations of some gases.

Finally, the experimental dates showed us that the most sensible pollute bioindicator was *Achilea millefolium,* showing great values due to metalic ions, the variability coefficient been greater in comparation with that of the other species. The variability coefficient have the following values: the length of the roots is 36.44 in the polluted zone and 26.25 in the non polluted zone; the diameter of the inflorescence is 43.20 in the polluted zone and 33.04 in the non polluted zone.

Most closed values regarding the variability coefficient is presented by *Pastinaca sativa* with the following values: root length in the polluted zone is 41.75 and in non polluted zone is 30.59, inflorescence length in the polluted zone was 34.09 and 34.15 in the non polluted zone.

The *Matricaria inodora* species proved to be the most resistant regarding the pollution with heavy metals.

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THE DETERMINATION OF THE SANOGENETIC POTENTIAL THROUGH THE AAS METHOD AT SOME DECORATIVE PLANTS

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KEY WORDS: *atmospheric pollutants, decorative plants, atomic absorption spectrophotometric*

ABSTRACT

A series of decorative plant species, especially in the urban area, fulfills along with an esthetic role a sanogenesis role by fixing different pollutants in the air. This characteristic is influenced by the species, the type of pollutant, the atmospheric air, etc. From the qualitative point of view, the atomic absorption spectrophotometric method has proved to be faster, precise and reproducible.

Applied in the ingathered pollen's analysis form 6 species, it has been determined that the measured values for heavy metals are far more beyond the recommended limit by the Romanian Pharmacopeia edition X (FR.X.). Toxic elements as: Pb, Ni, Cd are under the detection limit of the analyzing method. Some heavy metals as: Pb, Cd, Cr, Ni, Mn, can emanate from the soil or they can come from the atmospheric pollutants, burned gases respectively outlet gases.

INTRODUCTION

The research of the natural composition of water and air as well as of different pollutants, rises extremely difficult problems caused by the presence, at the same time, of a great number of composites, same as of the concentrations that spread between $0{,}1\text{-}10^{-9}$ (μg/g).

Ecotoxicology studies the noxious effects of the chemical pollutants on vegetal and animal ecosystems, terrestrial and aquatic. At the same time it studies the passing of the toxics along the alimentary chains with the possibility of accumulating at different levels of these. The persistence of the substance in the environment (air, water, soil) on which the duration of its effects depends, can be appreciated after its degree of degradability in the ecosystem.

From this point of view, there are substances and composites easily degradable or resistant at degradation under the influence of the microbic flora or at different environmental conditions (temperature, humidity, insolation, pH, etc.) (Schuurmann and Markert, 1997).

The pollution with toxic elements including the so – called heavy metals, with high density, as well as that with some lighter metals or of the toxic metalloids, constitutes

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a real threat given by the dissemination in the working environment, but also of the one in life, were air and foods frequently constitute the charging channel of the organism. Among the pollution modalities, the most frequent is through the water for irrigations (intensively polluted residual waters) or through the dust eliminated by the processing industries and the energy generating industries (thermal power stations).

Once they have reached the soil or on the plant's surface, the contaminants are absorbed by the plants and disseminated or concentrated in certain areas: **tubercles (**arsenic, selenium, molybdenum, chrome, etc.)**, leafs** (selenium, plumb, stibium, mercury, molybdenum, etc.), **fruit/pollen** (molybdenum, nickel, chrome, plumb, cadmium etc.) (Bargagli, 1998).

Lately, for establishing the pollution degree the instrumental methods have replaced more and more the classical volumetric and gravimetric methods for a large number of determinations. The superiority of these methods consists in the shortening of the execution duration of the determination and in the increasing of the result's accuracy.

The energetic atomic absorption and emission has become one of the analytical methods of quantitative and qualitative analysis.

Atomic absorption spectrophotometric (AAS) has become the most common technique in the determination of metals in the last 30 years.

It is the method that gives very good results for the determination of the microelements as well as for the other mineral elements. The celerity and sensibility of this method has determined its use in many laboratories. The method is very sensible and allows the determination of a great number of elements from a small volume of sample, through simple and reproducible operations (Morait and Roman, 1983).

Through this method there can be determined a number of 27 mineral elements among which: As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, Co etc. (Bungău et al., 2004).

MATERIAL AND METHOD

The vegetal material submitted for analysis

The samples submitted for analysis, were presented by the pollen coming from 6 vegetal species, that were harvested in the period of maximum inflorescence for each species, during March – May 2006, in Oradea, for the samples 1, 2, and 6, and from Satu Mare for the samples 3, 4, 5:

Sample 1 - *Juglans regia* pollen
Sample 2 - *Corylus avellana* pollen
Sample 3 - *Tulipa gesneriana* pollen
Sample 4 - *Populus nigra* pollen
Sample 5 - *Fraxinus ornus* pollen
Sample 6 - *Castanea sativa* pollen

The samples were gathered and dried on a glass plate in a kept off place from the possibility of a pollution, and have been operated under the form of an alcoholic extract (tincture)

Obtaining the tinctures

The vegetal product brought to the comminuted degree mapped out in the monography of each of the species (Eur. Arzn.), has been submitted, if the situation required, at a preliminary scouring.

The solvent used at extraction was thin alcohol.

The ratio between the mass of the vegetal product and the extraction solvent was of: 1:10 (m/m), for the tinctures that were made out of vegetal products that contain strongly active substances (FR.X.).

Over the vegetal product, brought to the comminuted degree mapped out in the monography, was added the solvent or the mixture of solvents stipulated, in a well closed vessel. It was kept at room temperature for ten days, and was shacked 3 – 4 times a day. The extractive liquid was decanted and the residue was pressed. In the case of the vegetal products that don't have their own monographies, has been worked with 1g of dried vegetal product (*Tulipa gesneriana*).

The obtained tinctures were liquid, clear, colored, with the smell and taste characteristic for the components of the vegetal product from which they were prepared and of the solvent used for extraction.

Limits admitted according to effectual standards are for iron: at the most 0,001% and for heavy metals: at the most 0,001% (FR.X.).

The analysis of the samples through the atomic absorption spectrophotometric method (abbreviated "AAS") can be realized through the direct aspiration method, without any preliminary arrangement (Skoong and Leary, 1998). The standards used are prepared from the alcohol used for preparing the tincture, in order to ensure the similitude of the matrixes with those of the samples. In the used standards an equilvalent quantity of ethyl alcohol is introduced.

Preparing the basic standards:

The Atomic absorption spectrophotometric method being an instrumental method that measures the physical parameter, the measurements must be compared to a standard, respectively with a solution that contains a known concentration from the elements that is being determined. For this purpose reagents of analytical purity were used, and the used dilutions were bigger, because the present impurities in the common reagents can affect the analysis's results.

Calibrating the gauges:

For the actual calibration of the spectrophotometer with atomic absorption, have been used sets of norms, chemicals from the basic standard solutions, through dilution. The basic standard solution is introduced in burette, from which increasing quantities are measured in balloons leveled at 50 or 100 cm^3. For the drawing of a calibration curve is necessary at least three standards, and in the execution of some measurements that can give certanty to the analyst that the obtained result is close to the real one with a probability higger than 95%, it is necessary that the mapping out of the calibration curve to be made at least out of 6 standards. The usage of this number allows not only the drawing of a calibration curve as precisely as possible, but also in establishing the spreading degree of the points (determinations) towards the most probabile bee line and so implicitely, the knowing of the precision in mapping out a standard curve.

Used reagents

- standard solutions for heavy metals **(**Cu, Zn, Fe, Mn, Ni, Cr, Cd, Pb,) 1000 mg/l Merck
- ethylic alcohol p.a.
- bidistillated water

The used installations

The spectrophotometer of atomic absorption with GBC type flame, Avanta. The parameters of the apparatus: air/acetylene flame, correction fund with deuterium flame, the

burner's length 10cm, optical system with double fascicle. The commanding of the apparatus and the processing of the data is done through the AVANTA software.

The absolute calibration of the apparatus: from the alternate standard solution (1g/l) is being prepared through the dilution of the working solution (100 mg/l). From this solution is being prepared the calibration standards with these concentrations: 0,5 mg/l; 1,0 mg/l; 3 mg/l; 5 mg/l; 10 mg/l using the equivalent quantity of ethylic alcohol.

Measuring the samples

The apparatus is being adjusted according to the working instructions. The wave length characteristic for the element is being established (**Table I**). The standard solutions and the tincture sample in being introduced in the apparatus through direct aspiration in the flame.

The determined metal concentrations are expressed in micrograms/ml solution for analysis (mg/l or ppm).

Table I.

Wave length characteristic for the element that is being determined

The element	Spectral line used
Cadmium	228,8 nm
Chrome	357,9 nm
Copper	324,8 nm
Mangham	279,5 nm
Nickel	232,0 nm
Plumb	217,0 nm
Zinc	213,9 nm

The metal concentrations determined were related to the unitary volume of tincture and have been expressed in micrograms/gram plant (ppm) and/ or micrograms/milliliter tincture. The obtained results are given in **Table II.**

Table II.

Metal concentrations determined from the samples submitted to the analysis expressed in µg/g plant

Microelements	Concentration (µg/g plant)					
	Sample1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Cu	< 0,02	0,370	0,234	1,560	1,689	< 0,02
Zn	< 0,01	< 0,01	0,516	5,590	4,623	< 0,01
Mn	< 0,03	0,133	0,293	0,710	< 0,03	< 0,03
Ni	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05
Cr	2,130	0,253	0,046	< 0,07	0,640	2,910
Cd	< 0,015	< 0,015	< 0,015	< 0,015	< 0,015	< 0,015
Pb	< 0,018	< 0,018	< 0,018	< 0,018	< 0,018	< 0,018

Table III.

Metal concentrations determined from the samples submitted to the analysis expressed in µg/g tincture

Microelements	Concentration (µg/g plant)					
	Sample1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Cu	< 0,002	0,0370	0,0234	0,1560	0,1689	< 0,002
Zn	< 0,001	< 0,001	0,0516	0,5590	0,4623	< 0,001
Mn	< 0,003	0,0133	0,0293	0,0710	< 0,003	< 0,003
Ni	< 0,005	< 0,005	< 0,005	< 0,005	< 0,005	< 0,005
Cr	0,2130	0,0253	0,0046	< 0,007	0,0640	0,2910
Cd	< 0,0015	< 0,0015	< 0,0015	< 0,001	< 0,0015	< 0,0015
Pb	< 0,0018	< 0,0018	< 0,0018	< 0,001	< 0,0018	< 0,0018

THE INTERPRETATION OF THE RESULS

In the gathered and processed samples, through spectrophotometric of atomic absorption analysis, the above mentioned concentrations were found. The maximum limit admitted for the content of heavy metals in tinctures according to the Romanian Pharmacopeia ed.X is 0,001%=10mg/l F.R.X). By totalizing the determination's concentrations resulted, the total content of heavy metals from the prepared tinctures, are by far under the limit recommended by FR.X. We can underline the fact that the toxic elements as: Pb, Ni, Cd are under the limit of detection of the analysis's method.

Some heavy metals as: Pb, Cd, Cr, Ni, Mn, can come from the soil or they can come from the atmospheric pollutants, because of the everyday circulation, meaning the exhausted gases. At the gathered samples from Oradea (samples 3, 4, 5) the determination's values for heavy metals are relatively increased, in relations with those taken from Satu-Mare (samples1, 2, 6), thing that indicates an increased concentration of the pollutants coming from the soil and/or the atmospheric pollutants in Oradea in comparison with Satu-Mare city.

At the same time, the choosing of the analysis method of the heavy metal content, and also in the manner of gathering, pretreatment and conservation of the samples subjected to the analysis, overwhelming contributes to the correctness of the obtained experimental data.

CONCLUSIONS

The performed analysis allows the interpretation of the phenomenon through which species without allergenic effects, in time, suffer modifications caused by the natural defending response against different toxins, thing that leads to important changes of their levels, at a morphological and cytological level.

Such a phenomenon has been registered at the pollen's level, where, a high quantity of protean and lipidic nature substances concentrate, but also the heavy metals can

come from different sources. These metals can modify the pollen's aspect and can form new enzymatic systems for neutralizing the toxins, phenomena that conducts at the transformation of the species in adapted forms at the new environmental conditions, in many time aggressive.

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PRELIMINARY PHARMACOBOTANICAL STUDY ON CULTURED PLEUROTUS SPECIES

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KEY WORDS: Pleurotus ostreatus L., cultured, qualitative and quantitative determination

ABSTRACT

Pleurotus ostreatus– the oyster mushroom, grows worldwide in the wild on deciduous trees in shelf-like clusters. The most important quantity of this mushroom comes from controlled cultures, grown on farms, from mycelia cultivated on liquid or solid substrates.

Taking into consideration its highly appraised nutritional value as well as the therapeutical properties of Pleurotus species, the aim of the present study is a preliminary analisys to establish the morphological and qualitative parameters and the content in protein and polysaccharides for the basidiocarp as a whole, and separately for the stipes.

INTRODUCTION

Many types of edible and poisonous mushrooms have long been used worldwide, but especially in the Middle East, for medicinal purposes. At present times, 80-85% of medicinal mushrooms products are obtained from cultured mushrooms. For the purpose of extracting the pharmacologically valuable compounds, generally whole mushrooms, extracts, or isolated compounds can be used.

These compounds can be obtained from mushrooms growing on farms or in their natural habitat, from mycelia cultivated on liquid or solid substrates. (Wasser 1999, Babitskatya 1999, Mateescu 1983). Pleurotus is one of the easiest mushrooms to cultivate, most often on straw or on logs with sawdust.

This species has become one of the most well known edible mushrooms, having a pleasant odour and taste. Nowadays about 1 million tons of Pleurotus mushrooms are produced, especially in China, Germany and the USA. In the world top of the mushrooms cultivated for culinary purposes, Pleurotus comes second after *Agaricus campestris* (*Agaricus bisporus*) (Weasel 1998).

The most cultivated species among the genus Pleurotus are: *Pleurotus ostreatus*, *Pleurotus florida, Pleurotus cornucopiae.* The most important goals of the cultivators are to ensure a superior quality and a homogeneity of composition for each harvest (Burzo 1980, Mateescu 1983).

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MATERIAL AND METHODS

The vegetal product subjected to analysis is represented by the fruiting body of the species *Pleurotus ostreatus* obtained from controlled cultures: I/4 series and II/4 series. The two series represent the species harvested in the first flush, on the fourth day, and on the fourteenth day from the basidiocarp formation period. The methods employed in determining the identity and the quality of a vegetal product subjected to analysis are the qualitative methods: macroscopic, microscopic, microchemical, referred to in the specialty books (Fasidi 1994, Parv 1996, R.F.X 1998).

Macroscopic examination of the basidiocarp

The macroscopic examination determines the main morphological characteristics of the fruiting bodies subjected to analysis: colour, fracture, odour, taste. The colour of the fruiting bodies is varied, being one of the main criteria in the macroscopic identification of the species. It differs from one species to another, and it is determined by the pigment in the cytoplasm and in the hyphal walls. Depending on the water solubility of the pigments, the fruiting bodies of some mushrooms change their colour on rainy days. Also, when touching, breaking or crushing, in contact with the air, the flesh or the external surface of some mushrooms change their colour to blue, green, yellow, brown or red as a result of oxidation reactions of some of the substances contained. The smell and the taste are characteristic. The taste is perceived differently: immediately or after a long while. The main characteristic of the fruiting body is spore production. At a certain stage of development a fertile area is individualized on the fruiting body – the hymeneal region which produces the basidia with the basidiospores. A palisade layer of prolonged cells is being differentiated, from which the basidia develop, and in which at the beginning the two adjacent nuclei form 4 exogenous spores disposed at the top of the sterigmata – the basidiospores.

Microscopic examination of the basidiocarp

The examination was carried out using an optical microscope Ultimate digital model MFL-85.

1. Species identification according to taxonomic position, using specific reagents

Melzer's reagent preparation

0,5 g iodine and 1,5 g potassium iodide are dissolved in 20 ml water and 20 ml chloral hydrate. The components are boiled in a Soxhlet installation for 40-60 minutes over a low flame, followed by filtration. The colour reaction of the spores with Melzer's reagent represents one of the main criteria in species determination. When identifying genera the colour of the spore dust is considered, which can be obtained by placing under a glass bell a sporocarp with the fertile region facing downwards on a black sheet of paper. The spores are laid down after a few hours, a sporogram being thus obtained.

Results: The spores obtained from *Pleurotus ostreatus* cultures are long, unicellular, light yellow. With Melzer's reagent the same blue coloration was obtained. The sporogram is identical. The elongated hyphae are fragmented, appearing as septate tubes. The spores are long unicellular with dimensions ranging between 0,2-0,5 cm, the colour is white-yellowish, ornamentation being characteristic.

Microscopic characterization of the analyzed series I/4 and II/4

Microscopic examination of the flesh distinguishes several types of hyphae: fundamental hyphae or inflated long-celled hyphae; generative hyphae or growing hyphae with side clamps; hyphae with a rich content of reserve substances; oliferous hyphae , rich

in oils and odoriferous substances; skeletal hyphae, with thick, woody or suberous walls. The fruiting bodies, represented by the mycelial hyphae, are differentiated at the two series by the density of the lax texture. Their dimensions range from a few millimeters to 1-2 cm. A secondary mycelium composed of septate hyphae with binuclear cells, with ramifications and strings is identified. The sterigmatae bear the basidiospores with 4 elongated basidia, of white colour on the coloured substrate represented by the primary mycelium, with numerous ramifications.

Determination of dry substance content and of residuum by calcination

The Romanian Pharmacopoeia Xth edition sets out the working method for establishing the mass of volatile compounds of any nature in the vegetal product subjected to analysis. The loss is calculated according to certain conditions of temperature, pressure and time. The results obtained are expressed in grams and are calculated in percents (% m/m). Determination is carried out in measuring vials whose diameter is chosen so that the layer is 1 cm thick. The measuring vial is brought to constant mass under the same conditions as the determination itself.

The samples to be analyzed are collected from the two series and are represented by the whole fruiting body and separately by the stipes, freshly harvested. 5 gram samples of the product, grounded to small fragments are measured on the analytical balance.

The measuring vial with the working sample is kept in the drying oven at 105ºC for 3-4 hours, cooled in a dessicator and measured. The drying process is furthered for periods of time of one hour, followed by cooling in the dessicator and measured until constant mass.

Determination of residuum by calcination

The residuum by calcination is the residuum obtained by the calcination of an organic substance. In the case of vegetal products the residuum obtained is referred to as ash. The results obtained are expressed in grams and calculated in percentages (% m/m).
The porcelain capsule used for the determination of the residuum by calcinations is brought to constant mass by maintaining it to the same temperature the calcination is to be carried out and it is cooled in the dessicator. 5 g samples are measured as follows: whole fruiting bodies harvested from the series I/4 and II/4, and fresh separate stipes from the same series. The sample grounded to small fragments is measured in the porcelain capsule on the analytical balance and heated on the wire gauze in low flame till the removal of water and other volatile substances. Heating is continued on a chamotte triangle, gradually increasing the temperature of the flame; heating is continued until carbonization. The capsule is cooled in the dessicator and weighed. Calcination is carried on, for 15 minutes followed by cooling in the dessicator and measuring till constant mass. To the residuum obtained from calcination 2-3 ml 100g/l hydrochloric acid is added. The capsule is covered with a watch glass and heated in the water bath for 10 minutes. 5 ml of 70 ºC heated water is added – also used for washing the watch glass-and the content is filtered using a microporous filter. The precipitate is brought on the filter and washed with 70 ºC heated water until the reactions of the wash waters with the chloride ion are negative. The filter containing the precipitate is dried at 105ºC, placed in the initially used capsule and calcined to constant mass.

Determination of proteins

More than half of the dry mass of the mushroom is formed of proteins containing all the essential amino acids. The mycelium uses sugars, organic acids as carbon source and components resulting from decomposition of proteins such as peptones, amino acids, urea and ammonia as nitrogen source.

Material and methods

Determination of overall protein content was carried out using the gravimetric method for the two representatives of I/4 and II/4 series.

The gravimetric method of measuring the protein content is based on the property of proteins to precipitate with salts of heavy metals (Hg^{2+}, Ag^{+}, Pb^{2+}), organic solvents such as ethanol, methanol, acetone or acid reagents (trichloroacetic acid, picric acid, phosphomolybdic acid). Extraction from the vegetal material can be performed using water, hydroalcoholic or saline solutions, phosphate buffer. The vegetal material was carefully treated not to damage the properties of the proteins.

Samples 1 and 2- whole fruiting bodies from series I/4 and II/4. Drying was performed under natural conditions keeping them from direct sunlight.

Samples 3 and 4– separate dry stipes from the two series

Working conditions

- extraction temperature 4-5ºC (refrigerator); separation by filtration; vacuum drying, maximum temperature 30 ºC; extraction solvent: phosphate buffer pH=7,4; precipitation solvent: methanol; precipitation time: 48 hours.

The vegetal materials are brought to the optimal grinding degree (sieve IV). 2,0 g samples are weighed and transferred to an adequate container, then 80 ml phosphate buffer pH=7,4 is added and stirred. The container is kept in the refrigerator at 4-5 ºC for 48 hours. It is stirred periodically then filtered through cotton wool. The residuum is washed twice with 10 ml phosphate buffer and 10 ml methanol is added by stirring. The mixture is kept at rest in the refrigerator for 24 hours followed by quantitative filtration, the filter is washed with methanol twice (maximum 10 ml). The filter with the precipitate is dried in the vacuum drying oven. The quantity of proteins resulted is determined by weighing. The obtained value is determined using the formula:

$$\text{proteins } \% = M_1\text{-Mo/a} \times 100$$

where:
M1= mass of filtrate with precipitate
M0= mass of filter
a= quantity of working vegetal material

Extraction of polysaccharides

There is a certain resemblance between the various methods employed for the extraction of antineoplastic polysaccharides from mushrooms. In the first stage, the dried powder from the mushrooms or from the mycelium is gradually heated in 80 % alcohol to extract and remove the substances with low molecular mass. Several gross fractions are obtained from the alcoholic residuum:

I. by water extraction at 100 ºC for 3 hours
II. 1% ammoium oxalate at 100 ºC for 6 hours
III. 5% sodium hydroxide at 80 ºC for 6 hours

The most effective method for extracting D glucan from *Pleurotus ostreatus* uses ethanol precipitation followed by lyophilization. Using a carbohydrate chromatography column the purity of the extract could be established, which is 87,5 % lentinan. The commercial value of this new method is of great importance as as it is more rapid, economically affordable and effective. The samples subjected to analysis are species of *Pleurotus* from I/4 and II/4 series. Determination of overall polysaccharide content was carried out at the Biology laboratory, University of Pecs, employing the *Yap* and *Neugemann* separation method.

RESULTS AND DISCUSSIONS

As a result of determinations the chemical content of the separate parts of *Pleurotus ostreatus* fruiting body could be established. The results are presented in table I. In the whole fruiting bodies, for both series, a quantity of over 40 % of protein from the overall dry substance was determined.

The species harvested in the first flush, on the fourth day of basidiocarp formation contain with 4 % more protein than those from the second flush, harvested on the 14th day. For the separate stipes, the determined values represent ¼ of the overall content of protein, for both series. The values obtained certify the homogeneity of the cultures and the high nutritional value of the analyzed *Pleurotus* species.

Table I.

Chemical content of parts of *Pleurotus ostreatus*

Analyzed vegetal material	Dry substance % m/m	Water and volatile compounds % m/m	Residuum by calcination % m/m	Proteins % m/m	Overall polysaccharide content % mg/g
Fruit I/4	9,842	90,158	6,321	46,22	325
Fruit II/4	9,456	90,544	6,073	41,13	318
Stipes I/4	7,230	92,770	4,643	11,55	-
Stipes II/4	7,441	92,559	4,779	10,28	-

CONCLUSIONS

Determination of chemical composition of the fruiting bodies involved:

Determination of content of dry substance, the values obtained ranging between 7,230% m/m and 9,842 % m/m, being lower for the stipes compared to the basidiocarp.

Determination of residuum by calcination (ash) led to lower values of 4,643 % m/m compared to the basidiocarp.

Determination of protein content certified the fact that more than half of the dry weight of the mushroom consists of proteins with all the essential amino acids. The mycelium uses sugars, organic acids as carbon source and components resulting from decomposition of proteins such as peptones, amino acids, urea and ammonia as nitrogen source.

The maximum values determined for the basidiocarp were 46,22 % m/m and 11,55 % m/m for the stipes. Separately for the stipes, the determined values represent ¼ of the overall content of proteins. The values obtained certify the homogeneity of the cultures and the high nutritional value of the analyzed *Pleurotus* species.

Extraction of polysaccharides employing a modern, easy to use, highly efficient method facilitated obtaining values of 318- 325 (mg/100g) overall purified polysaccharides. It can be stated that the period of harvesting does not significantly influences the content of overall polysaccharides, responsible for the antineoplastic and immunostimulant effects.

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COMPARATIVE STUDY ABOUT DIGIT RATIO IN TWO FEMININE POPULATIONS OF BIHOR COUNTY

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KEY WORDS: digit ratio, feminine populations, Oradea, Beiuş

ABSTRACT

This paper is about the differences of digit ratio in two human populations from two localities. These localities are: Oradea and Beiuş, from Bihor county. Oradea is a locality with over two hundred thousands of inhabitants, which means the variability of some phenotipical features must be a large one. Beiuş is a smaller locality (has under fifty thousands inhabitants), which means the variability of some phenotipical features is lower than in Oradea. This fact is showed in our study. We investigated 100 females in each locality. It were measured the lenghts of the digits 2, 3 and 4, and then we made the digit ratio. The results are important: the digit lenghts are very different in the two localities; the 2D:4D digit ratio, too.

INTRODUCTION

In a genetically heterogenous population, many genotypes will be formed by the processes of segregation and recombination. The study of inheritance in humans made necessary the appearance of a particular methods for genetic analysis (Maximilian et al, 1996). Making some special measurements is very important to detect some morphological traits (Hall et al., 1995). Some traits have a special inheritance which is not like a mendelian inheritance. We can mention some examples: colour of skin, eyes and hair, dermatogliphics, inteligence, height, weight etc. There are some factors which interact with the frequency of genes in a population. So, they can increase or decrease the alleles frequencies from a generation to an other. These factors are: non-randomised marriages, alteration of mutation rate, selection, small populations, genetical isolated population and migration (Applewhite, 1994; Batshaw, 1997; Behram et al., 1996). Human population can have some changes in sizes and traits (hair form, colour of eyes, hair, skin, lip firmness etc). These traits are determined by the interaction of genotype, environment, geographic area, climate conditions etc. (Ionescu and Drăgoi, 1974; Ionescu et al., 1994; Jones, 1996; Tomulescu, 2002).

The scientific study of papillary ridges of the hands and feet is credited as beginning with the work of Joannes Evangelista Purkinje, a czech psychologist and biologist in 1823. Then, in 1892 Sir Francis Galton published his classic treaties on fingerprints. He also studied the hereditary aspects of fingerprints, investigating

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comparisons of siblings, twins and genetically unrelated individuals and was the first to report concordance of papillary ridge patterns among relatives. Specifically, it is the ratio of the length of the index finger (digit 2, or "2D") and the ring finger (digit 4, or "4D") that is sexually dimorphic. Generally, males have a ring finger that is longer than their index finger. Females typically have index and ring fingers of about the same length. The ratio of index finger length to ring finger length is called the "2D:4D digit ratio," or more simply, the "digit ratio." Manning reports that, for males, the index finger is generally about 96 percent of the length of the ring finger, which gives an average digit ratio for males of 0.96. The digit ratio would be 1.00 if the ring and index fingers were the same length, and greater than 1.00 if the index finger was longer than the ring finger. Males generally have a digit ratio below 1.00 -- they have what is termed a "low digit ratio." Women generally have a digit ratio of about 1.00 (the index and ring fingers are of about equal length), or a "high digit ratio." Height, like digit ratio, is still sexually dimorphic. But the causes of between population variation in sexually dimorphic traits, such as digit ratio, is certainly puzzling, and it is a fertile area for future research.

MATERIAL AND METHODS

We investigated 200 individuals. We measured the length of the 2^{nd}, 3^{rd} and 4^{th} fingers of 100 from each locality (Oradea and Beiuş). The individuals were randomised choosed. We measured the length of the fingers, from the finger basis to the superior bound of the phalanx. Then we calculated the digit ratio: 2D:4D, 2D:3D and 3D:4D. Also, we calculated F distribution and z score.

Dictionary:
2D=index finger (second finger);
3D=median finger (third finger);
4D=ring finger (fourth finger);
2D:4D=ratio between index and ring finger length;
2D:3D= ratio between index and median finger length;
3D:4D= ratio between median and ring finger length;

RESULTS AND DISCUSSIONS

The results of our research are presented in the following tables. In table 1 are presented data about the feminine population of Oradea locality. The results obtained were analysed in tables 3, 4 and 5.

From table 1 we can observe in general the decreased variability of all digit ratio in Oradea locality. The observation is justified for all digit ratio, for both hands. So, there are no great variability of this feature. Making F distribution and z test (presented in table 2), we may strongly affirm that is no significant difference between two hands in population of Oradea. We analised 100 individuals. The critical value in table for F distribution in tha case of 100 subjects is between 1.43 and 1.53. For z test, the critical interval is between 1.96 and 2.58. In the both cases, our obtained values are lower, so, the differences are not significant (table 2).

In table 3, we can notice the small variability of the digit ratio in right and, separately, in left hand. 2D:4D digit ratio of right hand is near a value of moderate variability. But, in general we may say that the variability of all type of digit ratio is low.

Analysing the F distribution, we may say the two variances (of 2D:4D and 3D:4D digit ratio) of Beiuş are not identically, or we may say the values proceed from two very

different groups. After making the z-test, this showed that the averages are not significantly different (table 4). In the other words, comparing the two hands, we didn't noticed significant differences.

Table 1.

Presentation data about digit ratio of a female population in Oradea locality.

Parameters	***Right hands (RH)***			***Left hands (LH)***		
	2D:4D	2D:3D	3D:4D	2D:4D	2D:3D	3D:4D
Average	1.005442	0.916181	1.096054	1.005416	0.916155	1.095514
Standard deviation	0.038179	0.027684	0.034413	0.038106	0.028676	0.036938
Variance	0.001457	0.000766	0.001184	0.001452	0.000822	0.001364
Variation coefficient	3.797248	3.02172	3.13974	3.790104	3.130081	3.371791

Table 2.

Presentation of the Right hands-Left hands comparison
in a female population of Oradea.
F distribution and z-test.

Digit ratio	***Right hands-Left hands***	
	F	***z-test***
2D:4D	1,003443	0,00482
2D:3D	1,073107	0,006526
3D:4D	1,152027	0,107142

Table 3.

Presentation data about digit ratio of a female population in Beiuş locality.

Parameters	***Right hands (RH)***			***Left hands (LH)***		
	2D:4D	2D:3D	3D:4D	2D:4D	2D:3D	3D:4D
Average	0.995853	0.900252	1.096191	0.986399	0.903407	1.101221
Standard deviation	0.097525	0.034297	0.044432	0.034753	0.033367	0.093825
Variance	0.009511	0.001176	0.001974	0.001207	0.001113	0.008803
Variation coefficient	9.793126	3.80966	4.053339	3.523212	3.693518	8.520114

Analysing the F distribution for right hands of the two studied localities (table 5), we may say the three variances are not similar or very different, or we may say the values proceed from two very different groups. After making the z-test, this showed that the averages are not significantly different (table 5) in the cases of 2D:4D and 3D:4D digit ratio. In the other words, comparing the two hands, we didn't noticed significant differences in the cases of these two ratio in right hand. The difference was observed in the case of 2D:3D digit ratio. In the cases of left hands, the results are different. The F distribution shows that the data proceed from a very different populations in the cases of 3D:4D digit ratio. But, the z test in the case of this digit ratio didn't show a significant

difference between the the populations averages. The differences are significant in the cases of the 2D:4D and 3D:4D digit ratio.

Table 4.

Presentation of the Right hands-Left hands comparison
in a female population of Beiuş.
F distribution and z-test.

Digit ratio	***Right hands-Left hands***	
	F	***z-test***
2D:4D	7,879867	0,913253
2D:3D	1,056603	0,659489
3D:4D	4,459473	0,484539

Table 5.

Presentation of the Right hands and Left hands comparison
in the two female populations of Oradea and Beiuş.
F distribution and z-test.

Digit ratio	***Right hands (RH) Oradea - Beiuş***		***Left hands (LH) Oradea - Beiuş***	
	F	***z-test***	***F***	***z-test***
2D:4D	6.527796	1,0685	1.202982	3,68
2D:3D	1.535248	3,615297	1.354014	2,8985
3D:4D	1.667229	0,026570	6.453812	0,5660

There is a lack of information about digit ratio. Recently, some researchers start to investigate this feature of human race: digit length and digit ratio. Many of them linked the results of some environmental factors (geographic area, relatives etc) or other normal and abnormal human traits (dermatogliphics, inteligence, height, weight, malformations, sexual behaviour, autism, schizophrenia etc) (Arato et al, 2004; Wassink and Piven, 2000).

Manning links the proximate causes of digit ratio sexual dimorphism to the effects of sex hormones during early fetal development. He believes the evidence is persuasive, but not yet definitive, that higher levels of testosterone during this critical developmental stage facilitates the growth of the ring finger, while higher levels of estrogen facilitates the growth of the index finger. He also suggests that hypermasculinization increases the likelyhood of homosexuality or bisexuality, in both males and females. Somewhat surprisingly, the effect size for digit ratio between the sexes varies substantially as a function of geography and race.

Surprisingly, the females in some cultures may have a lower digit ratio than males of other cultures, although men have a lower digit ratio than women within populations in all cultures for which there is data. It is unclear why the effect size of the digit ratio of the sexes varies between different populations. This is a curious fact, one for which Manning provides little in the way of definitive conclusions -- and the reader may be left to wonder whether some of Manning's interpretations are threatened by this between population variability in effect sizes.

However, the fact that the average height of men of some populations is lower than women of other populations doesn't negate the sex difference in height, nor does the

fact that the gender effect size of height varies in different populations. It has been suggested that autism may arise as the result of exposure to high concentrations of prenatal testosterone. There is evidence that the ratio of the lengths of the 2nd and 4th digit (2D:4D) may be negatively correlated with prenatal testosterone (Maning et al., 2005). Voracek et al (2005) relate that neurohormonal theories of sexual orientation emphasize the organizational effects of testosterone on the developing brain. A recent suggestion, that the ratio of the length of the 2nd and 4th digits (2D:4D) is negatively correlated with prenatal testosterone, has led to a number of studies of 2D:4D in homosexual and heterosexual men and women.

The results have been mixed. In comparison to heterosexual men, mean 2D:4D in gay men has been reported to be hypermasculinized (lower 2D:4D), hypomasculinized (higher 2D:4D), or to show no significant difference. They report mean 2D:4D in Austrian homosexual and heterosexual men and found no significant difference between means for homosexual and heterosexual 2D:4D, with values for both falling between 0.96 to 0.97. There are now 6 reports of 2D:4D in heterosexual and homosexual men. Considering Caucasian men, the studies from the United States show low heterosexual mean 2D:4D, and homosexual mean 2D:4D is higher or similar to that of heterosexuals.

The European studies show high heterosexual mean 2D:4D, and comparisons with homosexuals reveal the latter to have lower or similar mean 2D:4D to that of heterosexuals. They discuss these results in relation to the suggestion that mean 2D:4D in heterosexual men differs across populations but mean 2D:4D in homosexuals shows less geographical variation (the "uniform mean hypothesis"). It is concluded that more data are required to clarify whether or not there is a 2D:4D effect for sexual orientation in men. The differentiation of the human brain is triggered by sexual steroid hormones in the fetus. The development of both the urogenital system and the appendicular skeleton are under common control by the HOX genes. Schizophrenic men and women showed a more "feminine" phenotype of the index and ring fingers in both hands than same-sex controls. This finding implies that low fetal androgen/estrogen ratio may have a predisposing role in the development of schizophrenia and points toward involvement of endocrine factors in the disturbed hemispheric lateralization attributed to the illness (Williams et al, 2003).

However, these data also draw attention to difficulties in the interpretation of results when somatic features are employed as biological markers of prenatal hormonal influences. The lack of consistency in the literature may be due to the differences in sample characteristics, methodology, or analytical techniques.

CONCLUSIONS

We can observe in general the decreased variability of all digit ratio in Oradea locality. The observation is justified for all digit ratio, for both hands. Z-test shows in the both cases the differences are not significant. In Beiuş, comparing the two hands, we didn't noticed significant differences. Analysing the F distribution for right hands of the two studied localities, we may say the values proceed from two very different groups. Comparing the two hands, we didn't noticed significant differences in the cases of these two ratio in right hand.

The difference was observed in the case of 2D:3D digit ratio. In the cases of left hands, the results are different. The F distribution shows that the data proceed from a very different populations in the cases of 3D:4D digit ratio. But, the z test in the case of this digit ratio didn't show a significant difference between the the populations averages. The differences are significant in the cases of the 2D:4D and 3D:4D digit ratio.

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CONTRIBUTIONS ABOUT THE COMPARATIVE DIGITAL MEASUREMENTS IN TWO MASCULINE POPULATIONS OF BIHOR COUNTY

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KEY WORDS: digit ratio, masculine populations, Oradea, Bulz

ABSTRACT

This paper is about the differences of digit ratio in two human populations of localities. These localities are: Oradea and Bulz, in Bihor county. Oradea is a locality with over two hundred thousands of inhabitants, which means the variability of some phenotipical features must be a large one. Bulz is a smaller locality (has under ten thousands inhabitants), which means the variability of some phenotipical features is lower than in Oradea.

This fact is showed in our study. We investigated 100 males from each locality. It were measured the lenghts of the digits 2, 3 and 4, and then we made the digit ratio. The results are important: the digit lenghts are very different in the two localities.

INTRODUCTION

Many traits that are important in human genetics are determined by the effects of many genes and by the environment. Many of these are influenced not only by the alleles of two or more genes but also by the effects of environment. Such traits are called quantitative traits, and with quantitative traits the phenotype of an individual is potentially influenced by genetic factors and environmental factors (Hartl et al., 1987; Maximilian et al, 1996). Making some special measurements is very important to detect some morphological traits (Hall et al., 1995).

Some traits have a special inheritance which is not like a mendelian inheritance. We can mention some examples: colour of skin, eyes and hair, dermatogliphics, inteligence, height, weight etc. There are some factors which interact with the frequency of genes in a population.

So, they can increase or decrease the alleles frequencies from a generation to an other. These factors are: non-randomised marriages, alteration of mutation rate, selection, small populations, genetical isolated population and migration (Applewhite, 1994; Batshaw, 1997; Behram et al., 1996; Chen, 1988). Human population can have some changes in sizes and traits (hair form, colour of eyes, hair, skin, lip firmness etc). These traits are determined by the interaction of genotype, environment, geographic area, climate conditions etc. (Ionescu and Drăgoi, 1974; Ionescu et al., 1994; Jones, 1996; Milcu, 1978; Tomulescu, 2002).

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There is a lack of information about digit ratio. Recently, some researchers start to investigate this feature of human race: digit length and digit ratio. Many of them linked the results of some environmental factors (geographic area, relatives etc) or other normal and abnormal human traits (dermatogliphics, inteligence, height, weight, malformations, sexual behaviour, autism, schizophrenia etc) (Arato et al, 2004; Wassink and Piven, 2000).

MATERIAL AND METHODS

We investigated 200 individuals. We measured the length of the 2nd, 3rd and 4th fingers of 100 from each locality (Oradea and Bulz). The individuals were randomised choosed. We measured the length of the fingers, from the finger basis to the superior bound of the phalanx. Then we calculated the average, standard deviation, variances, variation coefficient. Also, we calculated F distribution and z-test.

RESULTS AND DISCUSSIONS

The results of our research are presented in the following tables.

In table 1 are presented the results of data analysis in male population of Oradea. We notice the low variability of all fingers lengths. The phenomenon is observed either in right and left hand.

The F distribution of data from male population of Oradea (table 2), shows that the collected measurements proceed from very similar or identically populations. We may say that because the obtained values of F are situated under the critical value calculated for one hundred subjects for each populations, value which is between 1.53 and 1.43. Calculating the z-test, we noticed that the averages are not significant different. All values are lower than p=0.05 or p=0.01.

Table 1.

Presentation data about fingers length of a male population in Oradea locality.

Parameters	*Right hands (RH)*			*Left hands (LH)*		
	Index finger	Median finger	Ring finger	Index finger	Median finger	Ring finger
Average	7.504	8.176	7.694	7.625	8.166	7.645
Standard deviation	0.682082	0.631196	0.67117	0.750539	0.695036	0.62674
Variance	0,465235	0,398408	0,450469	0,563308	0,483075	0,392803
Variation coefficient	9.089584	7.720105	8.723286	9.843128	8.511337	8.198038

In table 3 are presented the results of data analysis in male population of Bulz. We can notice also the low variability of finger lengths.

In the male population of Bulz (table 4), the results show that data proceed from very similar or identical populations (right and left hands). All values are under de critical value situated under 1.53 and 1.43 for one hundred subjects in each population.

After the z-test was calculated for each finger, we noticed that there are no significant differences between the two hands. All values are lower than p=0.05 or p=0.01.

Comparing the finger lengths of the two populations, we obtained, in general, results alike those already related. The exception consist in the ring finger of left hands,

because we obtained a value of 2.454509, which exceeds the critical value situated between 1.53 and 1.43. So, we may say that the data of ring fingers proceeds from very different populations.

In the case of comparison of the two localities averages, the situation is very different than in each population, separately. After z-test calculation, we noticed that the two studied populations are very different, in all cases the obtained values exceeding the 2.58 value which coresponding to p=0.01.

Table 2.

Presentation of the Right hands-Left hands comparison
in a male population of Oradea.
F distribution and z-test.

Fingers	*Right hands-Left hands*	
	F	*z-test*
Index fingers	1,210803	1,193093
Median fingers	1,212513	0,106518
Ring fingers	1,146806	0,168738

Table 3.

Presentation data about fingers length of a male population in Bulz locality.

Parameters	*Right hands (RH)*			*Left hands (LH)*		
	Index finger	Median finger	Ring finger	Index finger	Median finger	Ring finger
Average	7.045	7.736	6.94	6.98	7.709	6.913
Standard deviation	0.622211	0.628212	0.627002	0.62845	0.629268	0.633677
Variance	0,387146	0,394650	0,393131	0,394949	0,395978	0,401546
Variation coefficient	8,831951	8,120633	9,034609	9,003583	8,162777	9,166454

Table 4.

Presentation of the Right hands-Left hands comparison
in a male population of Oradea.
F distribution and z-test.

Fingers	*Right hands-Left hands*	
	F	*z-test*
Index fingers	1,020155	0,735851
Median fingers	1,003365	0,303653
Ring fingers	1,021405	0,302881

Table 5.

Presentation of the Right hands and Left hands comparison
in the two male populations of Oradea and Beiuş.
F distribution and z-test.

Fingers	*Right hands (RH)*		*Left hands (LH)*	
	F	*z-test*	*F*	*z-test*
Index fingers	1,201704	4,971621	1,426281	6,589020
Median fingers	1,009522	4,940000	1,219954	4,874302
Ring fingers	1,145849	8,209300	**2,454509**	8,210000

Measuring people's finger patterns may reveal some surprising information. Animal models have indicated that androgenic steroids acting before birth might influence the sexual orientation of adult humans In women, the index finger (2D, second digit) is almost the same length as the ring finger, the fourth digit (4D), although it may be slightly longer or shorter; in men, the index finger is more often shorter than the fourth. The greater 2D:4D ratio in females is established in two-year-olds.

This sex difference in 2D:4D is greater on the right hand than on the left, indicating that the right-hand 2D:4D is more sensitive to fetal androgens than the left-hand ratio. The right-hand 2D:4D ratio of homosexual women was significantly more masculine (that is, smaller) than that of heterosexual women, and did not differ significantly from that of heterosexual men. Although it is possible that the maternal influence on finger growth of subsequent sons occurs after birth, a prenatal influence seems more likely because of the extensive physiological pairing of mother and fœtus. The locus of the maternal "memory" for previous sons, and the mechanisms by which fœtal development of subsequent sons is altered, remain unknown. The sex hormones are thought to govern brain development as well as finger length. Manning and Baron-Cohen found that autistic children had extremely long ring fingers compared with their index fingers. Children with Asperger's also had abnormal index-to-ring finger ratios, though less so than full-blown autistics. Even the unaffected siblings and parents of the autistic children had ratios that differed significantly from the normal controls. That may sound surprising, but high levels of testosterone in the womb have been linked to several other brain-related phenomena, including left-handedness, dyslexia and female homosexuality. Manning thinks that the families of autistic children are genetically predisposed to produce high levels of testosterone during early development. (The foetus makes most of the testosterone itself. In males, it comes from the testes and adrenal glands; in females from the adrenals alone. Only a small amount, if any, comes from the mother.)

The Beck Depression Inventory (BDI) - widely used to detect depression in the population as a whole, as well as psychiatric patients - was then deployed to identify those who suffered from depression, and to score the severity of their depression. The results showed that in men - but not women - a high BDI score was positively related to long digits, particularly the fourth digit (the ring finger). Dividing digit length by height, to take account of the fact that taller men tend to have longer limbs, fingers and feet, gave an even stronger predictor of high BDI scores in men. Fœtal testosterone concentrations are the most likely explanation, given the sex-dependent pattern of the data. Manning says "Testosterone has strong influences on the development of the male nervous system - not

all of them beneficial. It is believed that excess testosterone promotes the growth of the right hemisphere of the brain at the expense of the left hemisphere. This can lead to impaired reading ability, but also to enhanced mathematical and musical abilities. Unfortunately, there seem to be other, less welcome effects: excess testosterone has already been implicated in the origins of migraine, autism, stuttering, schizophrenia - and now depression, too. "Interestingly, the study's results suggest that depression in women has a different and as yet undetermined origin."

Having relatively long ring fingers does not necessarily mean that a man will, in fact, suffer from depression - just as people with high cholesterol levels do not necessarily have a heart attack. However, since the symptoms of depression can discourage sufferers from acknowledging their condition and seeking treatment, ring finger length could offer a simple, objective indicator of susceptibility in men.

Finger length patterns differ between men and women. In men, the 2nd finger is shorter than the 4th finger, while in women this difference is not evident, note Fleur R Cattrall from Monash University in Melbourne, Victoria, and her colleagues. The disparity might be explained by differences in the hormones that men and women are exposed to before birth, the researchers explain in their paper in the December issue of Fertility and Sterility. This idea is supported by the fact that women with conditions characterised by elevated levels of the male hormone testosterone during fœtal development often have a masculine finger length pattern. To determine whether women with PCOS (polychistic ovarian syndrom) have masculine finger length patterns, Cattrall's group conducted a comparison study involving 70 women with PCOS and 70 women without the condition, between the ages of 18 and 40. The investigators measured the 2nd to 4th finger length ratio on the palms of the left and right hand. Compared with the other women, PCOS patients had finger length patterns on the right hand that more closely resembled those of men. The team suggests that the masculinised finger length pattern they identified could be considered evidence of testosterone exposure in fetuses destined to develop PCOS.

CONCLUSIONS

We may conclude the following:

- low variability of all fingers lengths in male population of Oradea, for both hands. We noticed that the averages are not significant different.
- we can notice also the low variability of finger lengths in male population of Bulz. We noticed that there are no significant differences between the two hands. The exception consist in the ring finger of left hands.
- in the case of comparison of the two localities averages, the situation is very different than in each population, separately. After z-test calculation, we noticed that the two studied populations are very different, in all cases the obtained values exceeding the 2.58 value which coresponding to p=0.01.

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AEROMYCOLOGICAL STUDY ON *STEMPHYLIUM* CONIDIA IN FOUR ROMANIAN CITIES FOR 2005

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KEY WORDS: airborne spores, Stemphylium conidia

ABSTRACT

Airborne spores have been widely recognized as allergens, and were mostly linked with the etiology of asthma. Stemphylium conidia are typical airborne spores. The aim of the study was to analyse the Stemphylium conidia concentrations in Bucureşti, Braşov, Craiova şi Timişoara in the year 2005. Airborne spores sampling was carried out in this cities by employing volumetric sampling. Spores were identified at the genus level only. The greatest total concentrations were observed in Craiova and Bucuresti.

INTRODUCTION

Aeromycology, or the study of the biology of airborne fungal propagules, seeks to determine the dispersion, ecology and deposition patterns of phytopathogenic and non-phytopathogenic fungal spores. The capability of producing enormous numbers of spores is characteristic of most moulds (Reineria Diaz et al, 1998).

Most fungi belonging to the genus *Stemphylium* are saprophytes growing on dead plants and cellulose materials (Simmons, 1969). *Stemphylium* species are able to grow as endophytes in the living leaves of various plants (Sultanova et al., 2002). *Stemphylium vesicarium* (teleomorph *Pleospora allii*) causes severe epidemics in garlic, onion and leek crops in the main producing areas of the world (Miller et al., 1978; Aveling & Naude, 1992; Basallote Ureba et al., 1993; Suheri & Price, 2000; Prados-Ligero et al, 2003). In Southern Spain, symptoms of disease on garlic and onion crops include both white small oval lesions and large purple spots (Basallote et al., 1993).

The infection and colonisation of leaves by this fungus determine extensive necrosis followed by a premature dessication of the plants, which leads to important yield reductions (Miller et al., 1978; Aveling & Naude, 1992; Basallote et al., 1993; Suheri & Price, 2000). Relative humidity and/or duration of leaf wetness have been referred as determinant factors in the development of *Stemphylium vesicarium* in several hosts (Aveling & Naude, 1992; Basallote et al., 1993; Suheri & Price, 2000; Llorente and Montesinos, 2002).

The accumulation of short humidity periods along several nights in order to reach the total humidity period is required for the sporulation of *Alternaria porri* f.sp. *solani*,

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Stemphylium botryosum f.sp. *lycopersici* and *Alternaria dauci* (Strandberg, 1997). According to these results a similar process seems to occur in *Stemphylium vesicarium*. The results suggest that temperature is involved in the release of conidia. Temperature also plays an important role in infection and in the colonisation processes, with an optimal range of 18–26°C (Basallote et al., 1993).

Respiratory allergy to fungal spores may affect up to 30% of the allergic human population (Gravensen, 1979; Waisel et al., 1997). The literature that touches upon the occurrence of airborne spores in homes and in workplaces is rather extensive (Waisel et al., 1997). Airspora of several species of molds are known to induce numerous human diseases such as chronic bronchitis, asthma, fungal allergies and hypersensitivity pneumonitis. Tilak (1991) estimated that the between 2 and 30 % of respiratory allergies are due to fungal spores, and cited *Cladosporium* and *Alternaria* as the leading allergens (Pepeljnjak & Segvic 2003). Recent studies have shown that clinical sensitivity to *Cladosporium* and *Alternaria* can exacerbate asthma (O'Hollaren et al. 1991; Targonski et al. 1995). Airborne fungal spores originate from soil, plants, and vegetal and animal remains.

Therefore the fungal composition of a given area is dependent on its geographical location, meteorological factors, vegetation and human activity. An important interaction exists between some of these factors and the biological factors responsible for the production and dispersal of the conidial fungi.

The latter include according to some authors (Lyon et al. 1984), the conidia shape and size which, together with meteorological factors, are the most determining factors for the speed of dispersion and deposition of this type of propagulum. They require certain meteorological conditions for their development, and in different studies temperature and humidity have been shown to be factors conditioning their presence in the air. A significant increase in spore concentration after precipitation was determined. Since temperature and rainfall were low, spore concentrations were very low. There was a significant correlation between temperature and total spore concentration (Hjelmroos 1993). Friesen et al. (2001) estimated that wheat harvesting liberated immense numbers of fungal spores into the air many of which are carried long distances in wind currents.

MATERIALS AND METHODS

The concentrations of airborne *Stemphylium* spores from four urban areas in România (Braşov, Bucureşti, Craiova and Timişoara) were investigated. Lanzoni traps were used. Samplers functioned on a 220 V 50 Hz power source, and were adjusted to sample air at 10 L/min. All slides identified in this study are kept at West University, Department of Biology in Timisoara (România). All spore counts were obtained daily (7 days/week) at our institution during 15 May to 15 July 2005. The trapping surface was removed weekly and dissected for microscopic examination. Slides were covered with glycerine jelly mixed with basic fuchsin.

The slides were examined daily under the light microscope. The specific fungal spores were counted with ×400 magnification. Four equidistant transects across the long axis of the tape were scanned at 4 mm intervals and conidia observed in each 2 h transect were corrected for the proportion of the tape examined and the volume of air sampled: it was then expressed as trapped spores per m^3 of air over 2 h. The identification and counting of spores were limited to genus levels. Grant Smith (*Sampling and identifying allergenic pollens and molds – An Illustrated Identification Manual for Air Samplers,* 1990) was used as reference book for the identification and description of the fungal spore types.

RESULTS AND DISCUSSION

Stemphylium causes extended necrotic areas on leaves, shoots and fruits, which are unmarketable. Their aerial behaviour was frequently investigated in urban areas (Ibánêz-Henríquez et al., 2001), because of their allergenic effects on humans (Horner et al., 1995). Few works were aimed at studying dynamics of *Stemphylium* conidia in relation to plant disease development, with particular reference to garlic, leeks and asparagus (Suheri & Price, 2000; Prados-Ligero et al., 2003). Few information exists about spore dispersal in pear orchard affected by the brown spot disease (Rossi et al., 2005).

Stemphyllium Wallr. is the anamorph of *Pleospora* Rabehn. Its conidia are pale to mid dark or olivaceous brown with traverse and oblique or longitudinal septa. Their shape is variable but frequently they are ellipsoidal or ovoid and 20-80/µm long. Some species have one pointed conical apex and the other showing lateral conical protrusions. The walls are smooth, verrucose or echinulate and conidia are often constricted at one or more septa and cicatrized at the base (Reineria Diaz et al, 1998).

The aim of the present investigation was to monitor the *Stemphylium* airborne spores and to determine their intra-annual variations in four sites of România: Braşov, Bucureşti, Craiova and Timişoara. The totals of *Stemphylium* spores in the air recorded in this study show a considerable variation.

The highest level of conidia emission was recorded in Craiova with 763,3, the lowest value being recorded in Braşov with 334,9 spores. The daily mean concentrations of *Stemphylium* spores fluctuated between 5,4 – 12,31 spores/m^3. The highest concentration of *Stemphylium* spores, equal to 95,2 spores /m^3/24 h was noted in Bucureşti for 19 June. Differences between towns concerned total spore counts and numbers of peaks. Bugiani et al. (2004) introduced the concept of spore peak as a day with more than 30 conidia caught, and considered a peak as an indicator of the potential occurrence of the disease. Daily concentrations were very low, only exceeding 30 spores/m^3 on a few occasions.

Conidia are present airborne from early April to September. Conidia were particularly abundant at mid-June: more than 30% of the seasonal spores were trapped in the second 10 days of this month.

Aerial concentration of conidia showed a series of waves, with periods of spore abundance alternating with periods of spore scarcity. Conidial concentrations were low at the beginning of the sampling season, and progressively increased until a maximum density; afterwards, spore catches trended to reduce.

The diurnal periodicity of aerial conidia showed a peak around midday and low counts in the dark. The erratic daily distribution of conidia showed their highest concentrations between 12 and 18 h, with a pronounced peak between 14 and 16 h, and their lowest values at night (2–8 h).

The increase in spore concentration was significantly correlated with the reduction of relative humidity and wetness in early morning, and the increase of wind in late morning and afternoon. Favourable conditions are defined as a contemporaneous presence of temperature between 15 and 25°C and a high humidity, particularly a wetness duration longer than 10 h per day.

Air temperature limited the presence of conidia in the air in May and rarely in early-June. Humidity was the most limiting factor, being unfavourable in more than one half of the days considered, from early-May to August (Rossi et al, 2005). The dead plant material supporting fungal saprophytic growth can also contribute to the dose of airborne *Stemphylium* conidia.

Fungi are heterotrophic organisms. They obtain nutrients from dead or living organisms (being saprotrophs and parasites, respectively). To develop and complete their life cycle they are dependent on a ready access to organic material, which is more abundant in rural areas than in urban environments.

Other important factors influencing the occurrence of the spores in the air are weather and microclimatic factors. Cities have a specific microclimate which can be characterized by, among others, higher daily temperatures and intradiurnal temperature differences and a specific wind direction (Unger, 1999; Kasprzyk &Worek., 2006).

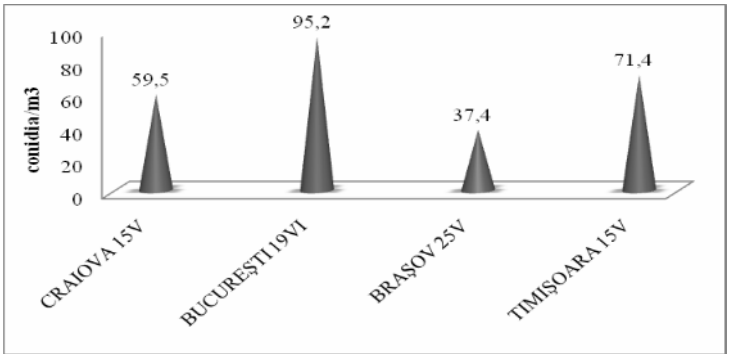

Figure 1. The peak daily concentration of *Stemphylium* conidia

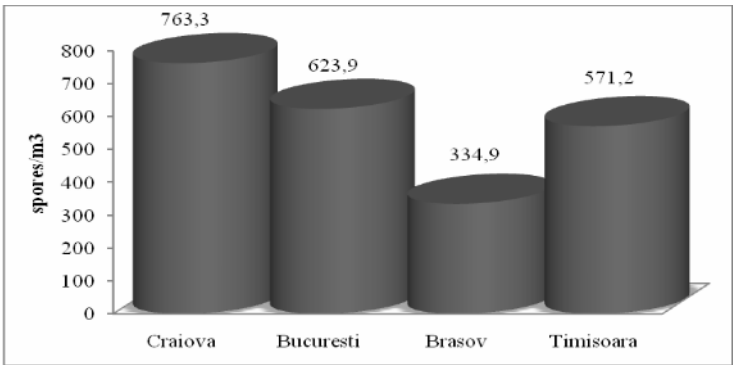

Figure 2. Totals of *Stemphylium* spores in the air recorded in four monitoring stations from Romania in 2005

CONCLUSIONS

Spore concentrations are known to show a considerable daily, seasonal and annual variability. *Stemphylium* is one of the most common atmospheric mould spores found in the România, the greatest numbers being found in Craiova and Bucureşti areas.

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THE ASSOCIATION SCIRPO – PHRAGMITETUM W. KOCH 1926 IN BANAT

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KEY WORDS: Scirpo – Phragmitetum, Banat

ABSTRACT

If in the past, Banat was considered to be a swampy area, today the naturally moist regions practically do not exist anymore, their place being taken by artificial accumulations. The hydro-improving works performed here in order to control the waters, have lead to the disappearance of these regions, along with which the characteristic vegetation having changed. The association under discussion, Scirpo – Phragmitetum W. Koch 1926, used to occupy significant surfaces. Although it is still frequent in the area, it appears more isolated and in greatly changed ecologic conditions. Between out phytocoenoses and the ones described by GRIGORE (1971) there are obvious differences regarding the floristic composition, the specters of the bioforms, the geoelements and the ecologic behaviour of the species.

INTRODUCTION

Situated in the South-Western part of the country, where many geographic elements merge and overlap, Banat benefits of a complex vegetation, which worked up the interest of many researchers.

Scirpo-Phragmitetum W. Koch 1926 is definitely one of the most studied paludicolous associations in the region. It was signalled by BOŞCAIU, 1966 (in Lugoj), GRIGORE, 1971 (interstream Timiş-Bega), RACLARU & ALEXAN, 1973 (at Pojejena), HOBORKA, 1980 (in the Dognecei Mountains), COSTE *et al.*, 1998-1999 (at Cenad, Satchinez, fishery Timişoara) etc. We have encountered it at Pişchia, Liebling, Sânandrei. The land improvement works executed in the area have determined severe modifications of the vegetation, certainly influencing and edified reed communities. In Banat, the association used to occupy considerable areas. Although it is quite frequent in the region, today it appears isolated and in much changed ecologic conditions. The main problem of these phytocoenoses is related to the lack of water, a fact which determined the reduction in the surfaces of places occupied, the modification of the floristic composition, the reduction in size of the edifying species, etc. Thus, in the paper there are mentioned some differences existing between our phytocoenoses and the ones described by GRIGORE (1971).

MATERIAL AND METHODS

The research has been performed in the period 2006-2007 in three accumulation lakes from Banat (Pişchia, Liebling, Sânandrei). The methodology of study approached is that of the central-European floristic phytocoenologic school. The association is analyzed

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from the point of view of chorology, of the floristic composition and of the sinmorphology, sinecology, cenotaxonomy, sindynamics and of the conservative value. Comparisons are also made between our phytocoenoses and those studied by GRIGORE (1971), in the same region.

RESULTS AND DISCUSSIONS

Chorology (SANDA, 2002): The association is widely spread in most accumulations of stagnant water, in all provinces of the country. For Banat, it is signalled by Boşcaiu, 1966 (Lugoj), Grigore, 1971 (Timiş-Bega), Raclaru, Alexan, 1973 (Pojejena), Hoborka, 1980 (Dognecei Mountains), Coste *et al.*, 1998-1999 (Cenad, Satchinez, fishery Timişoara). We have encountered edified cane phytocoenoses in the accumulation lakes Sânandrei, Pişchia şi Liebling.

Floristic composition and sinmorphology: Within the association we have identified 27 species. The edifying species is *Phragmites australis* (Cav.) Steudel. Among the species presented in te specialized bibliography as characteristic for the association and encountered by us, we mention: *Stachys palustris* L., *Glyceria maxima* (Hartman) Holmberg, *Typha angustifolia* L., *Lycopus europaeus* L., *Iris pseudacorus* L., *Symphytum officinale* L., *Calystegia sepium* (L.) R. Br. Other species which appear in the association, from the shore vegetation, are: *Bidens tripartita* L., *Calystegia sepium* (L.) R. Br., *Calamagrostis arundinacea* (L.) Roth etc. The 7 phytocoenoses studied by us are constituted by 27 species, while in the 12 phytocoenoses studied by GRIGORE (1971) are presented 54 species. This is due to the wide ecologic frame in which the association used to develop. Along with the edifying species, only 12 species are still common to both situations: *Bidens tripartita* L., *Butomus umbellatus* L., *Calystegia sepium* (L.) R. Br., *Lycopus europaeus* L., *Glyceria maxima* (Hartman) Holmberg, *Mentha aquatica* L., *Typha angustifolia* L., *Salix cinerea* L., *Stachys palustris* L., *Lythrum salicaria* L., *Symphytum officinale* L., *Xanthium strumarium* L. (tabelul 1). *Salvinia natans,* signaled by GRIGORE (1971) in the structure of the association, is today very rarely encountered in Banat. Also, the species *Lemna trisulca* L*., Lycopus exaltatus* L. and *Sagittaria sagittifolia* L., are less frequent than they used to be in the past.

Sinecology: The reed is frequent in swamps, in stagnant and slowly running waters, turf moors, on the shores of lakes and rivers, in ditches, depressions, moist meadows, in temporary flooded lands, on sandy cliffs. *Scirpo-Phragmitetum* W. Koch 1926 is an important association, with a characteristic aspect and a durable consistency, being considered a miniature forest. The reed multiplies vegetatively, through rhizomes, permanently generating an impressive number of individuals (clones). It prefers locations with stagnant waters, at depths of 0,8-1 m (1,5 m). At greater depths, the species disappears. Also, when the water level is very low, the phytocoenoses regress. The height of the vegetation varies depending on the water supply. The association presents a wide ecologic amplitude as to nutrients, developing both in clean waters and in those colmated, occasionally forming massive populations. It is a stable, stratified community, which creates for itself a micro-climate and which favours the apparition of submersed and natant associations in the places they occupy. GRIGORE (1971) mentions that in the Timis-Bega interstream area, the association is disposed under the shape of straps on the border of channels and ponds with over 1m deep water. The same layout is held by the phytocoenoses which we have also identified in three locations. The analysis of the main ecologic indexes shows that our phytocoenoses are mainly constituted by mesophilic and meso-hygrophilic (U) species, micro-mesothermic (T), amphitolerant and slight acid neutrophilic (R). Our phytocoenoses are, under the aspect of bioforms, mainly constituted

by terophyts and hemicryptophyts (with a participation of 37%, and 33,3% respectively), followed by helohydatophyts (22,2%). The phytocoenoses studied by GRIGORE (1971), under the aspect of bioforms, are well represented by helohydatophyte (46,2 %) and hemicryptophyte (38,8 %) species. The significant presence of the terophytes in our phytocoenoses indicate a high degree of their anthropization (table 2). At the same time, the presence of land-adjusted species is higher in our phytocoenoses because in the locations they occupy grass is being planted, due to the lack of water. Regarding the structure per geoelements, in the phytocoenoses analyzed by GRIGORE (1971), prevail, as in our case, the Eurasian and cosmopolite species (table 3). The circumpolar species are better represented in the phytocoenoses studied by GRIGORE (1971). In the structure of our phytocoenoses there are present, in reduced sizes, also adventive and European species; in the phytocoenoses studied by GRIGORE (1971), and also in reduced amounts, also participate species of Mediterranean and Pontic origin.

Table 1

The floristic composition of the phytocoenoses from the association *Scirpo-Phragmitetum* W. Koch 1926

No.	species	GRIGORE (1971)	NEACŞU *et al.* (2006-2007)
1.	*Abutilon theophrasti* Medik.	-	+
2.	*Achillea millefolium* L.	-	+
3.	*Agrostis alba* L.	+	-
4.	*Alisma lanceolatum* With.	+	-
5.	*Alisma plantago-aquatica* L.	+	-
6.	*Amaranthus retroflexus* L.	-	+
7.	*Arctium lappa* L.	-	+
8.	*Bidens tripartita* L.	+	+
9.	*Bolboschoenus maritimus* (L.) Palla	+	-
10.	*Butomus umbellatus* L.	+	+
11.	*Calamagrostis arundinacea* (L.) Roth.	-	+
12.	*Calystegia sepium* (L.) R. Br.	+	+
13.	*Carex acutiformis* Ehrh.	+	-
14.	*Carex vulpina* L.	+	-
15.	*Chenopodium album* L.	-	+
16.	*Cichorium intybus* L.	+	-
17.	*Daucus carota* L.	+	-
18.	*Datura stramonium* L.	-	+
19.	*Echinochloa crus-galli* (L.) Beauv.	-	+
20.	*Epilobium tetragonum* L.	+	-
21.	*Epilobium hirsutum* L.	+	-
22.	*Eupatorium cannabinum* L.	+	-
23.	*Galium palustre* L.	+	-
24.	*Glyceria maxima* (Hartman) Holmberg	+	+
25.	*Gratiola officinalis* L.	+	-
26.	*Eleocharis palustris* (L.) Roemer et Schultes	+	-
27.	*Iris pseudacorus* L.	-	+
28.	*Juncus articulatus* L.	+	-
29.	*Lactuca saligna* L.	+	-
30.	*Lemna minor* L.	+	-
31.	*Lemna trisulca* L.	+	-

32.	*Lycopus europaeus* L.	+	+
33.	*Lycopus exaltatus* L.	+	-
34.	*Lysimachia nummularia* L.	+	-
35.	*Lythrum salicaria* L.	+	+
36.	*Melilotus officinalis* Lam.	+	-
37.	*Mentha aquatica* L.	+	+
38.	*Mentha pulegium* L.	+	-
39.	*Myosotis scorpioides* L.	+	-
40.	*Oenanthe aquatica* (L.) Poiret	+	-
41.	*Phalaris arundinaceea* L.	+	-
42.	*Phragmites communis* (Cav.) Steudel	+	+
43.	*Plantago major* L.	+	-
44.	*Polygonum amphibium* L.	+	-
45.	*Polygonum hidropiper* L.	-	+
46.	*Pulicaria vulgaris* Gaertner	+	-
47.	*Ranunculus sardous* Crantz.	-	+
48.	*Rorippa amphibia* (L.) Besser	+	-
49.	*Rorippa austriaca* (Crantz) Besser	+	-
50.	*Rubus caesius* L.	-	+
51.	*Rumex obtusifolius* L.	-	+
52.	*Sagittaria sagittifolia* L.	+	-
53.	*Salix alba* L.	+	-
54.	*Salix cinerea* L.	+	+
55.	*Salvinia natans* (L.) All.	+	-
56.	*Schoenoplectus lacustris* (L.) Palla	+	-
57.	*Solanum dulcamara* L.	+	-
58.	*Sonchus arvensis* L.	+	-
59.	*Sparganium erectum* L.	+	-
60.	*Stachys palustris* L.	+	+
61.	*Symphytum officinale* L.	+	+
62.	*Taraxacum officinale* LWeber ex Wiggers	+	-
63.	*Teucrium scordium* L	+	-
64.	*Typha angustifolia* L.	+	+
65.	*Typha latifolia* L.	+	-
66.	*Urtica dioica* L.	-	+
67.	*Verbena officinalis* L.	+	-
68.	*Xanthium strumarium* L.	+	+

Table 2

Statistics of bioforms

bioforms	T	HH	H	G	Ph	Ch
GRIGORE (1971)	5,6	46,2	38,8	1,9	5,7	1,9
NEACŞU *et al.* (2006-2007)	37	22,2	33,3	3,7	3,7	-

Table 3

Statistics of floristic elements

geoelements	Eua	Adv	Circ	Cosm	Eur	Mdt	Pnt
GRIGORE (1971)	53,7	-	14,8	26	-	3,7	1,8
NEACŞU *et al.* (2006-2007)	55,5	3,7	7,4	25,9	7,4	-	-

Cenotaxonomy: The association belongs to the class PHRAGMITETEA Tx. Et Prsg. 1942, the order *Phragmitetalia* W. Koch 1926 emend. Pign. 1953, the alliance *Phragmition australis* W. Koch 1926. Frequently used synonyms: *Phragmitetum communis* (All. 1922) Pign. 1953 = *Phragmitetum australis* Schmale 1939 = *Scirpo – Phragmitetum austro-orientale* Soó 1957 = *Phragmitetum natans* (Borza 1960) Nedelcu 1967.

Sindynamics: The normal succession of rush-beds is towards associations of al. *Magnocaricion*. But the phytocoenoses are frequently invaded by gramineous meso-hygrophilic meadows, which in time tend to replace them. In order to keep under control the luxuriant growth of reed, grazing is often practiced. This practice determines the replacement of rush-beds with nytrophilic and ruderal associations, dominated by nettle, danewort, etc. The direction of evolution of our phytocoenoses depends on the water level, on the type of substratum and on the human intervention. While GRIGORE (1971) indicated a possible evolution of this association towards forestry meadow vegetation, in the cases analyzed by us this fact is excluded, only the evolution towards associations of meso-hygrophilic and mesophilic meadows being considered.

Importance: Among all the paludicolous associations, *Scirpo-Phragmitetum* W. Koch 1926 is the most studied. In our country, the first description is performed back in 1939, by PRODAN (*in* POPESCU *et al*., 1997). The reed has been mentioned in the literature ever since ancient times. The Italian naturalist MARSIGLI (1726, *in* POP, 1942) speaks in his work „*Danubius Pannonico-Mysicus*" (considered to be the first Danube monography), about the reed swamps in Moldova and Muntenia.

Concerning the importance, the cane insures mainly the protection and consolidation of the aquatic pools shores. It is renown for the capacity to concentrate heavy metals (Zn, Pb, Cd, Cu, Ni) being used in ecologic restoration activities through phytoremedy (YE *et al.*, 1998, *in* STĂNESCU, 2005). Also, it produces antibactericide substances, which, reaching the environment, favour the biologic purification of waters (ARDELEAN & KARACSONYI, 2002). It is successfully used in the industry of cellulose and paper. Other uses: in some light constructions (roofing, animal shelters, fences – reed plates are used, obtained by layering stalks), for braids, packaging, for fire. In the accumulation lakes studied by us, the reed is not correspondingly used.

Due to the well developed rhizome system and to the soil retained by the radicular system, the cane invades large areas and determines the acceleration of the colmating process of the water accumulations. When the rhizomes separate from the substratum, floating reed islets are formed, *Phragmitetum natans* (BORZA, 1960, *in* SANDA *et al*., 1998), which encumber fishing. On the other side, the fertilization of lands they occupy results in the etiolation of plants, the deterioration of the sclerenchymatic tissue, the cassation of above-ground stalks and the destruction of the rhizomes. The lack of water and the deposit of a algae crust on stalks, finally determines the degradation of these phytocoenoses. These communities offer relatively few sources of food for birds, but they represent a well protected shelter and nesting place.

The association presents a moderate *conservative value.*

(The authors can offer to the interested persons the synthetical tabel of the vegetal association which is not included into the paper because of its dimensions.)

CONCLUSIONS

1. The edified reed communities have represented a subject of study for numerous researchers. In Banat, due to the hydro-improving works, the association *Scirpo-Phragmitetum* W. Koch 1926 appears with a greatly changed isolated character, a fact which has determined us to study it too.

2. In the phytocoenoses analyzed, we have identified a number of 27 species. The edifying species is *Phragmites australis* (Cav.) Steudel. Among the species characteristic for the association, also encountered by us, we mention the following: *Stachys palustris* L., *Glyceria maxima* (Hartman) Holmberg, *Typha angustifolia* L., *Lycopus europaeus* L., *Iris pseudacorus* L., *Symphytum officinale* L., *Calystegia sepium* (L.) R. Br. The phytocoenoses described by GRIGORE (1971) are more complex from the point of view of the floristic composition, being constituted of 54 species. 13 species, including the edifying species, are found in both synthetic charts, the majority being characteristic for the association.
3. If the phytocoenoses described by GRIGORE (1971) were dominated by helohydatophytes, it is noticed that in our phytocoenoses the terophytes and the hemicryptophytes have a larger proportion. In the specter of floristic elements, the Eurasian and the cosmopolite species are dominant, followed by the circumpolar ones. In the structure of our phytocoenoses there are present, in reduced amounts, also adventive and European species; in the phytocoenoses analyzed by GRIGORE (1971), also in reduced amounts, participate species of Mediterranean and pontic origin.
4. Our association is edified (depending on the value of the ecologic indexes) by mesophilic and meso-hygrophilic species (humidity), micro-mesothermic (temperature), amphitolerant (soil reaction). Although there is no analysis per ecologic categories, it is obvious that the phytocoenoses analyzed by GRIGORE (1971) are mainly constituted by hygrophilic and meso-hygrophilic species, given the significant presence of helohidatophytes.
5. Together with the well-known economic importance, the association has a high economic significance, the cane being successfully used in activities of ecologic restoration, it also participates in the biologic purification of waters and it represents a well-protected shelter and nest place for birds. As well, it is worth mentioning the importance that it has in the succession of the vegetation. All these arguments indicate the value of these phytocoenoses, a fact which implies their protection.

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THE INFLUENCE OF WATER QUALITY ON THE FLORA IN SANANDREI ACCUMULATION (TIMIS COUNTY)

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KEY WORDS: water quality, diversity of the flora

ABSTRACT

The chemical analysis performed on water samples collected from the accumulation Sânandrei, emphasized the fact that the water is of a bad quality, due to the high nutrient level. This influences in a negative manner the diversity of aquatic and paludicolous flora. Out of the 112 species of cormophytes registered in the lake vicinity, only 36 are aquatic and paludicolous. The aquatic species mainly belong to the genus Lemna, Spirodella and Polygonum. The best represented genus among the species is Potamogeton, well being known that it develops well in waters rich in nutrients.

INTRODUCTION

The accumulation lake Sânandrei, created in 1971, is placed on the stream course of Valea Lacului, on the right side of the Timisoara – Arad road, at an altitude of 117m and coordinates 45^0 $54^{'}$ N, 21^0 $35^{'}$ E. The water volume is of 1.372 mil. m^3, and the surface of 50 ha. As it is not cadastred, the lake takes the cadastral code of the watercourse that it is placed on (Valea Lacului) – V-1.21.3 At present, the lake is leased to a trading company, which uses it mainly for fishing and recreation.

MATERIAL AND METHODS

The paper was centered on the study of flora around the accumulation Sanandrei, considering the water quality. The water sampling from the accumulation studied was performed according to the STAS in force (see table 1), and their analysis was performed based on the norm 161/2006 (issued by the Ministry of Environment). Regarding the study of flora, we initially made multiple field trips with a view to recording all the species in the lake vicinity.

The non-identified species were subsequently determined in the laboratory. After making the floristic summary, we analysed the species from the point of view of bioform and geoelement category and depending on their ecological behaviour. The aquatic and paludicolous species were extracted from the summary and analyzed separately. For determining the species we have used mainly Romanian Flora.

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RESULTS AND DISCUSSIONS

The results obtained for the water sample collected from the accumulation Sanandrei are presented in table 1 below.

Table1

The results of the chemical analysis performed for the water sample collected in the accumulation Sanandrei (according to analysis report AT 406, 30.10.2005)

No.	Parameters analysed	U/M	Method of analysis	Value obtained	Quality class – approved limits				
					I	II	III	IV	V
1.	ammonium nitrogen ($N\text{-}NH_4^+$)	mg N/l	SR ISO 7150/2	1,48	0,4	0,8	1,2	3,2*	> 3,2
2.	nitrits ($N\text{-}NO_2^-$)	mg N/l	SR ISO 6777/96	0,45	0,01	0,03	0,06	0,3	> 0,3*
3.	nitrates ($N\text{-}NO_3^-$)	mg N/l	SR-7890/3-2000	1,81	1	3*	5,6	11,2	> 11,2
4.	phosphates/ phosphor ($P\text{-}PO_4^{3-}$)	mg P/l	SR EN 1189-2000	0,01	0,1*	0,2	0,4	0,9	> 0,9
5.	dissolved oxygen (O_2)	mg O_2/l	SR EN 25813-2000	8,87	9	7*	5	4	< 4
6.	permanganate index (CCO-Mn)	mg O_2/l	SR EN ISO 8467/01	21,2	5	10	20	50*	> 50
7.	CBO_5	mg O_2/l	SR ISO 5815/89	0,76	3*	5	7	20	> 20
8.	pH	-	SR ISO 10523-97	8,55	6,5 – 8,5				
9.	suspensions	mg/l	STAS 6953-81	61	-				
10.	total hardness	^{0} Ge	STAS 3026-76	16,0	-				

Values of the parameters monitored:

- *ammonium nitrogen* ($N\text{-}NH_4^+$) is found in concentration of 1,48 mg N/l. The value is within the limits required by 4th quality class which has the limit 3,2 mg N/l (1,2 mg N/l is the value accepted for the 3rd quality class). This parameter was determined according to SR ISO 7150/2.

- *nitrites* ($N\text{-}NO_2^-$) have a value of 0,45 mg N/l. The determination was made in accordance with SR ISO 6777/96. The value determined corresponds to the 5th quality class (> 0,3 mg N/l).

- the concentration of *nitrates* ($N\text{-}NO_3^-$) was set to be of 1,81 mg N/l, being situated in the 2nd quality class (3 mgN/l). The method of analysis used was in keeping with SR-7890/3-2000.

- *phosphorous* ($P\text{-}PO_4^{3-}$) in water was determined by SR EN 1189-2000. It is encountered with a concentration of 0,01 mg P/l, corresponding to the 1st quality class (0,1 mg P/l).

- *dissolved oxygen (O_2)* found with the value of 8,87 mg O_2/l, according to SR EN 25813-2000. The value is situated in the 2nd quality class (7 mg O_2/l).

- *the biochemical consumption of oxygen* (CCO – Mn), determined by the potassium permanganate method according to SR EN ISO 8467/01, has the value of 21,2 mg O_2/l and corresponds to the 2nd water quality level, which has the admissible limit of 50 mg O_2/l (20 mg O_2/l is the value admissible for 3rd class).

- for CBO_5 (SR ISO 5815/89) it was registered a value of 0,76 mg O_2/l. This value corresponds to the 1st quality class (3 mg O_2/l).

- the water *pH* is of 8.55 and it is found within the limits required for the 5th quality class. This value shows a slightly basic reaction and a higher concentration of the cations in water.

- *the suspensions* in the water determined in keeping with STAS 6953-81, have a concentration of 61 mg/l. At the value of $16,0^0$ Ge it was determined, in accordance with STAS 3026-76, the total water *hardness,* a value probably influenced by the pH (12 – 18 dH^0 – moderately hard water).

From the analysis of these data it is found that the water from the accumulation Sanandrei is of a poor quality, being classified in the 5th quality group, as the value determined in the case of nitrites corresponds to this class. The other parameters have values which are situated in the 1st-4th quality classes.

Thus, it is noticed that nitrite-based nutrients are those which set the quality class. The higher values registered by these parameters can be explained by the fact that in the accumulation area there have been animal farms, where the discharge of waste was performed in an uncontrolled manner.

Also, in the close vicinity of the accumulation, there are cereal cultures, on which chemical fertilization is applied. For these reasons, we observed the reduction in the diversity of aquatic macrophytes and the development of the microflora (here we mainly refer to diatoms) responsible with the flourishing of waters. Therefore we recommend a close monitoring of the activities performed in the area, because that may negatively influence the status of the aquatic pools, including water quality, the flora and vegetation, as well as the fish populations.

Analysis of the flora in the accumulation Sanandrei area

The flora in the Sanandrei accumulation area is represented by 112 species, 98 genera and 43 botanical families. From the statistical study of the flora per categories of bioforms results that there are predominant the species: hemicryptophytes (H) 35,71 % and annual terophytes (Th) 32,14 %. The other categories have a reduced participation. In the case of the analysis fo flora per categories of geoelements, it is noticed that the majority of the species are Eurasian (Eua) 53,57 % and cosmopolite (Cosm) 19,64 %.

The presence of the other categories of geoelements is reduced. Depending on humidity, the species have the following distribution: 35,71 % xero-mezophytes, 26,78 % mezophytes, 16,96 % mezo-hydrophytes, 7,14 % amphitolerant, 6,25 % hydrophytes, 4,46 % ultra-hydrophytes 2,67 % xerophytes. For temperature, the distribution is the following: 56,24 % mezothermic, 24,10 % amphitolerant, 16,96 % moderately thermophilic, 1,78 % microthermic, 0,89 % thermophilic. As to the soil reaction, the species are: 42,85 % slightly acid-neutrophilic, 40,17 % amphitolerants, 14,28 % acid-neutrophilic, 1,78% neutro-basiphilic, 0,89 % acidophilic.

From the data previously presented, it results that the flora from the accumulation Sanandrei area is mainly constituted by hemicryptophyte, eurasian, xero-mesophyte and mesophyte species, mesothermic, slightly acid-neutrophilic and amphitolerant. As the aquatic and paludicolous flora is invaded by ruderal and segetal species (due to vecinity to the road and to the existence in the close vecinity of agricultural crops), we opted for its separate analysis.

An overview of the aquatic and paludicolous flora in Banat is enough to observe that it has suffered severe modifications throughout the time, its diversity being much reduced in our days. Many species have disappeared, others have considerably diminished their areas and are also threatened to be extinct.

The aquatic and paludicolous flora from the accumulation Sanandrei area was subject in time to an intense human influence, being invaded by numerous ruderal and segetal species (see the analysis of flora). It is constituted by the species comprised in table 2.

Table 2

Aquatic and Paludicolous Flora from the accumulation lake Sanandrei

No.	bioform	geoelement	humidity value	species
1.	H	Eua	4	*Alopecurus pratensis* L.
2.	Th	Eua	4,5	*Bidens tripartita* L.
3.	HH	Cosm	6	*Bolboschoenus maritimus* (L.) Palla
4.	HH	Eua	6	*Butomus umbellatus* L.
5.	H	Eua	4	*Calystegia sepium* (L.) R.Br.
6.	HH	Eua	5	*Carex riparia* Curtis
7.	TH	Eua	4	*Dipsacus laciniatus* L.
8.	Th	Cosm	4	*Echinochloa crus-galli* (L.) Beauv.
9.	HH	Cosm	5	*Glyceria maxima* (Hartm.) Holmberg.
10.	H	Cosm	4,5	*Juncus effusus* L.
11.	HH	Cosm	6	*Lemna minor* L.
12.	HH	Eua	5	*Lycopus europaeus* L.
13.	Ch	Eur	4	*Lysimachia nummularia* L.
14.	H	Eua	5	*Lysimachia vulgaris* L.
15.	H	Cosm	4	*Lythrum salicaria* L.
16.	HH	Eua	5	*Mentha aquatica* L.
17.	H	Eua	4,5	*Mentha longifolia* (L.) Hudson
18.	HH	Circ	5	*Phalaris arundinaceea* L.
19.	HH	Cosm	5	*Phragmites australis* (Cav.) Steudel
20.	G	Cosm	6	*Polygonum amphibium* L.
21.	Th	Eua	4,5	*Polygonum persicaria* L.
22.	MM	Eua	4	*Populus nigra* L.
23.	HH	Cosm	6	*Potamogeton crispus* L.
24.	HH	Cosm	6	*Potamogeton natans* L.
25.	HH	Cosm	6	*Potamogeton pectinatus* L.
26.	HH	Cosm	6	*Ranunculus aquatilis* L.
27.	H	Eua	4	*Ranunculus repens* L.

28.	H	Euc	4	*Rorippa austriaca* (Crantz) Besser
29.	H	Eua	4,5	*Rubus caesius* L.
30.	H	Eua	4	*Rumex crispus* L.
31.	MM	Eua	5	*Salix alba* L.
32.	M	Eua	5	*Salix cinerea* L.
33.	Ch	Eua	4,5	*Solanum dulcamara* L.
34.	HH	Cosm	6	*Spirodela polyrhiza* (L.) Schleichen
35.	H	Eua	4	*Symphytum officinale* L.
36.	HH	Cosm	6	*Typha angustifolia* L.

As it results from the chart, in the accumulation lake Sanandrei, there are 36 aquatic and paludicolous species. This floristic richness is related to the size of the accumulation surface and to the human influence manifested in the territory of the accumulation.

Among the frequently encountered species, we mention the followin: *Salix cinerea* L., *Ranunculus repens* L., *Polygonum amphibium* L., *Lythrum salicaria* L., *Typha angustifolia* L., *Carex riparia* Curtis, *Bidens tripartita* L., *Mentha aquatica* L. The best represented with aquatic species is the genus *Potamogeton*, whose species develop explosively, occupying considerable surfaces (*Potamogeton crispus* L., *Potamogeton natans* L., *Potamogeton pectinatus* L.).

This can be explained by the fact that the species of *Potamogeton* grow well in waters with a high nutrient level, just like the water from Sanandrei. It needs mentioning that among the species encountered , there are some whose existence is endangered and which are part of habitats with a high conservative value.

In this context, we mention again that the accumulation studied is mainly exploited for fishing and recreation and we consider that it is thus necessary a corresponding management, in order to preserve its resources.

CONCLUSIONS

1. Following the analysis performed on water samples collected from the accumulation lake Sanandrei, it was established that the water is of a poor quality.
2. This is due mainly due to the high content in nutrients and it can be explained by the fact that in the vicinity of the accumulation there are cereal cultures on which it is performed chemical fertilization and, also, there have been built animal farms where the waste discharge is made in an uncontrolled manner.
3. The poor water quality influences in a negative manner the diversity of the aquatic and paludicolous flora. Thus, there have been determined in the area around the accumulation lake, a number of only 36 aquatic and paludicolous species (out of the 112 identified), the best represented with species being the genera with a high nutrient level (e.g. *Potamogeton*).

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HEAVY METALS AND OTHERS TOXIC METALS CONTENT OF *MNIUM UNDULATUM* WHICH ARE GROWING NEAR TARGOVISTE TOWN

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KEY WORDS: heavy, toxic metals, Mnium undulatum

ABSTRACT

It is well known that generally, mosses are very good hyperaccumulators for heavy and others toxic metals. That's why is necessary to be well analysed their chemical content. This papers is about preliminary data obtained as part of a large research project about the monitoring of environmental pollution in Dambovita county using vegetation. The biological samples consist in some moss species which are growing in the sphere of influence of industrial objectives from Targoviste. The samples were analysed by PIXE (Particle Induced X-Ray Emission) method at IFIN – Horia Hulubei Bucuresti. In this work we presented the chemical content in heavy and others toxic metals in a very common moss species, Mnium undulatum. Inspite of the small size of this species, it is a very good accumulator for heavy and others toxic metals as manganese, chromium, magnesium, zinc, aluminium, strontium, and others metal species in trace.

INTRODUCTION

Today all over the world mosses became a very interesting subject for study because of their affinity for accumulating heavy and others toxic metals.

Mosses can indicate the presence of elements and their concentration gradients. Most methods in heavy metal monitoring employ mosses as bioaccumulators and involve sample collection followed by laboratory analysis techniques. A successfully series of studies using mosses as bioindicators were initiated in the Steinnes group [4, 5, 6, 7].

Why mosses as bioindicators?

We have checked the areas of distribution for species at the Dambovita county level and we have compared the efficiency of methods using different vegetal samples (mosses, lichens and tree leaves) [1, 3] collected from the same area. Preliminary results let us to think that the use of mosses constitutes an effective method in air pollution monitoring for several reasons:

- ❑ mosses are small and easy to handle;
- ❑ most of mosses species are evergreen and can be surveyed all year round;
- ❑ mosses lack a cuticle and a root system and obtain nutrients as particulates and in solution

directly from atmosphere. They have a good accumulation capacity, specialy for heavy metals, where metal concentrations reflect deposition without the complication of additional uptake via a root system;

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- the afinity of mosses to accumulate elements in very high concentrations aids chemical analyses of the tissues and may facilitate the detection of elements present in very low concentrations in the environment;
- comparisons of fresh samples with herbarium specimens enable retrospective analysis of metal pollution.

MATERIALS AND METHODS

Biological samples consisted in fresh moss plants of *Mnium undulatum* harvested from two places at different distances: one at 5 km from Targoviste (***M. undulatum* 1**), the second (***M. undulatum* 2**) at 8 km, both in the north-west part out of town. The samples were weight, then were drying at 105ºC one hour. The dried material was reduced to dusty and put on a special ray beam target. The powder was mixed with 2 μg of Yttrium (internal standard) in 150 μl demineralized water and depicted on Mylar support.

All samples were analysed by PIXE (Particle Induced X-ray Emission) method [2], at the National Institute of Nuclear Physics of Magurele, Bucuresti. Principle of PIXE method consist in ionization of the levels near the atomic nucleus. PIXE measurements of target elements were made using a 3 MeV proton beam extracted from the Tandem Accelerator FN-8. X-ray spectra were measured with a spectrometric chain with a CANBERRA Ge hyperpure detector with a 160 eV resolution at 6.4 KeV of Ka line of iron. This ionization is followed by a rearrangement of the electronic architecture with emission of characteristic X-ray. Detection of X radiation with Si(Li) or intrinsic Ge semiconductor detectors. The characteristics of the method: destructiveness, rapidity (15-30 minutes), easy preparation of the samples, multielementarity, determination of the most of elements with Z higher than 13, a good confidence, the sensibility of this method is 1ppm. The X-ray spectrum analysis were made off-line at Valahia University from Targoviste, using

LEONE fitting programs. The final resultes reprezent the average of some samples harvested from the same place, and were exprimated in ppm and related at dried substance.

RESULTS AND DISCUTIONS

Magnesium and iron were in a high quantity in plants of ***M. undulatum 1***(over 5000ppm), but reduced at half as values in ***M. undulatum 2*** (close to value of 2000ppm) (Fig. 1). Manganese and zinc were determinated in lower concentrations (under 500ppm) in both samples.

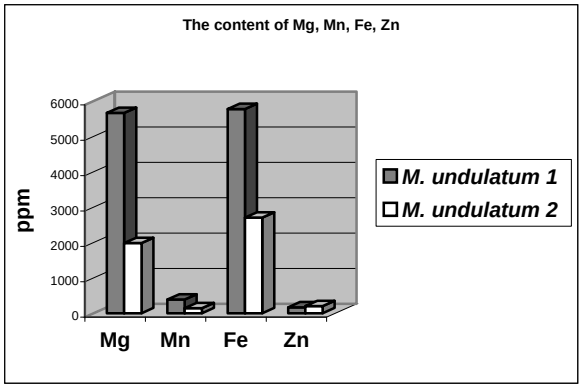

Fig.1. The concentration of some heavy metals in *Mnium undulatum*

In figure 2 it can see the highest concentrations of wolframium (between 3.5 and 4.5ppm) as well in ***M. undulatum 1*** as in ***2*** one, with a little diference (1ppm) between them. The content in cobalt was reduced within at half of wolfram level. The level of cadmium and mercury was under 0.5ppm in all samples analysed.

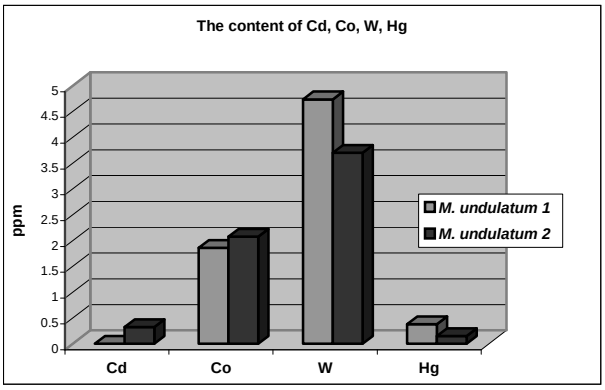

Fig.2. The concentration of some heavy and toxic metals in *Mnium undulatum*

Chromium level was highest (close to 40ppm) in ***M. undulatum 1*** samples (Fig.3) and lower in ***M. undulatum 2***. Vanadium and nickel concentration was a little higher in ***M. undulatum 1*** samples then in ***M. undulatum*** *2*, but lower than chromium level.

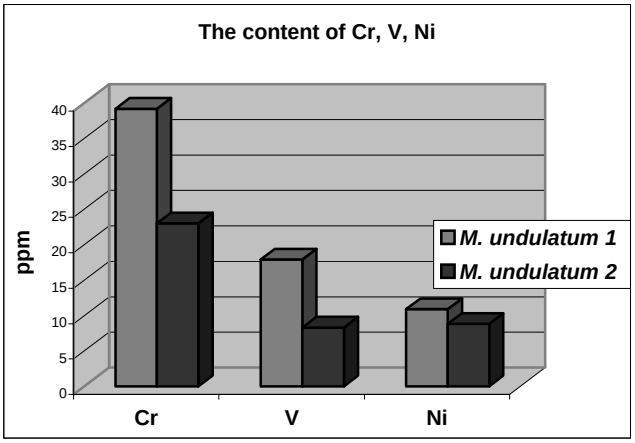

Fig.3. The concentration of some heavy and toxic metals in *Mnium undulatum*

In figure 4 on can see the level of concentrations of titanium, strontium, which were higher (over 70 and respectively 40ppm) than bromine, arsenic and uranium (all of them under 10ppm).

Strontium and uranium are radioactive elements but in very lower quantities.

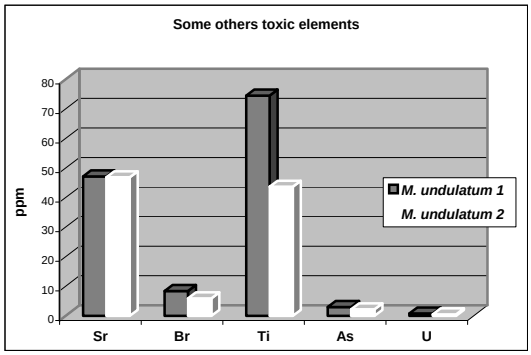

Fig.4. The concentration of some toxic metals in *Mnium undulatum*

CONCLUSIONS

- *Mnium undulatum 1* plants harvested from 5km distance from Targoviste, had higher quantities of Mg, Mn, Fe, W, Cr, V, Ni, Ti, Br and As than *M. undulatum 2.*
- *Mnium undulatum 2* plants contains more Zn, Cd, Co and Sr than *Mnium undulatum 1.*
- In higher concentration were determinated Mg and Fe, maximum being in *Mnium undulatum 1* samples.
- In moderate concentration were Cr, Ti, Sr in both type of samples, but V and Ni only in *Mnium undulatum 1* samples.
- A lower level had W and Co in all cases analysed.
- In trace were identified Cd, Hg, Br, but As and U had the lowest level in all samples.

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STUDIES REGARDING THE BIOLOGY AND ECOLOGY OF *TRITURUS DOBROGICUS* AND *TRITURUS VULGARIS* SPECIES FROM CAMPIA CERMEIULUI, ARAD COUNTY, ROMANIA

Cicort-Lucaciu Alfred-Ştefan[1], Covaciu-Marcov Severus-Daniel[2], Paina Cristiana[3], Radu Nicoleta-Reka[2], Toth Anamaria[2]

KEY WORDS: biology and ecology, Triturus dobrogicus, Triturus cristatus

ABSTRACT

In this research we have focused on the variation of the numbers of specimens during the aquatic period, in two cohabitant populations of Lissotriton vulgaris and Triturus dobrogicus. We have determined the size of populations, sex-ratio and age-ratio. The poulation of Lissotriton vulgaris is about three times larger than the population of Triturus dobrogicus. The value for sex-ratio for both populations is one male to three females, while age-ratio is one juvenile specimen to three/four adults. Although there was no noticeable difference recorded in the length of the reproductive activity of either of the two populations, we can definitely mention that Lissotriton vulgaris is repopulating the puddle later and depopulating it earlier than the other species. The juvenile species of Triturus dobrogicus are populating the aquatic habitat faster than the adults.

INTRODUCTION

The humidity and the temperature are the most important abiotic factors controlling the Amphibians' activity (Cogălniceanu, 2000). The temperature's influence on Amphibians is very evident, from cellular till the populational level (Rome et al, 1992). After the end of their hibernation, newts are part of the aquatic ecosystems only in the first stage of the aquatic period, leading afterwards a terrestrial existence (Fuhn, 1960). Their presence in the aquatic or terrestrial habitat is a consequence of the newts' trophic and reproductive activities.

Considering the fact that in Romania there are no researches done on the repopulation and depopulation of any aquatic habitat by newt species, we have decided to monitorize two populations in this regard, with specimens belonging to *Lissotriton vulgaris* and *Triturus dobrogicus.* These populations have their habitats near the locality of Cermei (Arad County). Besides the number of specimens, we have also recorded the stage of ontogenetical development, and the gender of each specimen.

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MATERIALS AND METHODS

Our research was done throughout four periods of 2006's spring: on the 6th of April, on the 5th of May, on the 21st of May, and on the 17th of June. We have studied 455 specimens, 131 belonging to *Triturus dobrogicus,* and 324 *Lissotriton vulgaris* specimens. In the areas where the water was not very deep and we could enter it using jackboots, we used rectangular shaped metal bordered dredges for capturing the specimens. In the areas where the water was deeper, we used bowls fitteded on long metal rods. Every captured speciemen was identified and immediately released back in its natural habitat.

The habitat near Cermei is located 20 meters away from the road which leads to Şomoşcheş, being actually parallel with this road on a distance of 70 meters in length. The habitat consists from a puddle located in the flood area of the river Teuz. This area is strongly dominated by Phragmites, which is completely covered by water in the rainy periods of the year. Being surrounded by agricultural fields, at the limit areas of the puddle, Juncus shrubbery is present instead of reeds. The plane feature of this location, having an altitude of 105m, has made the puddle to spread on a large area. The bank towards Şomoşcheş is abruptly ending into a ditch which is perpendicular on the road. The water's level is about 50 – 60 cm deep in this part, the maximum level exceeding 1 m. During summer, the puddle is not completely dry. The silt layer is very thin or completely missing. This habitat has a rich fauna, some species originating from the river (*Carassius auratus, Cobitis taenia*).

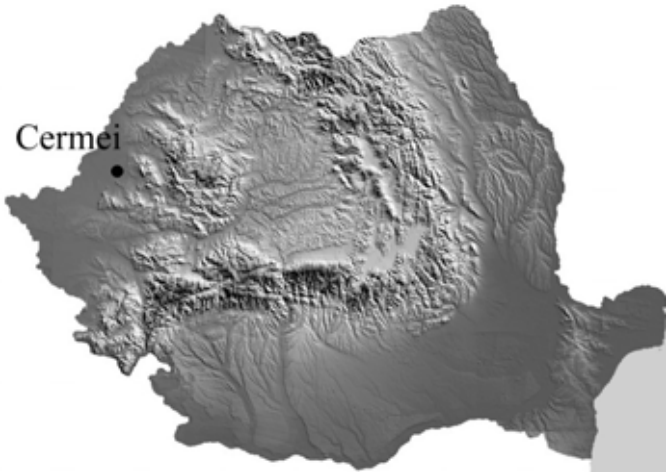

Photo no. 1. The habitat located near Cermei

Our objectives were as it follows: 1) to record the size of the populations, 2) to analyze the reproductive dynamic of the populations, based on the number of the captured specimens, their gender and ontogenetic stage, and 3) to record sex-ratio and age-ratio, observing the variation of these in time. We assessed the size of populations using the method of eliminating specimens (Cogălniceanu, 1997), actually counting all the specimens belonging to these populations.

RESULTS AND DISCUSSIONS

In the first period of the study, the newts were crowding under the scouring rush shrubbery, due to the unfavorable meteorological circumstances. In the second period (5th of May), we have found the newts even in the center of the puddle. In the areas where the water was deepest, newts were rare. An increasing in the temperature and the shrinking of their biotope were the factors which lead us to capture a much lower number of newts in the third period of the study (21st of May). The majority of specimens captured this time, had already had a rough tegument, while the male specimens' crests have considerably shrunk. In the last period, the 17th of June 2006, in spite of the fact that the puddle was not totally dry, no specimens from either of the newt populations were present in this habitat.

For both of the studied newt populations, during the period of research, we have recorded fluctuations in the number of specimens, gender and their ontogenetic developmental stage. These fluctuations were caused by changes in temperature and humidity, factors which have stabilized and optimized in the second period of the research.

Table 1

The number (*N*) of captured specimens, related to the period, their gender and ontogenetic developmental stage

Species	Gender / Ontogenetic developmental stage	6th of April 2006	5th of May 2006	21st of May 2006
Triturus dobrogicus	Total	39	62	30
	M	12	13	6
	F	14	35	19
	A	26	48	25
	J	13	14	5
Triturus vulgaris	Total	93	177	54
	M	24	37	13
	F	57	104	29
	A	81	141	42
	J	12	36	12

The recorded number of specimens is 62 for the dobrogean newt, and 177 specimens for the smooth newt. Considering the relation between the populations' size and the size of the habitat, it can be inferred that in this case mortality is high. Two of the major causes for this high value, are the lack of a terrestrial habitat, and the predators (ichthyofauna and other species feeding on eggs and larva). Out of 239 specimens, only 25,94% are represented by *Triturus dobrogicus,* this population being thus about three times smaller than that of the smooth newt. The cause for the difference in size of the two populations is most probably the larger size of the *Triturus dobrogicus* specimens on one hand, and on the other hand the big fluctuation of the water level during the year (the water level decreases considerably during summer).

In both populations, sex-ratio is in favor of femals, with a value of about 3:1 ($R_{Td\text{-}m}$ = 27,08%, $R_{Tv\text{-}m}$ = 26,24%). In this case, we consider that the intersexual numerical difference is caused by environmental factors. Researches assert that if the temperature is under a certain optimal value, it will lead to an increase in the number of female specimens in newt populations (Wallace & Wallace, 2000; Kinne, 2006); and also, mortality of the adults during winter time is higher among the males (Bell, 1977; Kinne, 2006).

The reproductive activity of Urodela group is very different from species to species, and from population to population (Duellman & Trueb, 1986). Althoug there are no such remarcable differences between the two studied populations, we have observed that there is a quantitative difference in the moment when the newts are leaving their aquatic habitat. *Triturus vulgaris* is repopulating the puddle later (P_{Td} = 62,90%, P_{Tv} = 52,54%), and is depopulating the puddle earlier (P_{Td} = 48,38%, P_{Tv} = 30,50%) than *Triturus dobrogicus* specimens.

Table 2

The ratio (*R*) of gender and age group.

Species	Gender / Ontogenetic developmental stage	6th of April 2006	5th of May 2006	21st of May 2006
Triturus dobrogicus	M / F	46,15 / 53,84	27,08 / 72,91	24 / 76
	A / J	66,66 / 33,33	77,41 / 22,58	83,33 / 16,66
Triturus vulgaris	M / F	29,62 / 70,37	26,24 / 73,75	30,95 / 60,04
	A / J	87,09 / 12,9	79,66 / 20,33	77,77 / 22,22

In the phase of repopulating the aquatic habitat, in both populations, there was a higher number recorded for males, than for female specimens ($P_{Td\text{-}m}$ = 92,30%, $P_{Td\text{-}f}$ = 40,00%, $P_{Tv\text{-}m}$ = 64,86%, $P_{Tv\text{-}f}$ = 54,80%). There are researches done which show that at the begining of their aquatic period, the smooth newt females, as well as the crest newt females, compete for assuring themselves a suplimentary sperm stock (Verrell & Halliday, 1985; Baker, 1992; Halliday, 1998). According to this theory, even if the females are getting into the water simoultaneously with the males, females dominate the habitat in the period of its repopulation. Most recently, another research on a crest newt population demonstrates that males get in the aquatic habitat before females do. Actually, preparing for the receival of females leads to a competition for teritory among the males in the aquatic habitat (Jalbă, 2008). We consider that in the case of our research, the first determinations were done in the second period of repopulating the aquatic habitat, and this is why the males were in higher number. Data from another research upon more newt populations habitting near Mădrigeşti locality, clearly distinguishes between these two distinct phases from the aquatic habitat's repopulating period (Cicort Lucaciu, unpublished data).

In both populations, juvenile specimens represent approximately the fifth part of the population ($R_{Td\text{-}j}$ = 22,58%, $R_{Tv\text{-}j}$ = 20,33%). In the first period of our research, the interspecific differences considering juveniles are insignificant (N_{Td} = 13, N_{Tv} = 12). Relating these values with the size of each population, *Triturus dobrogicus* juveniles dominate in number compared to the smooth newt juveniles ($P_{Td\text{-}j}$ = 92,85%, $P_{Tv\text{-}j}$ = 33,33%). It is worth mentioning that *Triturus dobrogicus* juveniles are repopulating the aquatic habitat faster than their adults ($P_{Td\text{-}j}$ = 92,85%, $P_{Tv\text{-}a}$ = 54,16%). This is an

interesting aspect because, usually, interests linked to sexual behavior determine the adults to enter earlier in the aquatic phase (the theory of male's competition for teritory, the theory of female's competition for the suplimentary sperm stock). Another implication of the sexual behavior is the difference between the young and the old adults: the young ones reach water after the old (Jalbă, 2008; Verrell & Halliday, 1985).

Table 3

The percentage variation of the number (*P*) of specimens, in accordance with gender and ontogenetic developmental stage

Species	Gender / Ontogenetic developmental stage	6th of April 2006	5th May 2006	21st of May 2006
Triturus dobrogicus	Total	62,90	100	48,38
	M	92,30	100	46,15
	F	40,00	100	54,28
	A	54,16	100	52,08
	J	92,85	100	35,71
Triturus vulgaris	Total	52,54	100	30,50
	M	64,86	100	35,13
	F	54,80	100	27,88
	A	57,44	100	29,78
	J	33,33	100	33,33

CONCLUSIONS

The size of population is represented by 62 specimens for *Triturus dobrogicus*, and 177 specimens for the smooth newt. The newts' presence in the aquatic habitat fluctuates in time: it is increasing till the begining of May, when it is the height of their reproductive activity, and decreasing afterwards. The aquatic period of the two newt populations started some time at the begining of March and ended at the end of May. The specimens occupied the aquatic habitat gradually, as with the warming of water.

As per the environmental factors effect, sex-ratio is dominated by females. In both populations, its value is approximately one male to three females.

Triturus vulgaris specimens repopulate the puddle later and depopulate it earlier than *Triturus dobrogicus* specimens.

In the first period of our reseach, males quantitatively dominate females. Based on other researches (Verrell & Halliday, 1985; Baker, 1992; Halliday, 1998; Jalbă, 2008), which indicate that males reach the aquatic habitat before females (due to the males' competition for teritory), and that females are in much higher number in the first period of repopulation (due to their competition for suplimentary sperm stock), we consider that the first determinations we made were done in the second period of the newts' repopulating the aquatic habitat.

In both populations, juvenile specimens show a reproductive success, representing approximately the fifth part of the population. Worth mentioning is the fact that *Triturus dobrogicus* juveniles are populating the aquatic habitat earlier than adults.

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VARIABILITY OF THE MAIN ANATOMICAL CHARACTERISTICS OF LEAVES AND FRUITS FOR SOME PEAR TREE VARIETIES AND HYBRID

Elena Săvulescu[1], Mihaela Ioana Georgescu[1], Elena Delian[1], Valeria Petre[2]

KEY WORDS: pear tree, epidermis, mesophyl, epicarp, collenchyma

SUMMARY

This study shows the comparative anatomical study for leaves and fruits of some pear tree varieties and hybrids, making evident the possible anatomical characters used in plant breeding to improve the passive resistance of the plant for field diseases attack.

The transversal section was provided in the median leaves blade to establish the epidermis and mesophyll thickness and in the fruit epicarp. The upper epidermis is thicker at the hybrids than to the Ervina variety. The mesophyll leaf thickness varies among varieties and hybrids studied,with average values between 174.5 μm and 234.5 μm.

Protective zone of the fruit done by the epicarp and wax layer exceeds 20 μm thickness in June and 25 μm in September.

INTRODUCTION

The plant response to the pathogens attack can be passive and active. The passive response is determined by the certain morphological and anatomical properties [1].

At the structural level, the types and size of tissue could have a significant influence on the resistance of the plant organs at the pathogen attacks.

The leaf has the biggest ecological plasticity and it reflect the species particularities concerning the species adaptability and sensitivity to the environmental conditions and attack of the pathogens on or into the leaf. From a structural point of view, the pear leaf has bifacial mesophyll and two epidermises, upper and lower [2, 4].

The extern part of the fruit, named epicarp, is covered from different size wax layer in term of the cultivated variety and hybrid properties and it represent a main barrier against pathogens attack [3].

MATERIALS AND METHODS

There was used leaves and fruits from six varieties/hybrids cultivated at the Research and Development Station for Tree Fruits – Voineşti, Dâmbovița. The samples of the leaves and fruits were collected at the beginning of Jun and September in 2007 and 2008.

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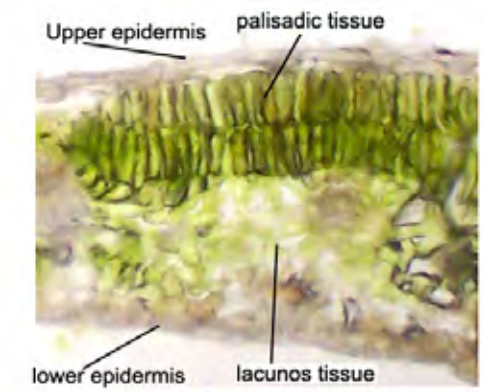

Figure 1. Transversal section through the pear blade leaf, variety Ervina

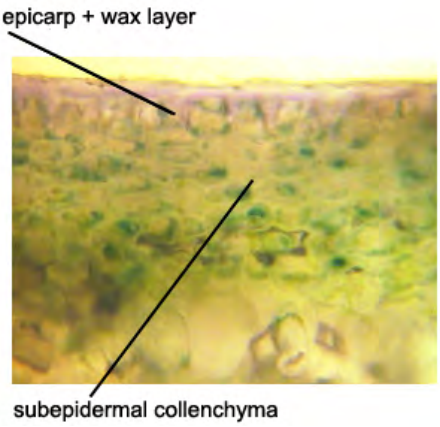

Figure 2. Transversal section through the pear fruit, variety Ervina

The observations and measurement were made on the transversal sections performed by the median leaf blade. The transversal sections of the fruits were made through the median zone. The anatomical sections were clarified 24 hours in chloral hydrate and after that there was washed and fixed into the gelatinous glycerin. The observations and measurements were making with the microscope - MC-7.

The anatomical measurements of the epidermis and mesophyll thickness were made at the blade of the leaves. The thickness of the epicarp with wax layer and subepidermal collenchyma were measures at the fruits. The results of the measurements were interpreted using analysis of variation.

RESULTS AND DISCUSSIONS

Upper epidermis of the leaf, formed by only one cell layer with external wall covered by the cuticle, was more thicker at some hybrids than the Ervina variety used as standard variant (table 1). All analyzed hybrids had thicker upper epidermis with the significant large values from H2/8-86 (26.5 μm), H12/83-79 (23.75 μm), H9/20-86 (22.6 μm) and H5/104-84 (20.9 μm). It can be seen in summary that, the hybrid H2/8-86 can demonstrate a good resistance to the diseases attack as a result of thicker epidermis and it should be confirmed in field trial. Taken into consideration the mesophyll thicker, the hybrid H12/83-79 can demonstrate a more intense photosynthesis and higher resistance in field compared to other analyzed hybrids.

The solely hybrid with thicker lower epidermis was H2/8-86 (18.5 μm). At the other hybrids, the differences between Ervina variety and the hybrids H9/20-86 and H12/83-79 was insignificant. The lower epidermis from H9/19-81 (12.75 μm) and H5/104-84 (12.8 μm) hybrids was thinner with significant difference compared to Ervina variety. The lower epidermis is thinner compared with the upper epidermis.

The bifacial leaf mesophyll (figure 1) had the average values between 174.5 μm and 234.5 μm. Only one hybrid H12/83-79 had the thicker significant mesophyll compared to the Ervina variety (234.5 μm). Only the H5/104-84 hybrid had the thinner significant mesophyll (174.5 μm).

Table 1

Variability of the thicker epidermis and mesophyll at the pear tree leaves

Variety/ Hybrid	LEAF								
	Upper epidermis (µm)			Mesophyl (µm)			Lower epidermis (µm)		
	2007	2008		2007	2008		2007	2008	
1. ERVINA	19.0	18.5	Check	200.0	191,0	Check	15.0	14,5	Check
2. H2/8-86	27.0	26.0	***	200.3	195,5	-	17.5	19,5	**
3. H9/19-81	20.0	19.0	-	190.0	184,5	-	12.5	13,0	0
4. H9/20-86	23.2	22.0	***	190.0	188,0	-	16.0	15,5	-
5. H12/83-79	24.0	23.5	***	235.5	233,5	***	14.0	14,5	-
6. H5/104-84	20.5	21.3	**	170.2	178,8	00	12.5	13,1	0
Dl 5% =	1.32 µm;			10.91 µm;			1.67 µm;		
Dl 1% =	2.08 µm;			17.11 µm;			2.62 µm;		
Dl 0.1% =	3.53 µm;			29.12 µm;			4.46 µm;		

At the analyzed hybrids, the protective zone formatted by the epicarp and wax layer exceeds thick of 20 µm in June, but the bigger values were measured at the Ervina variety (table 2). All hybrids had the epicarp and wax layer significantly thinner in Jun, possibly caused by more intense growing of the hybrids fruits.

The thick of the epicarp and wax layer at the fruit ripeness are considerate to be important for fruit storage and transport. The following hybrids had the thick epicarp and wax layer: H9/19-81 (38.85 µm) and H9/20-86 (33.0 µm).

The analyzed fruits of the hybrids, excepting the hybrid H9/19-81, had thicker subepidermal collenchyma tissue compared with standard variety, character that confers best resistance for the action of the mechanical factors and storage (figure 2).

Table 2

Variability of the thicker epicarp and subepidermal collenchyma at the pear tree fruits

Variety/ Hybrid	Fruits								
	Average of thick (µm)								
	epicarp + wax layer						collenchyma		
	Jun			September			September		
	2007	2008		2007	2008		2007	2008	
1. ERVINA	27,5	28,5	Check	31.0	30.5	Check	56.0	55.5	Check
2. H2/8-86	23,5	22,0	00	32.0	31.0	-	68.0	63.5	***
3. H9/19-81	26,4	25,9	-	39.2	38.5	***	46.5	43.5.	000
4. H9/20-86	23,5	22,5	00	33.5	32.5	**	62.5	60.0	**
5. H12/83-79	24,4	23,5	00	31.5	32.0	-	64.0	61.0	***
6. H5/104-84	19,0	20,6	000	25.5	26.0	000	62.4	60.0	**
Dl 5% =	2.24 µm;			1.27 µm;			2.35 µm;		
Dl 1% =	3.51 µm;			1.99 µm;			3.69 µm;		
Dl 0.1% =	5.98 µm;			3.38 µm;			6.28 µm;		

In general, it was noted that when the thick of the subepidermal collenchyma zone giving mechanical resistance is greater, the epicarp and wax layer are thinnest.

CONCLUSION

The hybrids have the upper epidermis of the leaf thicker than the Ervina variety.

The thick of the mesophyll leaf and lower epidermis are stable for phenologic characters under genotype influence;

The pear tree hybrids with thicker epicarp and subepidermal collenchyma tissue may develop resistance to the transport conditions and fruit storage and these issues would be studied in the future for quality and quantity of the yield.

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THE ASTERACEAE FAMILY FROM THE LOWER BASIN OF THE MOTRU RIVER (I)

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KEY WORDS: Asteraceae, Basin, flora, Motru

ABSTRACT

The paper presents the taxa from Asteraceae Family, which were identified in the Lower Basin of the Motru River. The Asteraceae Family has numerous taxa, being the most important family among the vascular plants from this zone.

INTRODUCTION

From the geographical point of view, the Lower Basin of the Motru River lies in the western part of the Getic Piedmont, with the coordinates: 44^0 55' north latitude and 23^0 45' east longitudes. The studied area is 691 Km^2. From the administrative-territorial point of view, the territory under research is located at the borderline between the counties of Gorj and Mehedinți – the borderline starts in the eastern part of the Negoiești Hills (Comăneşti-Mehedinți, altitude 388 m) and reaches Valea Jiului near Gura Motrului (altitude 110 m). Being situated in the south-western part of the country and of the Getic Piedmont, the studied area has a Central-European climate with sub-Mediterranean influences.

In the territory under research, there have been made floristic and phytosociological studies between 1997 and 2005, within the PhD thesis (COSTACHE I., 2005).

MATERIALS AND METHODS

The first stage in the research of the Lower Basin of the Motru River was represented by looking into the bibliographic material. Against the background of this bibliographic information there were carried out, several times, personal studies in the field, in order to identify and inventories all the species, to watch all their vegetation stages, to notice the biotope characteristics, to take photos, to collect and preserve the species for a thorough checking in the laboratory, as well as to enrich the Herbarium of the University of Craiova. The collected and preserved material was determined by using specialty literature: Romanian Flora, Flora Europaea, The Illustrated Flora of Romania (BELDIE AL., 1979; CIOCÂRLAN V., 2000 etc.) and others. The critical taxa were checked by Prof Dr. Vasile Ciocârlan and compared to other materials found in the great herbariums of the country (Bucharest, Cluj and Craiova). The terminology of the taxa was adopted according to ROTHMALER W., 2002; we have taken into

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consideration the International Botanical Terminology Index (Code de Tokyo, 1993). For the synonymies, we have taken into account the index worked out by KERGUÉLEN M., 1993.

The species were defined within the following systematical units: Phylum, Subphylum, Class, Order, Family, Underfamily and Genus (here, the species are alphabetically presented).

The description of the species includes: scientific name, author (authors), equivalent names, biological form, ecology, territories for rare species (for frequently met species there is no indication of locality), geoelement and coenotaxonomic belonging.

The coenotaxonomic belonging of the species, as well as their affiliation to high-leveled coenotaxonomic units, were made according to RODWELL & al. 2002 etc.

Abbreviations and conventional signs used in our paper:

Systematic units (taxa): Sp. - species; subsp.- underspecies; var. - variety.

Bioforms (biological forms, life forms): T. - terrophytes (annual plants which go over unfavorable conditions during winter or summer under the form of seeds); H. - hemicriptophytes (perennial plants whose regeneration organs are located at the soil level and are protected, during winter, by vegetal remains or snow).

Frequency (spreading level): Freq. - Frequent (numerous specimens with an odd spreading on large areas); Spor. - Sporadic (few specimens spread in few stations); R. - Rare (very few specimens spread in 1-2 stations).

Ecologic characterization: significance of the indexes U T R used in the paper in order to simplify the carrying out of the spectra when studying vegetation (according to POPESCU A. & SANDA V., 1998).

Soil humidity level (U_{1-6}): U_1 - xerophytes (they grow in dry soils and they can stand the prolonged dryness of the soil); U_2 - xeromesophyte; U_3 - mesophytes (in soils with average humidity, they cannot stand prolonged dryness); U_4 - mesohygrophyte; U_5 - hygrophyte (they grow in wet soils, and their roots are under water or in swampy areas); U_6 - hydrophyte (according to the author - ultrahydrophyte), (plants which grow in water, the regeneration organs are found under water); $U_{3(5)}$ - alternately hygrophyte (with oscillations of the humidity level during the plants' vegetation period); U_{1-5}, (includes U_{1-3}, U_{2-5} etc.) - eurip. = euriphyte (with large amplitude against the soil humidity).

Heat level (T): T_1 - hechistothermic; T_2 - microthermic; T_3 - mesothermic; T_4 - subthermophytes (moderately thermophytes); T_5 - thermophytes; T_{1-5} (include T_{1-4}, T_{2-4}, T_{2-5}, T_{3-5}) - euriterm. = euriterms.

Acidity level (R): R_1 - high acidophilus species; R_2 - acidophilus; R_3 - acido-neutrophyle; R_4 - low acid-neutrophyle; R_5 - neutral-basophile; R_{1-5} - (includes R_{1-4}, R_{1-3}, R_{2-5} etc.) - euriionic. Geoelement (phytogeographical elements, origin of the species): Adv.- adventive (species which appeared because of man's inconstant activity); Am. - America; As. - Asia; Atl.- Atlantic; Balc. - Balkan; Circ. - Circumpolar (spread in the northern part of Eurasia and North America); Cosm. - Cosmopolite (large spreading all over the world); Euras. - Eurasian; Euras. cont.- Eurasian continental; Eur. - European; Eur. centr. - Central European; Eur. cont. - European continental; Medit. - Mediterranean; Pont. - pontic (North of the Black Sea); Pan. - Panonic (In the Panonic Plain); Submedit. - SubMediterranean; Subatl. - Subatlantic. Other abbreviations: E. - east; GJ. - Gorj; MH. - Mehedinți; N. - north; Sol.- soils; S. - south; V. - west.

RESULTS AND DISCUSSION

The conspectus of the flora in the Lower Basin of the Motru River includes about 1.124 vascular taxa (COSTACHE I., 2005), among which, *Asteráceae* Family has

140 taxa, which were identified in the Lower Basin of the Motru River. In this part (I) we present 54 taxa from the *Asteroideaea* underfamily.

SPERMATÓPHÝTA
MAGNOLIOPHÝTINA (Angiospérmae)
MAGNOLIÓPSIDA (Dicotyledónatae)
ASTÉRIDAE
ASTERÁLES (COMPOSITÁLES)
Asteráceae (Compositae)
Asteroideae (Tubuliflórae)

1. *Achilléa asplenifólia* Vent. - H. Spor. $U_{3-4}T_4R_{4-5}$. **MH**: Broşteni (Grecescu 1898; Prodan 1931; Prodan & Nyárády 1964); Broşteni (Costache 2004), Comanda, Slătinicul Mare, Lunca Banului, Stângăceaua, Butoieşti, Buiceşti (Sat Mitulani); **GJ**: Văgiuleşti. Pan. Car. *Puccinellio-Salicornietea, Molinietalia, Alnion glutinosae*.
2. *A. collína* J. Becker. ex Rchb. - H. Frecv. $U_2T_4R_4$. Eur. cont. Car. *Festuco-Brometea, Festuco-Sedetalia, Convolvulo arvensis-Agropyrion repentis*.
3. *A. crithmifólia* Waldst. & Kit. - H. Spor. $U_{2-3}T_4R_{2-4}$. **MH**: Brezniţa (Prodan 1931; Prodan & Nyárády 1964). Balc.-Pan. Car. *Festuco-Brometea*.
   - **var. crithmifólia** - Spor. **MH**: Comăneşti; **GJ**: Glogova.
   - **var**. **gética** (Grec.) Borza [*A. gética* Grec.]- Frecv. **MH**: Comăneşti – Câmpu Mare, Strehaia, Slătinicul Mare, Lunca Banului; **GJ**: Glogova, Motru.
4. *A. millefólium* L. - H. Frecv. $U_{2-3(4)}T_4R_{3-4}$. Euras. Car. *Molinio-Arrhenatheretea*.
5. *A. pannónica* Scheele [*A. lanáta* auct., non Spreng., *A. millefólium* subsp. *pannónica* (Scheele) Hayek, *A. millefólium* var. *lanáta* W. D. J. Koch] - H., Frecv. $U_{2-3}T_4R_4$. Euras. cont. Car. *Festuco-Brometea, Festucetalia valesiacae, Geranion sanguinei*.
6. *A. roseo-álba* Ehrend. - H. **R.** $U_{2-3}T_{3-4}R_4$. **MH:** Slătinicul Mare, Stângăceaua (Poşta Veche-Pârlogeni), Gura Motrului. Eur. centr. şi de S. *Arrhenatheretalia, Festucion valesiacae*.
7. *A. setácea* Waldst. & Kit. - H. Frecv. $U_{2-3(4)}T_{3-4}R_{3-4}$. Euras. cont. Car. *Festuco-Brometea, Festucetalia valesiacae, Festuco-Sedetalia*.
8. *Ambrósia artemisiifólia* L. [*A. elátior* L.] - T. Frecv. $U_2T_{3-5}R_{2-5}$. Adv. (Am. by N.). *Sisymbrion officinalis*.
9. *Ánthemis arvénsis* L. - T. Frecv. $U_{2-3}T_3R_{3-4}$. Eur. Car. *Stellarietea mediae, Centaureetalia cyani, Sedo-Scleranthetalia.*
10. *Á. austríaca* Jacq. - T. Spor. $U_2T_4R_{4-5}$. Centr. eur.-pont. Car. *Stellarietea mediae, Sisymbrietalia, Caucalidion, Onopordion acanthii.*
11. *Á. cótula* L. - T. Frecv. $U_{2-3}T_4R_4$. Cosm. Car. *Stellarietea mediae, Sisymbrion officinalis, Centaureetalia cyani.*
12. *Á. ruthénica* M. Bieb. - T. Frecv. $U_{2-3}T_4R_4$. Centr. eur.-pont.-medit. *Sisymbrietalia, Onopordion acanthii*.
13. *Á. tinctória* L. - H. Spor. $U_2T_3R_4$. Euras. cont. Car. *Artemisietea vulgaris, Festucion valesiacae, Convolvulo arvensis-Agropyrion repentis, Dauco-Melilotion, Sisymbrion officinalis*.
14. *Árctium lápa* L. [*Lápa májor* Gaertn.] - Ht. Frecv. $U_{3-4}T_3R_4$. Euras. Car. *Artemisietea vulgaris, Arction lappae.*
15. *Á. mínus* (J. Hill) Bernh. - Ht. Frecv. $U_{2-3}T_3R_4$. Eur. Car. *Artemisietea vulgaris, Arction lappae*.
16. ***** *Artemísia abrótanum* L. - mPh., Subspont. **MH**: Lunca Banului. Eur. centr. and SE.
17. *A. absínthium* L. - H.(Ch.). Frecv. $U_{2-3}T_3R_4$. Euras. Car. *Artemisietea vulgaris, Arction lappae, Onopordion acanthii.*

18. *A. ánnua* L. - T. Frecv. $U_{2-3}T_4R_4$. Euras. cont. Car. *Stellarietea mediae, Sisymbrion officinalis*.
19. *A. scopária* Waldst. & Kit. - Ht. Frecv. $U_2T_3R_{2-5}$. **MH**: Broşteni (Grecescu 1898, Nyárády 1964). Euras. cont. Car. *Artemisietea vulgaris, Car. Festuco-Brometea, Festucion valesicae, Sisymbrion officinalis, Onopordion acanthii*.
20. *A. vulgáris* L. - H. Frecv. $U_3T_3R_4$. **MH**: Broşteni, Strehaia (Grecescu 1898). Circ. Car. *Artemisietea vulgaris, Arction lappae*.
21. *Béllis perénnis* L. - H. Frecv. $U_{3-4}T_2R_{2-5}$. Eur. Car. *Molinio-Arrhenatheretea, Cynosurion cristati*.
22. *Bídens cérnua* L. - T. Frecv. $U_5T_{3-5}R_{3-5}$. Euras. Car. *Bidentetea tripartiti, Bidention tripartiti*.
23. *B. frondósa* L. [*B. melanocárpa* Wiegand] - T. Spor. $U_5T_{3-5}R_{3-5}$. **MH**: Corcova, Strehaia, Argineşti. Adv. (Am. by N.). Car. *Bidentetea tripartiti*.
24. *B. tripartíta* L. - T. Frecv. $U_{4-5}T_3R_{3-5}$. Euras. Car. *Bidentetea tripartiti, Nanocyperion, Bidention tripartiti*.
25. *Cárduus acanthoídes* L. [incl. *C. camporum* Boiss.] - Ht. Frecv. $U_2T_3R_{3-5}$. Eur. Car. *Artemisietea vulgaris, Onopordion acanthii*.
26. *C. críspus* L. - Ht. Spor. $U_4T_3R_{3-5}$. **MH**: Lunca Banului ([CRA]-Leg. Prodan & al. 1954, Det. Costache 2004). Eur. Car. *Galio-Urticetea, Convolvuletalia sepium*.
27. *Carlína bieberstéinii* Bernh. ex Hornem. subsp. *brevibracteáta* (Andrae) K. Werner [*C. vulgáris* subsp. *brevibracteáta* (Andrae) Bornm., *C. intermédia* Schur, *C. vulgáris* subsp. *intermédia* (Schur) Hayek] - Ht. Frecv. $U_{2-3}T_3R_3$. **MH**: Albuleşti (Strehaia) (Nyárády 1964). Centr. eur. Car. *Festuco-Brometea, Festucetalia valesiacae*.
28. *C. vulgáris* L. - Ht. Frecv. $U_{2-3}T_3R_{3-4}$. Euras. Car. *Festuco-Brometea, Festucetalia valesiacae*.
29. *Carpésium cérnuum* L. - T.-Ht. Spor. $U_{3-4}T_4R_4$. **MH**: Lunca Banului ([CRA]-Prodan & al. 1954). Euras. by N. Car. *Querco-Fagetea*.
30. *Cárthamus lanátus* L. - T. Frecv. $U_{2-3}T_4R_{4-5}$. Pont.-medit. Car. *Artemisietea vulgaris, Festucion valesicae, Sisymbrion officinalis*.
31. *Centauréa apiculáta* Ledeb. subsp. *spinulósa* (Roch.) Dostal. [*C. spinulosa* Roch.] –H. Frecv. $U_2T_{3-4}R_{3-4}$. Eur. centr. and SE. *Danthonio-Brachypodion, Trifolion medii, Festucetalia valesiacae*.
32. *C. cýanus* L. [*Cýanus segétum* Hill]- T.-Ht. Frecv $U_{2-3}T_4R_{3-5}$. Medit. (Cosm). Car. *Stellarietea mediae, Centaureetalia cyani*.
33. *C. ibérica* Trev. ex Sprengel [*Calcitrápa ibérica* Schur] - Ht. $U_{2-3}T_4R_{2-5}$. **MH**: Broşteni ([CRA] Prodan & al. 1954), Corcova, Strehaia, Breznița, Butoieşti, Argineşti, Gura Motrului. Pont.-balc. Car. *Artemisietea vulgaris, Onopordetalia acanthii*.
34. *C. induráta* Janka - H. Spor. $U_{2-3}T_3R_3$. **MH**: Argineşti. Dacic. *Arrhenatherion, Festucetalia valesiacae*.
35. *C. jacéa* L. - H. Spor. $U_{2-3}T_4R_4$. **MH**: Comăneşti-Câmpu Mare; **GJ**: Glogova-Motru. Eur. Car. *Molinio-Arrhenatheretea, Arrhenatherion*.
36. *C. solstitiális* L. [*Cýanus solstitiális* Baumg., *Calcitrápa solstitiális* Schur] - Ht. Frecv. $U_2T_4R_{3-5}$. Medit. *Onopordetalia acanthii*.
37. *C. stenólepis* A. Kern.- H. Frecv. $U_{2-3}T_3R_3$. **MH**: Broşteni ([CRA]-Leg. Prodan & al. 1954, Det. Popescu 1993). Eur. centr. and by SE. *Origanetalia vulgaris, Arrhenatherion, Trifolion medii, Festucetalia valesiacae*.
38. *C. stoébe* L. [*C. rhenána* Boreau] subsp. **stoébe** - Ht. Frecv. $U_2T_{3-4}R_{4-5}$. Eur. centr. and SE. Car. *Festuco-Brometea, Festucetalia valesiacae*.

39. *C. s.* subsp. *micránthos* (Gugler) Hayek [*C. bieberstéinii* auct.] - Ht.-H. Frecv. $U_{1-2}T_4R_4$. Pont.-pan.-balc. Car. *Festuco-Brometea, Festucetalia valesiacae, Sisymbrion officinalis, Sedo-Scleranthetalia*.
40. *Círsium arvénse* (L.) Scop. - G. Frecv. $U_{2-4}T_3R_{2-5}$. Euras. Car. *Stellarietea mediae*.
41. *C. candelábrum* Griseb. - Ht. **R.** $U_{2-3}T_4R_4$. **MH**: Comăneşti (Valea Mare Valley). Balc. *Epilobietea angustifolii, Alno-Ulmion*.
42. *C. créticum* (Lam.) D`Urv. [*Cárduus créticus* Lam., *Círsium siculum* Grecescu non DC.]- G. Frecv. (**R. in National Red List**) $U_{4-5}T_4R_{4-5}$. **MH**: Broşteni, Strehaia (Grecescu 1898); Medit. Car. *Agrostion stoloniferae* (-*Magnocaricion elatae*, *Phragmition communis*).
43. *C. grecescui* Rouy - Ht. **R.** $U_{2-3}T_{3-4}R_4$. **MH:** Comăneşti; Broşteni ([CRA]-Leg. Prodan & al. 1954, Det. Costache 2003), Buiceşti, Argineşti, Gura Motrului; **GJ:** Steic, Motru, Văgiuleşti, Sat Cârciu. (Subendemit- subbalcanic element) Rom., Jug. *Onopordion acanthii, Festucion valesiacae*.
44. *C. oleráceum* (L.) Scop. - H. Spor. $U_{4-5}T_3R_4$. **MH**: Comanda, Corcova. Euras. Car. *Molinio-Arrhenatheretea, Calthion palustris, Alno-Ulmion*.
45. *C. vulgáre* (Savi.) Ten. [*C. lanceolátum* (L.) Scop., non Hill.] - Ht. Frecv. $U_{1-2}T_3R_{1-5}$. Euras. Car. *Artemisietea vulgaris, Onopordion acanthii, Festucion valesiacae*.
46. *Conýza canadénsis* (L.) Cronquist [*Erígeron canadénsis* L.] - T. Frecv. $U_{2-3}T_{2-5}R_{2-5}$. Adv. (Am. by N.). Car. *Stellarietea mediae, Atriplici-Chenopodietalia albi, Sisymbrion officinalis*.
47. *Echínops exaltátus* Schrad. [*E. commutátus* Jur.] - H. Spor. $U_{3-4}T_{2-5}R_4$. **GJ**: Văgiuleşti. Cont. euras. *Onopordion acanthii, Arction lappae*.
48. *E. sphaerocéphalus* L. - H. Frecv. $U_{2-3}T_{3-4}R_4$. **MH**: Broşteni ([CRA]-Leg. Prodan & al. 1954, Det. Costache 2003). Cont. euras. Car. *Artemisietea vulgaris, Agropyretalia repentis, Onopordion acanthii, Arction lappae*.
? *E. bannáticus* Rochel ex Schrad. - I don't find. Mentioned by Nyárády (1964) in **MH**: Lupşa de Jos, without herbarium materil.
49. *Erechtítes hieracifólia* (L.) Rafin. ex DC. - T. **R.** $U_3T_{2-5}R_{2-5}$. **MH**: Comăneşti Adv. (Am. by N. And S.). *Querco-Fagetea, Fagetalia sylvaticae*.
50. *Erígeron ácris* L. - Ht.-H. Frecv. $U_{2-3}T_3R_4$. Circ. *Festuco-Brometea, Sedo-Scleranthetalia*.
51. *E. ánnuus* (L.) Pers. [*Stenáctis ánnua* (L.) Less.] subsp. **ánnuus** - T.-Ht.-H. Frecv. $U_{2-3}T_{3-5}R_{2-5}$. Adv. (Am. by N). Car. *Artemisietea vulgaris, Sisymbrion officinalis, Convolvuletalia sepium, Arction lappae*.
52. *E. á.* subsp. *strigósus* (H. L. Mühl. ex Willd.) Wagenitz [*E. ramósus* (Walter) Britton, Sterns & Poggenb., *Stenáctis ramósa* (Walter) Domin] - Spor. $U_{3-4}T_{3-5}R_4$. Car. *Artemisietea vulgaris, Sisymbrion officinalis, Convolvuletalia sepium, Arction lappa.*

CONCLUSIONS

In the Lower Basin of the Motru River have been identified 140 taxa from *Asteráceae* Family, in this part (I) we presents 54 taxa (48 species, 4 subspecies and 2 varieties), among which, *Erechtítes hieracifólia* (L.) Rafin. ex DC., is rare in the Lower Basin of the Motru. *Achilléa roseo-álba* Ehrend., *Círsium créticum* (Lam.) D`Urv. (local it is frequent), *Círsium grecescui* Rouy, have been included in the National Red List.

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THE ASTERACEAE FAMILY FROM THE LOWER BASIN OF THE MOTRU RIVER (II)

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KEY WORDS: Asteraceae, Basin, flora, Motru.

ABSTRACT

The paper presents in continuation the taxa from Asteraceae Family, which were identified in the Lower Basin of the Motru River.

RESULTS AND DISCUSSION

SPERMATÓPHÝTA
MAGNOLIOPHÝTINA (Angiospérmae)
MAGNOLIÓPSIDA (Dicotyledónatae)
ASTÉRIDAE
ASTERÁLES (COMPOSITÁLES)
Asteráceae (Compositae)
Asteroideae (Tubuliflórae)

53. *Eupatórium cannábinum* L. - H. Frecv. $U_4T_3R_{4-5}$. **MH**: Lunca Banului ([CRA]-Prodan & al. 1954). Euras. *Epilobietea angustifolii, Convolvuletalia sepium, Atropion, Alnion glutinosae*.

54. *Filágo arvénsis* Lam. [*Lógfia arvénsis* (L.) Holub] - T. Frecv. $U_{1-2}T_3R_{2-5}$. Euras. by S. *Corynephoretalia, Festuco-Sedetalia, Thero-Airion*.

55. *F. vulgáris* Lam. [*F. canéscens* Jord., *F. germánica* L. non Huds.] - T. Frecv. paj., $U_{1-2}T_3R_{2-5}$. **MH**: Broşteni, Strehaia, Lupşa de Jos, Lupşa (Roşca Forest) (Nyárády 1964). Euras. by S. *Festuco-Sedetalia, Thero-Airion*.

56. *Galinsóga parviflóra* Cav. - T. Frecv. $U_{3-4}T_{3-5}R_3$. Adv. (Am. by S-Peru.) today Cosm. Car. *Stellarietea mediae, Atriplici-Chenopodietalia albi, Bidentetea tripartiti*.

57. *G. quadriradiáta* Ruiz & Pav. - T. Spor. $U_{3-4}T_4R_{3-4}$. **MH**: Strehaia (Huşniței Valley); **GJ**: in the Motrului Valley, Glogova place. Adv. (Am. by S.). Car. *Stellarietea mediae, Atriplici-Chenopodietalia albi, Chenopodion rubri.*

58. *Gnaphálium sylváticum* L. [*Omalothéca sylvática* (L.) Sch. Bip. & F.W. Schultz] - H. Spor. $U_3T_3R_3$. **MH**: Negoeşti, Comăneşti; **GJ**: Glogova (Cămuieşti). Circ. *Epilobietea angustifolii*.

59. *G. uliginósum* L. [*Filaginélla uliginósa* (L.) Opiz.] - T. Frecv. $U_{4-5}T_3R_4$. **MH**: Broşteni (Grecescu 1898). Euras. Car. *Isoëto-Nanojuncetea, Nanocyperion, Cyperetalia*.

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60. ***** *Heliánthus tuberósus* L. - G. Spor. subspont. **MH**: Strehaia (Huşniţei Valley); **GJ**: Cătunele. Am. by N. *Artemisietalia vulgaris*.

61. *Ínula británnica* L. - Ht. Frecv. $U_{3-4}T_3R_{2-5}$. **MH**: Broşteni ([CRA]-Prodan & al. 1954). Euras. Car. *Molinio-Arrhenatheretea, Molinietalia, Potentillion anserinae*.

62. *Í. conýza* DC. - H. Spor. $U_{2-3}T_4R_4$. **MH**: Corcova (Pârvuleşti); **GJ**: Văgiuleşti. Eur. centr. Car. *Trifolio-Geranietea sanguinei, Origanetalia vulgaris*.

63. *Í. germánica* L. - H. Frecv. $U_{1-2}T_4R_4$. Eur. centr. and SE. Car. *Trifolio-Geranietea sanguinei, Geranion sanguinei, Festucion valesicae*.

64. *Í. helénium* L. - H. Spor. $U_4T_3R_{3-4}$. **MH**: Broşteni ([CRA]-Prodan & al. 1954); Lupşa de Jos (Nyárády 1964); Lunca Banului (F.O.E. 1045/23.VII.1972 - Leg. D. & Mariana Cârţu - Centuria XI. Plantae Vascularis VIII - Păun, D. & Mariana Cârţu 1978). Euras. *Convolvuletalia sepium, Arction lappae, Alno-Ulmion*.

65. *Í. hírta* L. - H. Frecv. $U_2T_4R_4$. Cont. euras. Car. *Trifolio-Geranietea sanguinei, Geranion sanguinei, Festucetalia valesiacae*.

66. *Í. salícina* L. subsp. *salícina* - H. Frecv. $U_2T_4R_4$. **MH**: Strehaia, Breznița, Stângăceaua, Butoieşti, Argineşti, Euras. Car. *Festuco-Brometea, Festucion valesiacae, Geranion sanguinei*.

67. *Í. s.* subsp. *áspera* (Poir.) Hayek [incl. *I. sabuletorum* Czern. ex Lavrenko] - Frecv. $U_3T_3R_{3-4}$. **MH**: Comăneşti, **GJ**: Glogova. Eur. by S. Car. Festuco-Brometea.

68. *Leucánthemum vulgáre* Lam. [*Chrysánthemum leucánthemum* L.] - H. Frecv. $U_{2-3}T_3R_3$. Euras. Car. *Molinio-Arrhenatheretea, Arrhenatheretalia*.

69. *Matricária discoídea* DC. [*M. matricarioídes* auct., *M. suavéolens* (Pursh) Buchenau non L., *Chamomílla suavéolens* (Pursh) Rydb.] - T. Spor. $U_3T_{2-5}R_{2-5}$. **MH**: Lunca Banului, Butoieşti, Argineşti, Gura Motrului. Adv. (As. NE., Am. by N.?). Car. *Polygono arenastri-Poëtea annuae, Matricario-Polygonion avicularis*.

70. *M. perforáta* Mérat [*M. inodóra* L. nom. illegit., *Tripleurospérmum inodórum* (L.) Sch. Bip., *T. perforátum* (Mérat) M. Laínz] - T.-Ht. Frecv. $U_3T_{3-5}R_{3-4}$. Euras. *Centaureetalia cyani, Sisymbrion officinalis, Onopordion acanthii*.

71. *M. recutíta* L. [*M. chamomílla* auct. non L., *Chamomílla recutíta* (L.) Rauschert] - T. Frecv. $U_{2-3}T_{3-4}R_{3-4}$. Euras. Car. *Stellarietea mediae, Plantagini-Prunelletalia*.

72. *Onopordúm acánthium* L. - Ht. Frecv. $U_2T_4R_4$. Euras. *Onopordion acanthii*.

73. *Pulicária dysentérica* (L.) Berhn. - H. Frecv. $U_{3-4}T_3R_4$. **MH**: Butoieşti-Argineşti (F.O.E. 1047/25.VIII.1977 - Leg. D. & Mariana Cârţu, I. Teodorescu - Centuria XI. Plantae Vascularis VIII - Păun, D. & Mariana Cârţu 1978); Ohaba (Nyárády 1964). Centr. eur. *Molinietalia, Potentillion anserinae*.

74. *P. vulgáris* Gaertn. - T. Frecv. $U_4T_3R_3$. Euras. Car. *Bidentetea tripartiti, Potentillion anserinae, Nanocyperion*.

75. *Senécio erráticus* Bertol. [*S. erráticus* (Bertol.) Matthews, *S. aquáticus* subsp. *barbareifólius* (Wimm. & Grab.) Walters] - Ht. Spor. $U_{4-5}T_4R_4$. **MH**: Strehaia, Lunca Banului, Butoieşti, Argineşti, Gura Motrului. Centr. Eur. *Phragmitetea australis, Molinietalia, Calthion palustris*.

76. *S. erucifólius* L. - H. Frecv. $U_{2-3}T_3R_4$. Euras. Car. *Festuco-Brometea, Geranion sanguinei, Dauco-Melilotion, Convolvulo arvensis-Agropyrion repentis*.

77. *S. jacobǽa* L. - H. Frecv. $U_{2-3}T_3R_{3-4}$. Euras. Car. *Festuco-Brometea, Origanetalia vulgaris, Arrhenatheretalia, Dauco-Melilotion*.

78. *S. vernális* Waldst. & Kit. - T. Frecv. $U_{2-3}T_4R_{2-5}$. Euras. cont. *Stellarietea mediae*.

79. *S. viscósus* L. - T. Spor. $U_3T_{2-3}R_{2-5}$. **MH**: Comăneşti. Eur. *Epilobietea angustifolii, Galeopsidion segetalis, Sisymbrion officinalis*.

80. *S. vulgáris* L. - T. Frecv. $U_3T_3R_{2-5}$. Euras. *Atriplici-Chenopodietalia albi, Caucalidion, Aphanion*.

81. *Serrátula tinctória* L. - H. Frecv. $U_{3-4}T_3R_{2-5}$. Euras. Car. *Molinio-Arrhenatheretea, Alnion glutinosae*.

- var. **lancifólia** S. F. Gray [*S. t*. var. *integrifolia* Wallr., *S. indivisa* Poir.] - **MH**: Meriş; Broşteni (Nyárády 1964); Corcova, Lunca Banului, Argineşti; **GJ**: Văgiuleşti.
- var. **dissécta** Wallr. [*S. t*. var. *pinnatifida* Kit. ap Peterm.] - Frecv.

82. *Sigesbéckia orientális* L. - T. Spor. $U_2T_4R_4$. **MH**: Lunca Banului ([CRA] alt. 150 m.s.m., 13. VII. 1954 - Leg. Prodan I, Buia Al., Păun M. & al.), Cotoroaia: alt. 305 m, N 44^0 38' 505'', E 23^0 02' 230''). As. by V. (Cosm.). *Artemisietea vulgaris*.

83. *Solidágo gigantéa* Aiton subsp. *serótina* (Aiton) Mc Neill [*S. serótina* Aiton] - H. Spor. subspont. $U_{3-4}T_3R_3$. **MH**: Argineşti-Gura Motrului. Am. by N. *Artemisietea vulgaris, Onopordetalia acanthii, Convolvuletalia sepium*.

84. *S. virgáurea* L. subsp. *virgáurea* - H. Frecv. $U_{2-3}T_2R_3$. **MH**: Broşteni ([CRA]-Prodan & al. 1954). Circ. *Fagetalia sylvaticae, Trifolio-Geranietea sanguinei, Epilobietea angustifolii*.

- var. **latifólia** Koch. - **MH**: Miluţa (Strehaia), (Morariu & Nyárády 1964).

85. *Tanacétum corymbósum* (L.) Sch. Bip. [*Chrysánthemum corymbósum* L.] - H. Frecv. $U_{2-3}T_{2-3}R_3$. **MH**: Broşteni ([CRA] - Leg. Prodan & al. 1954). Euras. Car. *Trifolio-Geranietea sanguinei, Geranion sanguinei, Car. Quercetea pubescentis*.

86. *T. macrophýllum* (Waldst. & Kit.) Sch. Bip. [*Chrysánthemum macrophýllum* Waldst. & Kit.] - H. Spor. $U_3T_{3-4}R_3$. **MH**: Lunca Banului ([CRA] - Leg. Prodan & al. 1954). Carp.-balc-cauc. *Arction lappae*.

87. **T. parthénium* (L.) Sch. Bip. [*Chrysánthemum parthénium* (L.) Bernh.] - **e**,, H. Spor. subspont. $U_2T_{3-4}R_3$. **MH**: Comăneşti. Adv. (Medit. by E.). *Arction lappae*.

88. *T. vulgáre* L. [*Chrysánthemum vulgáre* (L.) Bernh.] - H. Frecv. $U_{3-4}T_3R_4$. Med., arom. **MH**: Broşteni ([CRA] - Leg. Prodan & al. 1954). Euras. Car. *Artemisietea vulgaris, Arction lappa*e.

89. *Tussilágo fárfara* L. - H. Frecv. $U_{3-4}T_3R_{3-4}$. Euras. Car. *Artemisietea vulgaris, Arction lappae, Convolvulo arvensis-Agropyrion repentis*.

90. *Xánthium itálicum* Moretti - T. Spor. $U_{3-4}T_4R_{2-5}$. Eur. by S. *Bidentetea tripartiti, Sisymbrion officinalis*.

91. *X. saccharátum* Wallr. - T. Frecv. $U_{3-4}T_4R_{2-5}$. Am. by N. Car. *Bidentetea tripartiti, Chenopodion rubri*.

92. *X. spinósum* L. - T. Frecv. $U_{2-3}T_4R_3$. Adv. (Am. by S.) today Cosm. *Atriplici-Chenopodietalia albi, Onopordion acanthii, Sisymbrion officinalis*.

93. *X. strumárium* L. - T. Frecv. $U_{3-4}T_{3-4}R_{4-5}$. **MH**: Argineşti (Nyárády 1964). Euras. today Cosm. *Atriplici-Chenopodietalia albi, Sisymbrion officinalis*.

94. *Xeránthemum ánnuum* L. - T. Spor. $U_2T_4R_3$. Pont.-Medit. *Festucetalia valesiacae*.

95. *X. cylindraceum* Sibt. & Sm. [*X. foétidum* auct. non Moench] - T. Frecv. $U_{1-2}T_4R_{3-4}$. **MH**: Lupşa de Jos (Strehaia), (Nyárády 1964). Pont.-medit. Car. *Festuco-Brometea, Festucetalia valesiacae*.

Cichorioideae (Liguliflorae)

96. *Chondrílla júncea* L. - Ht.-H. Frecv. $U_2T_4R_4$. Cont. euras. Car. *Artemisietea vulgaris*, Car. *Festuco-Brometea, Festuco-Sedetalia, Convolvulo arvensis-Agropyrion repentis, Dauco-Melilotion*.

97. *Cichórium íntybus* L. - H. Frecv. $U_{1-5}T_4R_{4-5}$. Euras. Car. *Artemisietea vulgaris, Arrhenatheretalia*.

98. *Crépis biénnis* L. - Ht. Frecv. $U_3T_3R_4$. Eur. Car. *Molinio-Arrhenatheretea, Molinietalia, Sisymbrion officinalis*.

99. *C. capilláris* (L.) Wallr. [*C. vírens* L.] - Ht. Spor. $U_{2-3}T_4R_3$. **MH**: Comăneşti, Stângăceaua, Butoieşti, Brezniţa; **GJ**: Glogova, Valea Perilor, Motru, Văgiuleşti. Eur. centr. and by S. Car. *Stellarietea mediae, Arrhenatheretalia, Dauco-Melilotion*.

100. *C. foétida* L. [*Barkhaúsia foétida* (L.) F. W. Schmidt] subsp. *rhoeadifólia* (Bieb.) Čelak. [*C. rhoeadifólia* Bieb., *Barkhaúsia rhoeadifólia* Bieb. ex Rchb.] - ⊙, T. Frecv. $U_{2-3}T_4R_{3-4}$. Pont.-medit. Car. *Artemisietea vulgaris, Onopordetalia acanthii, Sisymbrion officinalis*.

101. *C. púlchra* L. - T. Spor. $U_2T_4R_3$. **MH**: Brezniţa. Eur. by S. Car. *Stellarietea mediae, Sisymbrion officinalis, Onopordetalia acanthii*.

102. *C. setósa* Haller fil. [*Barkhaúsia setósa* (Haller fil.) DC.] - T. Frecv. $U_{2-3}T_4R_4$. **MH**: Broşteni (Grecescu 1898). Eur. centr. and by S. Car. *Artemisietea vulgaris, Atriplici-Chenopodietalia albi, Sisymbrion officinalis*.

103. *C. tectórum* L. - T. Frecv. $U_2T_{3-5}R_{2-5}$. Euras. Car. *Stellarietea mediae, Sisymbrion officinalis*.

104. *Hierácium bauhíni* Schult. in Besser subsp. *thaumasium* (Peter) P.D. Sell - H. Frecv. $U_{1-2}T_3R_{3-4}$. Centr. eur. and by E. Car. *Festuco-Brometea, Festucetalia valesiacae*.

105. *H. lactucélla* Wallr. [*H. auricula* auct. non L.] - H. Spor. $U_{2-3}T_{1-4}R_{3-4}$. Eur. *Arrhenatherion*.

106. *H. macránthum* (Ten.) Ten. [*H. hoppeánum* Schult. grex *macránthum* (Ten.) Nägeli & Peter] - H. Frecv. $U_{2-3}T_3R_4$. Eur. centr. and by SE. (mont.). Car. *Festuco-Brometea, Festucion valesicae*.

107. *H. murórum* L. [*H. sylváticum* (L.) L.] - H. Frecv. $U_3T_3R_{3-4}$. Eur. Car. *Querco-Fagetea, Trifolion medii*.

108. *H. piloséllа* L. - H. Frecv. $U_{2-3}T_{2-5}R_{3-4}$. Euras. *Festuco-Brometea, Sedo-Scleranthetalia*.

109. *H. racemósum* Waldst. & Kit. ex Willd. - H. Spor. $U_3T_3R_3$. **MH**: Comăneşti; Corcova (Pârvuleşti). Eur. centr. and by S. Car. *Trifolio-Geranietea sanguinei, Trifolion medii*.

110. *H. sabaúdum* L. [incl. *H. x platyphyllum* Arvet-Touvet (*H. racemosum* x *sabaudum*)] - H. Frecv. $U_3T_3R_{3-4}$. **MH**: Broşteni ([CRA] - Leg. Prodan & al. 1954, Det. Costache 2004). Eur. Car. *Querco-Fagetea, Origanetalia vulgaris*.

111. *H. umbellátum* L. - H. Spor. $U_{2-3}T_3R_3$. **MH**: Argineşti, Gura Motrului. Circ. Car. *Querco-Fagetea, Origanetalia vulgaris*.

112. *Hypochoéris maculáta* L. [*Achyróophorus maculátus* (L.) Scop.] - H. Spor. $U_3T_{2-5}R_4$. **MH**: Bala, Câmpu Mare, Stroeşti, Floreşti, Zegujani, Buiceşti (Mitulani); **GJ**: Negoeşti, Glogova, Cătunele, Motru, Văgiuleşti. Euras. Car. *Festuco-Brometea, Festucetalia valesiacae, Quercetalia pubescenti-petraeae, Danthonio-Brachypodion*.

113. *H. radicáta* L. - H. Frecv. $U_3T_3R_{3-4}$. Eur. *Arrhenatheretalia, Festuco-Sedetalia*.
- var. **híspida** Peterm. - Frecv.

114. *Lactúca quercína* L. [*L. stricta* Waldst. & Kit.] - Ht. Spor. $U_{2-3}T_4R_4$. **MH**: Brezniţa, Buiceşti (Mitulani), Argetoaia, Gura Motrului. Centr. eur. Car. *Quercetea pubescentis, Geranion sanguinei*.

115. *L. salígna* L. - T.-Ht. Frecv. $U_{2-3(4)}T_4R_4$. Eur. centr. and by S. *Agropyretalia repentis, Onopordion acanthii*.

116. *L. serríola* L. [*L. scaríola* L.] - Ht. Frecv. $U_{2-3}T_4R_{2-5}$. Euras. *Atriplici-Chenopodietalia albi, Sisymbrietalia, Convolvulo arvensis-Agropyrion repentis, Dauco-Melilotion*.

117. *Lápsana commúnis* L. subsp. *commúnis* - T.-H. Frecv. $U_{2-3}T_4R_4$. **MH**: Argineşti-Gura Motrului (Buia & al. 1961). Euras. Car. *Galio-Alliarion*.

118. *L. c.* subsp. *adenophora* (Boiss.) Rech. fil. - T. Frecv. $U_3T_3R_{3-4}$. **MH**: Comăneşti, Câmpu Mare, Corcova, Strehaia-Cerângani, Brezniţa; **GJ**: Negoeşti, Glogova, Cătunele. Trans. Balc. *Fagetalia sylvaticae*.

119. *Leóntodon autumnális* L. - H. Frecv. $U_3T_{2-5}R_{2-5}$. Euras. Car. *Molinio-Arrhenatheretea, Plantagini-Prunelletalia, Cynosurion cristati*.

120. *L. críspus* Vill. [*L. ásper* (Waldst. & Kit.) Poir., non Forskål, *L. crispus* subsp. *ásper* (Waldst. & Kit.) Rohlena] - H. Frecv. $U_2T_4R_4$. Euras. *Festuco-Brometea, Festucetalia valesiacae*.

121. *L. híspidus* L. - H. Frecv. $U_{2-3}T_{2-5}R_{2-5}$. Euras. Car. *Molinio-Arrhenatheretea*.

122. *Mycélis murális* (L.) Dumort. [*Lactúca murális* (L.) P. Gaertn.] - H. Frecv. $U_3T_3R_{3-4}$. **MH**: Broşteni (Grecescu 1898). Eur. Car. *Querco-Fagetea*.

123. *Pícris hieracioídes* L. subsp. *hieracioídes* - Ht.-H. Frecv. $U_{2-3}T_3R_4$. **MH**: Broşteni, (Grecescu 1898). Euras. Car. *Artemisietea vulgaris, Arction lappae, Sisymbrion officinalis*.

124. *P. h.* subsp. *grandiflóra* (Ten.) Arcang. [subsp. *auriculáta* (Sch. Bip.) Hayek] - Spor. $U_2T_4R_4$. **GJ**: Între Motru şi Văgiuleşti. Centr. eur. *Dauco-Melilotion, Arction lappae, Sisymbrion officinalis*.

125. *Scorzonéra laciniáta* L. [*Podospérmum laciniátum* (L.) DC.] - Ht.-H. Spor. $U_{2-3(4)}T_4R_4$. **MH**: Argineşti-Gura Motrului. Centr. eur.-medit. *Dauco-Melilotion, Agropyretalia repentis*.

126. *Sónchus arvénsis* L. subsp. *arvénsis* - G. Frecv. $U_3T_{2-5}R_{2-5}$. Euras. *Atriplici-Chenopodietalia albi, Convolvulo arvensis-Agropyrion repentis*.

127. *S. a.* subsp. *uliginósus* (Bieb.) Nyman [*S. uliginósus* Bieb., *S. a.* var. *uliginósus* (Bieb.) Grecescu] - Frecv. $U_{4-5}T_{2-5}R_{2-5}$. **MH**: Meriş, Broşteni, Comanda, Strehaia, Lunca Banului, Argineşti, Gura Motrului; **GJ**: Steic, Samarineşti (Horăşti). Euras. *Convolvuletalia sepium, Alnion glutinosae*.

128. *S. ásper* (L.) Hill subsp. *ásper* - T.-Ht. Frecv. $U_{2-3}T_{2-5}R_{2-5}$. Cosm. *Atriplici-Chenopodietalia albi*.

129. *S. á.* subsp. *glaucéscens* (Jord.) Ball [*S. glaucéscens* Jord.] - Spor. $U_{3-4}T_{3-4}R_4$. **MH**: Corcova, Butoieşti, Argineşti, Gura Motrului; **GJ**: Motru. Medit. *Isoëto-Nanojuncetea*.

130. *S. oleráceus* L. - T. Frecv. $U_{2-3}T_{2-5}R_{2-5}$. Cosm. *Atriplici-Chenopodietalia albi*.

131. *Taráxacum officinále* Web. ex Wiggers - H. Frecv. $U_{2-3}T_{2-5}R_{2-5}$. Euras. Car. *Molinio-Arrhenatheretea, Arrhenatheretalia, Plantagini-Prunelletalia*.

132. *Tragopógon dúbius* Scop. [*T. májor* Jacq.] - T.-Ht. Frecv. $U_{2-3}T_4R_{2-5}$. Centr. eur.-medit. Car. *Artemisietea vulgaris*.

133. *T. praténsis* L. - Ht.-H. Spor. $U_3T_2R_3$. **MH**: Corcova. Euras. Car. *Molinio-Arrhenatheretea, Arrhenatherion, Sisymbrion officinalis*.

134. *T. orientális* L. [*T. praténsis* subsp. *orientális* (L.) Čelak.] - Ht.-H. Frecv. $U_{2-3}T_3R_4$. Eur. centr. and by E. Car. *Molinio-Arrhenatheretea, Arrhenatheretalia, Sisymbrion officinalis*.

CONCLUSIONS

In the Lower Basin of the Motru River have been identified 140 taxa from *Asteráceae* Family, among which, the *Asteroideae* (Tubuliflórae) subfamily has 100 taxa (89 species, 6 subspecies and 5 varieties) and *Cichorioideae* (Liguliflórae) subfamily has 39 taxa (32 species, 6 subspecies and 1 varieties). In this part (II) we present 86 taxa (74 species, 8 subspecies and 4 varieties).

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CHOROLOGY OF SOLITARY FLOWERS VERONICA SPECIES IN ROMANIA (I)

Amira Răduțoiu[1]

KEY WORDS: chorology, Veronica, Romania

ABSTRACT

The present paper is part of a synthesis representing the doctorate thesis of the present work's author, titled: Morphology, anatomy, chorology and economic importance of solitary flowers Veronica species in Romania. Of the total of Veronica species of Romanian Flora, the following species have been considered for the study: Veronica acinifolia L., V. agrestis L., V. arvensis L., V. dillenii Cr., V. filiformis Sm., V. hederifolia L., V. opaca Fries, V. praecox All., V. peregrina L., V. persica Poiret, V. polita Fries, V. triphyllos L. and V. verna L.

The mjority is formed of annual species, whose biological cicle take place during one single vegetation period, sometimes even shorter, only Veronica filiformis being perenial. Among these species are also found adventive taxons (Veronica persica, V. peregrina, V. filiformis), some of them being rarely found in the Romanian Florai (Veronica peregrina, V. filiformis).

INTRODUCTION

A large part of cultivated terrains are left as fallow fields either for lack of financial support for the initiation of new cultures, or in order for the soil to ‚rest', in the conditions of low fertility. Although these terrains are left to ‚rest', they still produce a large quantity of green mass made up of weeds. The majority of weeds that grow during this phase, such as: *Veronica hederifolia*, *V. persica*, *V. polita*, *Stellaria media*, *Lamium purpureum*, *Capsella bursa pastoris* and so on, is not consumed by farm animals, no matter their development stage, except some of them, like the Canada thistle (*Cirsium arvense*), which are consumed only in their young stages. Others are consumed no matter the stage of development, like the field bindweed, for example. The purpose of the present paper is the better knowledge of chorology ecology and importance (where it exists) of the species analyzed for e better rationalization of their use, thus combining scientific research with practical demands.

MATERIAL AND METHODS

Starting from bibliografic information, I have repeatedly conducted personal studies on site, in order to identify species, observe them in all vegetation stages, marking the biotope's characteristics, taking photographs, collecting and conserving speies for minute laboratory analysis. The photographs were taking with a Panasonic digital camera.

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For the realization of the chorology for the species analyzed all the country's herbariums, for a clearer image on these species distribution in Romania. All the collected data have been introduced in this paper, together with the personal data.

For every consulted species the locality, county, station, altitude, date, the collector and the person responsible for the determination, the herbarium's acronym and inventory number (where case be).

The country's herbarium acronyms are the following: **CRA** – University of Craiova Herbarium; **I** – Herbarium of "A.I.Cuza" University Iaşi; **CL** – Herbarium of "Babeş Bolyai" University in Cluj Napoca; **BUC** –Bucharest University Herbarium; **BUCA** – Herbarium of the Romanian Academy Biology Institute; **CLA** – Herbarium of the Cluj Napoca University of Agricultural Sciences and Veterinary Medicine, and **IAAG** – Herbarium of the Iaşi University of Agricultural Sciences and Veterinary Medicine.

Where the material was part of the Flora Olteniae Exiccata or Flora Romaniae Exiccata, the inventory number of the file was mentioned.

RESULTS AND DISSCUSIONS

Veronica filiformis Sm.

In Romania's Flora volume VII this taxon is not mentioned. Only after synonymies for the *Veronica persica* Poiret species *Veronica filiformis* Baumg. et auct transs., non Sm. Is mentioned, but in this case a different taxon is mentioned.

It's possible that up to that period, the presence of this taxon in Romania's flora was uncertain. A few years later, Al. Beldie signals the presece of this taxon in Romania's Flora, Illustrated Determinator of Vascular Plants, volume II (1979).

Flora Europaea situates this taxon in the North-Western and central parts of Europe, Romania not being mentioned at chorology.

It can be easily mistaken for the *Veronica persica* Poiret. The main character for its recognition is: *V. filiformis* is a perenial species, whereas *V. persica* is annual. The following are added to the above-mentioned:

1a. Robust stems, leaves crenate- dentated on the sides; 7-15 mm wide corolla, 7-10 mm wide capsule formes an obtuse angle at side-level.……...**Veronica persica** Poiret

1b. thin and soft stems. Leaves with barely-crenated sides; corolla with a 5-8 mm diameter, and 4-5 mm wide capsule and forms a sharp angle at side-level.....**Veronica filiformis** Sm.

Chorology

Mentioned localities in Romania: Cluj Napoca – Cluj county.

Herbarium material from Craiova (CRA): Transylvania, Cluj, in the "Al. Borza" Botanic Garden, Cluj Napoca. Altitude of approx. 400 m.s.m. 09.IX.2007. Leg. et det. D. Răduțoiu.

Herbarium material from Iaşi (I): there is no hermarium material from Romania.

Herbarium material from Cluj (CL):

Cluj, in orchards' grass along Bisericii Ortodoxe Street. Altitude of approx. 400 m.s.m. 16.VI.1958. Leg. et det. D. Pázmány. CL 560584.

Ecology and geo-element. Lawns in Cluj Napoca. Frequent sterility is observed with this species, apparently associated with the vigorous development of a single exemplary. Adv. (Cauc. and N. Anat.).

Veronica peregrina L. (*Veronica romana*) L.

Chorology

Mentioned localities in Romania: Rupea –Braşov county; Biertan – Sibiu county; Şura Mică, Şura Mare –Sibiu county; Puțeni – Galați county; Popricani, Stânca – Iaşi county; Rast – Dolj county (Romania's Flora volume VII.).

Herbarium material from Craiova (CRA): Craiova region, Calafat sector, locality of Desa, at the Prundul cel mare on the Danube. Altitude approx. 55 m.s.m. Leg. Al. Buia et L. Pop.

Oltenia, Dolj county, locality of Rast, in easily flooded areas, on ditch sides. Altitude approx. 40-50 m.s.m. Leg. D. Rădutoiu and Amira Rădușoiu.

Herbarium material from Iaşi (I):

Muntenia, Ilfov county, Mânăstirea locality, in the riverside coppice. 11.VI.1963. Leg. et det. N. & Şt. Roman. 87014.

Herbarium material from Cluj (CL):

Banat, Caraş Severin county, Moldova Veche. 1966. Leg. I. Morariu, M. Danciu.

Ecology and geo-element. Along riversides, in marshy and cultivated areas, gardens, and within ditches. Meso-hygrophil species, hygrophil at times. Adv. (South America).

Veronica persica Poir. – Ventrilical. (*Veronica byzantina* (Sibth. & Sm.) Degen, *V. tournefortii* C.C. Gmel. pro parte, non Vill., *V. buxbaumii* Ten., non F.W. Schmidt).

Chorology.

Mentioned localities in Romania: Villages around the country.

Herbarium material from Craiova (CRA):

Oltenia, Dolj county, Radovan village, in the Radovan forest. Altitude approx. 60 m.s.m. 10.VI.1949. Leg. Al. Buia et A. Popescu;

Oltenia, Dolj county, Bucovăț village, in the Bucovăț forest. Altitude approx. 100 m.s.m. 10.IV.1949. Leg. Al. Buia et A. Patega et A. Popescu.; 2 files.

Oltenia, Calafat district, around the village of Ciupercenii Noi. Altitude aprox. 50 m.s.m. 11.IV.1967. Leg. M. Păun et G. Popescu.

Oltenia, Dolj county, Craiova, in the "Poporului" Park. Altitude approx. 60 m.s.m. 29.IV.1949. Leg. Al. Buia et A. Popescu;

Oltenia, Dloj county, Craiova, in the "Poporului" Park. Altitude approx. 60 m.s.m. 15.VI.1949. Leg. Al. Buia et A. Popescu;

Oltenia, Dolj county, Craiova, in the "Lascăr Catargiu" neighbourhood. Altitude approx. 85 m.s.m. 03.V.1951. Leg. Al. Buia et M. Trică.

Transylvania, Braşov county, Braşov, in ruderal areas. 07.V.1966. Leg. M. Danciu.

Oltenia, Dolj county, Craiova, in the Obedeanu neighbourhood. Altitude approx. 85 m.s.m. 22.IV.1965. Leg. et det. D. Cârțu. Flora Olteniae Exiccata no. 1008.

Oltenia, Dolj county, Radovan, Valea Rea. Altitude approx. 90 m.s.m. 08.VI.2001. Leg. Gh. Popescu, I. Costache & D. Rădușoiu

Oltenia, Vâlcea county, Oteteliş, in ruderal areas. Altitude approx. 178 m.s.m. 04.IV.2001. Leg. D. Rădutoiu.

Oltenia, Vâlcea county, locality of Fârtățe ti, in corn- cultivated areas. Altitude approx. 180-200 m.s.m. 04.IV.2001. Leg. D. Rădușoiu.

Oltenia, Vâlcea county, locality of Rusăneşti, in ruderal areas. Altitude approx. 200 m.s.m. 284.IV.2004. Leg. D. Rădușoiu.

Herbarium material from Iaşi (I):

Moldavia, Bacău county, locality of Răcăciuni. 25.VI.1970. Leg. et det. N. Barabaş & D. Mititelu. [I 49180].

Transylvania, Cluj county, locality of Cojocna. Leg. I. Prodan. [I 119235].

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Moldavia, Iaşi county, Iaşi, Copou. III.1932. The collector is not mentioned. [I 13690].

A file where there is no mention made for the locality, station and the collector. 20.VII.1936. Det. C. Burduja. [I 13692].

Moldavia, Bacău county, locality of Buciumi, residue deposit, 06.VII.1999. Leg. et det. M. Gurău. [I 114990].

Moldavia, Iaşi county, locality of Mirceşti. 06.XI.1968. Leg. et det. V. Slonovschi. [I 28715].

Muntenia, Ilfov county, locality of Văcăreşti, Văcăreşti meadow. 10.VIII.1954. Leg. et det. Al. Borza. [I 39710].

There is no mention of the locality and county. Valea Vămăşoaiei. 18.X.1969. Leg. et det. M. Barcan. [I 67407].

Moldavia, Vaslui county, locality of Bolaţi. 08.VII.1972. Leg. C. Dobrescu, det. I. Sârbu. [I 68612].

Moldavia, Suceava county, locality of Horodnic. 20.V.1971. Leg. et det. T. Chifu, N. Ştefan & D. Florea. [I 93690].

Valea Dâmbovicioarei. 01.VII.1966. Leg. M. Danciu. [I 49181].

Moldavia, Suceava county, locality of Vişina. 20.X.1968. Leg. et det. S. Hobincu. [I 17731].

There is no mention of the locality and county. 14.VIII.1936. Det. C. Burduja [I 13693].

Moldavia, Iaşi county, locality of Ţibana, Valea Sacovăţului. 28.VIII.1969. Leg. et det. C. Dobrescu. 78040.

Moldavia, Neamţ county, Nemţişor rivulet. 23.X.1968. Leg. et det. T. Chifu & N. Ştefan. [I 93688, 93689].

There is no mention of the locality and county; between Dârste and the marshalling yard. 16.VI.1963. Det. P. Ularu & M. Danciu. [I 49183].

Muntenia, Ilfov county, locality of Comana. 05.V.1954. Leg. et det. Al. Borza. [I 39711].

Moldavia, Neamţ county. There is no mention of the locality. 16.VI.1916. Leg. C. Petrescu. Det. El. Eftimie. [I 27402, 27403].

There is no mention regarding the station and county; locality of Stânca. 24.IV.1916. Leg. C. Petrescu. Det. El. Eftimie. [I 27432].

Moldavia, Bacău county, locality of Asău, common pasture. 12.VII.2001. Leg. et det. L. Gorea. [I 100698].

Moldavia, Galaţi county, locality of Nicoreşti. 25.IV.1955. Leg. et det. Ad. Oprea. [I 98202, 98203].

Moldavia, Botoşani county, locality of Şendriceni. 11.VI.1967. The collector is not mentioned. [I 17732].

Moldavia, Botoşani county, locality of Suharău. 20.VII.1966. Leg. et det. Gh. Mihai. [I 29361].

Moldavia, Neamţ county, locality of Boteşti. 25.VII.1969. Leg. et det. M. Bursuc. [I 19996].

Oltenia, Olt county, locality of Morunglav. 11.IV.1970. Leg. et det. Şt. Ciorică. [I 28008].

Valea Dâmbovicioarei. 1.VII.1966. Leg. M. Danciu. [I 49182].

Moldavia, Iaşi county, Iaşi, Via IAS Bucium, dl. "Doi Peri". 09.VIII.1968. Leg. et det. I. Sârbu. [I 41051].

Herbarium material from Cluj (CL):

Transylvania, Harghita county, locality of Tuşnad Băi, in Mr. Blaga's garden. 07.VII.1937. Leg. M. Şerban. [CL 643758].

Satu Mare district, in cultivated areas. Turț. V.1976. Leg. I. Pop. [CL 612470].

Transylvania, Cluj county, locality of Cluj Napoca, Cetățuia Park. 30.IV.2004. Leg. et det. Filipaş Liviu. [CL 657011].

Transylvania, Sibiu district, Sadu, ploughed field towards Şuvară. 14.IV.1980. Leg. C. Drăgulescu. [CL 629108].

Moldavia, Iaşi district, in the Botanic Garden. Altitude approx. 90. m.s.m. 04.V.1939. Leg. et det. M. Răvăruț. [CL 615907].

Banat, Timiş county, Lugoj, Airport towards Armadia. 20.V.1969. Leg. E. Vicol. [CL 652197].

Cluj region, between Apahida and Moci. Altitude approx. 350 m.s.m. 24.X.1960. Leg. et det. E. Vicol. [CL 646308].

Moldavia, Bacău county, Răcăciuni village, in cultivated areas. Altitude approx. 120 m.s.m. 25.VI.1970. Leg. et det. N. Barabaş et D. Mititelu. [CL 596131]. Flora Exiccata Districti Bacoviensis no. 99.

Transylvania, Braşov county, locality of Braşov, in ruderal areas. 07.V.1966. Leg. M. Danciu. [CL 567903].

Moldavia, Iaşi district, in the Botanic Garden. Altitude approx. 90. m.s.m. 04.V.1939. Leg. et det. M. Răvăruț. [CL 197495]. Flora Romaniae Exiccata no. 2065.

Transylvania, Odorhei district, locality Mereşti. Altitude approx. 700 m.s.m. 08.VII.1929. Leg. E.I. Nyárády. [CL 192974].

Banat, Timişoara district, locality of Liebling, in ruderal areas. Altitude approx. 94-97 m.s.m. 07.VIII.1949. Leg. V. Soran. [CL 17270].

Transylvania, Cluj region, Câmpeni district, alongside the "Drumul Moților" path, village of Lupşa-Mânăstire. Altitude approx. 540 m.s.m. 29.V.1953. Leg. V. Soran. [CL 219202].

Romania, Maramureş county, locality of Vişeul de Jos. Altitude approx. 454 m.s.m. 27.XI.1988. Leg. A. Coman. [CL 448864].

Banat, Timiş-Torontal district, on the roadside in the « Casa Verde" forest. Altitude approx. 90 m.s.m. 12.IV.1941. Leg. I. Todor. [CL 505181].

Transylvania, Cluj district, ruderal areas, on the side of the industrial railway, on the Drăganului Valley. Altitude approx. 700 m.s.m. 16.VI.1950. Leg. V. Soran. [CL 214502].

Transylvania, Bihor county, locality of Salonta, roadside between the villages of Salonta-Mădăraş and Marțihaş. 20.IV.1951. Leg. I. Pop. [CL 216381].

Transylvania, Cluj district, Izvoru Crişului. 18.IV.1943. Leg. R. Soó, det. I. Gergely. (01.IX.1969). [CL 594717].

Bucovina, Suceava district, locality of Bosanci, in different gardens. VIII.1921. Leg. G. Bujorean. [CL 152620].

Transylvania, Covasna county, locality of Hajongard. 19.V.1900. Leg. A. Richter. [CL 20280].

Ecology and geo-element. Cultivated and ruderal areas starting from lane areas up to hillside and sub-Carpathian depressions level; ploughfields, gardens, vineyards; on mezobasic- eubasic soils, slightly acid-neutral, usually low in humus. It is a mezophil species. Adv. (South- West Asia).

Importance. In some parts of the country it is used by the people in order to feed farm animals. Due to the large number of individuals on fallow fields in springtime, it is grazed by the sheep.

CONCLUSIONS

In order to realize the chorology of the analyzed species, all the country's herbariums have been consulted to have a clearer image of the distribution of these species in Romania. All collected data have been inserted in the present paper. From the totality of the species considered for the study, only three were presented in the present paper (*Veronica filiformis*, *V. peregrina* and *V. persica*), the rest of them going to be published in future papers.

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20. CL - Herbarul Universităţii "Babeş Bolyai" Cluj Napoca.
21. BUC – Herbarul Universităţii din Bucureşti.

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CHOROLOGY OF SOLITARY FLOWERS VERONICA SPECIES IN ROMANIA (II)

Amira Răduțoiu[1]

KEY WORDS: chorology, Veronica, Romania

ABSTRACT

The present paper is part of a synthesis representing the doctorate thesis of the present work's author, titled: Morphology, anatomy, chorology and economic importance of solitary flowers Veronica species in Romania. Of the total of Veronica species of Romanian Flora, the following species is considered for the study: Veronica hederifolia L. It is an annual plant, whose biological cicle takes place during a single vegetation period, and sometimes even shorter than that.

INTRODUCTION

The *Veronica* genus is appreciated as being one of the richest of Romania's spontaneous genus (and not only). In the Flora Europaea 62 species with numerous infraspecifical taxons are presented, and in Romania's Flora – volume VII – 41 species and 3 hybrids. Recent works on Romania's Flora present only 40 species.

The important character of *Veronica* species from among the *Scrophulariaceae* is the androcecium made up of 2 epipetal stamens.

Data referring to the *Veronica* species of Romanian flora are sporadic, until the publishing of the „Conspectul Florei României"work (D. Grecescu 1898 – page 435-442).

According to D. Grecescu's research, floristic data have a fragmentary character and are entirely insignifiant. In Romania's Flora (volume VII, 1960 – page 505-565), coordinated by the great academician Tr. Săvulescu, E. Ghişa cites 41 spontaneous *Veronica* species.

Al. Beldie (1977, 1979) in his work "Flora României - determinator ilustrat al plantelor vasculare" (Romania's Flora- illustrated determinator of vascular plants) (pages 102-111) cites 36 species, 19 subspecies and 2 hybrids.

V. Ciocârlan (2000) in the "Romanian Illustrated Flora *Pteridophyta* & *Spermatophyta*" signals the presence of 40 species and 15 subspecies (pages 696-705).

MATERIAL AND METHODS

The first phase in researching the solitary flowers *Veronica* species in Romania was represented by the research of the bibliographic material.

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Starting from these bibliographic information, I have repeatedly conducted personal on-site studies, in order to identify species, analyze them in all vegetation stages, marking the bitopoe's characteristics, taking pictures, collecting and conserving the species for minute laboratory analysis, and for the realization of a botanic herbarium which would bring new information regarding the chorology of these species in Romania.

RESULTS AND DISSCUSIONS

Due to the numerous mentions and vast bibliographic material, in this paper only the chorology of the *Veronica hederifolia* L.– Doritoare will be presented.

Chorology.

Mentioned localities in Romania: Villages throughout the country, from the field up to the mountain areas.

Herbarium material from Craiova (CRA):

Oltenia, Băileşti district, locality of Radovan, next to the forest. Altitude approx. 190 m.s.m. 16.IV.1963. Leg. Al. Buia, D. Cârțu, C. Maloş, V. Panait. Flora Olteniae Exiccata no. 383;

Transylvania, Cluj district, Hajongart, in cultivated places. Altitude approx. 410 m.s.m. 23.IV.1964. Leg. E.I. Nyárády. Flora Romaniae Exiccata no. 1760a; 3 files.

Moldavia, Iaşi district, in the „Aroneanu" Forest. Altitude approx. 175 m.s.m. 25.III.1937. Leg. C. Burduja et M. Răvăruț. Flora Romaniae Exiccata no. 1760b; 3 files.

Oltenia, Dolj district, in the Leamna forest. Altitude approx. 80 m.s.m. 04.IV.1950. Leg. A. Popescu; 3 files.

Oltenia, Dolj district, in the Vlădila forest. Altitude approx. 60 m.s.m. 01.IV.1954. Leg. Al. Buia;

Craiova region, Gura Jiului district, Piscul Sadovei. Altitude approx. 80 m.s.m. 04.V.1954. Leg. Al. Buia, N. Gagiu et M. Trică;

Oltenia, Dolj district, in the Perişor forest. Altitude approx. 60 m.s.m. 16.III.1950. Leg. Al. Buia et A. Popescu;

Oltenia, Vânju Mare district, Burila Mare village. Altitude approx. 65 m.s.m. 21.IV.1966. Leg. M. Păun, C. Pavel et D. Cârțu.

Craiova region, Gura Jiului district, around the village of Tâmbureşti. Altitude approx. 95 m.s.m. 04.V.1954. Leg. Al. Buia, I. Safta, N. Gagiu et M. Trică;

Oltenia, Craiova region, in the Leamna forest. Altitude approx. 80 m.s.m. 16.IV.1952. Leg. Al. Buia et M. Păun;

Craiova region, Filiaşi district, Coțofenii din Față village. Altitude approx. 100 m.s.m. 20.IV.1954. Leg. M. Păun;

Craiova region, Craiova district, between the Bucovăț and Leamna forests. Altitude approx. 100 m.s.m. 11.V.1955. Leg. Al. Buia, N. Gagiu et V. Năzdrăvan;

Craiova region, Caracal district, around the village of Drăghiceni. Altitude approx. 80 m.s.m. 07.V.1955. Leg. M. Păun;

Craiova region, Calafat district, the Danube's meadow at Başcov. Alt. cca. 55 m.s.m. 23.IV.1955. Leg. M. Păun;

Oltenia, Craiova region, in the Jiu meadow between the villages of Malu Mare and Secui, in the point called Zăvoi. Altitude approx. 75 m.s.m. 19.IV.1952. Leg. M. Păun;

Oltenia, Dolj district, in the Bucovăț forest. Altitude approx. 90 m.s.m. 15.IV.1950. Leg. Al. Buia et A. Popescu;

Oltenia, Dolj-Calafat district, on the side of the Danube. Altitude approx. 40 m.s.m. 08.V.1951. Leg. Al. Buia;

Oltenia, Craiova region, the forest on the Church's hill, close to the Livezi village. Altitude approx. 95 m.s.m. 13.IV.1952. There is no mention of the collector;

Oltenia, Dolj district, in the Glavacioc forest. Altitude approx. 60 m.s.m. 14.IV.1950. Leg. Al. Buia et M. Trică.

Oltenia, Dolj district, locality of Coțofenii din Față. Altitude approx. 85 m.s.m. 09.V.1950. Leg. Al. Buia et A. Popescu.

Herbarium material from Iaşi (I):

Moldavia, Iaşi county, Iaşi, via IAS Bucium, La greci point. 17.IV.1968. Leg. et det. I. Sârbu. [I 41059].

Moldavia, Vaslui county, locality of Brăhăşoaia, Hârboanca forest. 25.IV.1966. Leg. et det. C. Dobrescu. [I 78085].

Moldavia, Iaşi county, locality of Grajduri. 18.IV.1973. Leg. C. Dobrescu., det. I. Sârbu. [I 68230].

Moldavia, jud. Iaşi, Iaşi, via IAS Bucium, La greci point. 17.IV.1968. Leg. et det. I. Sârbu. [I 41060].

Moldavia, Bacău county, locality of Răcăuți. 25.IV.2000. Leg. et det. M. Gurău. [I 114989].

Moldavia, Iaşi county, locality of Bârnova. 22.IV.1972. Leg. et det. C. Dobrescu. [I 68901].

Moldavia, Iaşi county, locality of Iaşi, "Doi Peri" hill, via IAS Bucium, Talaşmani forest, in the oak wood, towards the end of the forest. 25.IV.1968. Leg. et det. I. Sârbu. [I 41061].

Moldavia, Galați county, locality of Bereşti. 04.V.1974. Leg. et det. I. Sârbu. [I 59983].

Moldavia, Buzău county, Dumbrava forest. 24.X.1956. The collector is not mentioned. [I 84726].

There is no mention of the county and locality. Codrului forest. 03.V.1958. Leg. et det. Gh. Mihai. [I 76406].

Moldavia, Galați county, locality of Zărneşti, Ilieş forest, towards the end of the forest. 22.V.1974. Leg. et det I. Sârbu. [I 59984].

Moldavia, Iaşi county, locality of Bârnova, in the forest. 12.IV.1971. Leg. et det. C. Dobrescu. [I 67905].

There is no mention of the county and locality. 24.III.1914. Leg. C. Petrescu, det. El. Eftimie. [I 27391].

Moldavia, Iaşi county, Iaşi, Copou. 01.IV.1916. Leg. C. Petrescu, det. El. Eftimie. [I 27392].

Dobrogea, Tulcea county, the place is not mentioned. 23.IV.1912. Leg. C. Petrescu, det. El. Eftimie. [I 27393].

Muntenia, Ilfov county, locality of Comana, Câlnişte forest. 06.V.1954. Leg. et det. Al. Borza. [I 39705].

Muntenia, Ilfov county, locality of 30 Decembrie, in the riverside coppice. 27.IV.1954. Leg. et det. Al. Borza. [I 39703].

Moldavia, Iaşi county, Iaşi, Bucium. 16.IV.1968. Leg. et det. T. Chifu. [I 93671].

Moldavia, Iaşi county, Voineşti, Voineşti forest. 08.IV.1975. Leg. et det. T. Chifu. [I 93672].

Moldavia, Vaslui county, Brăhăşoaia, Hârboanca forest. 02.V.1967. Leg. et det. C. Dobrescu. [I 78056].

There is no mention of the county, locality, or collector. 15.IV.1947. [I 61806].

Moldavia, Botoşani county, locality of Conceşti, Conceşti forest. 21.IV.1966. Leg. et det. Gh. Mihai. [I 29350].

Moldavia, Iaşi county, locality of Breazu, Breazu forest. 24.IV.1968. Leg et det. T. Chifu. [I 93670].

Muntenia, Ilfov county, Comana. 17.IV.1954. Leg. et det. Al. Borza. [I 39704].

Moldavia, Suceava county, locality of Vişina. 06.IV.1968. Leg. et det. S. Hobincu. [I 17714].

Oltenia, Dolj county, Radovan. 16.IV.1963. Leg. Al. Buia, D. Cârţu, C. Maloş & V. Panait. [I 49163]. Flora Olteniae Exsiccata, no. 383.

Moldavia, Iaşi county, locality of Aroneanu, Aroneanu forest. 25.III.1937. Leg. C. Burduja & M. Răvăruţ. [I 49164]. Flora Romaniae Exsiccata, no. 1760b.

Moldavia, Iaşi county, localitatea Iaşi, Bucium. 25.IV.1968. Leg. et det. I. Sârbu. [I 41058].

Moldavia, Galaţi county, Suceveni, Pogăneşti forest, opening. 17.IV.1969. Leg. et det. I. Sârbu. [I 41247].

Transylvania, Cluj county, Cluj-Napoca, "Hajongard". 26.IV.1924. Leg. E. I. Nyárády. [I 49165]. Flora Romaniae Exsiccata, no. 1760a.

Moldavia, Galaţi county, Ţepu de Jos locality. 02.IV.1995. Leg. et det. Ad. Oprea. [I 98197].

Moldavia, Iaşi county, Răchiteni, on the ditch's side. 05.V.1969. Leg. et det. V. Slonovschi. [I 28711].

Dobrogea, Constanţa county, Canaraua Fetii. 21.V.1963. Leg. et det. Gh. Mihai. [I 27969].

Moldavia, Iaşi county, locality of Uricani. 05.IV.1959. The collector is not mentioned. [I 21284].

Moldavia, Iaşi county, Miroslava locality. 2.V.1969. The collector is not mentioned. [I 21283].

Moldavia, Iaşi county, locality of Dumbrava, Dumbrava forest. Leg. et det. T.S.D. [I 17715].

Moldavia, Iaşi county, Iaşi, Copou Garden. 25.III.1894. Leg. I. C. Constantineanu. [I 13640, 13641, 13646, 13647].

Moldavia, Iaşi county, Aroneanu, Dorobanţ lake. 25.VI.1965. Leg. et det. M. Teodorescu. [I 63500].

Moldavia, Iaşi county, Iaşi, Cetăţuiei hill. 03.IV.1897. The collector is not mentioned. [I 13643].

Moldavia, Botoşani county, Şendriceni locality, Pădureni forest. 10.05.1965. [I 17716].

Moldavia, Iaşi county, there is no mention of the locality. 03.IV.1897. The collector is not mentioned. [I 13645].

Moldavia, Iaşi county, Niţelea station, 03.IV.1897. The collector is not mentioned. [I 13642].

Moldavia, Iaşi county, locality of Aroneanu. 07.V.1924. 13638.

Moldavia, Iaşi county, Iaşi. 09.IV.1938. The collector is not mentioned. [I 13639].

Moldavia, Vaslui county, Hoceni locality. 20.V.1967. The collector is not mentioned. [I 15808].

Dobrogea, Constanţa county, locality of Murfatlar. IV.1897. The collector is not mentioned. [I 13644].

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Transylvania, Mureş county, Sighişoara. 1817. Leg. J.C.Baumgarten;

Transylvania, Cluj county, Cluj - Dl.Becaş. 1943. Leg. R. Soo;
Transylvania, Cluj county, Bontida locality. 1944. Leg. R. Soo;
Transylvania, Cluj county, Cluj –Făget forest. 1988. Leg. Gh. Groza;
Banat, Timiş county, locality of Făget. 1973. Leg. F. Tauber;
Transylvania, Bihor county, locality of Vadu Crişului. 1965. Hort.Bot.Cluj;
Dobrogea, Constanța county, Eforie Nord. 1963. Leg. I. Morariu, P. Ularu;
Oltenia, Dolj county, locality of Rodovan – forest. 1963. Leg. Al. Buia.
Moldavia, Iaşi county, locality of Țibăneşti –Domnița forest. 1971. Leg. C. Dobrescu;
Transylvania, Bihor county, locality of Suncuius - Vl.Misid. 1985. Leg. Gh. Groza.
Transylvania, Sibiu county, locality of Tălmaci. 1980. Leg. C. Drăgulescu;
Transylvania, Cluj county, Cluj –Hajongard. 1924. Leg. E.I. Nyárády;
Transylvania, Sibiu county, Sibiu – Guşterita. 1966. Leg. E. Schneider;
Transylvania, Alba county, locality of Colţeşti. 1956. Leg. I. Gergely;
Moldavia, Iaşi county, Aroneanu forest. 1937. Leg. C. Burduja, M. Răvăruț;
Transylvania, Cluj county, Cluj- Mănăştur. 1924. Leg. E.I. Nyárády;
Banat, Timiş county, locality of Foieni. 1943. Leg. Al. Borza, Al. Buia;
Moldavia, Galați county, locality of Galați. 1928. Leg. Al. Borza;
Dobrogea, Tulcea county, Cocos Monastery – Isaccea. 1924. Leg. Al. Borza;
Transylvania, Cluj county, Turda - Băile Sărate. 1941. Leg. I. Todor;
Transylvania, Hunedoara county, Poiana Ruscă Mountains – Vulcez. 1943. Leg. P. Pteancu.
Muntenia, Ilfov county, Bucureşti – Băneasa. 1950. Leg. I. Todor;
Transylvania, Cluj county, Cluj Napoca. 1901. Leg. Al. Richter;
Transylvania, Bistrița Năsăud county, The year is not mentioned. Leg. F. Porcius;
Transylvania, Bistrița Năsăud county, locality of Chiochiş. 1897. Leg. I. Prodan.
Transylvania, Braşov county, Brasov – Kisfuggoko. 1905. Leg. G. Moesz;
Transylvania, Cluj county, Turda. The year is not mentioned. G. Wolff .

Veronica hederifolia subsp. ***triloba***. Dobrogea, Constanța county, Băneasa locality. 22.V.1963. Leg. et det. Gh. Mihai. [I 27968].

Ecology and geo-element. It is present in ruderalized places, with grass, bushes and forest clearings, riverside coppices, ploughfields, locust tree plantations, gardens, vineyards, on eubasic-mezobasic soils, slightly acid- neutral, mellow. Euras.

CONCLUSIONS

Veronica hederifolia L. is a frequent species in Romanian flora, in ruderalized places, with grass, bushes and forest clearings, riverside coppices, ploughfields, locust tree plantations, gardens, vineyards, on eubasic-mezobasic soils, slightly acid- neutral, mellow. The chorological herbarium data is numerous, and so are the literature mentions. Practically, there is no territory in Romania where this species cannot be found.

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21. BUC – Herbarul Universităţii din Bucureşti.

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NEW CHOROLOGIC DATA IN THE REGION OF OLTENIA

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KEY WORDS: chorology, rare species, Oltenia

ABSTRACT

The paper presents new chorological data of 9 taxons identified in the Otenia region, a part of them being put down on the Red National List.

The new dates want to complete the sozologic table of the taxons. Also, the emphasis of the dynamics of the populations of those in time and space gives us precios signs in the sozologic evaluation. Interesting is the expansiveness of the populations of the following taxons: Achillea roseo-alba, Cirsium creticum, Sedum cepaea, inclusive the presence, in premiere for Oltenia, of the endemic taxon Dactylorhiza cordigera subsp. siculorum (in the Ranca Mountain, Parang Masiv) and of the species Veronica peregrina (in the Rast).

INTRODUCTION

Oltenia is situated in the south-western part of Romania, bordering the Olt River to the East, the Cerna River to the West, the Danube to the South and the Meridional Carpathians to the North. Although the region is not so large, as compared to others at a national level, it shelters an extremely rich and various floras. Among the taxa which are present in this part of the country, there are some rare ones, included in the national lists.

The necessity to know the complete chorology of the rare taxa constitutes a compulsory premise for setting their spreading area.

This information is also necessary in order to have a clear and objective view on their sozologic framing. Otherwise, we may become subjective in assigning certain sozologic framing of the above mentioned taxa. Therefore, this new chorology information comes to sustain the previous desideratum.

METHOD OF RESEARCH

The method of research used was the observation. During the research in the field the identified species were collected and there were mentioned the following: the collecting date, the locality as well as the stational conditions. Subsequently, the material was examined in details, both rough and preserved, using the binocular magnifying glass. The determinations were established according with the specialty literature found in the bibliography. The detailed images were taken by a digital camera provided with a magnifying device.

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RESULTS AND DISCUSSIONS

1. *Achillea roseo-alba* Ehrend.– Fam. *Asteraceae* (*Compositae*).

Sozologic characterization. In Oltenia is rare, beeing mentioned just in the Inferior Basin of Motru **[6, 7]**. At national level is rarelly mentioned in ruderalization meadows around Constanța city **[3, 4, 9]**.

Ecology and geoelement. Xeromes.-mesophile species, termophile. Central Europe and South.

Coenology. Xeromesophile meadows (*Festucion valesiacae*).

New locations: Seaca de Câmp - FP 76, Rast - FP 86 (Dolj).

2. *Cirsium creticum* (Lam.) D' Urv. – Fam. *Asteraceae* (*Compositae*)

Sozologic characterization. In Oltenia is relatively frequent in the south-western part, with preponderance in the Inferior Basin of Motru **[6, 7]**. At national level is mentioned: **R [1, 3, 4, 8, 12]**; **VU [5]; V/R [10].**

Ecology and geoelement. The species is located in wet places (mesohygr.-hygrophile) with a low salinity. Mediterranean.

Coenology. Mesophile meadows (*Agrostion stoloniferae*) forming subassociationes (*Caricetum hirtae* Soó 1927 *cirsietosum creticae* Costache 2005).

New locations: Novaci – GR 00/10, Tg. Cărbunești – FQ 98, Rovinari – FQ 77, between Turburea – FQ 95/GQ05 and Țânțăreni – FQ 94 in Gilort Valley (Gorj), Tâmburești – GP 37, Râpa Roșie - Terpezița –FQ 90/GQ00 (Dolj).

3. *Dactylorhiza cordigera* (Fries) Soó subsp. ***siculorum*** – Fam *Orchidaceae*

Sozologic characterization. Until now it doesn't exist dates about the presence of this taxon in Oltenia. At national level it is mentioned just in some mountain tops of the Estern and Northen Carpathians. Also it is mentioned in the Red National List **[10]**. It is thought that it is endemic.

Ecology and geoelement. Wet meadows, marshes and peatery from boreal level. Endemic Carpathian.

Coenology. In habitates characteristic to *Scheuchzerio-Caricetea fuscae* class.

New locations: The top Rânca from Parâng Massif.

4. *Hordeum bulbosum* L. – Fam. *Poaceae* (*Gramineae*)

Sozologic characterization: In Oltenia this taxon is rare, beeing mentioned from the localities: Radovan **[11]**, Bucovăț – Bucovăț Forest, Coțofenii din Față, Mofleni, Jiului Valley, Popoveni, Verbicioara, Desa, Pisculeț (Dolj), Orșova on Alion Hill (Mehedinți) **[16]**. At national level is rare in Delta Danube **[1, 3, 4, 8, 10]**.

Ecology and geoelement. In xerophile meadows and the edges of cultivable fields. Mediterranean.

Coenology. *Sisymbrion*.

New locations: Râpa Roșie - Terpezița – FQ 90/GQ00 (Dolj).

5. *Ranunculus constantinopolitanus* (DC) D´ Urv. – Fam. *Ranunculaceae*.

Sozologic characterization: In the center and southern part of Oltenia is frequent. At national level it is mentioned: **R [1, 3, 4, 10, 12, 15]**; **V [8**].

Ecology and geoelement. Mesohygrophil, subterm.-termophil species, on brown soils, mesobasic or gleic, low on acid (pH = 6-6.6), from river meadows and low terraces, temporary floodable. Balkan.

Coenology. In meadow woods (*Carpinion betuli*).

New locations: Râpa Roșie - Terpezița – FQ 90/GQ00 (Dolj).

6. *Sedum cepaea* L. – Fam. *Crassulaceae*.

Sozologic characterization: In Oltenia this taxon is rare, local it may be abundant **[6, 7]**. At national level it is mentioned: **R [1, 3, 4, 10, 12, 16]**.

Ecology and geoelement. Xeromesophile species. Mediterranean.

Choenology. In Turkey oak and Hungarian oak forest (*Quercetum frainetto-cerris*).

New locations: Sarului Forest - KK 61/71 (Olt).

7. *Veronica catenata* Pennell – Fam. *Scrophulariaceae*

Sozologic characterization: In Oltenia this taxon is mentioned from the localities: Craiova – Botanical Gardens „Al. Buia" **[13]** Giuleşti, Pojogi-Cerna, Cireşu, Stroeşti, Mogeşti and Obroceşti **[14]**. The species is sporadically mentioned at the national level.

Ecology and geoelement It is found on borders, in places where the water maintains a long time. Circumpolar.

Coenology. *Glycerio-Sparganion*.

New locations: Rast - FP 86 (Dolj), Balş - KK 61/71 (Olt).

8. *Veronica peregrina* L. – Fam. *Scrophulariaceae*

Sozologic characterization: Until now it doesn't exist dates about the presence of this taxon in Oltenia. At national level it is rare **[1, 3, 4]**, is mentioned from the localities: Rupea, Biertan (Mediaş), Şura Mică, Şura Mare (Sibiu), Puțeni (Tecuci), Popricani, Stânca (Iaşi) **[16]**.

Ecology and geoelement. Mesohygrophil species, from river meadows and low terraces, temporary floodable and the cultivable fields. Adventiv (America) .

Coenology. *Nanocyperion*.

New locations: Rast - FP 86 (Dolj).

9.*Vaccaria hispanica* (Mill.) Rauschert – Fam. *Caryophyllaceae*

Sozologic characterization: In Oltenia this taxon is mentioned from the localities: Craiova, Caracal **[16]** and Valea Mare **[14]**. The species is sporadically mentioned at the national level, in the north part of the country. Also it is mentioned in the Red National List: **V [2]**.

Ecology and geoelement Mesoxerophil species, is found on sown field, marginal road, cultivable fields and vines. Eurasian.

Coenology. *Stellarietea mediae*.

New locations: Rast - FP 86, Ciupercenii Noi – FP 56 şi Desa – FP 65/66 (Dolj).

CONCLUSIONS

The paper presents new chorological data of 9 taxons identified in the Oltenia region. The taxons were identified under the form of isolated specimens in few localities within the studied area, a part of them being put down on the Red National List.

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SPONTANEOUS PLANTS WITH POTENTIAL IN THE OBTAINMENT OF PHYTOGENICAL ADDITIVES

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KEY WORDS: phytogenical additives, microelements, spontaneous plants

ABSTRACT

Because it has been found that through the utilization of the chemical premixes in the porcines' food a considerable part of the microelements is removed together with the animal dejection, it is tried the replacement of these premixes with phytogenical additives obtained from spontaneous plants. In this way it is reduced the pollutant impact, especially with heavy metals, against the soil quality and the surface water.

The researches belong to the research contract CEEX – Programe 4 – Partnerships in prioritary domains: " The reduction of the mineral microelements level from the pig dejections through the replacement of the chemical premixes with phytogenical additives and enzymatic preparations, way of promotion of the sustainable agriculture".

INTRODUCTION

The necessity of obtaing phytogenical agents and enzymatic preparations to replace the chemical premixes utilizated in the animal nutrition derived from the relevancy of the fact, experimentally proved, that a part of the microelements and heavy metals contained in these chemical premixes are eliminated with the dejections. So the soils fertilized with organic fertilizers, constituted from this kind of dejections, reached in the course of 25-30 years to accumulate considerable concentrations of heavy metals, concentrations that turn out to be pollutant for the respective soils.

To avoid the elimination of these microelements and heavy metals in the dejections it is necessary the replacement of the chemical premixes with fodder additives of phytogenical nature and enzymatic preparations which will reduce the inputs of minerals on long term, in this way counteracting the pollutant impact of the raisment of domesticated animals sector against the quality of the soil and the surface water.

In the first phase takes place the identification and characterization of the spontaneous plants with high potential in the creation of the mechanisms of implementation of solutions for the nutrition and obtainment of new products which will result of this research project.

These desideratums constitute a part of the principles which are the basis of the realization of a sustainable agriculture.

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MATERIAL AND METHOD

The work method has ascertained in displacements on the field in the period optimum for the collecting of the vegetal material took into study. After the collecting, the plants have been dried at shade, naturally, with the view of avoiding the fast dehydration. The obtained material is improved by the contract partners in order to obtain solutions regarding the nutrition which should replace the chemical premixes.

RESULTS AND DISCUSSIONS

Although in the spontaneous flora exist numerous species with potential for the phytogenical additives obtainment, in the study it have been taken just the ones with high capacity. Among these we present:

1. Wild rose (*Rosa canina* L. s.l. - Fam. Rosaceae) - fig. 1.

Fig. 1. *Rosa canina*

Popular denominations: englantine**,** wild rose, briar, dog-rose, sweet brier, hip rose, hip tree, bramble tassels, crow's bramble, magpie's bramble, apple rose, cow's bramble, wild bramble, rose, field rose, pile shrub, brier rose, dogberry, eglantine gall, hep tree, hip fruit, hop fruit, hogseed, wild brier, witches' brier, brier hip.

Presentation: The brier is a ligneous arbustus with shrub form, whose branches have spines with curved extremity. The leaves are odd pennate, with oval or elliptic folioles, having the stipes well visible at the petiole's base. The flowers are beautiful, red, pink or sometimes also white. They are fragrant. The receptacle is ovoid, globulous, ellipsoidal, long of 1.5-2 cm. They are popullary named rose hips.

The brier is known from ancient times as a medicinal herb.

Spreading: It grows in the plain and hill regions, forming shrubs covered by pink flowers. In autumn mature the red "fruits". It must be collected the receptacle with fruits in autumn before the hoarfrost's fall.

Geoelement: European.

Used part: *Fructus cynosabi* - false fruit.

Collecting : in august-september, before the hoarfrost's fall, when the rose hips get a dark red color.

Drying: it is made artificially, at 80-85^{0}C, entire or cutted, and after that the achenes are separated by the pulpy part of the receptacle. From approximately 2 kg a fresh fruit is obtained 1 kg of dry product.

Chemical composition: His fruits - *Fructus Cynosbati* – contain 250-500 mg% ascorbic acid, 2,55-6,18 mg% diverse carotenoids (provitamin A), vitamins B_1, B_2, PP and K, vitamin P as rutoside, hiperoside, 20% glucides, pectines, tanins, malic and citric acid. The terpenoids are represented by glycosides of the the oleanolic acid, b-sitosterol (the glycosides A, B and C), linalool, citronelol, geraniol, nerol, nicotinic acid (antipellagra vitamin).

Pharmacodynamic action – therapeutic use: Because of the high content of ascorbic and dehidroascorbic acid which form a « redox » system, the pharmaceutical and alimentary products play an important part both in the biological oxydoreduction and in the

cellular respiration. Because of the other vitamins and especially the vitamin P they have the property to diminish the capillaries' permeability and fragility. They supply a contribution of salts necessary for the organism and they have diuretic action. They are recommended for avitaminosis C, hepatic and renal affections as diuretic. It falls under the composition of the aromatic tea, hepatic nr. 2, appetizer tonic tea and the achenes in the diuretic nr. 2 tea. From the pseudofruits' pulp are preparated sirups (brier sirup), « Sambucovit ». For not losing vitamin C during the preparation, there are recommended unoxidable recipients and nitrogen atmosphere.

The rose hips have the property to increase the biliary secretion, being proper for the liver afections. Some author's recomend the brier fruits for the intestinal inflammations and others for the kidney and vesicle stones.

Because it doesn't produce any irritation, it is recommended for the urinary ducts and kidney diseases for a long treatment.

For the rose hips preparations it will be used only glazed vessels, because the metals decompose the vitamin C.

The brier fruits, from which have been removed the achenes, puverized and mixed with bee honey are recommended for the elimination of the intestinal worms.

The oil obtained from this plant's seeds has in his composition active principles, with multiple therapeutic properties. Used in dermatology, nutrition, the rose hips oil contains essential fatty acids.

2. Oregano or **pot marjoram** (*Origanum vulgare* L. - Fam. Lamiaceae) - fig. 2.

Presentation: It has a horizontal rhizome in the soil, with subterranean stolons and numerous filiform adventive roots. The stalk is erect, lignified at the base, ramified, ± pubescent and brown-russet colored. The leaves are ovoid, entire or weakly serrated, obtuse at the apex and suddenly attenuated in petiole. The flowers are purple, rarely white, and grouped in corymbiform inflorescences formed of contracted cymes. The fruit is a tetraachene.

The used part of the plant: *Origani herba* - formed of the upright, ramified stalks, leaves and the inflorescence. The smell is aromatic, characteristic, the taste bitter-aromatic.

Collecting: It is collected the bloomed plant, from the point where ramifies. Preferably in the morning, because the plant contains the biggest cantity of ethereal oil.

Drying: it is made in thin layers, the plant being laid on frames, or in bunches hanged on strings. Artificially, the temperature musn't pass 35^0C.

From 2-3 kg fresh plant is obtained 1kg dry product.

Fig. 2. *Origanum vulgare*

Chemical composition: The aerian parts collected during the flowering contain 0,15-1% volatile oil with high thymol and carvacrol content; cimol and smaller amounts of a-tuiona (origanen), dipenten, selinen, a-terpinen and other terpenes; tanoids formed from cafeic acid and their depsides(about 8%), ursolic acid, bitter substances with yet unestablished structures, etc.

Pharmacodynamic action – therapeutic use: Because of the volatile oil's components it has an antispastic action on the flat musculature and sedative on the central nervous system and especially on the respiration centers; it produces an easy bronchio-

dilatation. Because of the bitter substances and the tanoids it has a tonic action – bitter and weakly astringent, carininative. It is used in tracheitis and bronchitis and some stomachal disease, especially anacide gastritis.

3. The stinging nettle (*Urtica dioica* L. - Fam. Urticaceae) - fig. 3.

Description: The stinging nettle is a herbaceous plant, that grows maximum 150 cm height, having in the ground a thin, cylindrical, whitish, long and ramified rhizome. The stalks are upright, with 4 edges covered with opposite leaves, serrated on the edges. Both the stalk and the leaves are provided with stinging hairs. At the base, the leaves are corroded. The stinging nettle has male and female flowers on diferent stalks (dioecious).

Spreading: The stinging nettle grows everywhere, in cultivated and uncultivated places, in ditches, aside the roads, on the edges of waters, in forests and fat places where were sheepfolds.

Used parts of the plant: *Herba Urticae,* constituted from young stalks collected before or during the flowering time, having oval serrated-edged leaves, 7-14 cm long, 2-4 cm wide, petiole, with pointed apex covered with harsh hairs.

Urticae radix - rhizome and thin roots combination, cylindrical brown coloured - light at the exterior. No smell, weakly astringent taste.

Geoelement: Cosmopolitan.

Collecting: From the stinging nettle are collected the leaves, from may until the end of the autumn.

Drying: The leaves are layed in thin layers, in well aired rooms. The drying can be made even in open-air, in places hidden from intense light. The drying with hot air is made at a temperature of 50-60^{0}C. From 4.5-5.5 kg of fresh leaves is obtained 1 kg dry product.

Chemical composition: The plant contains proteinic substances, having a high amount of amino acids, glucidic substances, amines, sterols, cetones, (methylheptenone and acetophenone), volatile oil, fatty substances, sitosterols, formic and acetic acid, vitamins C B2 and K (aproximatively 400 units per gram), pantotenic acid, formic acid, clorophyl 0.3-0.8%, protoporphyrin and coproporphyrin, ß-caroten, Ca Fe Ma Si salts, phosphates, etc. The vesicant substance for the skin of the fresh plant is made of formic acid, an enzyme and a toxalbumine. By drying, these subsances are lost or they transform, therefore the vesicant properties dissapear.

From the roots it have been isolated: the phenyl derivates - propanic, fatty acids, phytosterols (free ß - sitosterol and glycoside, ? -5- free sterols and glycosides), cumarine (scopoletin), a lectin complex (UDA -*Urtica Dioica Aglutinina*), lignans, prosaccharides.

Fig. 3. *Urtica dioica*

Pharmacodynamic action - therapeutic use: In the traditional medicine the stinging nettle has been used for her antianaemic, hemostatic, antidiabetes, diuretics and cholagogue properties. But more important is for the extaction of the ß-caroten as source of provitamin A and for obtaining clorophyle. The last one is used rarely as such, more often is utilized under the form of his products of hydrolytic degradation, process in which the clorophyle is transformed in chlorophyllins. These, as sodium chlorides are soluble in water and are used as antituberculars, antianaemics, cicatrizing (at burns, dermatosis), but especially as deodorizers in all sorts of medicinal and cosmetic extracts as pomades, aerosols,

toothpastes, chewing gum, deodorant sprays. The copper chlorophyllin, green coloured, is also very used as green dyestuff or deodorant. Besides the mentioned substances, the stinging nettle contains histamine, formic acid and an urticant toxin whom's composition is still unidentified (1/10.000.000 of this toxine produces the stinging at the level of the skin). In the young stinging nettles, the production of the toxin is not having place yet. Because of that there won't be used old or mature stinging nettles, that even after boiling, the liquid and the vegetal integrated part, lead to the aparition of a gastrical irritation, sensation of burning at the level of skin, edemas, urinating impossibility. Regarding the diuretic action, it must be specified that the stinging nettles' leaves are used especially for the metabolic diseases' treatment, as rheumatism and gout it has been demonstrated that the stinging nettle extracts provoke an abundant renal elimination of uric acid, and on the other side displaces the uric acid from the tissues, putting it in the sanguine circulation. As important as this is the hematopoietic action, as the action of the spinach and the iron preparations. It determines a hypertensive action on the cardiovascular device. Interesting are the galactogogue and of stimulation of the pancreatic secretion properties. It has been described a hypoglycemiant effect too, but as in other cases, beside the hypoglycemiant principle it exist a hyperglycemiant one. The infusion is used 5-10%, the decoct 3-10%, and the extract 1:2 in alcohol at 70 °C. For the treatment of entero-colitis it is administrated a spoon of infusion at each 1-3 hours, or 2-5 drops of alcoholic extract. As a purgative it is administrated a little glass of decoct, in the morning, or 5-10 drops of alcoholic extract of 2-4 times a day, 5-15 days a month.

These researches reduce onself to the demonstration of the hemostatic, astringent, hemopoietic, weakly hypoglycemiant, diuretic or externally antiseptic and stimulator of the teguments' epitelialization action. The watery extract inhibits the evolution of the pathogenic agents (*Shigella, Staphylococcus, Pasteurela*, etc.). The roots have an antiinflammator decongestive and imunomodulator action. The root tincture is empirical used in lotions in combination with other plants against hair falling. Clinical studies have proved that the effectiveness of the tincture and decoct of stinging nettle root in the adjuvant treatment of the prostate adenoma and in the treatment of BPH (benign prostatic hyperplasia, stages I-II). The action is owed to the phytosterols, UDA and polysaccharides, that act through different mechanisms. The isolectins (UDA) tie to the T- lymphocytes, activates the T3-T1 complex and this way inhibits the prostaglandins, having as a consequence an antiphlogistic. The polysaccharidic fraction stimulates the T-lymphocyts, having a contribution at the antiinflammator action, and the phytosterols interfere with the DHT (dihydrotestosterone). It has been also proved that a dry extract of stinging nettle root, significantly decreases the serumal SHBG (sexual hormone binding globulin), globulin that inactivates the androgyne hormones that at the old people are found in bigger amounts.

4. Bilberry (*Vaccinium myrtillus* L. - Fam. Ericaceae) - fig. 4.

Description: Is a bowery, branchy little shrub. Superficial, very dense, intertwined like a felt, usually devoid of absorbent hairs roots. Very branched, green, long of about 30-60 cm, with angular branches. Green, glabrous, with knee form and sharp selvage stems. Alternated, small, flattened, joined to the stem buds. Round-ovoid to ellipitical, acuminate, finely serate, caducous leaves. Pale-pink, alone, androgyny, pendulum, with gamosepal calyx, globular-urceolat (eyesore) flower; androecium of 10 stamens. Spherical berry, black-bluish, frosted, juicy, sourish taste, eatable fruits.

Used parts of the plant: the fruits (bacels), the leaves and the root.

Geoelement: Circumpolar.

Colectare: The material has been collected from the Rânca (Parâng Mountains).

After the collecting it have been put to dry in well airy rooms.

Chemical composition: The leaves contain: tanin, arbutozida, neomirtilozida, mirtilozida, flavin derivati, hydrochinon, vacinina, ericolin, quininic acid, quinic acid.

The fruits contain: tanin, pectines, mirtilozida, sugars, acids : citric, malic, oxalic, succinic, lactic, antociani, 86% water, 13% carbohydrates, mineral substances as potassium chloride (65 mg%), calcium (10 mg%), sodium (1 mg%), phosphorus (10 mg%), iron (0,7 mg%). The content of provitamin A and aproximatively 20 µg%, vitamines B1 and B2 and niacina between 0.02 and 0.04 mg% each, vitamine C aproximatively 15 mg%.

Pharmacodynamic action – terapeutics use: Absorbant, antiatherogen, antibiotic, antidiabetical, antidiarrheaic, antidysenteric, antiemetic, anthelmintic, antihemorrage, antihemorroidal, antiinflammator, antipuride, antisecretive, antiseptic, astringent, bactericidal, bacteriostatic, cicatrizing, coagulating, disinfecting, diuretic, disolves calculus of uric acid and urates, hypocholesteroleminat energizer, hypoglycemiant, refreshing, regulates seeing problems (including adapting at seeing in the dark), tonic, trophic.

Fig. 4. The fruition degree
Vaccinium myrtillus

CONCLUSION

We considerate that the 4 species took into study have a high potential for reaching the objectives proposed in the complex researches regarding the reduction of the mineral microelements level from the pig dejections through the replacement of the chemical premixes with phytogenic additives and enzymatic preparations.

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COMPARATIVE MORPHO-ANATOMICAL STUDIES TO LEAVES OF PHOENIX DACTYLIFERA ATTACKED AND UNATTACKED BY GRAPHIOLA PHOENICIS (MOUG.) POITEAU

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KEY-WORDS: parasite, leaves, epidermis, mesophile

SUMMARY

In this paper are presented comparative data regarding the morphology and anatomy of Phoenix Dactylifera leave unattacked and attacked by Graphiola Phoenicis. The leaves attacked by the parasite present on both sides black pustules which turn later into red-brown. In transversal section the unattacked leaves and those with initial attack have an homogenous mesophil. As the attack advances, the abaxial epidermis and the mesophile situated above it are destroyed. In tangent sections the unattacked leaves have cells longed of adaxial epidermis, with lateral walls slightly greasy, a parasitic type stomata, and those attacked by the parasite have cells of the adaxial epidermis destroyed and looking like a macerate surface.

INTRODUCTION

The disease produced by Graphiola Phoenicis to Phoenix Dactylifera is called the palm trees smut and it is spread mostly in Europe, Asia, Africa and America attacking all species of palm trees grown in green houses. The attack leads to drying of leaves.

MATERIAL AND METHOD

For the biological material we used whole leaf of Phoenix Dactylifera unattacked and attacked by Graphiola Phoenicis taken fron the collection of palm trees belonging to The Botanical Garden "Al. Buia" of University of Craiova.

The cropped leaves have been analyzed macroscopically in fresh stage, and a part of them have been conservated in a mixture made in equal parts from etilic alcohol, glycerin, and distillated water in order to make the anatomical analysis. The transversal and tangent sections made through unattacked and attacked leaf by the parasite have been analysed with a Nikon microscope and then photographed. In this paper we used the terms V_M = individual maximum value, V_m = individual minimum value, $\bar{X}$ = average individual values.

RESULTS AND DISCUSSIONS

The attack of the Graphiola Phoenicis fungus to the leaves of Phoenix Dactylifera is content in the category of smuts. The disease is frequently spread in poorly aired green houses, very humid and it is demonstrated on the both sides of the leave under the aspect of

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black pustules, in the beginning covered by epidermis, which turn later into dusty leaves and finally they become red-brown (figure 1). In the pustules the telio spores of the pathogen are differentiated. After vacuuming the pustules the telio spores are staying on the leaves hard coats in the shape of craters.

To reveal the modifications that are happening at cell level and tissues of the leaves attacked by the parasite we have done transversal and tangent sections through areas which did not represented attack symptoms, but also through areas in which the pathogen pustules where present.

In transversal section the unattacked leave has a homogeneous mesophile (figure 2) and thickness of 450 µm.

The adaxiale epidermis is mono stratum and has a thickness of 11, 25 µm and a cuticule of 4,5 µm. Under this epidermis are found packages of sclerenchyma which alternates with parenchyma assimilator. The length of sclerenchyma packages is V_M = 117 µm, V_m = 45 µm, $\bar{X}$ = 84,15 µm and the thickness of: V_M = 90 µm, V_m = 35 µm, $\bar{X}$ = 61,65 µm.

The cells of the homogenous mesophile are oval shape, with spaces in between and those in the median area of the leave are much bigger.

The abaxial epidermis is also monostratum and it has a 9 µm thickness with a thick cuticule of 2,25 µm. Above the abaxial epidermis there are packeages of sclerenchyma which alternates with parenchyma assimilator. The length of those aphetes of sclerenchyma is: V_M = 72 µm, V_m = 29,25 µm, $\bar{X}$ = 52,65 µm and the thickness of: V_M = 69,75 µm, Vm = 22, 25 µm, $\bar{X}$ = 45,68 µm.

The main nervations of the leave are very developed, sometimes they reach the abaxial epidermis (figure). The leading fascicles from nervations are closed co-lateral type and are completely surrounded by a coat of multistrata sclerenchyma figure 2). The diameter of phloem vessels from the leading fascicles of the main nervations are: V_M = 18 µm, V_m = 9 µm, $\bar{X}$ = 12,6 µm and of the nerves vessels are V_M = 72 µm, V_m = 13,5 µm, $\bar{X}$ = 27,45 µm.

The initial parasite attack stage leaves have in transversal section almost the same structure like the unattacked leaves (figure 3).

In transversal section, in areas where the leave was strongly attacked by parasite we can notice that the abaxial epidermis and the homogenous mesophile are destroyed to the zone of leading fascicles by the pathogen agent (figure 4).

The tangent sections cut on the upper part of the leaves unattacked and attacked by the parasite show the the adaxial epidermis of the leaves unattacked by the parasite is formed by prolonged cells in the growth direction of the leave, have greasy lateral walls, they do not have any spaces between them and do not hold chloroplasts inside.

The cells of the adaxial epidermis situate between the main nervations of the leave have the length of: V_M = 126 µm, V_m = 27 µm, $\bar{X}$ = 72,45 µm, the width of: V_M = 13,5 µm, V_m = 6,75 µm, $\bar{X}$ = 9,23 µm and the density of: V_M = 5486,97 cells/mm², V_m = 587,89 cells/mm², $\bar{X}$ = 1495,41 cells/mm². Among epidermis cells there are stomata of parasitic type which have the average length of 24,75 µm.

The tangent sections done through the upper side of the attacked leaves by the parasite show out that the cells of the adaxial epidermis have been destroyed by the parasite and appear as a macerated area (figure 6).

Tangent sections done through attacked zones by parasite on the back side of the leaf are not possible to be done because the attack manifests more intense on this side producing the breaking and maceration of the cells of the abaxial epidermis.

Fig. 1. Phoenix dactylifera leaves - attacked by Graphiola phoenicis

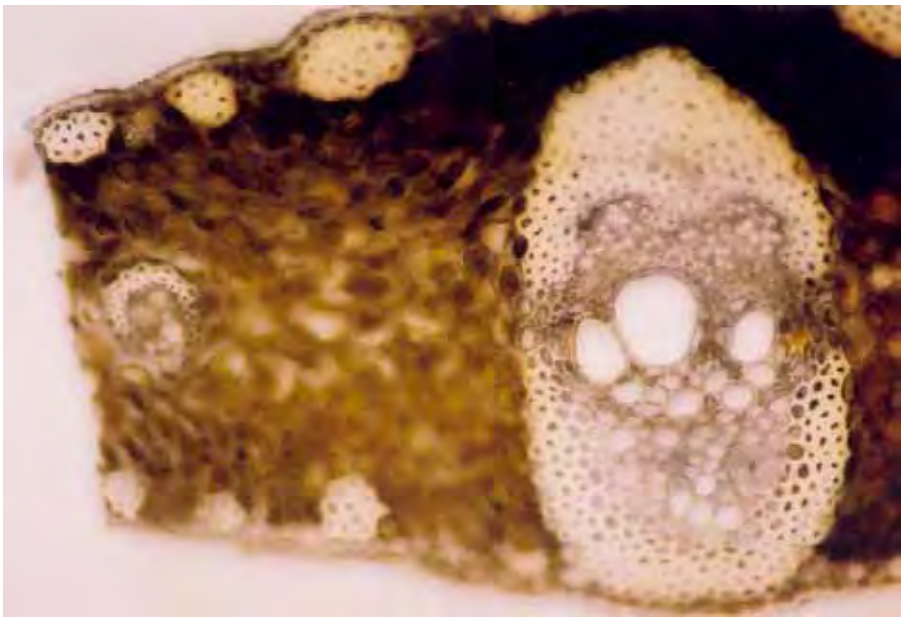

Fig.2. Transversal section through non-attacked Phoenix dactylifera leaf

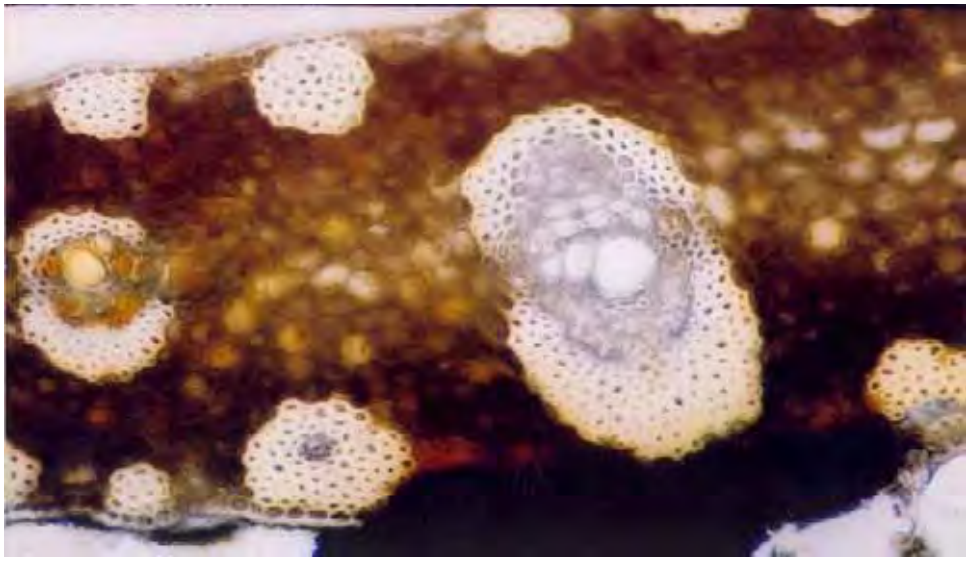

Fig. 3. Transversal section through Phoenix dactylifera leaf with Graphiola phoenicis incipient attack

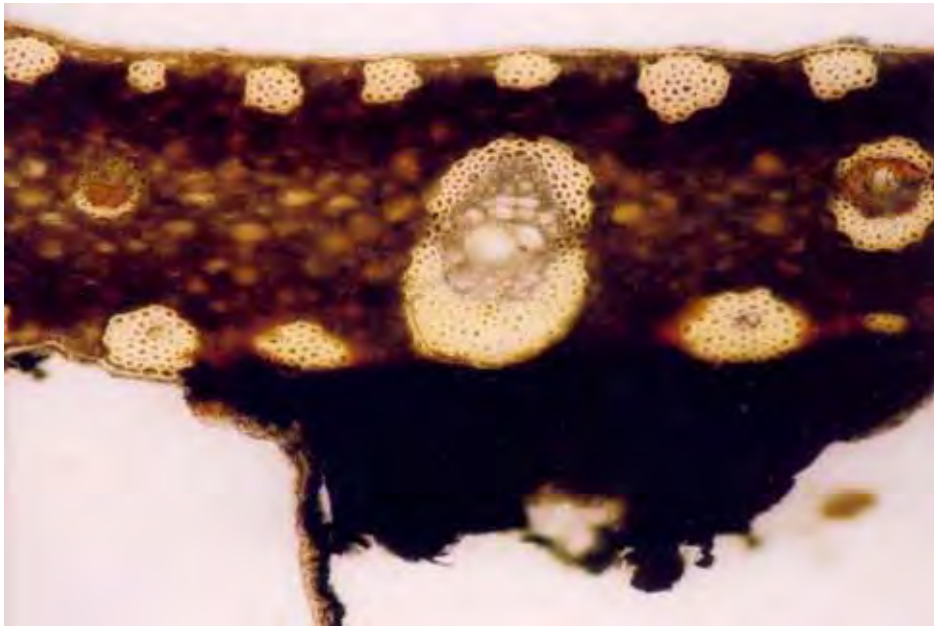

Fig. 4. Transversal section through Phoenix dactylifera leaf- strongly attacked by Graphiola phoenicis

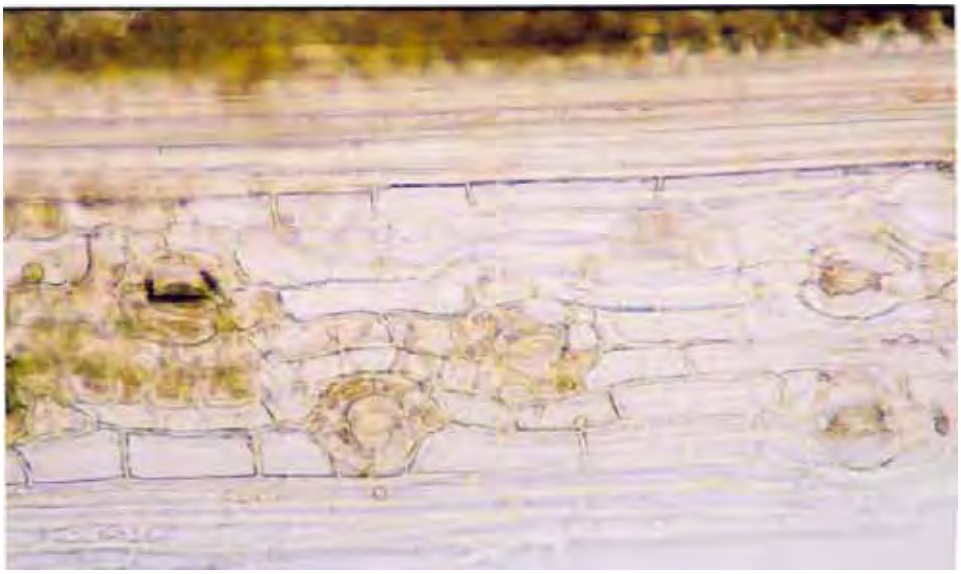

Fig. 5. Adaxial epidermis of non-attacked Phoenix dactylifera leaf

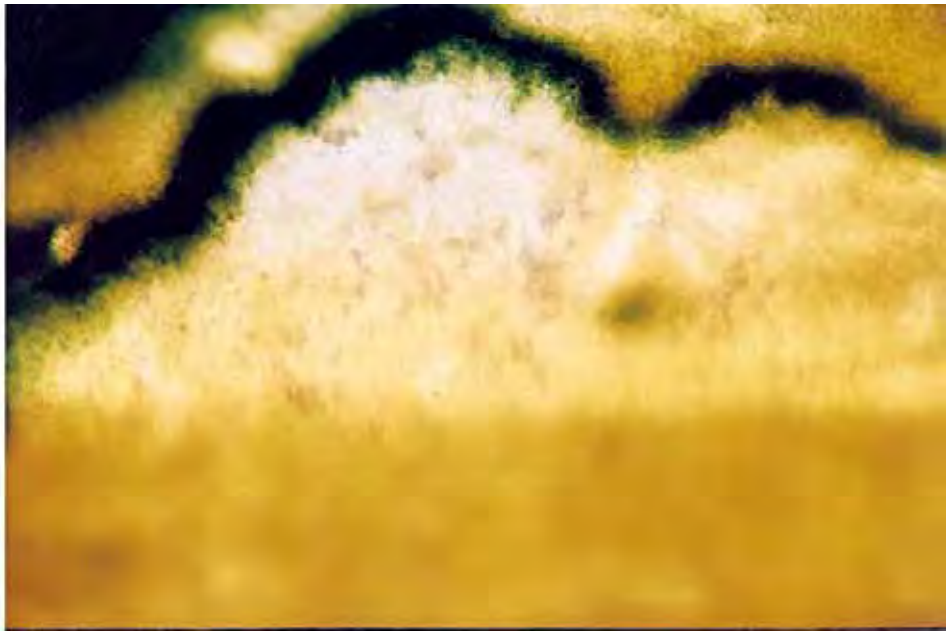

Fig.6. Adaxial epidermis of Phoenix dactylifera leaf - attacked by Graphiola phoenicis

CONCLUSIONS

From the comparative analysis of unattacked and attacked Phoenix Dactylifera leave by Graphiola Phoenicis we turn out to the following conclusions:

1. The disease is manifested on both sides of the leaf in the form of black pustules which turn later into re-brown.
2. In transversal section the unattacked leaves and those in initial stage of attack have a homogenous mesophil , and in the areas strongly attacked the abaxial epidermis and the mesophil situated in the proximity above are immediately destroyed.
3. In tangent sections where the leaf is strongly attacked it is noticed that both epidermis are destroyed by the parasite.

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Vol. XIII (XLIX) - 2008

MORPHO-ANATOMIC MODIFICATION CAUSED BY THE VENTURIA INEQUALIS (COOKE) WINT. MASHROOM ON THE MALUS DOMESTICA BORKH. LEAVES.

Simeanu C. G.[1], Simeanu Camelia – Ecaterina[2]

KEY WORDS: parasite, leaf, epidermis, mesophil

SUMMARY

The paper presents the comparative results regarding the morphology and the anatomy of the leaf limb of Malus domestica, the fonathan type both attacked and anattacked by the Venturia inequalis mashroom. The leaves attacked by the mashroom present spots more or less circular, being more obviovs on the inferior side of the leaf. In cross section the leaf presents bifacial structure. The limb of the leaf attacked by the parasite loses it`s bifacial structure as the attacked goes on. It is the abaxal epidermis which is destroyed first and then the gapped parenchyma, the palisade one and the adaxle epidermis. The tangent section made through the limb of the leaf attacked by the parasite point out that the cells of the abaxale epidermis deform first, thing which goes on as far as the mesophil level, which makes the limb of the leaf thinner at the level of the attacked zones.

INTRODUCTION

The brownish stain of the leaves, fruit and the disease of the apple-tree branches is considered in our country the most damaging disease of the apple-tree and it is met in all tree basins every year. The disease weakens the trees very seriously and makes them bear less and less fruit. The Venturia inequalis mashrooms attacks the leaves, blooms, fruit and branches. The leaves are most damaged and that is why we have researched them only.

MATERIALS AND METHODS

As biological material we have used full leaves of Malus domestica, the Jonathan type both attacked and unattacked by the parasite, and cultivated in Banu Maracine Didactic region, Craiova. The leaves were analysed macriscopically, in fresh condition, and part of them were preserved in a mixture of equal parts of absolute ethy/alcohol, glycerine and distillate water. The cross and tangent sections made through the limb of the attacked and unattacked leaves were analyzed whith a Nikon microscope and then photographed.

RESULTS AND DISCUSSIONS

The attack of the Venturia inepualis mashroom on the leaves of Malus domestica the Jonathan type is part of the stain cathegory and manifestsitself since spring to autumn as stains more or less circular. The stains appear on both sides of the leaves, but the ones on the inferior side are prevailing.(fig, 1, 2).

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In order to point out the anatomic modifications which led to the stain symptom, we analysed comparatively section made through the limb of the leaves attacked and unattacked by the parasite.

In cross section through the limb of the unattacked leaf and of the leaf with the attack in the beginning phase we can notice that the leaf is bifacial (fig. 3). At the same figure we can also notice that both epidermises as well as the mesophil have cells with intact sides, but the mycelium of the mashroom broke the cuticula of the abaxle epidermis and is starting to affect the external side of the cells of this epidermis where it develops into stroma.

The cross section made through the limb of the leaves with advanced attack (the spore moment) point out that in this phase the external sides of the abaxle epidermis cells are disorganized and the asexuate fruit of the mashroom make their appearance (fig. 4). We mention that in this phase of the attack the leaves macrosopically analyzed presented brown-olivestains with slightly velveted aspect on the inferior side of the limb.

In phase even more advanced of the attack, due to the spore of the mycelium, numerous cells of the abaxle epidermis lose their individuality (fig. 5). Still in this phase of the attack the deforming of the cells of the gapped parenchyma takes place which results in making the leaf thinner in the affected zones (fig. 6).

In tangent section through the superior side of the limb og Malus domestica leaf, the Jonathan type, unattacked by the parasite, we can notice that the cells of the adaxle epidermis are polygen shaped, with vertical sides or slighthy sinuous, no gaps between them, and they contain severa chloroplasts in the interior (fig. 7).

The tangent sections made through the superior side of the limb of the leaf attacked by the parasite point out that in the attacked zones the cells of the adaxle epidermis change their shape, become less obvious, and in their exterior we can see the conidia of the pathogene (fig. 8).

The tangent sections made through the inferior side of the leaf limb show that the abaxle epidermis from the zones attacked by Venturia inepualis changes it`s sides and in some places these ones get macerated, while the areas not attacked by the parasite the epidermic cells preserve their individuality and resemble the ones of the adaxle epidermis (fig. 9).

CONCLUSIONS

By comparatively analyzing the limb of the Malus domestica leaves, the Jonathan type, attacked and unattacked by the Venturia inequalis we can rach the following conclusions:

1. The parasite attack all the surface organs of the plants except for the trunk.
2. The strongest attack is suffered by the leaves, especially their limb and takes place throughout the vegetation time.
3. At the level of the leaf limb the attack starts with the distraying of the cuticula of the abaxale epidermis, it moves further on to the cells of the epidermis, gapped parenchyma and finally it reaches the palisade parenchyma and the adaxale epidermis.
4. The morpho-anatomic modificationsuffered by the leaf during the pathogenesis, in association with the action of the toxins secreted by the pathogene, explain the necrosis of the tissues in the attack zones, which leads to the diminishing of the leaf surface in the metabolic processes.

Fig. 1. Malus domestica, Jonathan type, attacked by *Venturia inequalis*

Fig. 2. Leaves of Malus domestica, Jonathan type, attacked by *Venturia inequalis*

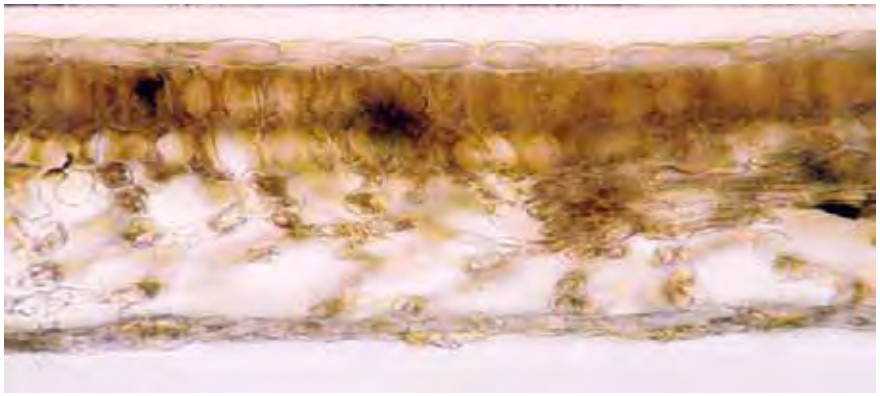

Fig. 3. Cross section through the limb of the *Malus domestica* limb, Jonathan type, with attack in the beginning phase of *Venturia inequalis*

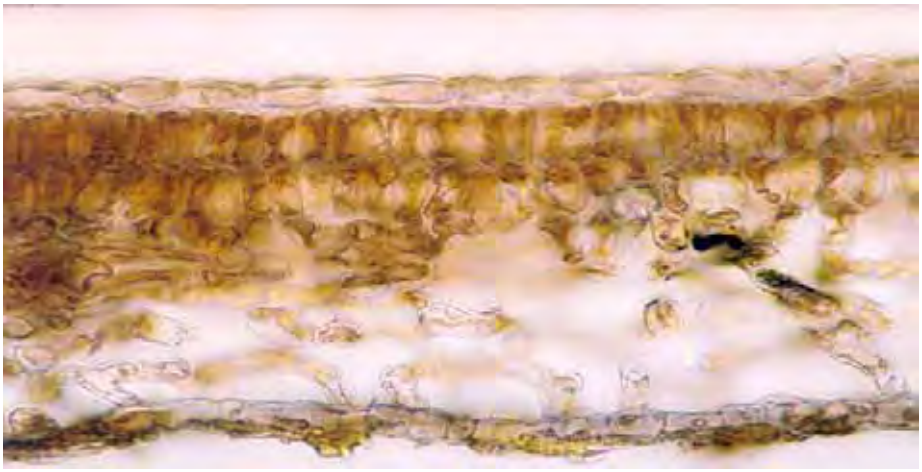

Fig. 4. Cross section through the limb of *Malus domestica* leaf, Jonathan type, attacked by *Venturia inequalis*, the beginning of the spore phase

Fig. 5. Cross section through the limb of *Malus domestica* leaf, Jonathan type, attacked by *Venturia inequalis*, the apparition of Conidiophorums with conidia

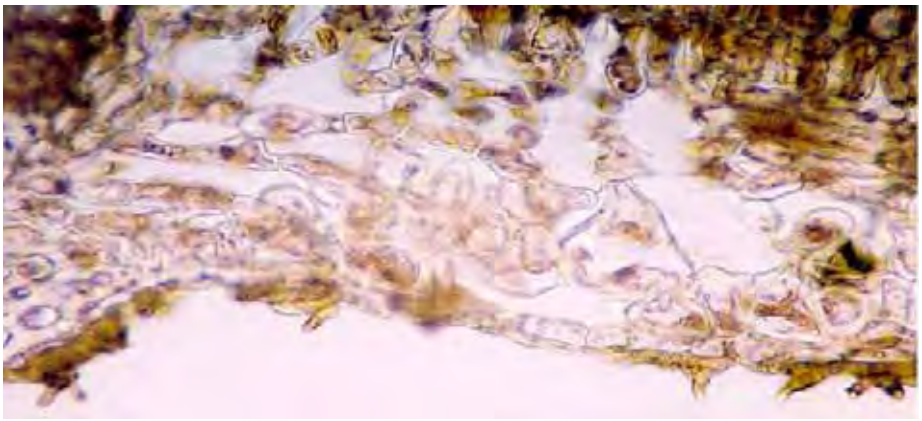

Fig. 6. Cross section through the limb of *Malus domestica* leaf, Jonathan type, strongly attacked by *Venturia inequalis*, the deforming of the gapped parenchyma cells

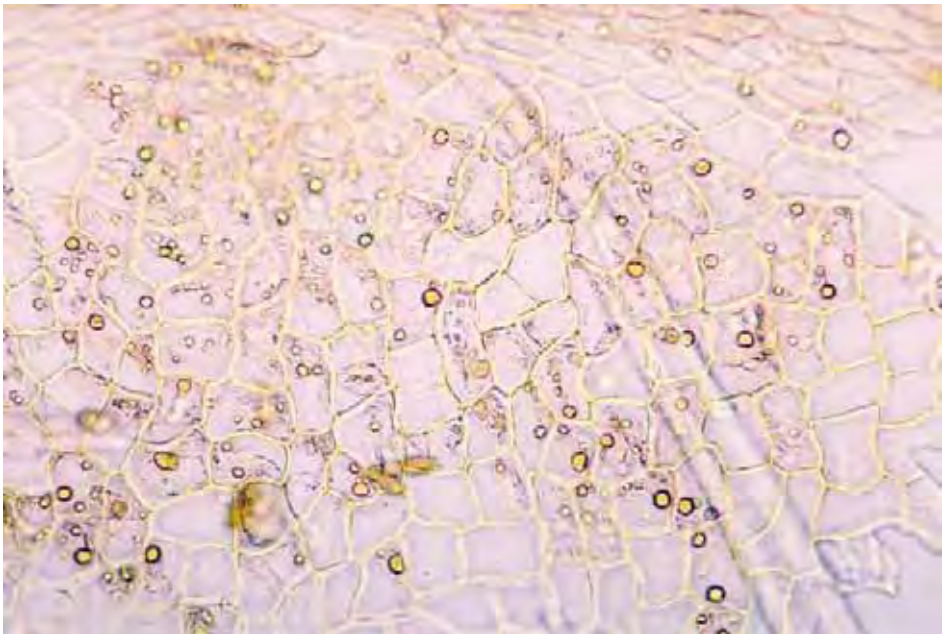

Fig. 7. The adaxle epidermis of the limb of *Malus domestica* leaf, Jonathan type, not attacked by the parasite

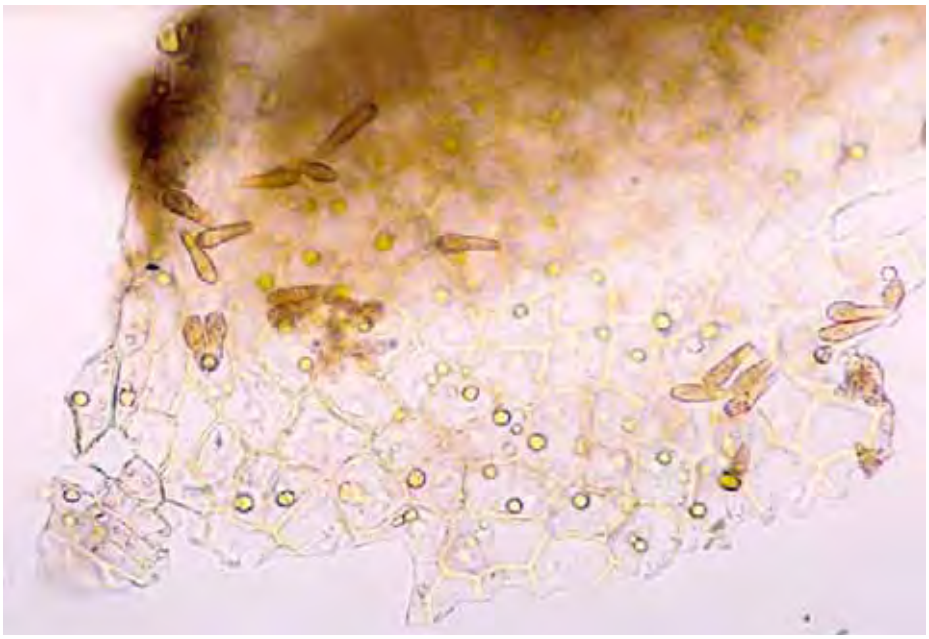

Fig. 8. The adaxle epidermis of the limb of *Malus domestica* leaf, Jonathan type, attacked by *Venturia inequalis*

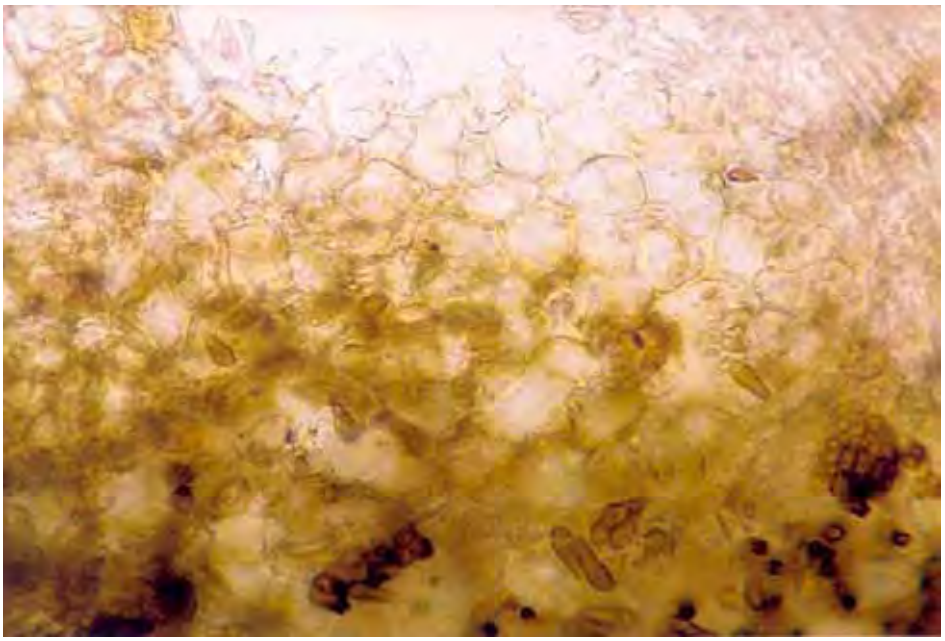

Fig. 9. The abaxle epidermis of the limb of *Malus domestica* leaf, Jonathan type, with areas attacked and unattacked by *Venturia inequalis*

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PHYSIOLOGICAL RESEARCH REGARDING THE TOMATO PLANTS ATTACKED BY PATHOGENIC FUNGUSES

I. Nicolae[1]

KEY WORDS: pathogen, healthy plants, attacked plants, tomato.

ABSTRACT

At the tomato plants attacked by the pathogens one can observe the diurnal dynamics of the photosynthesis and of transpiration presents a minimum in the morning, a maximum after lunch and a minimum toward the evening, is similar to that in healthy plants but presents specific variations of the pathogen actions. One can also observe the decrease of the total water contents determines the withering and premature drying of the plants and the decrease of the contents in chlorophyllian pigments because of the intensification of the chlorophylases and the deterioration of the chloroplasts. The plants attacked present an increase of the concentration of the cellular juice which is manifested by the increase of the osmotic pressure of the cells.

INTRODUCTION

During the vegetation tomato plants are attacked by numerous pathogenic funguses which cause physiological, morphological or mechanical modifications that have repercussions both on the span of life as well as on the production potential of these.

MATERIAL AND METHOD

Research regarding some physiological modifications produced by the pathogenic agents was made in *Buzău* tomato plants cultivated under field conditions (Işalnița -Dolj).

The pathogenic factors frequently met at tomato crops have been represented by *Phytophthora infestans* (Mont.) de Bary, *Botrytis cinerea* Pers. and *Alternaria solani* Sorauer.

For the estimate of the attack was made using the calculation formulae (Săvescu A., Rafailă C.). The diurnal dynamics of photosynthesis and transpiration was established non-destructive method with the analyzer LCi (Ultra Compact Photosynthesis Measurement System).

The intensity of the total water contents and of dry substance were determined by the help of the drying stove - gravimetric method, the concentration of the cell juice was determined by of the Abbe - Zeiss refractionmeter. The contents of the chlorophyllian pigments was estimates by of the analizor portable SPAD 502 (the use of the chlorophyll meter SPAD is non-destructive method and permits repeated measurements).

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RESULTS AND DISCUSSIONS

The tomato plants were analyzed macroscopically and microscopically and after signaling the attack and identifying the pathogen, the dynamics of the physiological processes was established.

The pathogenic agent *Phytophthora infestans* (Mont.) de Bary has a hyaline mycelium that is non-septated and is developed intercellularily.

On the leaves, the attack manifests itself through the appearance of several large, hydrated, dark green-grey stains on the edge and especially the tip of the folioles. Under conditions of maximum atmospheric humidity, on the inferior side of the leaf, on the edge of the stains there appear the fructifications of the fungus under the shape of a lax, white puff. In the area of the stains, the tissues are not turgescent anymore, they turn dry, brown and the folioles turn their edges to the upper side (Fig. 1).

The *Botrytis cinerea* Pers. has a mycelium that is developed in the intercellular spaces of the tissues and is made up of septate, ramified, and grey-olive hifes.

At first the attack appears on the dead leaves that are placed at the bottom of the plant, that are covered by a grey-white felt on which there are formed the fungus spores . On the stalks, the disease manifests itself by the appearance of several elliptical, grey, slightly hollowed stains that progress concentrically, the tissues become rotten, the circuit of water and nutritious substance supply is interrupted, the plant withers and dies (Fig. 2).

The *Alternaria solani* Sorauer present small, septate conidiophores, colored in brown - olive with conidia that are disposed in a chain, colored in light brown.

The most frequent and obvious symptoms manifest on the leaves of the plants, the attack starting on the mature basic leaves and extending to the top of the plants. On the leaves there appear brown stains, of a more or less round shape, placed circularly. The stains grow, intersect, and gradually the leaves dry. On the stalk, there appear stains of dark brown color, well contoured, elliptical, slightly depressionary that stay at the level of the superficial tissues (Fig. 3). The dynamics of the physiological processes at the *Buzău* tomato plants was established, according to the frequency, the intensity and the degree of attack, but also by the climatic conditions, on July 15th 2007 conditions presented in Table no. 1.

Estimating the attack caused by the pathogen is presented in Fig. 4.

Fig. 1. The tomato plants attacked by the *Phytophthora infestans.*

Fig. 2. The tomato plants attacked by the *Botrytis cinerea.*

Fig. 3. The tomato plants attacked by the *Alternaria solani.*

Table 1

The analyzed Climatic Factors	The Hours of the Analyses	The Climatic Data
The Temperature (^oC)	9^{00}	23.0
	13^{00}	30.6
	17^{00}	34.2
The Lightness (Luxes)	9^{00}	35,000
	13^{00}	89,400
	17^{00}	75,200
Atm. Humidity (%)	9^{00}	86
	13^{00}	80
	17^{00}	75

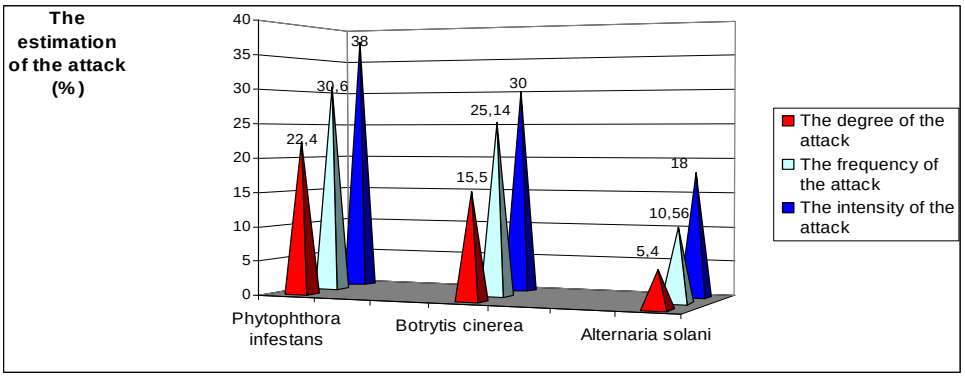

Fig. 4. The estimate of the attack produced by the pathogen agents at the tomato plants

The diurnal dynamics of photosynthesis and transpiration follows a unimodal curve which in the morning presents a minimum in the morning, a maximum after lunch and a minimum toward the evening with specific variations in the plants attacked.

The diurnal dynamics of photosynthesis in the attacked plants is similar to that in healthy plants but the recorded values are lower in comparison with these as a result of the reduction of the assimilation surface through the deterioration chloroplasts, as well as the inhibition of several biochemical reactions of the photosynthesis (Fig. 5).

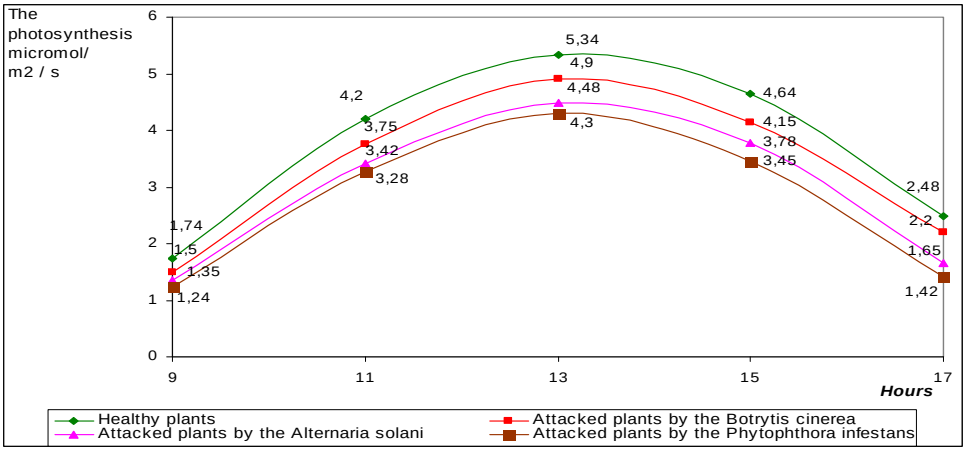

Fig. 5. The diurnal dynamics of photosynthesis at the tomato plants attacked by the pathogens.

The diurnal dynamics of transpiration in the attacked plants is similar to that in healthy plants, but the recorded values are lower in comparison with these as a result of the reduction of the transpiration surface, the occlusion of the stomates by the mycelium of the fungus and the malfunctioning of the stomatic apparatus (Fig. 6).

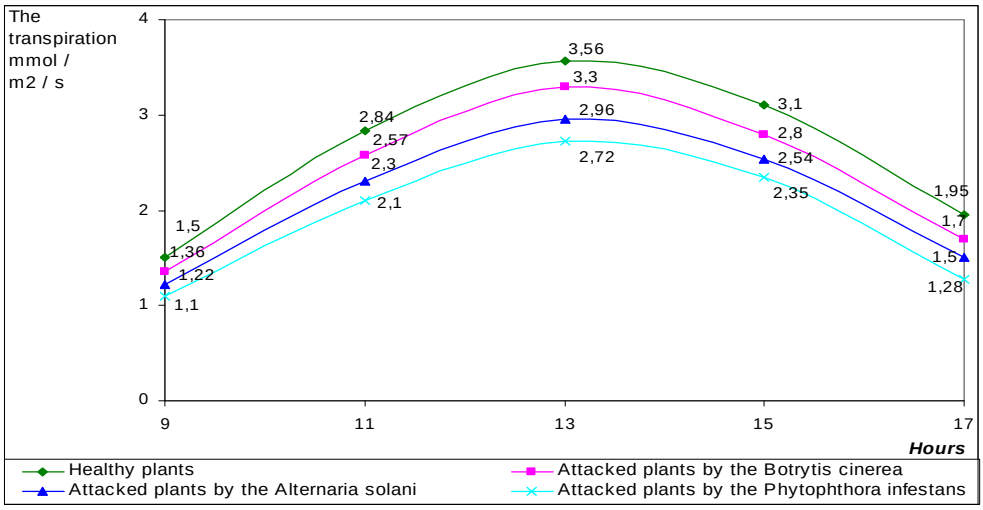

Fig. 6. The diurnal dynamics of transpiration at the tomato plants attacked by the pathogens.

In order to present a very correct symptomatological picture, along side the diurnal analysis of the photosynthesis and transpiration, there were also determined: the total water contents, the dry substance contents, the concentration of the cellular juice and the contents in chlorophyllian pigments and the obtained resulted are presented in Fig. 7-10.

The total water contents. There can be seen a decrease of the total water contents by 1.42 % at the tomato plants attacked by the *Phytophthora infestans*, by 1.54 % at the plants attacked by the *Botrytis cinerea* and by 1.0 % at the plants attacked by the *Alternaria solani* which is manifested by the occurrence of some hydric unbalances, the decrease of the cellular turgor, the withering and premature drying of the plants (Fig 7).

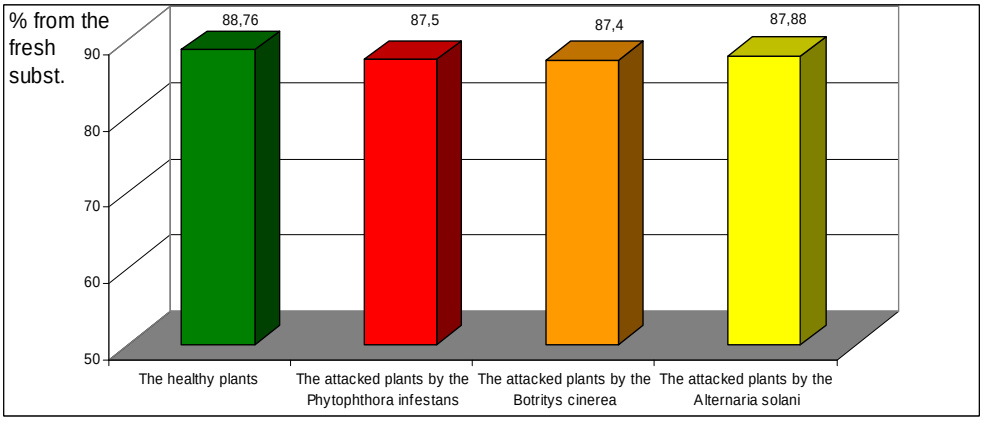

Fig.7. The total water contents.

The dry substance contents. In the tomato plants there can be seen an increase of the dry substance contents by 11.20 % at the tomato plants attacked by the *Phytophthora infestans*, by 12.09 % at the plants attacked by the *Botrytis cinerea* and by 7.82 % at the plants attacked by the *Alternaria solani* as a result of the malfunctioning of the closing and opening mechanisms of the stomates, which is manifested by the decrease of the cellular turgor, the withering and premature drying of the plants (Fig 8).

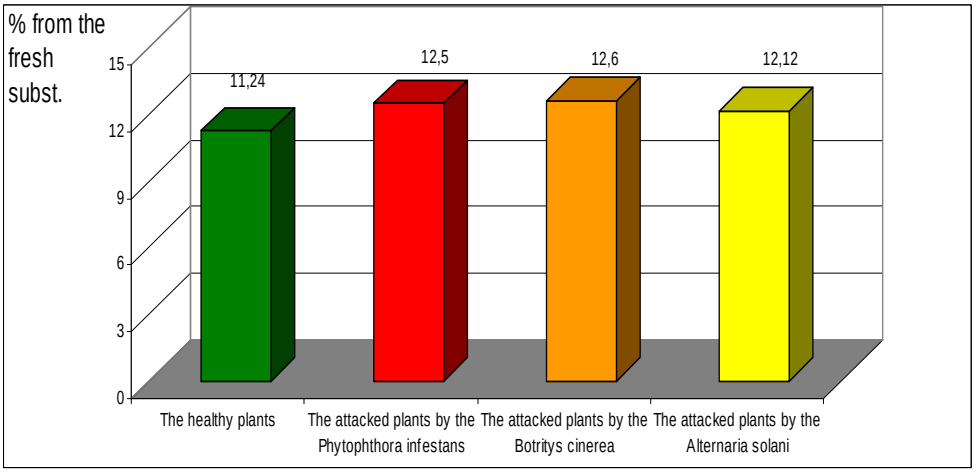

Fig. 8. The dry substance contents.

The concentration of the cellular juice. In the tomato plants there can be seen an increase of the concentration of the cellular juice by 12.0 % at the tomato plants attacked by the *Phytophthora infestans*, by 14,6 % at the plants attacked by the *Botrytis cinerea* and by 8.0 % at the plants attacked by the *Alternaria solani* which is manifested by the increase of the osmotic pressure of the cells (Fig. 9).

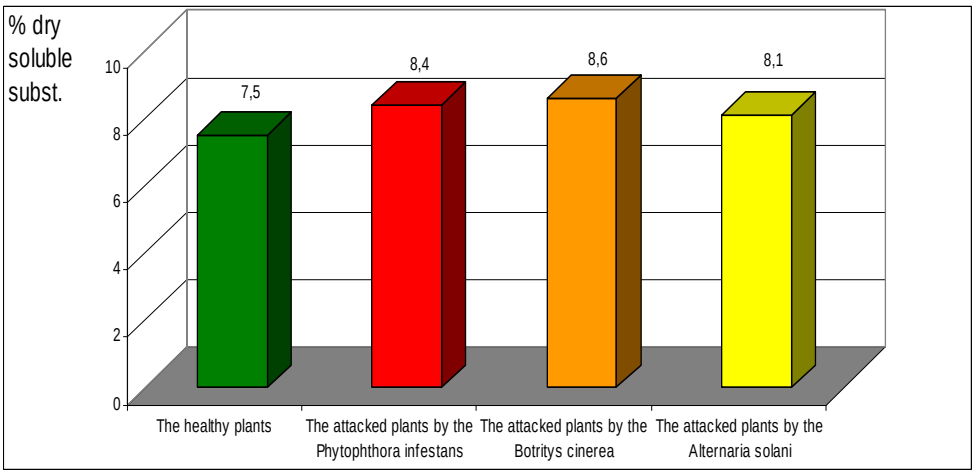

Fig. 9. The concentration of the cellular juice.

The contents of the chlorophyllian pigments. There can be seen a decrease of the of the contents of chlorophyllian pigments by 29.53 % at the tomato plants attacked by the *Phytophthora infestans*, by 13.66 % at the plants attacked by the *Botrytis cinerea* and by 25.10 % at the plants attacked by the *Alternaria solani* as a result of the intensification of the chlorophylases and of the deterioration chloroplasts (Fig. 10).

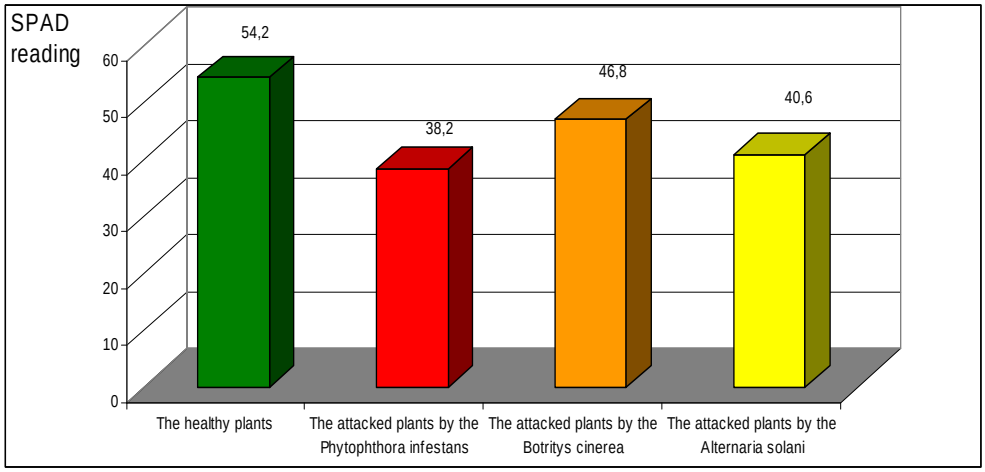

Fig. 10. The contents of the pigments.

CONCLUSIONS

The following can be observed in relation to the tomato plants attacked by the pathogens: the diurnal dynamics of the photosynthesis and of transpiration presents a minimum in the morning, a maximum after lunch and a minimum toward the evening, with specific variations in the plants attacked, the decrease of the total water contents wich determines of the withering and premature drying of the plants; the decrease of the contents in chlorophyllian pigments because of the intensification of the chlorophylases and deterioration of the chloroplasts.

The physiological modifications produced in the attacked plants determine several metabolic unbalances, with implications on the growth and development.

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PHYSIOLOGICAL RESEARCH REGARDING SOME PLANTS FROM THE POLLUTED AREAS

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KEY WORDS: polluting agents, atmospheric pollution, plants, physiological processes

ABSTRACT

The research regarding several physiological modifications caused by the atmospheric pollution has been done for the ligneous plants living in the area of the chemical factory Işalniţa, Dolj.

As a consequence of the atmospheric pollution the organs of the plants suffer physiological and biochemical modifications which have repercussions on the growth and the development of the plants. The physiological processes that are mostly affected, that also influence the productivity of the plants are photosynthesis, respiration and transpiration.

In connection with the climatic conditions, as a result of the action polluting agents one can observe the diminution of the photosynthesis, of the total water content. One can also observe the rise of the respiration and the transpiration with specific variations according to the analysed plants.

INTRODUCTION

Atmospheric pollution causes modifications in the physiological processes of the plants in different ways, according to the nature of the polluting factor, the species involved and the climatic conditions in the respective area. There are also affected the processes of growth and development, the process of the photosynthesis and the water regime, all of these being of maximum importance for the productivity, the vitality and the existence of the vegetation.

MATERIAL AND METHOD

Research regarding some physiological modifications produced by the atmospheric pollution has been done on the following plants: *Salix alba, Robinia pseudoacacia, Populus alba, Acer negundo* and *Juglans regia.*

The intensity of the photosynthesis was determined through Ivanov method, of the respiration through Boysen - Jensen metod and of the transpiration through the method of rapid weighings by of the torsion scale (Huber and Ivanov). The total contents of water and of dry substance was determined by the help of the drying stove - gravimetric method.

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RESULTS AND DISCUSSIONS

Industrial factories represent the most important source of pollution of the air in the cities. Thanks to the small dimensions, industrial powders are scattered up to high altitudes and are maintained for a long time in the atmosphere.

The main polluting factors present in the atmosphere are the carbon monoxide, the carbon dioxide, the sulphur dioxide, the sulphuric hydrogen, the halogen derivatives (hydrochloric acid, chlorine, bromine , fluorine, etc), the oxides of the nitrogen (N_2O, NO, NO_2), the hydrocarbons, the heavy metals, as well as some polluting agents such as pollen, fumes and the dust raised by the wind.

The atmospheric polluting agents produce symptoms visible mostly on the leaves of the plants. According to the action of the polluting agent the leaves may have stains, decolourization, lesions, necroses and burnings more or less profound. In conditions of repeated pollution takes place the precocious ageing of the foliar apparatus.

The atmospheric pollution cause:

- the decolourization of the leaves at the *Acer negundo* (which is made through the appearance of stains inside the limb or through junctions of the affected areas) and at the *Salix alba* (the leaves present decolourizations that later become necroses) - Fig. 1 and Fig. 2.

- the defoliation at the *Populus alba*, *Robinia pseudoacacia* (at first the leaves become yellow and later they fall) - Fig. 3. and Fig. 4.

Fig. 1. Decolourizations at the *Acer negundo*

Fig. 2. Decolourizations at the *Salix alba*

Fig. 3. Defoliation at the *Populus alba*

- the necroses and defoliation at the *Salix alba* (the apical and the marginal side of the limb suffer a necrosis, the tissues restrain and the leaf rolls around the main nerve, the necroses have a dark colour, almost black) - Fig. 5 and Fig. 6.

The penetration of polluting agents inside the plants takes place mostly through the ostioles of the stomates and in a smaller quantity through the other cells of the cuticle. The polluting agents may interfere in controlling the opening and the closing of the stomates, thus modifying the exchange of gases between plants. Some gases react with the plasma lemma, with the cellular wall or with some enzymatic systems causing

modifications of the semi permeability and of the exchange surface between the cell and the outer world.

Fig. 4. Defoliation at the *Robinia pseudoacacia*

Fig. 5. Necroses at the *Salix alba*

Fig. 6. Necroses at the *Juglans regia*

As a consequence of the atmospheric pollution the photosynthesis is reduced, the respiration of the aerial organs is intensified and the transport of what is being assimilated towards the roots is reduced. The influence over the photosynthesis is done mostly by affecting the chloroplasts, thus being disturbed the synthesis of the organic substances thanks to the modification of the selectivity of the plasmatic membranes. The pollution diminishes the resistance of the plants to drought by creating disequilibrium between transpiration and water absorption.

The symptoms of the atmospheric pollution are different and they manifest through the slowing or the inhibition of the plant growing, through stopping the penetration of the light into the tissues because of the thick layer of powder set on the leaves which represent an impediment for the photosynthesis and the nutrition of the plants and which also provokes the intoxication of the plants with chemical and solid substances or with gases. These penetrate into the tissues; they scatter inside the cells and disturb their metabolism or their cytological structure. The physiological determinations were made according to the climatic conditions on June 26th 2007, conditions presented in Table no. 1.

Table 1

The analyzed Climatic Factors	**The Hours of the Analyses**	**The Climatic Data**
The Temperature (^oC)	9^{00}	24.2
	13^{00}	26.6
	17^{00}	28.1
The Lightness (Luxes)	9^{00}	32,600
	13^{00}	88,700
	17^{00}	54,800
Atm. Humidity (%)	9^{00}	87
	13^{00}	85
	17^{00}	76

The intensity of photosynthesis. It can be noticed at the plants affected by the pollution the diminishing of the photosynthesis intensity that has different values according to the analysed plants, as a result of the reduction of the assimilation surface by the deterioration of the chloroplasts, the malfunctioning of the mechanisms of closing and opening of the stomates, as well as the inhibition of some biochemical reactions of the photosynthesis, which determines the decrease in the quantity of nutritive substances assimilated and accumulated in the plants (Fig. 7.).

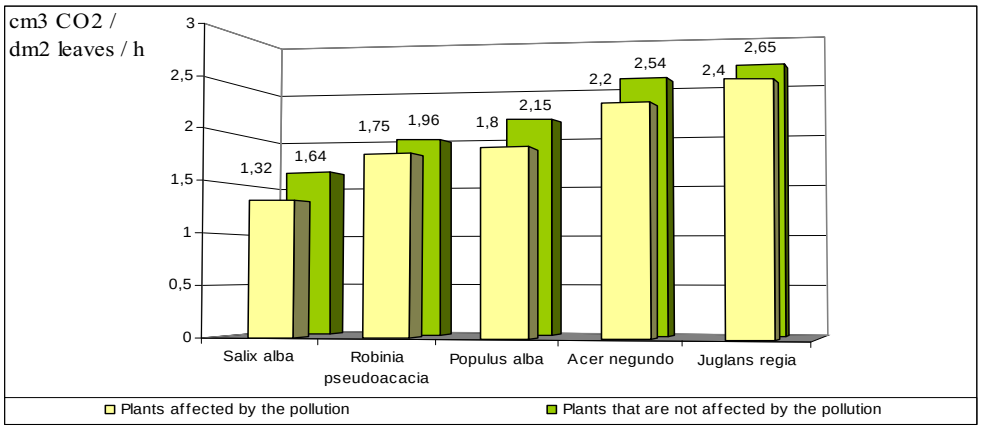

Fig. 7. The intensity of photosynthesis.

The intensity of respiration. The atmospheric pollution causes important modifications of the respiration intensity. For plants affected by the pollution the respiration is intensified by the growing income of the oxygen, by the rise of the temperature as a defence reaction of the plants against the action of the polluting agents and the intensification of the activity of the respiratory enzymes (Fig. 8.).

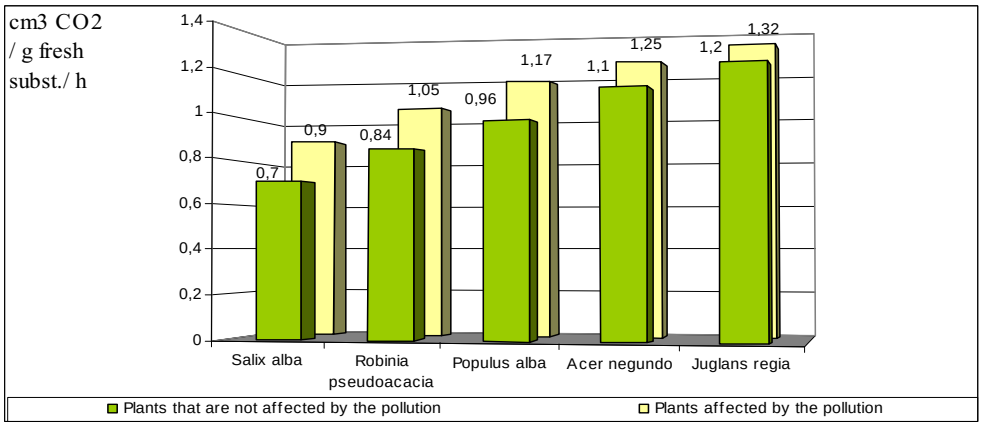

Fig. 8. The intensity of respiration.

The intensity of transpiration. The plants affected by the pollution present a growing of the transpiration, especially thanks, the malfunctioning of the stomatic apparatus (Fig. 9).

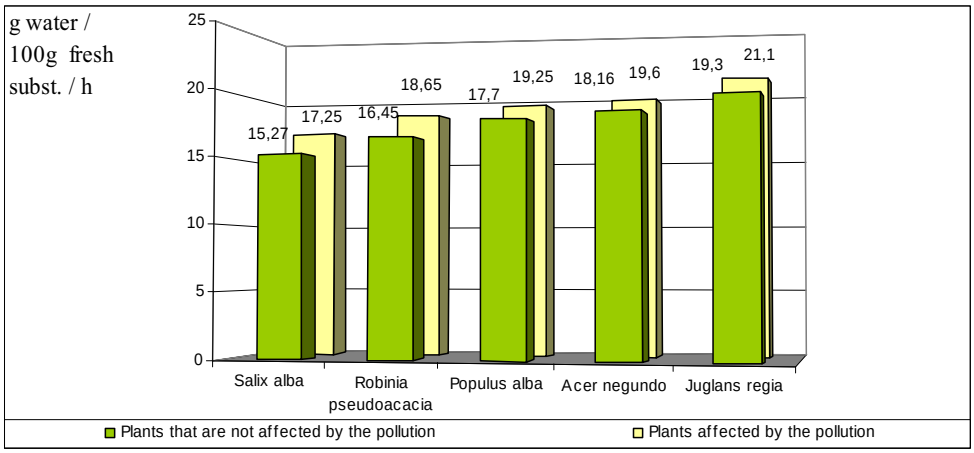

Fig. 9. The intensity of transpiration.

The total water contents and the dry substance contents. In the plants affected by the pollution there can be seen a decrease of the total water contents and an increase of the dry substance contents, as a result of the lesion leaf tissues, the modification of the permebiality of the cellular membranes and the malfunctioning of the closing and opening mechanisms of the stomates, which is manifested by the decrease of the cellular turgor, the withering and premature drying of the plants (Fig. 10 and Fig. 11).

Knowing the resistance of the plants in front of the polluting gases in the atmosphere is very important when the matter of planting plants in areas with a developed industry is taken into consideration. Certain species are more sensitive and can serve as bioindicators of the atmospheric pollution, while other species are more resistant and can serve as acumulators, which incorporate great quantities of pollution whitout affecting their growth and the development of the plants.

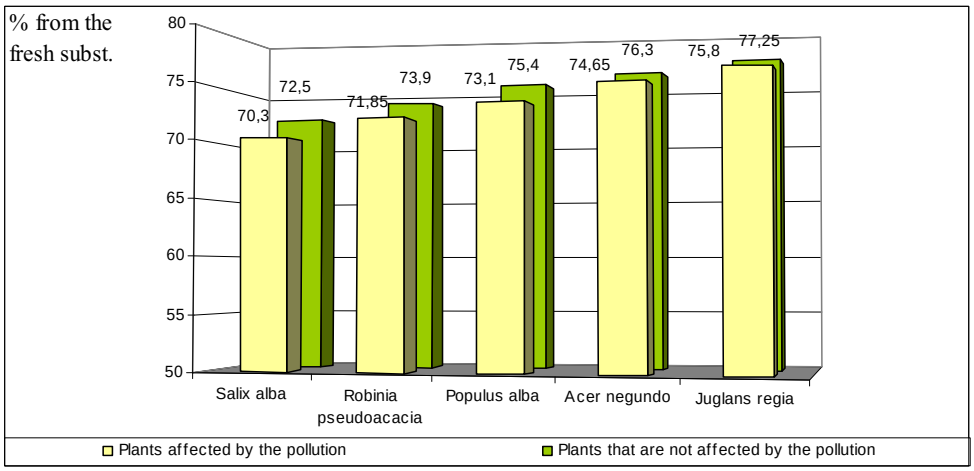

Fig. 10. The total water contents.

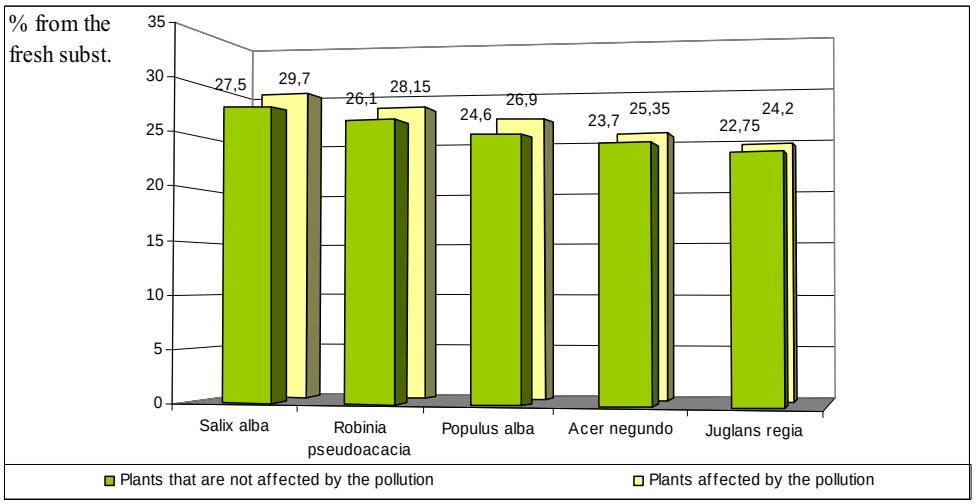

Fig. 11. The dry substance contents.

CONCLUSIONS

After the physiological research regarding some plants affected by the pollution the following have been discovered: the decrease of the photosynthesis as a result of the reduction of the assimilation surface and the inhibation of some biochemical reactions and the increase of respiration due to the deterioration of the integrity of the defense tissues, the intensification of the activity of the respiratory enzymes, which determines a reduced accumulation of organic substances and a deterioration of the plants with consequences on the growth and on the development; the increase of the transpiration and the decrease of the total water contents, witch determines a decrease of the cellular turgescence the withering and premature drying of the plants.

The target of all the investigations regarding the reaction plants have to different polluting agents, to the concentration of nixes in the entire environment of the phytocoenosis and ecosystems, is that of saving the vegetation and one way of doing this represents selecting those species that are more resistant.

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PRELIMINARY DATA ABOUT THE ICHTHYOFAUNA OF THE AMARADIA RIVER

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KEY WORDS: ichthyofauna, the Amaradia River, protection

ABSTRACT

The present paper renders the results of the ichthyologic observation for the Amaradia River during 2006-2007. The almost complete lack of data in the area made us gather ichthyologic information. Thus, on their base, we elaborated a preliminary list of 19 fish species (2 acclimatized) grouped into 3 orders: Cypriniformes (16 species), Siluriformes (one species), Perciformes (2 species) and 5 families (Cyprinidae, Cobitidae, Siluridae, Percidae, Centrarchidae). Among these, 9 species can be found on different national and international lists, the purpose of this action being the protection of the species, as well as of their geographical area (Gobio kessleri, Leuciscus leuciscus, Rhodeus sericeus amarus, Alburnoides bipunctatus, Chondrostoma nasus, Barbus barbus, Cobitis taenia, Misgurnus fossilis, Silurus glanis).

INTRODUCTION

The Amaradia River springs from the Oltenia sub-Carpathian hills (Cărbuneştilor Hills – The Getic sub-Carpathians) from an altitude of about 490-549 meters. The surface of the drainage area reaches about 879 square kilometers and its length is of 106 kilometers; the river crosses Gorj and Dolj counties. It is a left-side tributary of the Jiu and the river mouth is located near Troaca village (Işalnița commune), North of Craiova city. The Amaradia River flows from North to South and receives many tributaries. Thus, its drainage area is quite complex as the river crosses many landforms.

The main tributaries of the Amaradia are: the Gâlceşti stream, the Negreni stream, the Totea stream – on the right, the Slăvuța, the Amărăzuia, the Plosca – on the left. The Amaradia River has a relatively low discharge. The mean multiannual discharge at the river mouth is of 3.20 cubic meters/second (Ghinea D., 2002). The bedrock is sandy and rocky. Its depth varies between 10 and 70 centimeters, but, from place to place, it can be more than 1 meter. During winter months, it can freeze.

The Amaradia River was not very well studied till now from the ichthyofauna point of view; the only references are the quotations of the fish species in the area (Bazilescu Elena & al., 1980). The present paper renders the partial results of the research regarding the ichthyofauna of the lower and middle course of the Amaradia River.

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MATERIAL AND METHOD

The collecting of the ichthyologic material from the Amaradia River was achieved in four sampling points, in February, August, and October 2006 and in July, September, and October 2007. The sampling points are located on the main course of the Amaradia River: Cruşeț (Gorj), Melineşti, Negoieşti, Şimnicu de Sus (Dolj).

The collecting was made with simple fishing rods with cork, easy semi-automatic fishing rods, and bagnet (sacket).

For the elaboration of this paper, I want to thank to: Ms. Gavrilescu Magdalena for supplying valuable information regarding the ichthyofauna of the Amaradia River; Family Ciobănescu Petre and Gheorghița from the National Administration Romanian Waters – Craiova who offered me the possibility of consulting important data regarding the present state of the Amaradia River; the amateur fishermen from the area, who gave me the ichthyologic material, as well as numerous valuable data referring to the presence of fish in the Amaradia.

RESULTS AND DISCUSSIONS

The collected species were identified on the base of the morphologic characters, using the determination keys for each systematic unit and the description of the respective species found in the literature in the field (Bănărescu P., 1964).

We drew up the systematic list of fish species of the Amaradia River. It was noted: the zoo-geographical origin, their distribution and frequency in the Amaradia. At the same time, there were specified the species found on different protection lists, which present a certain conservation status.

SUPRACLASS: Pisces
CLASS: Osteichthyes (Goodrich, 1909)
SUBCLASS: Actinopterygii (Cope, 1871)
INFRACLASS: Teleostei
I. Order Cypriniformes (Goodrich, 1909)
Suborder Cyprinoidei (Berg, 1940)
1. Family Cyprinidae (Yordan et Evermann, 1896)

(1) *Alburnus alburnus* (Linnaeus, 1758) -bleak-

It is a central East European element (Vasiliu and Şova, 1968). It is a common and numerous species. The species presents an increasing tendency. There are not stipulated special protection measures.

(2) *Alburnoides bipunctatus* (Bloch, 1782) -spirlin, schneider-

It is of European-Ponthic-Caspian-Aral origin (Vasiliu and Şova, 1968). It is a rare species along the lower and the middle course of the river. The collecting was made in the area of Negoieşti and Melineşti. Although we have some information about its presence in the upper course, its number considerably reduces. It is a protected species.

(3) *Barbus barbus* (Linnaeus, 1758) -barbell-

It is a Danube West-European element (Vasiliu and Şova, 1968). It is a quite frequent species in the lower and middle course of the river.

(4) *Carassius auratus gibelio* (Bloch, 1782) -gold fish-

It is an East-Asian element (Vasiliu and Şova, 1968). It is numerous in the lower and middle course of the river.

(5) *Chondrostoma nasus* (Linnaeus, 1758) - nase, sneep-

It is a Northern and central European species (Vasiliu and Şova, 1968). It is a rare
species, noticed in the lower course of the river, 5-10 kilometers away of the confluence of the Amaradia with the Jiu. It presents a small number.

(6) *Cyprinus carpio* (Linnaeus, 1758) - carp -

It is an European species (Vasiliu and Şova, 1968). The wild form originates in the Ponthic-Caspian-Aral basin. The carp appears all along the river and it is quite numerous. It has a lower frequency than that of the gold fish.

(7) *Gobio gobio obtusirostris* (Valenciennes, 1844) -gudgeon-

It is an endemic element for the Danube's basin (Vasiliu and Şova, 1968). It was fished in the area of Cruşet. The fishermen sustain the existence of the species in the upstream part of the river and in the Amărăzuia stream. It is characterized by a great morphologic variability.

(8) *Gobio kessleri* (Dybowsky, 1862) -kessler' s gudgeon-

It is of South-East European origin (Vasiliu and Şova, 1968). Samples of Keller's gudgeon have been captured at Negoieşti. Its number is reduced.

(9) *Leuciscus cephalus* (Linnaeus, 1758) -chub-

It is a Central European species (Vasiliu and Şova, 1968). Common species, it appears all along the Amaradia River. It is appreciated by the sportive fishermen as for its capturing there are used a wide range of natural baits.

(10) *Leuciscus leuciscus* (Linnaeus, 1758) -dace-

It is an European element (Vasiliu and Şova, 1968). It appears less than the chub.

(11) *Pseudorasbora parva* (Temmink & Schlegel, 1842) -topmouth gudgeon-

It is a species of East-Asian origin. It was introduced after 1990. It is frequently met in the river and it displays a great number.

(12) *Rhodeus sericeus amarus* (Bloch, 1782) - bitterling -

It is a Palearctic element (Vasiliu and Şova, 1968). It was noticed 10-20 kilometers upstream the river mouth. Its number is reduced in the lower course of the river.

(13) *Rutilus rutilus* (Linnaeus, 1758) -roach-

It is a Danube endemic element (Vasiliu and Şova, 1968). The species appears in the lower and middle course.

(14) *Scardinius erythrophthalmus* (Linnaeus, 1758) -rudd-

It is an element of Central and East European origin (Vasiliu and Şova, 1968). The species displays quite a great number of individuals in the lower river.

2. Family Cobitidae

(15) *Cobitis taenia* (Linnaeus, 1758) -spiny loach, spined loach-

It has a Palearctic origin (Vasiliu and Şova, 1968). It is quite an frequent species. It is active especially during the night.

(16) *Misgurnus fossilis* (Linnaeus, 1758) –weatherfish, weather loach-

It is an European-Ponthic-Caspian-Aral species (Vasiliu and Şova, 1968). It is rare in the lower river.

II. Order Siluriformes (Berg, 1940)

3. Family Siluridae (Regan, 1911)

(17) *Silurus glanis* (Linnaeus, 1758) -wels catfish-

It is an East European-Ponthic-Caspian-Aral species (Vasiliu and Şova, 1968). Rare species, the wels catfish is reduced as number in the lower river.

III. Order Perciformes (Bertin et Arambourg, 1958)

4. Family Centrarchidae (Regan, 1913)

(18) *Lepomis gibbosus* (Linnaeus, 1758) -pumpkinseed-

It is a species of North American origin. It is noticed in shallow areas with vegetation. The species is increasing in number. It is captured in shallow areas with vegetation and in areas where the flow is reduced. From the acquired information, it seems that the species appeared in the Amaradia in 1980-1985.

5. Family Percidae (Jordan et Evermann, 1898)

(19) Perca fluviatilis (Linnaeus, 1758) -perch-

It is an European-Siberian species (Vasiliu and Şova, 1968). The perch is numerous in the middle and lower course of the river. The fishermen informed me that it can be found in the upper course, as well.

In the systematic catalogue of the vertebrates' collection from the Oltenia Museum (Bazilescu Elena & al., 1980), it is mentioned that three fish species were collected from the Amaradia River. These species are: *Leucaspius delineatus* – a protected species (the collecting point Goeşti – Dolj County in 1974), *Carassius carassius* – endangered species (Stoina – Gorj County in 1967), *Sabanejewia romanica* – vulnerable species (Căpreni in 1967, Târgu Logreşti in 1975 – Gorj County and Goeşti in 1974 – Dolj County). I did not noticed them in the above-mentioned collecting points.

Upon the Amaradia River there act a series of environmental and man-induced factors that endanger the fish within the studied area. Among these, we mention drought, pollution, water catchments, construction of bridges, deviations of the river course, excessive deforestation, floods caused by rains and snow thawing, colmatation, poaching and illegal fishing etc. A major negative role upon the aquatic fauna is played by pollutants. We mention the industrial pollutants from Işalnița Chemical Plant or S.C. Petrom S.A. For example, in November 2007, 1,000-1,500 liters of oil flew in the Amaradia River, Gorj County, due to a S.C. Petrom pipe breaking in the proximity of Stoina settlement. The river was polluted on 5 kilometers and about 800 squire meters of a terrain were covered by this product. The accidental pollutants are represented by the substances used for sprinkling the crops in the area. The pollution with domestic waste, garbage, has been much frequent in the last years. The water catchment necessary for Işalnița Chemical Plant leads to the decrease of the river discharge. In dry years, this fact makes the river get dry in certain sector. All these bring to the reduction of fish species, which become vulnerable to poaching and the reproduction do not occur in optimal conditions any more. The land improvement works led to the cutting of numerous trees. The construction of bridges in different areas along the river brought to the retreat of certain fish species from the respective area. The deforestation of the banks generates a more intense evaporation along the river, which means a decrease of the discharge, lack of oxygenation in the water, and, of course, the reduction of the fish number. Sometimes, there occur floods, which are accompanied by landslides. During floods, the fish place of refuge and protection disappears leading thus to the
reduction of the fish populations.

The reduction of the number of certain fish species during the last years imposes the elaboration of a complex program of measures, which is achievable if local factors are involved, as well: the reduction of pollution, the monitoring of the river in order to preserve the water quality, the preservation of certain long sectors of the river under its natural regime, the suspension of the construction of new bridges, the reforestation of the riverside coppices, the control of illegal fishing, the prohibition of fishing small fish etc.

Of the total number of species identified in the Amaradia River, 9 species are found on different national and international lists as they have a certain degree of endangerment (Table 1). The references were made taking into account the following documents: - Berne Convention from the 19th of September 1979 on the protection of wildlife and European habitats, implemented through Law 13/1993, where in Annex 3 there are rendered the protected species; - Habitats Directive from the 21st of May 1992 referring to the conservation of the natural habitats and of the wild flora and fauna, where: Annex 2 – comprises species of community interest for the conservation of which there are necessary special areas of conservation, Annex 5 – comprises species of community interest the exploitation and drawing of which may represent the object of the management measures; - The Urgent Decree no. 57 from the 20th of June 2007 regarding the regime of natural protected areas, the conservation of natural habitats, of wild flora and fauna, where Annex 3 comprises species the conservation of which requires the denomination of special conservation areas, Annex 5A – comprises species of community interest the drawing and exploitation of which make the object of certain management measure; - The Red Book of Vertebrates from Romania (Botnariuc N. and Tatole V., 2005).

Table 1

Fish species in the Amaradia River that are found on different protection lists

Nr. Crt.	Species	The document where the species is included	Annex/Category of endangerment
1.	*Gobio kessleri*	Berne Convention Urgent Decree Red Book of Romania	Annex 3 Annex 3 Vulnerable species
2.	*Leuciscus leuciscus*	Red Book of Romania	Endangered species
3.	*Rhodeus sericeus amarus*	Berne Convention Habitats Directive Urgent Decree	Annex 3 Annex 2 Annex 3
4.	*Alburnoides bipunctatus*	Berne Convention	Annex 3
5.	*Chondrostoma nasus*	Berne Convention	Annex 3
6.	*Barbus barbus*	Habitats Directive	Annex 5
		Urgent Decree	Annex 5A
7.	*Cobitis taenia*	Berne Convention Habitats Directive Urgent Decree	Annex 3 Annex 2 Annex 3
8.	*Misgurnus fossilis*	Berne Convention Habitats Directive Urgent Decree	Annex 3 Annex 2 Annex 3
9.	*Silurus glanis*	Berne Convention	Annex 3

CONCLUSIONS

In the four collecting points, there were identified 19 species of teleost fish that belong to 3 orders (Cypriniformes, Siluriformes, and Perciformes) and to 5 families (Cyprinidae, Cobitidae, Siluridae, Percidae, Centrarchidae). Of the total number of species, 2 are acclimatized: *Pseudorasbora parva* and *Lepomis gibbosus*

The species with a great number of individuals are: *Alburnus alburnus, Carassius auratus gibelio, Pseudorasbora parva, Scardinius erythrophthalmus*, *Rutilus rutilus,, Cobitis taenia, Perca fluviatilis*. Among the species that are rarely met, we mention:

Alburnoides bipunctatus, *Chondrostoma nasus, Gobio kessleri, Rhodeus sericeus amarus, Misgurnus fossilis, Silurus glanis*.

From the zoo-geographical point of view, there predominate the European species followed by those of Euro-Asian, Asian, Palearctic, and North-American origin.

Among the species identified in the Amaradia River, 9 are found on different national and international lists, which represent instruments of the strategies for the conservation of the natural environment at a national, European, and global level (*Gobio kessleri, Leuciscus leuciscus, Rhodeus sericeus amarus, Alburnoides bipunctatus, Chondrostoma nasus, Barbus barbus, Cobitis taenia, Misgurnus fossilsi, Silurus glanis)*.

Upon the Amaradia River, there act a series of both environmental and man-induced factors, which determine important changes of the ichthyofauna and of the aquatic habitat. The development and maintenance of the fish effectives within the Amaradia River may be achieved by respecting the legal norms related to environment protection and by increasing the level of ecologic consciousness of the population in the area.

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THE INCIDENCE OF THE *STAPHYLOCOCUSCUS AUREUS* INFECTION IN A NEWBORN SECTION

Daniela Ciupeanu[1]

KEY WORDS: Staphylococuscus aureus, infection, newborn

ABSTRACT

In our days the nosocomial infections have an universal character, these being considered to be one of the major problem of populational health for all the medical-sanitary or medical-social services.

Staphylococuscus aureus it is the most encountered germ, being implyed in a percentage of 80% in the human festering infection. Due to the tropism marked for the dermic tissue, the pathogen Staphylococus produce especially infection at the tegument level and skin annexes, but they can invade any other tissue or organ.

The study focused on the isolation of the Staphylococus strains incriminated as posible ethyologic agent of some infections develop at the hospitalized patients. For this there has been made a case selection, collection of the pathological products, their processing and incubation in order to isolate the germs in pure culture.

The second stage consist in the identification of the isolated germs and their differentiated dyagnostication with the related species, and in the third stage there has been made the differentiation, from the epidemiological point of view of the identified strains.

INTRODUCTION

In our days the nosocomial infections have an universal character, these being considered to be one of the major problem of populational health for all the medical-sanitary or medical-social services.

The apperanace of the microbian resistance to the antibiotics has constitute an alarm signal with multiple resonances, between these the reconsideration of some eronated preventive concepts like „the antibiotics resolve all the problems of the infection.

Starting from 1960, there has been appear more and more information regarding the isolation of resistant strains to the antibiotics. In this period *Staphylococuscus aureus* resistent to meticiline, was allready implyed in the nosocomial infection from Europe and U.S.A.. During the decade 1965-1975 over 50% from the epidemic outbreak of nosocomial infection had as ethiology, various species of *Enterobacteriaceae* species multiple resistant.

Staphylococuscus aureus it is the most encountered germ, being implyed in a percentage of 80% in the human festering infection. Due to the tropism marked for the dermic tissue, the pathogen *Staphylococus* produce especially infection at the tegument level and skin annexes, but they can invade any other tissue or organ.

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MATERIAL AND METHOD

The study focused on the isolation of the *Staphylococus* strains incriminated as posible ethyologic agent of some infections develop at the hospitalized patients. For this there has been made a case selection, collection of the pathological products, their processing and incubation in order to isolate the germs in pure culture.

The second stage consist in the identification of the isolated germs and their differentiated dyagnostication with the related species, and in the third stage there has been made the differentiation, from the epidemiological point of view of the identified strains.

The hemoculture samples, has been incubated for 24 hours at 37^0C, after that there has been made native smear, and Gram coloured smear. The microscopical examination has been followed to a prime passing on solide medium .

The inseminated medium has been introduce in the thermostate, at 37^0C and incubated for 24 hours. The samples has been checked daily, making subcultures in the days 3, 5, 7, and 14 after collection.

RESULTS AND DISCUSION

Staphylococcus aureus has been the germ isolated prevalent in the microbian flora evidentiated in the salubrity samples from the researched section of new borns, reaching a maximum percentage of (66,66%) in september, comparative with the next months.

The insufficient space (crowded ward), with a big number of beds (10 wards with over 25 beds/ward) has repercussion on the high percentage of samples nonconform of aeromicroflora at the level of new borns section during 2007 (Table 1).

Table 1

The control of the aeromicroflora at the level of new borns section (01.09.2007-31.12.2007)

Month	Nr. prevailed samples	Nr. of nonconform samples	% of nonconform samples
September	12	8	66,66
October	9	2	22,22
November	8	3	37,5
December	6	1	16,66

From the data presented in the table it come out that the microbiological tests of the salubrity control and the aeromicroflora from the new born section have given the alarm signal for the appearance of the conditions with infectious potential..

The microbial portage

In the hotbed, there has been prevailed sample (pharyngeal and nasal exudate) from new borns, mothers and medical personnel (Table 2).

The portage of *Staphylococcus aureus* at the new borns from the hotbed bacteriological investigated has been high (62,79%) comparatively with the mothers and medical personnel evidentiating the susceptibility of the new borns to the staphylococ infection. At mothers the portajul of *Staphylococcus aureus* (35,48%) has not presented statistically significant differences with the portage of this germ at the level of the medical personnel.

Table 2

The portage of *Staphylococcus aureus* in the epidemic hotbed (pharingeal and nasal exudate)

The provienence of the sample	Nr. of prevailed samples	Nr. of positive samples with *S. aureus*	%
New borns	16	9	62,79
Mothers	11	4	35,48
Medical personnel	8	3	29,26
Total	35	16	46,5

The microbiological investigation of the hotbed

There has been send for bacteriological investigation to the Cantacuzino Institute Bucureşti 70 strains of *Staphylococcus aureus* proceed from: children (38 strain), mothers (18 strain), medical personnel (10 strains) salubrity samples (2 strains), aeromicroflora (1 strain), powder milk (1 strain) . The presence of the *Staphylococcus aureus h*as been identified in the salubrity samples at the level of the following surface: dipers box (1 strain), powder (1 strain), nursing equipment (1 strain) and incubator (1 strain).

From the 70 strains send for identification, has been confirmed (through laboratory investigation) the presence of the *Staphylococcus aureus* positive-coagulated at 63 from these ones (92,15%), in 3 cases (2,94%) being isolated *Staphylococcus aureus* negative-coagulated and in 4 cases (3,92%) Gram negative bacilus.

The number of the confirmed samples with the presence of *Staphylococcus aureus* has been higher in the pharyngeal exudate (35 positive samples) comparatively with nasal exudate (19 negative samples) both at the children and at the mothers or medical personnel.

The antibiotic sensibility

The antibiograms made through the difusimetric method at the Cantacuzino Institute has emphasized that the germs sensibility to the antibiotic has been quite good. In the studied hotbed 86.02% of the staphylococs has been sensible to Meticiline, 81.72% has been sensible for Gentamicine and 100% has been sensible to Vancomicine .

Codiță I. And others has emphasized variable percentage of *Staphylococcus* resistant to Meticiline function the hospital, ranged between 7.43% and 39.70, with a resistance to Licine over 50% .

Isolated in the hotbed, the 93 tested strains of *Staphylococcus aureus* have presented a very high resistance to Peniciline, Tetracicline, Kanamicine, with a sensibility of the staphylococs determined through difusimetric method of: 3.22%, 19.35 respectively 24.73, these antibiotics being practic ineficient in the staphylococus infection.

Regarding the repartition of the isolated strains of *Staphylococcus aureus,* function the sensibility to Oxaciline and aminoglicozide the distribution has been the following: 77.41% strains sensible to Meticiline, sensible to Aminoglicozide, 8.60% strains resistant to Aminoglicozide, 4,3% strains resistant to meticiline and resistant to Aminoglicozide and 9.67% strains very resistant Meticiline and Aminoglicozide.

The staphylococus sensible to Meticiline isolated in the studied hotbed has been resistant to Gentamicine only 10% comparative with the Staphylococs resistant to Meticiline from the same hotbed that have presented a much higher resistant to Gentamicine 69.24%.

We continued the observation of the staphylococus strains isolated from the hotbed with resistance to Meticiline under the aspect of their apartanenace to the phagic group, the sample proveninence and their sensibility to other tested antibiotics.

The most of the *Staphylococcus aureus* strain with resistance to Meticiline has been isolated at the children (46.15%), at the medical personnel (30,76%) and in percentage of 23.07% at mothers.

The Staphylococus resistant to Meticiline has been sensible to Gentamicine only in a percentage of 1.76%, presenting a total resistance to Peniciline, Streptomicine, Tetracicline. The sensibility for Eritromicine (38.46%) and Cefuroxine (69.23%) has been reduce comparatively with the Staphylococs sensible to Meticiline but there has been maintained a percentage of 100% for Kinolone, Vancomicine, Sydic acid and quite good to Clindamicine.

According to these data, there are many studies that consider the resistance to Meticiline as a marker of poli-resistance to antibiotics including cephalosporine and Eritromicine.

CONCLUSIONS

The microbiological sample collected from the hospital environment have identified the risk situation for the appearance of the nosocomial infection and the type of the microbian agents in each medical unit.

The presence of the pathogen agents at the hand levell, as a risk factor known in the criss cross transmition of the nosocomial infection, has been identified in a higher percentage on the mothers hand comparatively with the medical personnel hands in the newborn sections.

The study of the epidemical hotbed has identified the following causes favorisant for the appearance of the nosocomial infection under the epidemical form

- crowded ward with high number of beds and inadequate compartimentation;
- deficiencies in the cyclic disinfection of the ward;
- unaccordingly isolation of the cases;
- the presence of the carriers of pathogens germs;
- the presence of the pathogen germs at the levell of the surfaces in the hospital environemnt;
- the uneficient sanitary education reagrding the risk of the disease transmition.

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SONOGRAPHIC AND HISTOLOGIC CORRELATIONS OF THE HYPERPLASTIC ENDOMETRIUM

Diana Olimid, D. A. Olimid[1]

KEY WORDS: endometrium, hyperplasia, ultrasonography, biopsy

ABSTRACT

Transvaginal ultrasonography has a significant role in the assessment of the endometrium and it can help in the differentiation between benign and malignant conditions. The main indication for transvaginal US evaluation of the endometrium is abnormal premenopausal or postmenopausal bleeding. A correlative sonographic and histopathologic analysis was performed in 64 women with a histologic diagnosis of endometrial hyperplasia.

INTRODUCTION

Transvaginal ultrasound (US) is now the standard investigative tool for evaluating the endometrium. The use of transvaginal US has significantly improved the ability to resolve the normal and abnormal endometrial echotexture. It has been to be sensitive in depicting endometrial abnormalities.

An abnormal endometriale stripe was defined as either irregular in shape or echotexture. The cardinal sonographic sign of endometrial hyperplasia or carcinoma is an abnormally thickened endometrium.

Endometrial thickness varies throughout the menstrual cycle, ranging 3 to 14 mm and a thickened stripe may not indicate pathology. In postmenopausal women, endometrial stripe measurements by US are used to exclude endometrial abnormalities, especially endometrial neoplasia.

A sonographically thin (less than 5 mm) endometrial stripe has been correlated microscopically with inactive postmenopausal endometrium. Transvaginal US permite accurate measurement of the endometrial stripe and detailed analysis of endometrial echotexture. US is used in the evaluation of abonormal uterine bleeding. The major clinical application of transvaginal US of the endometrium is which women with abnormal uterine bleeding should undergo endometrial biopsy.

MATERIAL AND METHODS

The study was performed on a series of 64 women with abnormal uterine bleedings, who had undergone transvaginal ultrasound before endometrial biopsy.

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Tissue for histologic examination was available in all 64 pacients. The specimens consisted of endometrial curettage tissue and uteri from hysterectomies. In all instances, the microscopic slides were correlated with the sonographic appearance of the endometrium.

Transvaginal US was performed by using a 6,5 MHz endovaginal trasducer. The uterus was scanned in the sagittal plane from the lateral aspect on one side to the lateral aspect on the other side. The endometrial stripe was measured at its maximum anteroposterior thickness along the longitudinal axis of the uterine body.

The measurement included both anterior and posterior endometrial layers. It was obtained by placing electronic calipers at the anterior and posterior uterine walls at the margins of the basal layer of the endometrium delineated by the echogenic interface between endometrium and inner myometrium. In the presence of intrauterine fluid, the thickness of endometrial layers were measured separately and summed. Care was taken not to include the hypoechoic subendometrial halo. The following endometrial findings were evaluated: endometrial thickness, echogenicity, smoothness or irregularity of the contour, definition of the contour, and the presence of cystic areas.

We evaluated, also, for each case, the ovarian follicular activity.

RESULTS AND DISCUTIONS

The endometrial thickness ranged from 5 to 20 mm, with a mean of 13 mm. Analysis of endometrial echotexture demonstrated three distinct sonographic patterns.

Pattern 1

The most common ultrasonographic pattern was represented by an inhomogeneous thickned endometrium. In the saggital plane, the endometrium appears like an echogenic triangle with hyphechoic areas within, which represents infoldings of the mucosa (fig. 1, 2).

Correlation with the pathologic specimens showed a simple hyperplasia in 5 cases and a complex hyperplasia in 6 cases.

Pattern 2

Another sonographic pattern was represented by an echogenic, relatively homogeneous endometrium, corresponding to the median line of the uterine cavity. The appearance was similar to that from the secretory phase of the menstrual cycle (fig. 3, 4).

Pathologic correlation revealed the presence of simple hyperplasia in 19 cases, complex hyperplasia in 8 cases and one case of atypical hyperplasia. In three cases fluid distended the endometrial cavity (fig. 4).

Pattern 3

The third pattern was represented by an echogenic endometrium with clearly anechoic areas (small cysts). The cysts varied in size from 1 to 5 mm and in number and it represent the dilated endometrial glands (fig. 5). Pathologic correlation revealed a complex hyperplasia in 13 cases, simple hyperplasia in 9 cases and atypical hyperplasia in one case.

In a percentage of 35,94% we noticed the presence of cystical formations at the ovary level, their existence being correlated with the appearance of hyperplastic lesions.

Endovaginal scanning combined with color Doppler evaluation of the endometrial blood flow has shown the absence of endometrial vascularity in all cases of endometrial hyperplasia. The Doppler evaluation of the uterine artery shown values of resistance index over value of 0,5 (fig. 6).

The presence of low-impedance blood flow within the endometrium indicate tumor neovascularity associated with endometrial cancer. Therefore Doppler evaluation has been advocated as a method to differentiate benign endometrial lesions from endometrial malignancies.

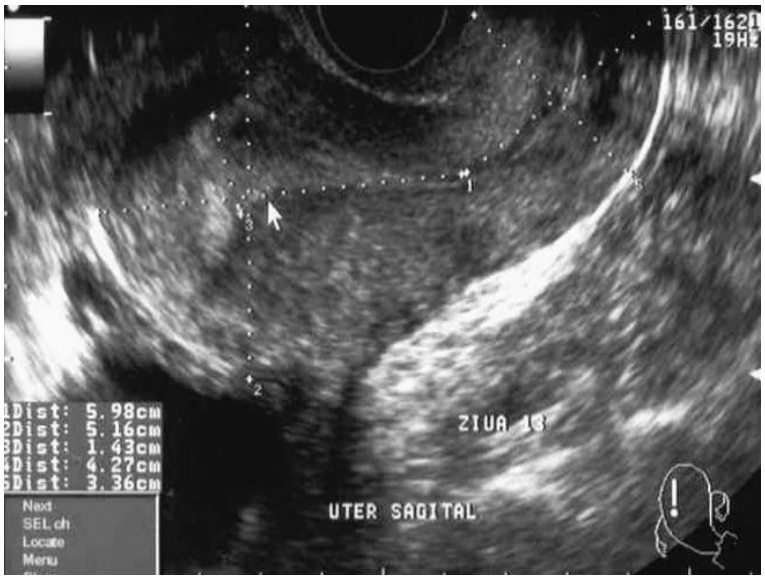

Fig. 1. Sagittal endovaginal sonogram of the uterus - complex hyperplasia

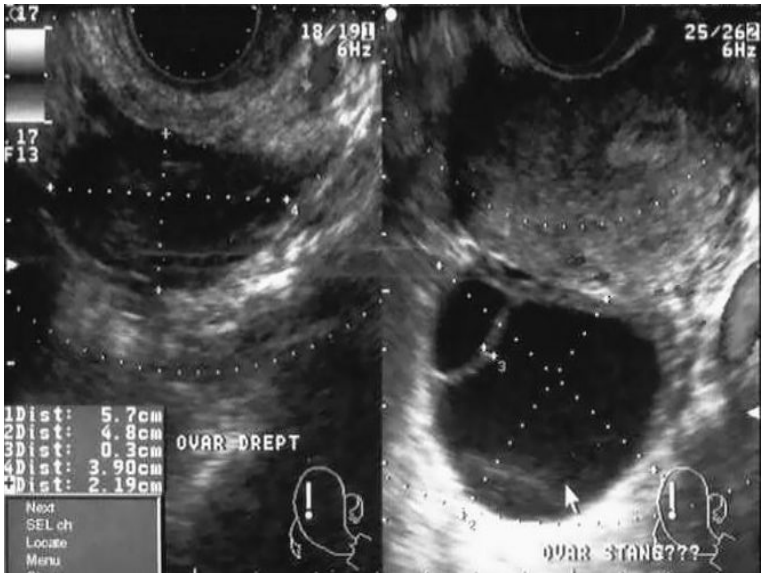

Fig. 2. The same case - the appearance of ovaries

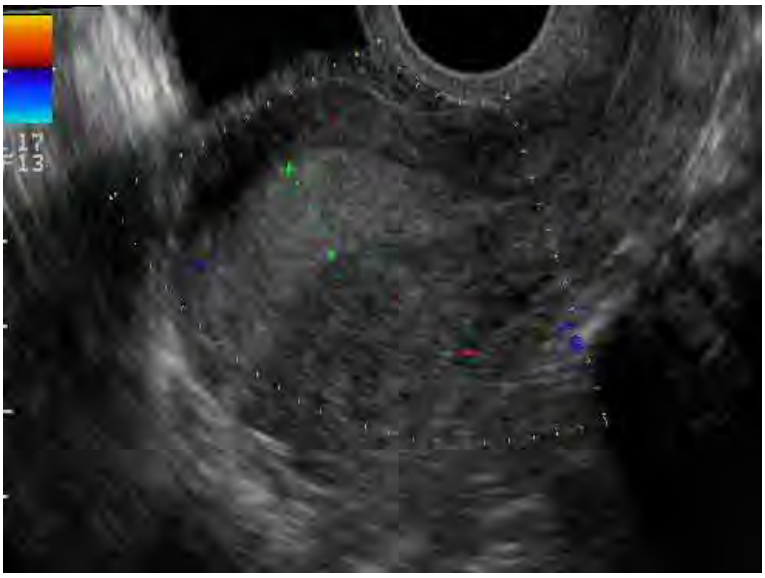

Fig. 3. Sagittal endovaginal sonogram of the uterus - simple hyperplasia

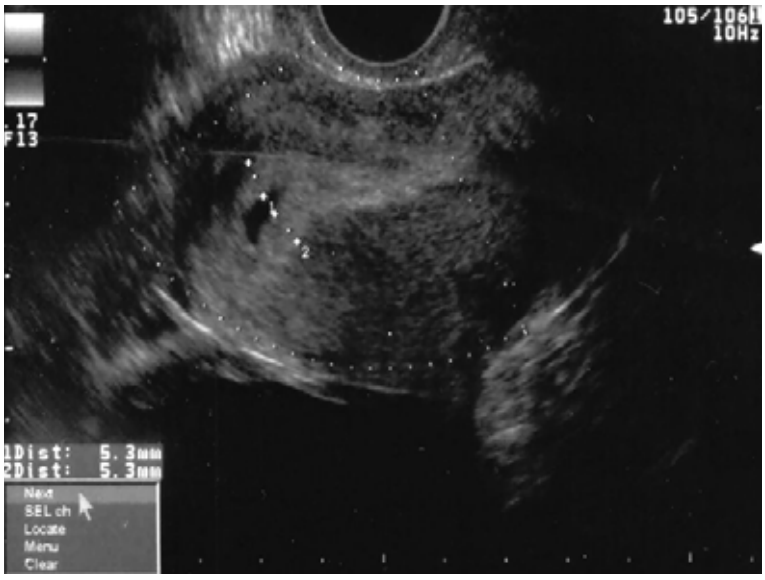

Fig. 4. Sagittal endovaginal sonogram of the uterus -
endometrial cavity distended with fluid, complex hyperplasia

Fig. 5. Sagittal endovaginal sonogram of the uterus - complex hyperplasia with cystically dilated glands

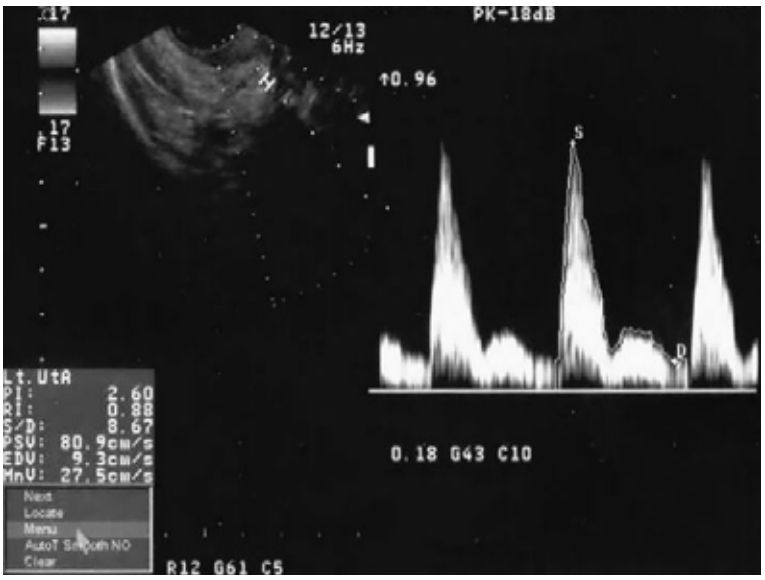

Fig. 6. Doppler flux - uterine artery (resistance index = 0,88)

CONCLUSIONS

The ultrasonographic appearance correlated with the histological shape of the lesions was variable, the most frequent aspect encountered in the case of simple hyperplasia being represented by the hyperecogenic diffuse and homogenous thickening of the endometer, similar to that from the secretory phase of the menstrual cycle; for the forms of complex hyperplasia, the predominant aspect was that of an hyperecogenic and non-homogenous endometer characterized by the presence of regulated anecogenic phases, with the diameter between 1-5 mm.

Atipical hyperplasia did not present from an ecographic point of view nothing special in comparison to the other forms of hyperplasia.

The endometrial stripe thickness alone should not be used to exclude benign endometrial masses in premenopausal women.

We conclude that sonographic evaluation of abnormal uterine bleeding should be following by performing an endometrial biopsy.

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ANALYSIS OF PROLIFERATING CELL NUCLEAR ANTIGEN EXPRESSION IN HYPERPLASTIC AND MALIGNANT ENDOMETRIUM

Diana Olimid, D.A. Olimid[1]

KEY WORDS: endometrium, immunochemistry, PCNA

ABSTRACT

Cell proliferation is a fundamental biological activity, which plays an important role in both physiologic and pathologic processes. Quantification of the PCNA protein with immunohistochemical techniques is thought to provide a measure of cell proliferation. We studied the proliferative activity of normal, hyperplastic and neoplastic endometrium in an attempt to understand the mechanism of endometrial progression to malignancy.

INTRODUCTION

Various aspects of proliferation within tissues can now be assessed directly or indirectly by a multitude of modalities in tissue sections. These methods include the determination of proliferating cell nuclear antigen (PCNA) by immunohistochemistry.

Proliferating cell nuclear antigen is a nuclear protein that is expressed in the late G_1 phase, peaks in the S phase and persists in the G_2M phases of the cycle. PCNA is a 36-kd DNA polymerase delta auxiliary protein.

It is involved in the proliferation of neoplastic as well as noneoplastic cells and is specifically expressed in proliferating cell nuclei. Immunohistologic methods of assessing cell proliferation have particular advantages because of the maintenance of cellular morphology and tissue architecture, the simplicity of the methodology and the rapidity of the results.

The endometrium is subject to rapid cycling with proliferation, differentiation and breakdown under hormonal influences. The mechanism of carcinogenesis is associated in the most cases with estrogen influence on the mucosal turnover.

It has been suggested that are many steps involved in the progression to malignancy. Molecular changes occurring in the premalignant and malignant conditions have been proposed as an adjunct to diagnosis.

To identify patients with a high risk of recurrence and to prevent unnecessary treatment in patients with low risk disease, prognostic factors need to be defined clearly. PCNA is an independent prognostic factor which correlates significantly with overall survival and recurrence free survival.

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MATERIAL AND METHODS

The material used in this study consisted of 49 endometrial biopsy specimens. The cases studied were divided into the following groups: simple hyperplasia (20 cases), complex hyperplasia (5 cases), complex atypical hyperplasia (2 cases), well-differentiated endometrial carcinoma (12 cases), poorly-differentiated carcinoma (7 cases) and undifferentiated carcinoma (3 cases).

All specimens were fixed in formalin, embedded in paraffin and cut in 3-4-μm-thick sections. An immunoperoxidase method using an avidin-biotinylated horseradish peroxidase complex was used to detect PCNA expression in deparaffinized tissue sections.

The set of slides was incubated with a monoclonal antibody (PC10, Dako, Denmark) diluted 1:50. Positive control tissue sections were prepared from specimens recommended by the manufacturers.

PCNA index was defined as the percentage of cells showing positive nuclear staining. We used 2 chromogens, hematoxylin, which stains all nuclei, and diaminobenzidine, which stains only the nuclei that express PCNA. Proliferating nuclear antigen index was obtained nuclear antigen index was obtained by calculating the percentage of positively staining cells per total of 1000 counted cells. The results were further divided into those cases in which only scattered cells were positive or the majority of cells were positive.

RESULTS AND DISCUTIONS

The study of PCNA expression indicated a positive nuclear staining in all examined cases, but PCNA index was different between the various histologic subgroups, indicating a higher degree of PCNA expression in the worse prognostic categories.

The results of PCNA staining in the various groups studied are presented in table 1.

Table 1

PCNA epithelial index according to the histopathologic type

Lesional type	PCNA index
Simple hyperplasia	30%
Complex hyperplasia	44%
Complex atypical hyperplasia	53%
Well-differentiated endometrioid carcinoma	60%
Poorly-differentiated endometrioid carcinoma	72%
Undifferentiated carcinoma	84%

PCNA positivity differed among the various diagnostic groups.

The hyperplasia groups shows the lowest PCNA count of all groups, with increased values from simple hyperplasia to complex atypical hyperplasia.

Simple hyperplasia had a mean proliferation rate of 30%. The percentage of positive proliferating cells was higher for complex hyperplasia (44%) and complex atypical hyperplasia (53%). The cases of atypical endometrial hyperplasia showed patchy staining. Morphologic observations have suggested that atypical hyperplasia is a patchy process, and this is supported by the finding of patchy immunostaining for PCNA in glands predominantly in the areas with the most marked cytologic atypia (fig. 1, 2, 3).

In normal cycling endometrium, the highest proliferative activity it shows during the proliferative phase and early secretory phase. At stromal level, proliferative activity is low during all phases of the menstrual cycle.

For endometrial carcinoma, the study of PCNA expression showed higher glandular PCNA values then the histopathologic group of endometrial hyperplasia. The morphometric analysis of PCNA showed a significant positive correlation with the endometrioid carcinoma differentiation grade and the endometrial carcinoma subtype.

Expression of PCNA was higher in the poor prognostic categories, represented by undifferentiated carcinoma (84%) and poorly-differentiated endometrioid carcinoma (72%). Staining tented to be uniformely distributed in the glands of adenocarcinoma (fig. 4, 5, 6).

The results coincide with those of others studies, which found that PCNA – positive staining was focally distributed in atypical endometrial hyperplasia and was diffuse in endometrial adenocarcinoma.

In an attempt to understand the mechanism of progression to malignancy, the proliferative activity of normal and neoplastic endometrium has been studied extensively. It was shown that it is as high in benign proliferative endometrium as in endometrial carcinoma. The proliferative activity of endometrial hyperplasia was reported to be variable. PCNA is involved in DNA repair and there is evidence that PCNA immunostaining may occur in cases in which DNA repair rather then proliferation occurs.

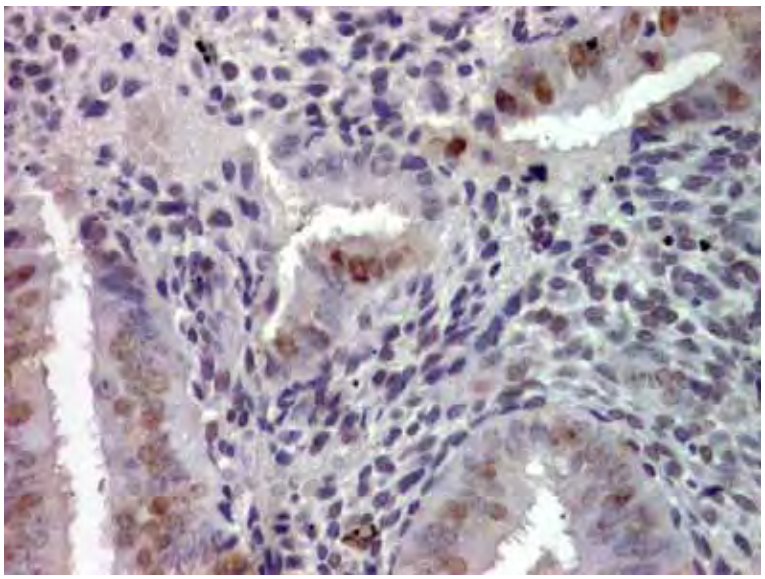

Fig. 1. Immunostaining for PCNA,
simple hyperplasia, X 100

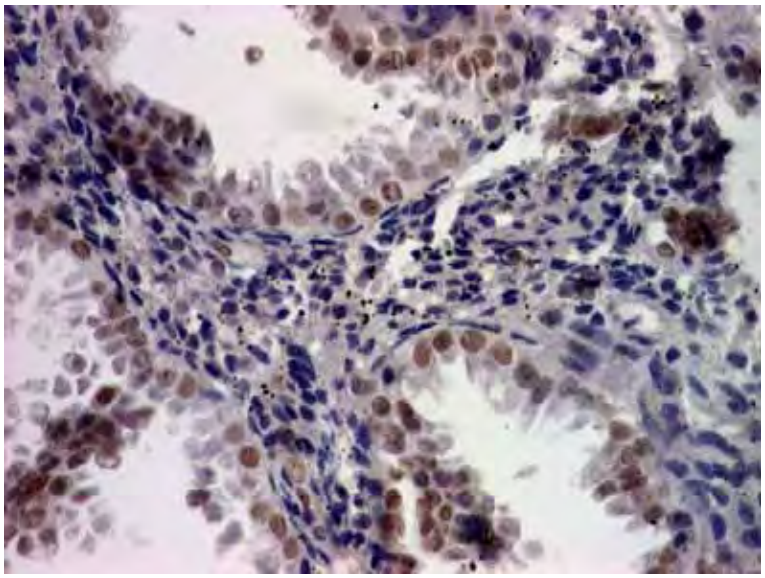

Fig. 2. Immunostaining for PCNA,
complex hyperplasia, X 100

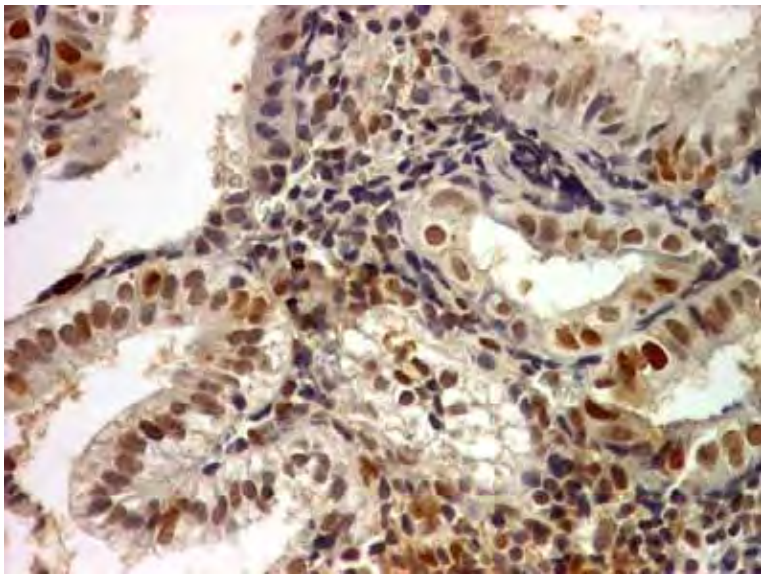

Fig. 3. Immunostaining for PCNA,
complex atypical hyperplasia, X 100

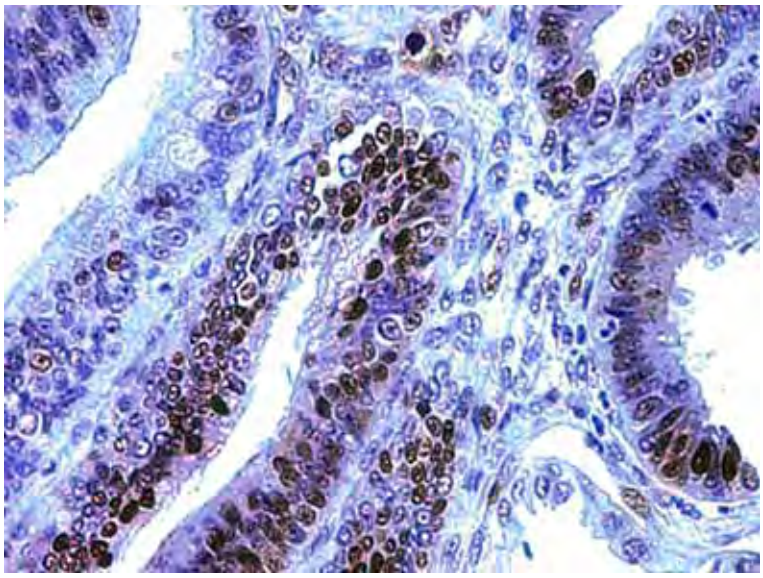

Fig. 4. Immunostaining for PCNA,
well-differentiated endometrioid carcinoma, X100

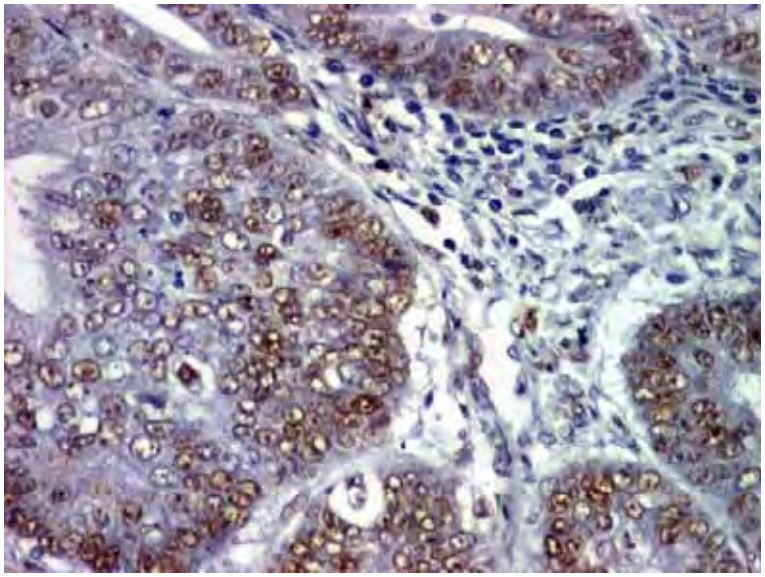

Fig. 5. Immunostaining for PCNA,
poorly-differentiated endometrioid carcinoma, X100

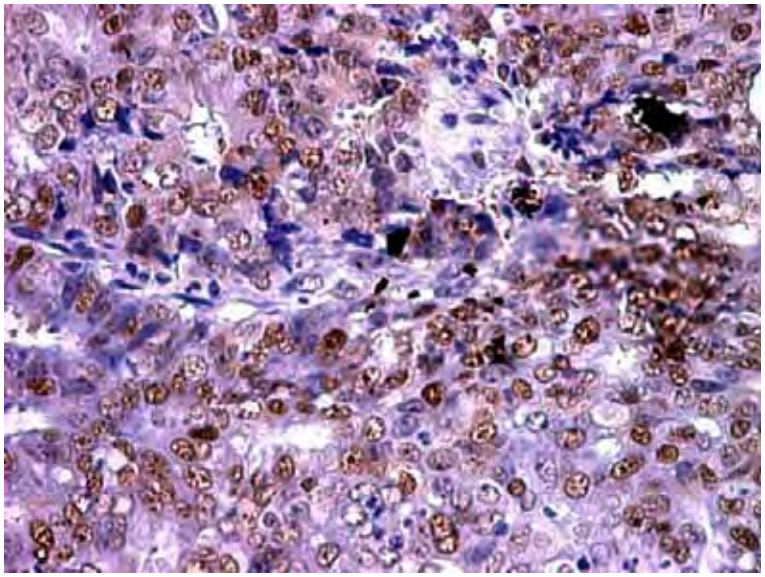

Fig. 6. Immunostaining for PCNA,
undifferentiated carcinoma, X 100

CONCLUSIONS

The immunohistochemical study made on a number of 27 endometrial hyperplasias and 22 endometrial carcinomas intented to emphasize the degree of endometrial proliferation by estimating the PCNA index.

Endometrial hyperplasia PCNA values were the lowest among all the groups. Carcinomas showed higher PCNA values. Expression of PCNA was higher in the poor prognostic categories. The values of PCNA index correlates with the morphologic groups of endometrial pathology. PCNA analysis can be useful in cases of diagnostic problems. The immunohistochemical determination of proliferative activity could contribute to the identification of a high risk subgroup of endometrial carcinomas.

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A CLINICAL-STATISTICAL STUDY OF SCUAMOUS CARCINOMAS OF THE ORAL MUCOSA

D. A. Olimid, Diana Olimid[1]

KEY WORDS: oral cancer, ulcerous, vegetative, infiltrative

ABSTRACT

In this study entitled "A clinical-statistical study of squamous carcinomas of the oral mucosa" for the interval of 4 years between 2003-2006, we noticed an alarming increase of the frequency of oral squamous carcinomas, with a maximum incidence in the 6th and 7th decades of life, affecting especially males (88,62%).One of the macroscopic parameters studied was the shape of malignant neoplasias. In relation to this criterion, we classified the tumours analyzed in one of the following four categories: ulcerous, vegetative, ulcerous – vegetative and infiltrated. The majority of patients were diagnosed in advanced stages of carcinomatous disease, the metastatic adenopathies being present at the first clinical examination in 62,88 % of the patients. We notice the fact that a percentage of 37,72 % of malignant tumours were ulcerous.

INTRODUCTION

Squamous Cell Carcinoma represents more than 90 percent of all head and neck cancers. In this study we analyze a case series of patients with squamous cell carcinoma of the lip and oral cavity, and try to determine the distribution of SCCs according to age, gender, clinicopathological parameters, treatment, recurrence, and survival with statistical methods. Find significant associations between epidemiological, clinical data and survival and find associations between risk factors and prognosis with statistical methods.

MATERIALS AND METHODS

Patients with primary SCC treated between 2003 and 2006 in the Department of Oral and Maxilo-Facial Surgery, Faculty of Dentistry, Craiova, were studied, through direct clinical examination. All tumors were classified according to the International Union Against Cancer (UICC) TNM classification.

Histological grading was done according to WHO classification. Clinicopathological information on each case, including age, gender, tumor size, nodal status, location, treatment, presence or absence of tumor recurrence and survival was obtained from patient record files.

[1] University of Craiova

RESULTS AND DISCUSSION

In this study, for the interval of 4 years, between 2003-2006, we noticed an alarming increase of the frequency of oral squamous carcinomas, with a maximum incidence in the 6th and 7th decades of life, affecting especially males (88,62%) (fig. no. 1).

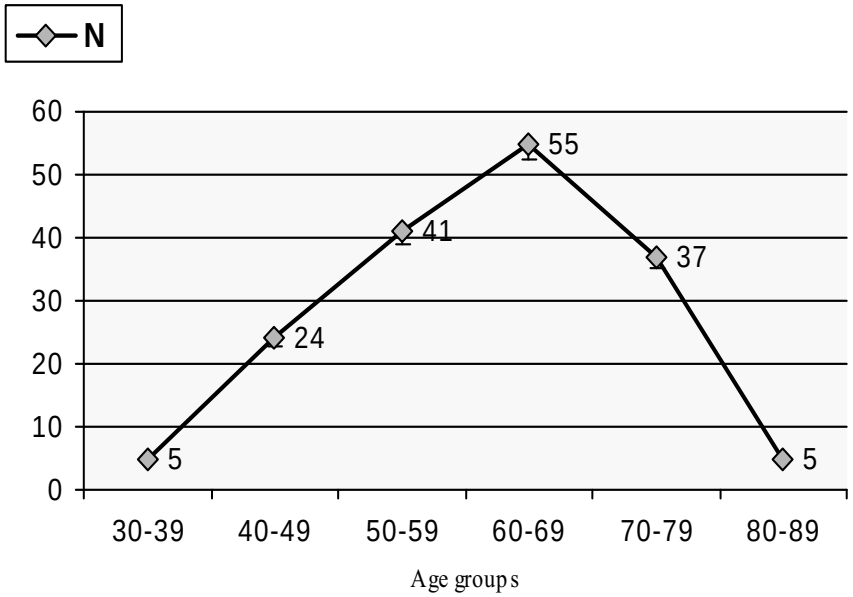

Fig. no. 1. The incidence of oral carcinomas on age groups

Among the risk factors are distinguished their associations, especially alcohol and tobacco, but also alcohol, tobacco, the inflamatory factors and the pre-cancer lesions, in connection to a precarious oral hygiene encountered in 65,88% of the members of the group studied. The investigated tumours presented a frequency of localization in lips (41,32%), followed by localization in tongue (26,94%) and that at the level of the floor (19,76%) (table no.1).

Table no. 1.

Distribution of lesions in connection to topography

Localzation	Lips	Tongue	Palate	Gum	Floor of the mouth
No. of cases	69	45	6	14	33
Percentage%	41,32	26,94	3,60	8.38	19,76

One of the macroscopic parameters studied was the *shape* of malignant neoplasias. In relation to this criterion, we classified the tumours analyzed in one of the following four categories: ulcerous, vegetative, ulcerous – vegetative and infiltrated (table no. 2).

Table no. 2.

The shape of oral malignant tumours

Shape of tumours	Ulcerous	Vegetative	Ulcerous-vegetative	Infiltrated
No. of cases	63	47	39	18
Percentage %	37,72	28,14	23,36	10,78

We notice the fact that a percentage of 37,72 % of malignant tumours were ulcerous**.** We encountered this aspect in 63 cases, with localization in tongue and at the level of lips, especially the inferior lip.

The majority of patients were diagnosed in advanced stages of carcinomatous disease, the metastatic adenopathies being present at the first clinical examination in 62,88 % of the patients.

CONCLUSIONS

- The results of this study made on a number of 167 oral squamous carcinomas selected in an interval of time of 4 years between 2003-2006 shows an alarming increase of the frequency of oral malignant neoplasias especially in the last year.
- The oral malignant neoplasias were encountered in all age groups, with maximum incidence in the 6^{th} and 7^{th} decade, affecting especially the males (88,62%).
- The main environment of origin of patients from the group was the urban environment (67,66%), with a ration of 2/1 towards the rural one , explained by the different diet of the two groups of population, as well as by a more intense action of carcinogens in the urban environment.
- We noted the plurifactorial etiology of oral malignant neoplasias, by distinguishing the association of risk factors, especially alcohol and tobacco, but also alcohol, tobacco, inflamatory factors, pre-cancerous lesions, in the context of a precarious hygiene encountered in 65,88% of the members of the studied lot.
- The interval between the appearance of the lesion and the medical consultation of patients with malignant tumours of the oral cavity was big, taking into account the gravity and the implications of the neoplasia phenomenon on their personal and social life, the majority of patients came to the doctor between 6-12 months from the beginning (42,51%) and over 12 months (38,93%), the patients belonging to social defavoured categories, uninformed regarding the risks they are exposed to or the chronic consumers of alcohol.
- The most encountered macroscopic aspect in the case of oral malignant neoplasias at the beginning, as well as in the status period was the aspect of ***ulceration***, due to direct action of carcinogens at the level of oral mucosa.
- The oral malignant neoplasias were frequently localized at the level of lips (41,32%), tongue (26,94%) and bridging (19,76%).
- The majority of patients came to the doctor in advanced stages of the disease, most of them being diagnosed with tumours T2 and T3 representing 70,59%.

- The tumours had a generally reduced consistence, even friable, the aspect on a section being associated with the presence of necrosis and hemorrhage areas, association present in 22,85% of the patients, in the cases of tumours with a vegetative and ulcerous-vegetative development pattern and rarely in the infiltrated one.
- The metasthatic or reactive adenopathies were present at the first clinical examination in 62,88% of the patients, showing the agressive and lymphophile character of oral carcinomas.

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A HISTOPATHOLOGICAL STUDY OF SQUAMOUS CARCINOMAS OF THE ORAL MUCOSA

D. A. Olimid, Diana Olimid[1]

KEY WORDS: pre-cancer lesions, dysplasia, basal layer

ABSTRACT

In this study we analyzed from a histopathological and histochemical point of view the common and particular aspects of of oral squamous carcinoma researching the histopathological type and the degree of differentiation, the stage of tumour progession, the analysis of the surgical safety margins, the presence of pre-cancerous lesions (dysplasias of different degrees) at the level of epithelium adjacent to areas of carcinoma, coilocytosic modifications associated to neoplasias. The various hyitopathological aspects analyzed are subsequently compared to the results from literature, obtained in other studies; in the final of this study, we underlined the results with relevant aspects.

INTRODUCTION

More than 90% of malignant neoplasms of the oral cavity and oropharynx are squamous cell carcinomas of the lining mucosae with relatively rare neoplasms. The diagnosis of oral precancer and cancer remains a challenge to the dental profession, particularly in the detection, evaluation and management of early phase alterations or frank disease. The majority of cases of SCC present no difficulty in diagnosis for the experienced pathologist. However, the recognition of the earliest stages of invasion can be problematic.

The deepest layers of the epithelium and the interface between the epithelium and the lamina propria need to be examined in detail.

MATERIALS AND METHODS

The formalin-fixed, paraffin-embedded blocks were retrieved from the surgical pathology archives of the Department of Pathology from Emergency Hospital of Craiova. All tumors were classified according to the International Union Against Cancer (UICC) TNM classification. Histological grading was done according to WHO classification. Clinicopathological information on each case, including age, gender, tumor size, nodal status, location, treatment, presence or absence of tumor recurrence and survival was obtained from patient record files. From the risk factors we investigated smoking and alcohol intake history, oral status and urban vs. rural residence. Serial thick sections were cut from the tissue blocks and mounted on silanized slides. One section was stained with hematoxylin-eosin and examined to confirm the original diagnosis and tumor grade.

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RESULTS AND DISCUSSION

From histopathologycal and evolutiv point of view, in direct corelations with the prognostics, the 167 studied cases were represented in 2 cases from carcinoma "in situ"and in 165 cases from invasive scuamous cell carcinoma(table 1).

Table 1.

Distributions of SCC according to invasions depth

Tumor type	**Carcinoma „in situ"**	**Invasive carcinoama**	
		Microcarcinoma	**Invasive carcinoma SCC**
Nr. cazuri	2	3	162
Percentage %	1,2	1,8	97

From the point of view of the histological subtype, most of the net invasive squamous carcinomas were typical non-keratinised and keratinised forms, followed in the order of frequency by the acantholitic form and rarely by variants of these such as: basaloyde carcinoma, carcinoma with the spindle shape and verucous carcinoma (table 2).

Table 2.

Distributions of cases according to histopathologycal type

Carcinoma type	**CS keratinised**	**CS non keratinised**	**CS basaloid**	**CS adenoid**	**CS with fusiforme cells**	**C verucous**
No. cases	65	76	2	16	2	1
Percentage %	40,20	46,90	1,20	9,90	1,20	0,60

In relation to the differentiation degree of carcinomas studied, we noticed the predominance of well differentiated tumours (42,6 %), followed by low differentiated tumours (33,4 %) and the moderately differentiated tumours (24 %) (table 3).

Table 3.

Distributions of SCC according to the differentiation degree

Differentiation degree	**CS well diferentiated**	**CS moderately diferentiated**	**CS low diferentiated**
Nr. cazuri	69	39	54
Procente %	42,6	24	33,4

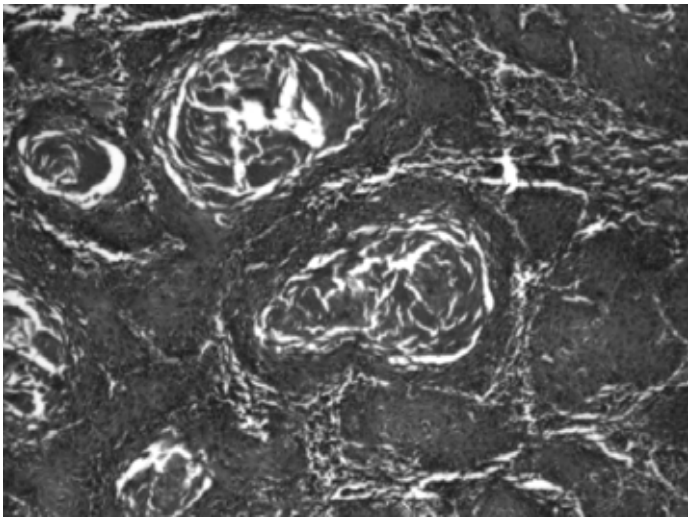

Fig. 1. SCC well differentiated

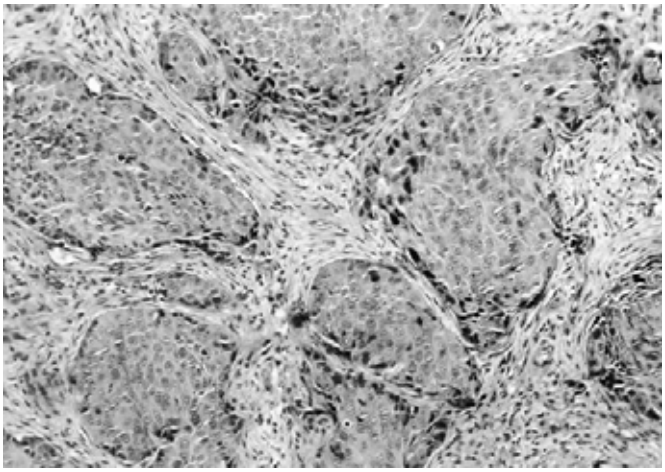

Fig. 2, SCC moderately differentiated

CONCLUSIONS

- Regarding the *tumour progression*, we noticed that the majority of investigated tumours were franc invasive squamous carcinomas without metasthatic adenopathy (93 cases), followed by the ones with metasthatic adenopathy (6 cases), microcarcinomas (3 cases) and in other 2 cases in situ carcinomas. The invasion in adjacent structures intersected the muscles, nervs, salivary glands, bone. The pattern of the invasion was varied, corresponding to irregular and non-cohesive neoplasia belts to isolated infiltrated cells, compressive tumoral margins or round invasise neoplasia islands.

- The analysis of *the surgical safety margins* indicated indemn resection limits in 69,6 % of the cases and the presence of tumour invasion in 30,4 % out of which, at the level of one surgical safety margin at 18,6% of the cases, and the rest of 11,8% for both margins of surgical safety.
- The presence of *pre-cancerous lesions* (dysplasia of various degrees) at the level of adjacent epithelium to carcinoma areas was distinguished at a number of 22 cases.

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THE PHYTOCOENOLOGICAL AFFILIATION OF *ALCHEMILLA* SPECIES FROM PARÂNG MOUNTAIN UNIT (1)

Violeta Boruz[1]

KEY WORDS: Alchemilla, the phytocoenologyc conspectus, Parâng Mountain Unit

ABSTRACT

The research concerning the Alchemilla genre focus on the detailed knowledge of Alchemilla species from the Meridional Carpathians, especially those in the Parâng Mountain Unit. From 20 species of Alchemilla of the Romanian Flora, quoted by V. Ciocârlan (2000) in the Parâng Massif, 11 species were found (T. Pócs 1962) and some of them were refound and others reviewed. The paper presents phytocoenotaxons, vegetation floors and the massif mountains, where species of Alchemilla were registered. For the vegetal associations, well outlined from the floristic and ecological point of view their names were adopted after the Roumanian authors, also according with the latest synthesis at the European level.

INTRODUCTION

The *Alchemilla* species are, mostly, mountain, subalpine, alpine, perennial plants, from small to medium size. The research related to *Alchemilla* genre followed the detailed knowledge of the species from the Meridional Carpathians, with a special referring to the Parâng Massif. The well known botanist from Craiova (even if born in Ardeal) A. Buia worked here, monograph of the *Alchemilla* genre from the Romanian flora. An exhaustive work on the Parâng flora was published by T. Pócs in 1962, where 11 species of *Alchemilla* were recorded.

To correlate the phytocoenological data, obtained after the field trip in Meridional Carpathians, with those from Romanian botanical literature, I have consulted the research on flora and vegetation published so far in Romania. In many publications, the *Alchemilla* species are often quotet generally over the name of "*A. vulgaris*" and "*A. hybrida*". Very few publications have information of identification of the microspecies, which are partially not sustained by herbarium material and thats why at the critical species must be seen with a certain reservation. In this respect, we can say that many things are unknown about the phytocoenology of the *Alchemilla* microspecies from Romania.

MATERIAL AND METHOD

The research was done on itinerary in the Parâng Massif where *Alchemilla* species present in other mountains from Carpathians, too were collected and identified. There have

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been done research trips starting from May until October, for six years. The research trips in other mountains proved to be very necessary to complete the personal collection of herbarium or living plants for comparative research. In this way, the research trips were extended in Căpățânii, Lotru-Latorița and Vâlcan Mountains.

On the field, after the elaboration of the surveying of *Alchemilla,* there have been made complex stational, ecological and phytocoenological observations on *Alchemilla* species.

For vegetal associations, well outlined from the floristic and ecological point of view, their noming was adapted after Romanian authors (A. Buia, A. Borza, N. Boşcaiu, I. Pop, V. Sanda and so on) but according to the stipulations of the Phytocoenology Nomenclature Code (J. J. Barkman, J. Moraveç & S. Rauschert 1986).

RESULTS AND DISCUSSIONS

The Phytocoenologyc conspectus:

I. Cl. MOLINIO - ARRHENATHERETEA Tx. 1937

Ord. Molinietalia W. Koch 1926

Al. *Calthion palustris* Tx. 1937

As. *Scirpetum sylvatici* Ralski 1931, Maloch 1935 em. Schwick. 1944

The beech forest sub-floor: Parâng Mountains - Romanului Valley (*Alchemilla connivens*). Căpățânii Mountains - Piatra Mountains and Ştevioara Mountains (*A. connivens*). The Lotru Mountains - towards the Vidra Dam and around the Villa Complex of Vidra (*A. connivens, A. glabra*).

Al. *Telekion* Morariu 1967

As. *Carduo personatae - Petasitetum hybridi* Oberd. 1957

The beech forest sub-floor, in the Căpățânii Mountains: the Bistriței-Vâlcii Valley, Olăneşti, Cheia - in alder tree fields of *Alnus incana* (*A. crinita*).

As. *Calthetum laetae* V. Krajina 1933

The common spruce floor in Parâng Mountains: Dâlbanul, Şaua Caprei (*A. connivens*). The juniper floor (the subalpine) in Parâng Mountains: Paltinul, Tidvele Hole (*A. connivens*, *A. crinita*).

As. *Calamagrostietum pseudophragmites* Beldie 1967

The common spruce floor: Lotru Mountains - Lotrului Valley at Obârşia Lotrului (*A. connivens*, *A. micans*).

Al. *Agrostion stoloniferae* Soó (1933) 1971

As. *Festucetum pratensis* Soó 1938

The beech forest sub-floor: Lotru Mountains - towards Vidra Dam (*A. connivens*).

As. *Ranunculo strigulosi - Equisetetum palustris* G. Pop. (1974) 1975

The beech forest sub-floor and the common spruce floor: Lotru Mountains - Vidra Dam (*A. connivens*). Căpățânii Mountains - Ştevioara Mt. (*A. connivens*).

Al. *Filipendulo - Petasition* Br. - Bl. 1947

As. *Carduetum personatae* Dihoru (1965) 1975

The beech forest sub-floor: Parâng Mountains - Rânca on the rivulet Valley Romanului and Dâlbanu (*A. connivens*); Mountain Botanical Garden „M. Păun'' (*A. connivens*). Căpățânii Mountains - Bistriței-Vâlcii Valley, the rivulet Valley Mânzu - Olăneşti (*A. connivens*).

As. *Telekio speciosae - Petasitetum albae* Beldie 1967

The beech forest sub-floor: Parâng Mountains, Vâlcan Mountains and Căpățânii Mountains along the mountains valleys (*A. connivens, A. crinita*).

As. *Filipenduletum ulmariae* W. Koch 1926

The beech forest sub-floor and the common spruce floor: Lotru Mountains at Obârşia Lotrului (*A. connivens*).

As. *Chaerophylletum hirsuti* (Soó 1927) Krajina 1933

The common spruce floor: Lotru Mountains towards Câlcescu Lake around a slope spring in the spruce fir (*A. micans, A. connivens, A. glabra, A. crinita*).

As. *Angelico - Cirsietum oleracei* R. Tx. 1937

The beech forest sub-floor: Căpăţânii Mountains - the rivulet Valley Mânzu - Olăneşti (*A. connivens*).

Ord. Arrhenatheretalia Pawł. 1928

Al. *Cynosurion cristati* Br. - Bl. et Tx. 1943 em. Jurko 1969

As. *Agrosti capillaris - Festucetum rubrae* Horv. (1951) 1952

The beech forest sub-floor and the common spruce floor: Parâng Mountains - Ghereşului Valley (*A. connivens*, *A. crinita*, *A. micans*, *A. monticola, A. glabra*); Groapa Seacă - Zănoaga Sliveiului (*A. connivens*, *A. monticola, A. crinita, A. micans, A. glabra*); Groapa Seacă (*A. xanthochlora*); Mountain Botanical Garden „M. Păun" Rânca (*A. connivens*, *A. crinita*); Narrow Path Jieţ (*A. connivens*); Ciobanul Mare Mountain şi Huluzu Mt. (*A. xanthochlora*); Şaua Caprei (*A. micans, A. glabra, A. crinita*); Badea Mt. (*A. glaucescens, A. crinita*); Găuri Sheepfold (*A. crinita*). Lotru Mountains - Vidra Dam and the Villa Complex of Vidra (*A. connivens, A. crinita, A. monticola, A. micans, A. glabra*); Obârşia Lotrului (*A. connivens*, *A. crinita*, *A. micans*); Aviatorului Chalet (*A. monticola*); „Cascada Dracului" (*A. crinita*, *A. connivens*). Căpăţânii Mountains - on Piatra Mt. (*A. connivens*, *A. crinita*), Ştevioara Mt. (*A. connivens, A. crinita, A. micans, A. glabra*); Albu Mt. (*A. xanthochlora, A. crinita, A. micans*). Vâlcan Mountains - Constantinescu Plateau, Piatra Vindereului (*A. monticola*).

II. Cl. PLANTAGINETEA MAJORIS Tx. et Prsg. 1950

Ord. Plantaginetalia majoris Tx. (1947) 1950

Al. *Polygonion avicularis* Br. - Bl. 1931 em. Tx. 1950

As. *Lolio - Plantaginetum majoris* (Linkola 1921) Beger 1930

The beech forest sub-floor: Lotru Mountains - near Voineasa rescont (*A. connivens*, *A. crinita*). The common spruce floor: Lotru Mountains - Dam and the Villa Complex of Vidra, Obârşia Lotrului by degradation of the *Agrosti - Festucetum rubrae* meadow (*A. connivens, A. crinita, A. micans*). Căpăţânii Mountains - Văleanu Mountain (*A. connivens*).

As. *Juncetum tenuis* (Diemont, Siss. et Westhoff 1940) Schwick. 1944

The common spruce floor: Parâng Mountains - Groapa Seacă Pass (*A. connivens*). The juniper floor (the subalpine): Parâng Mountains - Mija Lake (*A. connivens*).

Al. *Agropyro - Rumicion crispi* Nordh. 1940

As. *Junco - Agrostetum capillaris* Resm. 1970

The common spruce floor: Parâng Mountains - Ghereşului Valley (*A. crinita*). Lotru Mountains - Obârşia Lotrului, Glacial Circle Câlcescu (*A. connivens, A. micans, A. crinita*).

As. *Juncetum conglomerati* Prodan 1939 (Syn. *Juncetum effusi* Soó (1931) 1949)

The common spruce floor in Lotru Mountains: Aviatorului Chalet towards „Cascada Dracului", Obârşia Lotrului (*A. connivens*, *A. crinita*, *A. xanthochlora*, *A. glabra*).

III. Cl. PHRAGMITETEA Tx. et Prsg. 1942

Ord. Nasturtio - Glycerietalia Pign. 1953

Al. *Phalarido - Glycerion* Pass. 1964

As. *Equisetetum fluviatilis* Steffen 1931

The common spruce floor: Lotru Mountains - between Aviatorului Chalet, "Cascada Dracului" and Câlcescu Lake (*A. connivens, A. crinita*).

Al. *Glycerio - Sparganion* Br. - Bl. et Sissingh ex Boer 1942

As. *Veronico beccabungae - Glycerietum plicatae* Soó 1971

The common spruce floor: Parâng Mountains - Groapa Seacă Chalet (*A. connivens*); Zănoaga Sliveiului, Ghereşului Valley (*A. connivens, A. crinita*); Parângul Mic - Badea Mt., I.E.F.S. Chalet (*A. connivens, A. glabra, A. crinita*).

IV. Cl. QUERCO - FAGETEA Br. - Bl. et Vlieger 1937 em. Borhidi 1996

Ord. Fagetalia sylvaticae (Pawł. 1928) Tx. et Diem. 1936

Al. *Alno - Ulmion* Br. - Bl. et Tx. 1943 em. Müller et Görs 1958

As. *Alnetum incanae* (Brockman 1907) Aichinger et Siegrist 1930

The beech forest sub-floor: Parâng Mountains - Narrow Path Jieț (*A. connivens*, *A. crinita*, *A. micans, A. glabra*); Dâlbanu Rivulet, Galbenu Rivulet (*A. connivens*). Lotru Mountains - towards Obârşia Lotrului (*A. connivens*).

V. Cl. VACCINIO - PICEETEA Br. - Bl. 1939 em. Pass. 1963

Ord. Junipero - Pinetalia mugi Boşcaiu 1971

Al. *Pinion mugi* Pawł. 1928

As. *Pinetum mugi carpaticum* (Soó 1930) Szafer, Pawł. et Kulcz. 1931

The juniper floor (the subalpine): Parâng Mountains - Paltinul Mountain, Tidvele, Păpuşa, Roşiile, Mija (*A. connivens*). Lotru Mountains: along the paths towards Câlcescu Lake (*A. crinita, A. connivens*).

Al. *Rhododendro - Vaccinion* Br. - Bl. 1926

As. *Rhododendro myrtifolii - Vaccinietum* Borza (1955) 1959 em. Boşcaiu 1971

The juniper floor: Parâng Mountains - Păpuşa Peak (*A. crinita, A. connivens*).

As. *Rhododendro myrtifolii - Pinetum mugi* Borza 1959 em. Coldea 1985

The juniper floor: Parâng Mountains at Mândra Lake, between cliffs (*A. connivens*).

Al. *Vaccinio - Juniperion* Pass. et Hofm. 1968

As. *Vaccinio - Juniperetum sibiricae* Br. - Bl. 1930

The juniper floor: Parâng Mountains on Paltinul Mountains (*A. flabellata*, *A. connivens*, *A. crinita*); Găuri Mt. (*A. crinita, A. flabellata, A. incisa, A. connivens*).

Al. *Junipero - Bruckenthalion* (Horv. 1949) Boşcaiu 1971

As. *Campanulo abietinae - Vaccinietum myrtilli* (Buia et al. 1962) Boşcaiu 1971

The common spruce floor and the juniper floor: Parâng Mountains - Dâlbanul Mountain, Paltinul, Sălişteni Leg, Crucii Slope, Păpuşa Mt. (*A. connivens*); Sălişteni Leg, Corneşul Mic, Corneşul Mare, Dengherul (*A. flabellata*).

As. *Antennario dioicae - Bruckenthalietum* I. Şerbănescu 1961

The juniper floor: Parâng Mountains - Roşiile Sheepfold (*A. connivens*); Crucii Slope Mountain (*A. connivens*, *A. crinita*); Paltinul (*A. connivens*, *A. crinita, A. flabellata*); Sălişteni Leg (*A. crinita*); Dengherul (*A. crinita, A. flabellata*); Dâlbanul, Păpuşa (*A. flabellata*).

As. *Juniperetum sibiricae* Rațiu 1965

The juniper floor: Parâng Mountains on Găuri Mt. (*A. flabellata*).

VI. Cl. NARDO - CALLUNETEA Prsg. 1949

Ord. Nardetalia Oberd. 1949

Al. *Potentillo - Nardion* Simon 1959

As. *Poetum mediae* Csűrös et al. 1956

The juniper floor: Parâng Mountains - Tidvele and Tidvelor Hole, Sălişteni Leg, Rus Slope, Caprei Saddle (*A. connivens*); Paltinul (*A. connivens*, *A. crinita*); Crucii Slope, Urdele (*A. crinita*). Latoriței Mountains - Cărbunele (*A. crinita*).

As. *Festuco rubrae - Nardetum* Csűrös et Resm. 1960 (syn. *Nardus stricta - Festuca rubra fallax* Puşc. et al. 1959)

The juniper floor: Parâng Mountains – Tidvelor Hole, Paltinul, Rânca, Dâlbanul, Păpuşa, Dengherul, Mija Peak, Capra Peak and Caprei Saddle, Parângul Mic (the Parâng Villa, the telechair terminus stop), Ciobanul Mic, Scovarda, Pravăţul (*A. connivens, A. crinita*); Badea Mt. at I.E.F.S. Chalet (*A. glaucescens*); Rus Slope (*A. acutiloba*, *A. connivens*, *A. crinita*, *A. monticola*). Căpăţânii Mountains: Văleanu Peak (*A. conivens*, *A. xanthochlora*); Zănoaga Peak (*A. connivens*).

As. *Scorzonero roseae - Festucetum nigricantis* (Puşcaru et al. 1956) Coldea 1978

The common spruce floor and the juniper floor: Parâng Mountains - Parângul Mic, Badea Mt., Mija (*A. crinita, A. monticola, A. connivens, A. flabellata, A. glaucescens*); Rânca, Rus Slope, Roşiile (*A. monticola*); Dâlbanul, Paltinul, Crucii Slope, Săliştenilor Leg, Tidvele (*A. glabra, A. crinita*). Munţii Căpăţânii - Văleanu Mt. (*A. monticola*, *A. micans*, *A. connivens*, *A. crinita, A. glabra*); Rodeanu Mt. (*A. crinita*); Zănoaga Peak (*A. xanthochlora, A. crinita, A. micans*). Vâlcan Mountains - Straja Peak (*A. crinita*, *A. monticola*); Constantinescu Plateau and Mutului Peak (*A. monticola, A. glabra*).

As. *Violo declinatae - Nardetum* Simon 1966

The common spruce floor and the juniper floor: Parâng Mountains - Badea Mountain, Parângul Mic towards Cârja (*A. crinita, A. flabellata*); Parângul Mic, Badea Mountain (*A. micans, A. glaucescens*); I.E.F.S. Chalet (*A. micans, A. glaucescens, A. glabra, A. flabellata*); Dengherul Hole, Mija Mt., Găuri Mt., Rus Slope (*A. flabellata*). Vâlcan Mountains - Straja, Constantinescu Plateau (*A. flabellata*). Căpăţânii Mountains - Văleanu Peak (*A. crinita*).

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THE PHYTOCOENOLOGICAL AFFILIATION OF *ALCHEMILLA* SPECIES FROM PARÂNG MOUNTAIN UNIT (2)

Violeta Boruz[1]

KEY WORDS: Alchemilla, the phytocoenologyc conspectus, Parâng Mountain Unit

ABSTRACT

The research concerning the Alchemilla genre focus on the detailed knowledge of Alchemilla species from the Meridional Carpathians, especially those in the Parâng Mountain Unit. From 20 species of Alchemilla of the Romanian Flora, quoted by V. Ciocârlan (2000) in the Parâng Massif, 11 species were found (T. Pócs 1962) and some of them were refound and others reviewed. The paper presents phytocoenotaxons, vegetation floors and the massif mountains, where species of Alchemilla were registered. For the vegetal associations, well outlined from the floristic and ecological point of view their names were adopted after the Roumanian authors, also according with the latest synthesis at the European level.

RESULTS AND DISCUSSIONS

The phytocoenologyc conspectus:

VII. Cl. MONTIO - CARDAMINETEA Br. - Bl. et Tx. 1943
Ord. Montio - Cardaminetalia Pawł. 1928
Al. *Cardamini - Montion* Br. - Bl. 1925

As. *Chrysosplenio - Cardaminetum amarae* (Tx. 1937) Maas 1959
The beech forest sub-floor in the Parâng Mountains: Rânca, Galbenu Valley, Dâlbanu Valley (*A. connivens*). The common spruce floor: Parâng Mountains: Dâlbanul, Paltinul (*A. connivens, A. glabra*); Dengherul, Tidvele (*A. glabra*); Groapa Seacă - Zănoaga Sliveiului (*A. connivens*, *A. crinita*, *A. straminea*, *A. xanthochlora, A. micans, A. glabra*); Săliştenilor Leg (*A. connivens*, *A. crinita*). The juniper floor: Parâng Mountains - Paltinul, Parângul Mic towards Cârja (*A. crinita*).

As. *Philonotido - Saxifragetum stellaris* Horv. 1949
The common spruce floor and the juniper floor: Parâng Mountains - Săliştenilor Leg, Paltinul and Crucii Slope, Muşătoiu Rivulet, Romanu Rivulet (*A. connivens*); Sheepfold Rânca Tail, Dengherul Hole, Tidvele Hole, between Parângul Mic and Mija (*A. crinita, A. micans, A. connivens, A. glabra*);

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between Parângul Mic and Cârja, Roşiile Sheepfold (*A. micans*); Slope rivulet between Parângul Mic and Cârja (*A. glabra*); Roşiile Lake (*A. connivens*). Căpăţânii Mountains - Văleanu Mountain (*A. connivens*, *A. micans*); Zănoaga Mountain (*A. connivens*); Smeurăt (*A. micans*).

VIII. Cl. BETULO - ADENOSTYLETEA Br. - Bl. 1948

Ord. Adenostyletalia Br. - Bl. 1931

Al. *Calamagrostion villosae* Pawł. 1928

As. *Phleo alpini - Deschampsietum cespitosae* (Krajina 1933) Coldea 1983

The juniper floor: Parâng Mountains - Mija Lake (*A. crinita*); Sheepfold and Roşiile Peak (*A. crinita*, *A. connivens*, *A. xanthochlora*); High Valley of Slivei (*A. crinita*, *A. glabra*, *A. connivens*, *A. incisa*).

As. *Carici leporinae - Deschampsietum cespitosae* (Borza 1934) Beldie 1967

The common spruce floor: Parâng Mountains - Ciobanul Mic, Ciobanul Mare (*A. connivens*, *A. monticola*); Tidvele, Tidvele Hole, Dengherul Hole, Sălişteni lor Leg (*A. crinita, A. monticola*); Caprei Saddle, High Valley of Slivei (*A. connivens*, *A. crinita*, *A. monticola, A. glabra*); Rus Slope (*A. glabra*); Roşiile Lake (*A. connivens*, *A. crinita*, *A. glabra*); Rânca, Paltinul (*A. crinita*, *A. connivens*, *A. glabra*); Ghereşului Valley (*A. glabra*, *A. connivens*, *A. micans*, *A. crinita*); towards Găuri Peak (*A. glabra*). Lotru Mountains - Dam and the Villa Complex of Vidra (*A. connivens*, *A. monticola, A. crinita, A. glabra*); Lotrului Valley (*A. glabra*); Obârşia Lotrului (*A. xanthochlora*, *A. connivens, A. glabra, A. crinita, A. micans*); Aviatorului Chalet (*A. micans, A. glabra, A. crinita*); at "Cascada Dracului" (*A. glabra, A. micans, A. crinita*); towards Câlcescu Lake (*A. crinita*, *A. glabra*). Căpăţânii Mountains - Văleanu Mt., Zănoaga Mt. (*A. connivens, A. crinita, A. monticola, A. micans*); Smeurăt (*A. connivens, A. crinita, A. monticola*). Vâlcan Mountains - Straja Mt., Constantinescu Plateau (*A. monticola*, *A. crinita*, *A. micans, A. flabellata*).

Al. *Rumicion alpini* (Rübel 1933) Klika 1939

As. *Urtico dioicae - Rumicetum alpini* (I. Şerbănescu 1939, Todor et Culică 1967) corr. Oltean et Dihoru 1986

The common spruce floor: Parâng Mountains – Tidvele Sheepfold, Paltinul, Roşiile Sheepfold (*A. connivens*, *A. glabra*, *A. crinita*); Caprei Saddle (*A. connivens, A. crinita, A. glabra*); Ciobanul Mic Mt. (*A. crinita*); Suspended Refuge (*A. connivens*); High Valley of Slivei (*A. connivens*, *A. micans*). Latoriţei Mountains - Muntinul Mic, Muntinul Mare (*A. connivens*). Căpăţânii Mountains - Zănoaga, Văleanu, Albu (*A. connivens*).

The juniper floor, Lotru Mountains: near the Glacial Circle Câlcescu (*A. glabra, A. connivens, A. crinita*).

As. *Trifolio repenti - Poetum annuae* Todor et Culică 1967

The common spruce floor: Parâng Mountains – Tidvelor Leg, High Valley of Slivei (*A. connivens*); Tidvelor Hole, Păpuşa, Dengherul (*A. crinita*). Căpăţânii Mountains – Rodeanu Mt., Zănoaga Mt. (*A. connivens, A. crinita*); Văleanu Mt., Zănoaga Mt., Govora, Rodeanu Mt. (in near the sheepfolds) - *A. connivens*, *A. glabra, A. crinita*. Vâlcan Mountains: Straja (*A. connivens*). Latoriţei Mountains: Muntinul Mic (*A. connivens*).

Al. *Adenostylion alliariae* Br. - Bl. 1925

As. *Petasito - Cicerbitetum* Tx. 1937

The juniper floor: Parâng Mountains - at Mija Lake, Dâlbanu Rivulet (*A. connivens*).

As. *Aconitetum taurici* Borza 1934

The common spruce floor and the juniper floor: along the slope springs in all the mountains (*A. connivens*).

As. *Saliceto silesiacae - Alnetum viridis* Colič et al. 1962

The common spruce floor: in all the mountains (*A. connivens*).

Al. *Calamagrostion arundinaceae* (Luquet 1926) Jenik 1961

As. *Calamagrostio - Spiraeetum chamaedryfoliae* Resm. et Csűrös 1966

The beech forest sub-floor: Căpăţânii Mountains - the Bistriţei-Vâlcii Gorges, the Gorges "Cheii Olăneşti" (*A. connivens*). Parâng Mountains: Narrow Path of Jieţ on the slopes (*A. micans*).

As. *Digitalo - Calamagrostetum arundinaceae* Sillinger 1933

The beech forest sub-floor: in forest clearings, on Bistriţei-Vâlcii Valley, Olăneşti, Olteţ (*A. connivens*).

IX. Cl. SCHEUCHZERIO - CARICETEA NIGRAE Nordh. 1936

Ord. Scheuchzerio - Caricetalia nigrae (W. Koch 1926) Müller et Görs ex Oberd. 1967

Al. *Caricion canescenti - nigrae* (W. Koch 1926) Nordh. 1936

As. *Caricetum canescenti - nigrae* Vlieger 1937

The common spruce floor and the juniper floor: Parâng Mountains - Sheepfold Rânca Tail, Tidvele Sheepfold, Caprei Saddle (*A. connivens*); Rus Slope (*A. connivens*, *A. crinita*). Lotru Mountains: Dam and the Villa Complex of Vidra (*A. connivens*).

As. *Carici echinatae - Sphagnetum* (Balázs 1942) Soó 1955

The common spruce floor and the juniper floor: Parâng Mountains - Tidvele, Tidvele Hole (*A. connivens*, *A. crinita*); Sheepfold Rânca Tail (*A. connivens*); Caprei Saddle (*A. connivens, A. crinita, A. glabra*); Mija Lake (*A. glabra*); Paltinul, Roşiile Sheepfold, Rus Slope (*A. crinita*). Căpăţânii Mountains - Văleanu, Zănoaga (*A. crinita*). Lotru Mountains - Vidra Dam (*A. connivens*).

As. *Caricetum rostratae* Rübel 1912

The juniper floor: Parâng Mountains - Roşiile Sheepfold and Roşiile Lake (*A. connivens, A. crinita, A. micans*); Ciobanu Mt., Rus Slope (*A. crinita*).

As. *Valeriano simplicifoliae - Caricetum davallianae* (Kulcz. 1928) Moraveč 1966

The common spruce floor: Parâng Mountains - between Sheepfold Rânca Tail and Tidvele Sheepfold (*A. connivens*). The juniper floor: Parâng Mountains - Rus Slope (*A. connivens*).

As. *Carici dacicae - Plantaginetum gentianoidis* Boşcaiu et al. 1972

The juniper floor: Parâng Mountains - Rânca, Tidvele, Tidvele Hole, Dengheru (*A. connivens*).

Ord. Tofieldetalia Prsg. apud. Oberd. 1949

Al. *Eriophorion latifolii* Br. - Bl. et Tx. 1943

As. *Carici flavae - Eriophoretum latifolii* Soó 1944

The common spruce floor: Parâng Mountains - between Sheepfold Rânca Tail and Tidvele Sheepfold (*A. connivens, A. crinita*). The juniper floor: Parâng Mountains – Dengheru Mt., Tidvele Mt. (*A. connivens, A. crinita*), Rus Slope (*A. connivens*, *A. crinita*, *A. glabra*).

As. *Juncetum filiformis* Tx. 1937

The juniper floor: Parâng Mountains - Tidvele, Tidvele Hole (*A. connivens, A. crinita*); Muşetoiu (*A. crinita*). Lotru Mountains: at Vidra Dam (*A. connivens*).

X. Cl. SESLERIETEA ALBICANTIS Br. - Bl. 1948 em. Oberd. 1978

Ord. Seslerietalia albicantis Br. - Bl. 1926

Al. *Festuco saxatilis - Seslerion bielzii* (Pawł. et Walas 1949) Coldea 1984

As. *Festucetum saxatilis* Domin 1933

The juniper floor: Căpăţânii Mountains - Piatra Mountain (*A. connivens, A. crinita, A. monticola, A. flabellata*) and Albu Mountain (*A. connivens, A. crinita, A. monticola*).

As. *Seslerio bielzii - Caricetum sempervirentis* Puşcaru et al. 1956

The juniper floor: Parâng Mountains - Cioara Peak (*A. crinita*); Păpuşa Mountain (*A. connivens, A. crinita, A. flabellata*); Crucii Slope, Sălişteni lor Leg (*A. flabellata*); Găuri Sheepfold (*A. connivens, A. crinita*); Rus Slope (*A. connivens*, *A. crinita*, *A. incisa, A. flabellata*). Căpăţânii Mountains - Piatra Mt., Albu Mt. (*A. xanthochlora*).

Al. *Bellardiochloion (Poion) violaceae* Horv. 1937

As. *Poetum violaceae* (Răv. et Mit. 1958) Resm. 1973

The juniper floor: Căpăţânii Mountains - Piatra şi Albu (*A. connivens*).

XI. Cl. THLASPIETEA ROTUNDIFOLII Br. - Bl. 1926

Ord. Androsacetalia alpinae Br. - Bl. 1926

Al. *Veronicion baumgartenii* Coldea 1991

As. *Saxifrago carpathicae - Oxyrietum digynae* Pawł. et al. 1928

The juniper floor: Parâng Mountains - Cârja towards Verde Lake (*A. incisa*).

XII. Cl. SALICETEA HERBACEAE Br. - Bl. 1947

Ord. Salicetalia herbaceae Br. - Bl. 1926

Al. *Salicion herbaceae* Br. - Bl. 1926

As. *Arenarietum biflorae* Voik 1976

The juniper floor: Parâng Mountains - along the road between Păpuşa Peak, Cioara, Dengherul, and Urdele (*A. connivens, A. crinita, A. glabra*); between roks along a path in juniper tree field towards the Roşiile Lake (*A. flabellata*).

Ord. Arabidetalia coeruleae Rübel 1933

Al. *Salicion retusae* Horv. 1949

As. *Dryadetum octopetalae* Csűrös et al. 1956

In the alpine meadows from Căpăţânii Mountains on Piatra Peak (*A. flabellata*).

XIII. Cl. JUNCETEA TRIFIDI Klika et Hadač 1944

Ord. Caricetalia curvulae Br. - Bl. 1926

Al. *Caricion curvulae* Br. - Bl. 1925

As. *Primulo - Caricetum curvulae* Br. - Bl. 1926 em. Oberd. 1957

Alpine meadows floor: Parâng Mountains - Păpuşa Peak (*A. connivens, A. crinita*); Cioara and Urdele (*A. connivens*). Latoriţei Mountains: Cărbunele Peak (*A. flabellata*).

As. *Potentillo ternatae - Festucetum supinae* Boşcaiu 1971

The juniper floor (rarely) and alpine meadows: Parâng Mountains - Păpuşa, Cioara (*A. connivens, A. flabellata*); Urdele, Cărbunele, Mohorul (*A. connivens*); Rus Slope (*A. incisa*, *A. flabellata*); Găuri Sheepfold (*A. incisa*, *A. xanthochlora, A. crinita, A. flabellata*); Crucii Slope (*A. flabellata*); Mija Peak towards Mija Lake (*A. flabellata*); Parângul Mic (*A. flabellata, A. glaucescens*); Parângul Mic near a fire place in this association area (*A. crinita*).

Al. *Loiseleurio - Vaccinion* Br. - Bl. 1926

As. *Cetrario - Loiseleurietum procumbentis* Br. - Bl. et al. 1939

Alpine meadow floor: Parâng Mountains - Cioara Peak, Păpuşa, Dengherul, Muntinul Mic (*A. connivens*); Găuri Peak, Iezerul Peak (*A. flabellata*).

CONCLUSIONS

Following our own research, in this area there were identified 11 species of *Alchemilla* in 57 associations.

Among them, *Alchemilla connivens, A. crinita* and *A. glabra* have a larger ecologic amplitude, being met in several vegetal associations. The species *Alchemilla glaucescens, A. straminea* and *A. xanthochlora* are characterized by fewer ecologic requirements and they are found in fewer associations.

Vertically, the most numerous species are spread from the lower mountain floor to the lower alpine one.

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MACROMYCETES FROM THE AS. ALNETUM *INCANAE* (OLTEȚ RIVER HALLOW - CĂPĂȚÂNII MOUNTAINS)

Ciortan Ioana[1]

KEY WORDS: macromycetes, phytocoenoses, association, saprotroph

ABSTRACT

The paper presents a number of 33 species of macromycetes from phytocoenoses belonging to the association Alnetum incanae Aich. et Siegr. 1930. Phytocoenoses are situated along Olteț River, where mycological researches were made in June 2008.

INTRODUCTION

River Oltet, an affluent on the right side of Olt River, has a narrow valley in the mountain and a cliff of 40-50 km, the basin is 175 km long. It flows in the Olt River in Oltenia's Plain, at Brâncoveni, south of Balş town.

At the exit from the Carpathian's area, Olteț River cuts Jurassic limestones and it forms the gorges from Polovragi, were it separates Parâng Mountains from Căpățânii Mountains.

The Olteț Gorges have a status of natural area reservation at a national level (100 % area protégée).

Botanical researches in this territory were made by the specialists from Craiova University Centre (Păun M., Popescu Gh., 1971, 1973; Popescu Gh. et al., 2005) [3, 4, and 5]. The mycological data inserted in the paper are preliminary of a larger study which will finalize with the PhD thesis and are also the first mycological data published from this territory.

MATERIAL AND METHOD

The mycological researches were made in June 2008, beginning from approximately 5 km from the exit of Olteț Gorges, on a distance of 15 km.

The mycological material was collected while itinerary trips.

For identification we use macroscopic and microscopic features. Macromycetes were investigated macroscopically fallowed substratum, consistency, trama color and taste, odor, color of the spore print. The microscopically study was effected with the use of an x8-x10 hand lens. The species of macromycetes are presented in the paper in alphabetically order, in table no.1.

[1] Botanical Garden "Al. Buia" of Craiova

RESULTS AND DISCUSSIONS

The researches made had the purpose of identifying the macromycetes species from the as. *Alnetum incanae* Aich. et Siegr. 1930. The arborescent layer is dominated by *Alnus incana* (L.) Moench, and also we can find the species *Fraxinus excelsior* L., *Salix caprea* L., *Fagus sylvatica* L., *Picea abies* (L.) Karsten. The arbustive layer is poor represented and we can find here also *Lonicera xylosteum* L., *Spiraea chamaedryfolia* L., *Evonymus europaeus* L., *Corylus avellana* L., *Daphne mezereum* L. etc.

In the phytocoenoses of the association, installed on the valley were differentiated 2 sub associations: *typicum* which phytocoenoses of *Alnus incana* which homogenous floristic compositions, characterized from the presence of characteristic mezohygrophile flora and *petasitetosum hybridi* Coldea, 1991 with new ground coenoses in which herbaceous synusium are predominant *Petasites hybridus*.

This floristic composition influences the mycosynusium's dynamic and structure.

Table no 1.

Macromycetes species collected in the as. *Alnetum incanae*

B. f.	E. g.	Species	As. *Alnetum incanae*	Spruce fir tree
Gm	M	*Amanita vaginata* (Bull.) Lam.	+	
Gs	St	*Clitocybe gibba* (Pers.) P. Kummer	+	
Th	Sh	*Coprinus atramentarius* (Bulliard) Fries	+	
EPx	Sl	*Daldinia concentrica* (Bolton) Ces. & De Not.	+	
Ex	Sl	*Fomes fomentarius* (L.) J.J. Kickx.	+	
Ex-EPx	SPl	*Fomitopsis pinicola* (Sow.) P. Karst.	+	
Gs	St	*Hygrocybe conica (Scop.)* P. Kummer	+	
EPx	Sl	*Hypholoma fasciculare* (Huds.) P. Kummer	+	+
EPx	Sl	*Hypoxylon fragiforme* (Pers.) J. Kickx f.		+
EPx	SPl	*Kuehneromyces mutabilis* (Schaeff.) Singer & A. H. Smith (Fig. 2.)	+	+
Gm	M	*Inocybe geophylla* var. *lilacina* Gillet	+	+
EPx	Sl	*Lentinellus ursinus* (Fr.) Kühner.	+	
EPx	Sl	*Lentinus tigrinus* (Bull.) Fr.	+	
Gs	Sh	*Lepista flaccida* (Sowerby) Pat.		+
EPx	Sl	*Megacollybia platyphylla* (Pers.) Kotl & Pouzar.		+
Gs	St	*Melanoleuca evenosa* (Sacc.) Konrad.		+
Gs	Sf	*Micromphale perforans* (Hoffm.) Gray		+
Gs	Sf	*Mycena crocata* (Schrad.) Fr.	+	
EPx	Sl	*Mycena galericulata* (Scop.) Gray		+
Gs	Sh	*Mycena galopus var. galopus* (Pers.) P. Kummer	+	
Gs	Sh	*Mycena haematopus* (Pers.) P. Kummer	+	
Ex-EPx	SPl	*Mycena inclinata* (Fr.) Quél. (Fig. 1.)	+	
Gs	Sf	*Mycena pura* (Pers.) P. Kummer		+
Th	Sc	*Panaeolus papilionaceus* (Bull.) Quél.	+	

		var. *papilionaceus*		
Gs	St	*Psathyrella candolleana* (Fr.) Maire	+	
Gs	Sh	*Rhodocollybia butyracea* (Bull.) Lennox, f. *butyracea*	+	+
Ex-EPx	SPl	*Schizophyllum commune* Fr.	+	
Ex-EPx	SPl	*Stereum hirsutum* (Wild.) Pers.	+	
Ex-EPx	SPl	*Stereum insignitum* Quél.	+	
Gs	Sl	*Strobilurus tenacellus* (Pers.) Singer	+	
Gs	Sh	*Stropharia coronilla* (Bull.) Quél.	+	
Gm	M	*Russula lepida* Fr.	+	
EPx	Sl	*Trametes hirsuta* (Wulfen) Pilát	+	

B. f. – biological form; E. g. – ecological category

In terricolous synusium were identified 18 macromycetes species from which 2 mycorrhizal species (*Russula lepida*, characteristic species from spruce fir forest and *Inocybe geophylla*, frequent species in deciduous and coniferous wood), 2 therophytic and 14 saprotrophic species.

In epixylous synusium we have identified 15 macromycetes species.

Fig. 1. ***Mycena inclinata***

Fig. 2. ***Kuehneromyces mutabilis***

Fig. 3. - ***Megacollybia platyphylla***

Fig. 4. ***Melanoleuca evenosa***

Lepista flaccida, Megacollybia platyphylla (Fig. 3.), *Melanoleuca evenosa* (Fig. 4.) and *Micromphale perforans* are characteristic macromycetes species from coniferous forests and they are identified under spruce fir tree.

CONCLUSIONS

In contrast to plants, fungi are not capable of photosynthesis: they cannot make carbohydrates from sunlight and carbonic acid. Fungi lack the necessarily chlorophyll or leaf green. They therefore have to obtain the substances necessary for their growth and existence from living or dead organic matter, vegetable or animal.

It can be observed from table no.1 that the numerous species are saprotrophs: geosaprotrophs species (Gs: Sh, St, Sf) - 14 species) as well as lignicolous species (Ex-EPx: Sl, SPl) - 15 species.

The small number of identified species is justified for this part of the year, Jully being the first month of phenophasis of many terricoulous species which maximum of development is in August-September and beginning of October.

Another factor of major importance in the apparition and development of the basidiomata of macromycetes is the soil humidity determined of the precipitation levels. Either, 2008 was excessively dry, which took to the decrease in the number of species and basidiomata (Fig. 5. – Olteț Valley aspects, 20.VI.2008).

Fig. 5. – Olteț Valley aspects, 20.VI.2008

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DATA CONCERNING THE HISTO-ANATOMICAL KNOWLEDGE OF THE SPECIES *OCIMUM BASILICUM* L. (LAMIACEAE)

Bejenaru Cornelia, Bejenaru Ludovic Everard[1]

KEY WORDS: Ocimum basilicum, structures, root, stem

ABSTRACT

Objective: To characterize the histo-anatomical structure of the root and the stem of the species Ocimum basilicum L.

Material and methods: The vegetal material was harvested from the Craiova University Botanical Garden and was preserved using a 70° ethanol solution. The sections through the root were coloured with Congo red and chrisoidine, and the sections through the stem were coloured with green methyl and fucsine and after that there were photographied with binocular microscope type Krüss (objectives x4, x10, x40) with a system Soligor SR 350 .

Results and discussions: The structure of the root and the stem is a secondary type. The root has the primary bast situated to the outline making some insular zones. The primary wood from the centre is less developed than the secondary wood situated above this. The secondary bast, situated above the bast-ligneous cambiu, is well developed. The stem has from place to place stomats, secretory peri, tector peri bi- and threecells on epiderma. The pith is a cellulosic-parenchima type.

Conclusions: The root has a secondary structure. The stem has secondary conducting tissues.

INTRODUCTION

Ocimum basilicum L., sweet basil (Lamiaceae), is an annual herbal plant originary from India and China, cultivated on whole Globe.

The leaves, the flowers and the young branches contain essential oil and the seeds contain mucilages.

The aerial parts of the plant (Basilici herb) and used in therapeutics for the treatment of digestive apparatus diseases, the bronchitis, the gripe, the infections of urinary tract, aphta, cold, and externaly is used in the treatment of the wounds [1, 2, 3, 4].

MATERIAL AND METHODS

The vegetal produce was harvested in June 2004 from the Craiova University Botanical Garden – Dolj district. The vegetal material was preserved using a 70˚ ethanol solution.

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The transversal sections were efectuated with an anatomical razor through the root and the stem. After washing with distilled water, these swctions were clarified using10 % potassium hypochlorine solution (Javel water).

The succesive wash of the sections was necessary for the elimination of the clarifying agent. The sections through the root was coloured with Congo red and chrisoidine, and the sections through the stem was coloured with green methy and fucsine. The coloured and fixed sections were studied using an binocular microscope type Krüss (objectives x4, x10, x40) and after that these were photographied with an system Soligor SR 350 [5, 6].

RESULTS AND DISCUTIONS

The anatomical structure of the root

The rot has a circulary outline. In transversal section we can observed the next succesion of tissues:

Epiderma maked from one layer with isodiametrical cells; it is continued with exoderma. There is cortical parenchyma under exoderma. This is continued from cells with thin walls and irregular aspect with spaces berween them.

The last layer of the bark (endoderma) is disposed alternatively with the first layer of the central cylindre is maked from word and bast. The bast creates an outline cordon and the wood fills the central zone of the structure.

The conducting tissue contains secundary elements. The primary bast is situated to the outline making some insular zones. There is secondary bast under the conducting tissue who is continually. The primary wood from the centre is less developed then the secondary wood situated above this, under the secondary bast and bast-ligneous cambiu it is well developed [7, 8, 9].

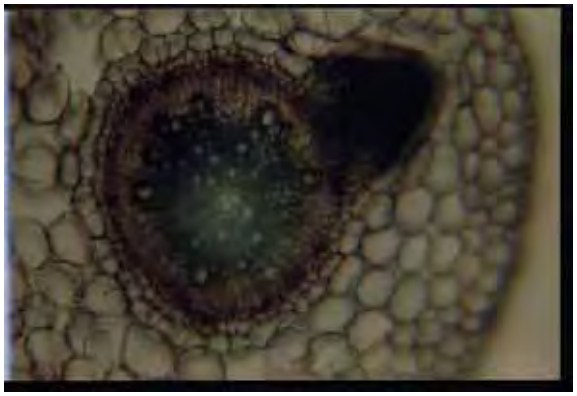

Figure 1. Transversatial section through the root of the species *Ocimum basilicum* L. (green iodine alum coloration, ob. x 10)

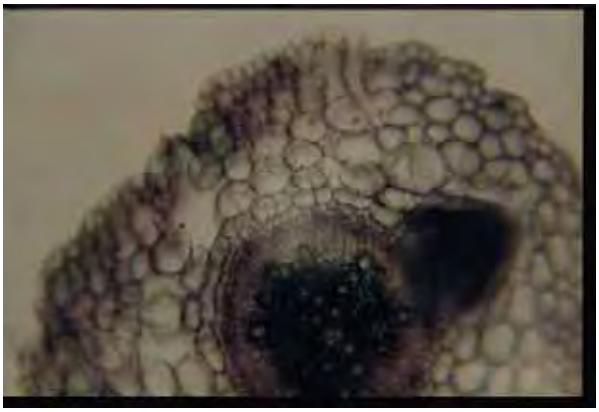

Figure 2. Transversatial section through the root of the species *Ocimum basilicum* L. (green iodine alum coloration, ob. x 10)

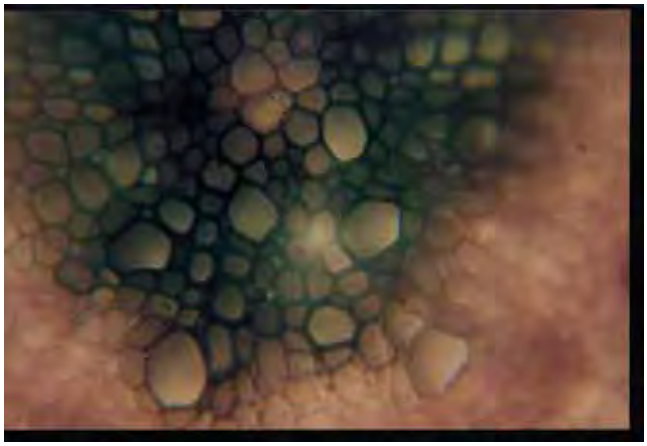

Figure 3. Transversatial section through the root of the species *Ocimum basilicum* L. (green iodine alum coloration, ob. x 40)

The anatomical structure of the stem

The stem presents on the transversal section a square contour in which the four sides are proeminenthy. The epiderma presents the approximate isodiametrical cells with intern and extern tangential walls who are mild tickened. The outline wall of the epidermic cells is tickener than the others walls. It is covered with a thin cuticula with a toothed relief. There are from place to place stomats, secretory peri, tector peri bi- and threecells on epiderma. There are cordons with angular colenchyma in the four sides of the stene. The endoderma is a primary type. The most conducting tissues have a secondary origin. The bast-libneous fascicles from the four sides are bigger than the other fascicles.

The bast is maked from riddled tubes, annexed cells and parenchyma cells. The wood is thicker than the bast; it is constitued from dispersed vessels who are disorderly in the fundamental mass of the basted tissue. To the primary wood there are vessels who are

maked from radial rows separated by cellulosic parenchyma. In the very big fascicles, the wood is wander by numerous uniseriated parenchyma beams. The pith is a cellulosic-parenchima type [7, 8, 9].

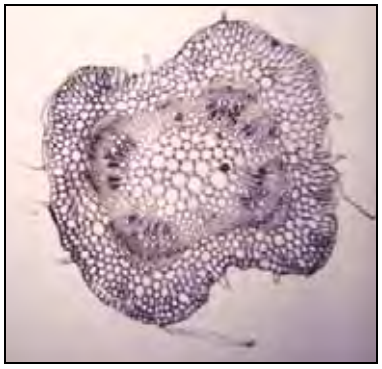

Figure 4. Transversatial section through the stem of the species *Ocimum basilicum* L. (green iodine alum coloration, ob. x 4)

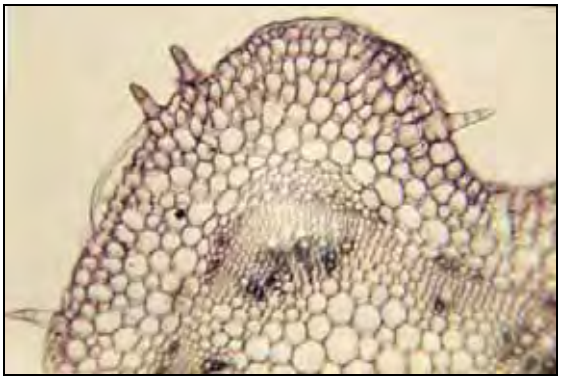

Figure 5. Transversatial section through the stem of the species *Ocimum basilicum* L. (green iodine alum coloration, ob. x 10)

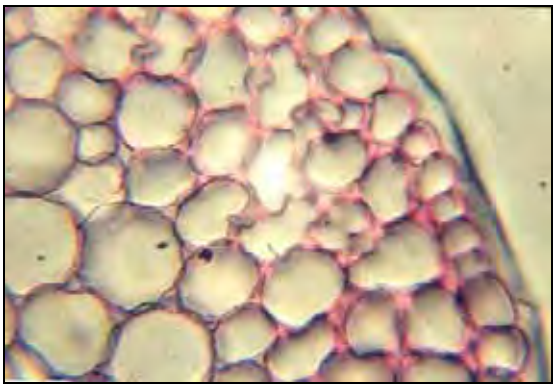

Figure 6. Transversatial section through the stem of the species *Ocimum basilicum* L. (green iodine alum coloration, ob. x 40)

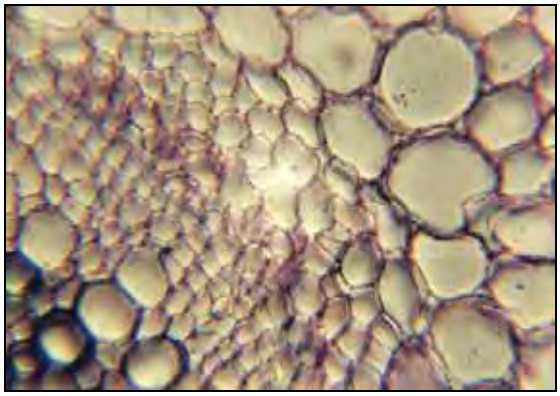

Figure 7. Transversatial section through the stem of the species *Ocimum basilicum* L. (green iodine alum coloration, ob. x 40)

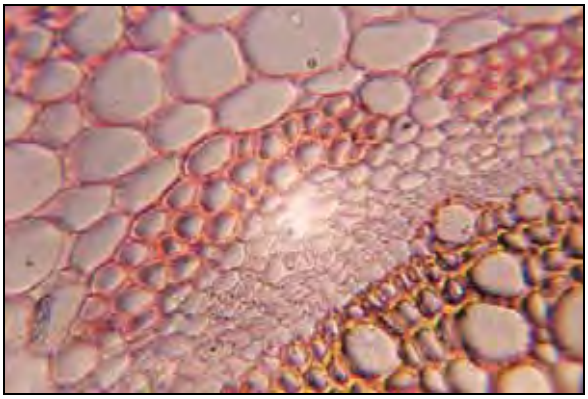

Figure 8. Transversatial section through the stem of the species *Ocimum basilicum* L. (Congo red and chrisoidine coloration, ob. x 40)

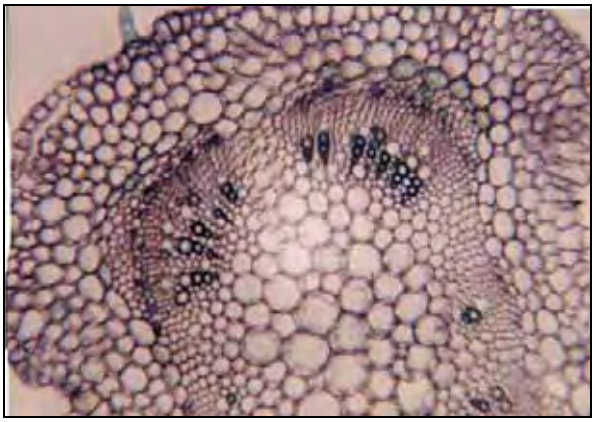

Figure 9. Transversatial section through the stem of the species *Ocimum basilicum* L. (green iodine alum coloration, ob. x 40)

CONCLUSIONS

1. In this work are analyzed the histo-anatomical strustures of the root and stem.
2. The root has a secondary structure due to the activity of bast-ligneous cambiu.
3. The stem has bi- and threecells peri, secretory peri, angular colenchyma and secondary conducting tissues.

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THE PRELIMINARY PHYTO-CHEMICAL ANALYSIS OF THE AERIAL PARTS HARVESTED FROM MEDICINAL SPECIES FROM DOLJ

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KEY WORDS: active principles, preliminary analysis, extract

ABSTRACT

Material and methods: the vegetal produce was harvested from species such as: (aerial bloomy parts), (branches with leaves).

To extract the active principles there have been used three solutions with different polarity: etilic ether, methanol and water. The identification was achieved through specific features to each group of active principles.

Results and discussions: the active elements found in the etheric extract were: essential oil, sterols, triterpenes, fatty and resinic acids, saponozides, carotenoids, alkaloids, flavonic aglycones, coumarinas. The methanolic extract contained: cathetic tannin, reducing compounds, alkaloids, amino acids, coumarinas, triterpenes, saponosides, flavonoides.. In the aqueous extract there were identified: polyuronides, reducing compounds, glucids, saponosides, cathetic tannin, alkaloids.

Conclusions: the preliminary phyto-chemical analysis of some medicinal species in Dolj will support the cromatographic analysis (CSS, HPLC, MS-GC). The nine analysed species' chemical composition is different and may be analysed in comparison, in the shown tables.

INTRODUCTION

In Dolj there are 268 plants which have therapeutic activity: 229 of them grow spontaneously and only 39 are cultivated. These species are framed in 79 botanical families. From these medicinal species 48 are wooden species and 220 are grassy ones.

In therapeutics there are often used the aerial parts. Knowing the medicinal plants in an area allows harvesting them for their use in therapeutics.

In order to treat efficiently it is necessary having a scientific confirmation of the pharmacological activity but also identifying the active principles.

Among plants in Dolj with therapeutic properties I studied nine of them on which I made a preliminary phytochemical analysis to identify the active principles[3,4,7,10].

MATERIALS AND METHOD

The vegetal produces were represented by the aerial parts or by branches with leaves (for the wooden species) of: *Consolida regalis, Tribulus terestris, Oenothera biennis, Lycopus europaeus, Solanum nigrum, Daucus carota subsp. carota, Hedera helix, Physalis alkekengi, Taxus baccata* [1,2,8,9].

[1] University of Medicine and Pharmacy, Faculty of Pharmacy, Craiova

The harvesting was made in 2003-2007 from around Craiova. Using different polarity solvents to successively and selectively extract I separated the groups of active compounds.

The vegetal produces were naturally aired in thin layer and then preserved, until extraction, in paper bags.

By successive and selective extraction with different polarity solvents I separated groups of active principles.

The vegetal produces were first extracted with an unpolar solvent (etilic ether), then with a polar one (methanol) and, finally, with water. There were obtained three extractive solutions: etheric, alcoholic, aqueous one[4,5,6,7].

The etheric extract analysis

Obtaining the etheric extract: one gram of vegetal produce pulverized using sieve V was extracted through manual, intermittent stir, after a three days rest with fifty millilitres etilic ether, until the etheric solution left no residue when evaporated.

After filtering the etheric extract I proceeded identifying the active substances: essential oil, fatty substances, sterols, carotenoids, triterpenes, fatty acids, resinic acids, alkaloids base, flavonic aglycones, emodols, coumarines.

The methanolic extract analysis

The vegetal produces left from the etheric extraction were put in a balloon with ascendent refrigerator. I added one hundred fifty milliliters of methanol and then extracted, using temperature, on the water bath, for 20-40 minutes.

The alcoholic extract was concentrated to fifty millilitres in a device called rotary evaporator.

Methanol extracts from the skimmed vegetal produce the following types of active substances: polyphenols and polyphenolic glicozidas (antracenozids, coumarines, flavonoids), demulsifing agents, alkaloids, amino acids, sterolic glicozids and triterpenic ones. The active substances are identified through typical reactions made on the alcoholic extract as such or initially hidrolysated.

The aqueous extract analysis

The vegetal produces left from the methanolic extraction were dried and then extracted through boiling, with fifty-one hundred millilitres distilled water, for fifteen minutes.

The filtered aqueous solution was then concentrated to fifty millilitres.

In this solution there are identified the following active substances: glucids, heterozides, tannins, proteic substances, alkaloids sales.

RESULTS AND DISCUSSION

Tables 1-3 show the results of the preliminary phytochemical analysis of the etheric, methanolic and aqueous extracts from the aerial parts of *Consolida regalis, Tribulus terestris, Oenothera biennis, Lycopus europaeus, Solanum nigrum, Daucus carota subsp. carota, Hedera helix, Physalis alkekengi, Taxus baccata.*

CONCLUSIONS

It was made the preliminary phytochemical analysis of the aerial parts of *Consolida regalis, Tribulus terrestris, Oenothera biennis, Lycopus europaeus, Solanum nigrum, Daucus carota subsp. carota, Hedera helix, Physalis alkekengi, Taxus baccata.*

Separating different groups of active principles was made by successively and selectively extracting the vegetal produces with different polarity solvents:etilic ether (unpolar sovent), methanol (polar solvent) and, finally, with water.

In the etheric extract there were positive reactions for: essential oil, sterols, triterpenes, saponosides, carotenoids, fatty acids, flavonic aglycones, coumarins, alkaloids. The alcoholic extract gave positive reactions for: catethic tannin, reducing compounds, coumarins, flavonoids, alkaloids, amino acids, saponosides, triterpenes.

In the aqueous extract there were identified: polyuronids, reducing compounds, oozes, saponosides, cathetic tannin, alkaloids.

As a result of the preliminary analysis of the nine phytoextracts there were not identified the following groups of active principles: emodols, antocianosides, anthracenes, cardiotonic glycosides.

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Table 1

The preliminary phytochemical analisys of the etheric extract

Crt. nr	Active principles	Identification reactions	1	2	3	4	5
1.	Essential oil	Method NeoClevenger	(+)	(+)	(+)	(+)	(+)
2.	Sterols, triterpenes	r. Liebermann-Burchard	(+)	(+)	(+)	(+)	(+)
3.	Carotenoids	r. Carr-Price	(-)	(-)	(-)	(-)	(+)
4.	Fatty acids	after soaping	(-)	(+)	(+)	(-)	(+)
5.	Resinic acids	r. Hirschsohn	(-)	(-)	(-)	(-)	(-)
6.	Alkaloids base	r. Mayer / Bertrand	(+)	(+)	(-)	(-)	(-)
7.	Flavonic glycones	r. Shibata / intense yellow in alkalin medium	(+)	(+)	(+)	(+)	(+)
8.	Emodols	r. Bornträger	(-)	(-)	(-)	(-)	(-)
9.	Coumarins	Fluorescence UV /r. Feigl-Frehden-Anger	(-)	(-)	(-)	(+)	(+)
Crt. nr.	**Active principles**	**Identification reactions**	**6**	**7**	**8**	**9**	
1.	Essential oil	Method NeoClevenger	(+)	(-)	(-)	(-)	
2.	Sterols, triterpenes	r. Liebermann-Burchard	(+)	(+)	(-)	(+)	
3.	Carotenoids	r. Carr-Price	(+)	(+)	(+)	(+)	
4.	Fatty acids	after soaping	(+)	(+)	(-)	(-)	
5.	Resinic acids	r. Hirschsohn	(-)	(+)	(+)	(+)	
6.	Alkaloids base	r. Mayer / Bertrand	(-)	(+)	(+)	(+)	
7.	Flavonic glycones	r. Shibata / intense yellow in alkalin medium	(+)	(+)	(+)	(+)	
8.	Emodols	r. Bornträger	(-)	(-)	(-)	(-)	
9.	Coumarins	Fluorescence UV / r. Feigl-Frehden-Anger	(-)	(+)	(-)	(-)	

Table 2

The preliminary phytochemical analisys of the methanolic extract

Crt.nr.	Active principles	Identification reactions	1	2	3	4	5
1.	Cathetic tannins	r. cu $FeCl_3$ / r. Styassny	(+)	(+)	(+)	(+)	(+)
2.	Reducing compounds	r. Fehling	(+)	(+)	(+)	(+)	(+)
3.	Alkaloids base	r. Mayer / Bertrand	(+)	(+)	(-)	(-)	(-)
4.	Alkaloids cuaternary base of ammonia	r. Mayer / Bertrand	(+)	(+)	(-)	(-)	(-)
5.	Amino acids	r. Ninhidrină	(-)	(+)	(+)	(-)	(+)
6.	Anthracenes	r. Bornträger	(-)	(-)	(-)	(-)	(-)
7.	Coumarines	Fluorescence UV /r. Feigl-Frehden-Anger	(-)	(-)	(-)	(+)	(+)
8.	Cardiotonic heterozides	r. Kedde / r. Legal	(-)	(-)	(-)	(-)	(-)
9.	Saponosides, triterpenes	r. Liebermann-Burchard / foamy test	(+)	(+)	(+)	(+)	(+)

10.	Flavonoids	r. Shibata / intense yellow in alkalin medium	(+)	(+)	(+)	(+)	(+)
11.	Proantocianidols	r. with HCl conc., t^0 C	(+)	(-)	(-)	(-)	(-)
12.	Antocianosides	Red solution in acid medium, with turn of colour	(-)	(-)	(-)	(-)	(-)
Crt. nr	**Active principles**	**Identification reactions**	**6**	**7**	**8**	**9**	
1.	Cathetic tannins	r. cu $FeCl_3$ / r. Styassny	(-)	(+)	(+)	(+)	
2.	Reducing compounds	r. Fehling	(+)	(+)	(+)	(+)	
3.	Alkaloids base	r. Mayer / Bertrand	(-)	(+)	(+)	(+)	
4.	Alkaloids cuaternary base of ammonia	r. Mayer / Bertrand	(-)	(+)	(+)	(+)	
5.	Amino acids	r. Ninhidrină	(-)	(+)	(-)	(-)	
6.	Anthracenes	r. Bornträger	(-)	(-)	(-)	(-)	
7.	Coumarines	Fluorescence UV /r. Feigl-Frehden-Anger	(-)	(+)	(-)	(-)	
8.	Cardiotonic heterozides	r. Kedde / r. Legal	(-)	(-)	(-)	(-)	
9.	Saponosides, triterpenes	r. Liebermann-Burchard / foamy test	(+)	(+)	(-)	(+)	
10.	Flavonoids	r. Shibata / intense yellow in alkalin medium	(+)	(+)	(+)	(+)	
11.	Proantocianidols	r. with HCl conc., t^0 C	(-)	(-)	(-)	(-)	
12.	Antocianosides	Red solution in acid medium, with turn of colour	(-)	(-)	(-)	(-)	

Table 3

The preliminary phytochemical analisys of the aqueous extract

Crt. nr	**Active principles**	**Identification reactions**	**1**	**2**	**3**	**4**	**5**
1.	Polyuronids	Precipitate in acetone and colouring with methylen blue	(-)	(-)	(+)	(+)	(+)
2.	Reducing compounds	r. Fehling	(+)	(+)	(+)	(+)	(+)
3.	Oozes	r. timol	(+)	(+)	(+)	(+)	(+)
4.	Saponosides	r. Liebermann-Burchard / foamy test	(-)	(+)	(-)	(-)	(+)
5.	Cathetic tannins	r. cu $FeCl_3$ / r. Styassny	(+)	(+)	(+)	(+)	(+)
6.	Alkaloids salt	r. Mayer / Bertrand	(+)	(+)	(-)	(-)	(-)
Crt. nr	**Active principles**	**Identification reactions**	**6**	**7**	**8**	**9**	
1.	Polyuronids	Precipitate in acetone and colouring with methylen blue	(-)	(+)	(+)	(-)	
2.	Reducing compounds	r. Fehling	(+)	(+)	(+)	(+)	
3.	Oozes	r. timol	(+)	(+)	(+)	(+)	
4.	Saponosides	r. Liebermann-Burchard / foamy test	(+)	(+)	(+)	(-)	
5.	Cathetic tannins	r. cu $FeCl_3$ / r. Styassny	(+)	(+)	(+)	(+)	
6.	Alkaloids salt	r. Mayer / Bertrand	(-)	(+)	(+)	(+)	

1- *Consolida regalis (herba)*, 2 - *Tribulus terrestris (herba)*, 3 - *Oenothera biennis (herba)*, 4 - *Lycopus europaeus (herba)*, 5 - *Solanum nigrum (herba)*, 6 - *Daucus carota subsp. carota (herba)*, 7 - *Hedera helix (folium)*, 8 - *Physalis alkekengi (herba),* 9 - *Taxus baccata (folium)*.

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THE PRELIMINARY PHYTO-CHEMICAL ANALYSIS OF THE BLOOMY AERIAL PARTS HARVESTED FROM DIFFERENT SPECIES OF *EPILOBIUM*

Bejenaru Ludovic Everard, Bejenaru Cornelia, Neamțu Johny[1]

KEY WORDS: Epilobium, active principles, preliminary analysis, extract

ABSTRACT

Material and methods: to accomplish the preliminary analysis I harvested the bloomy aerial parts of Epilobium hirsutum, E. collinum, E. parviflorum, E. montanum, E. angustifolium .

In order to separate different groups of active principles, the vegetal produces were successively and selectively extracted with different polarity solvents: etilic ether, methanol and water. Results and discussions: the preliminary analysis results are presented in comparative tables, being identified the following active principles: essential oil, sterols, triterpenes, carotenoids, fatty acids, flavonic aglycones, coumarines (etheric extract); cathetic tannin, reducing compounds, coumarines, flavonozides (methanolic extract); polyuronides, reducing compounds, glucids, cathetic tannin (aqueous extract).

Conclusions: Epilobium species have the same active elements. The chemical composition of Epilobium sp. is complex, for a detailed analysis being necessary the preliminary analysis. Some of the new discovered active principles have never been mentioned in the reference materials: sterols, carotenoids, lipids, glucids. Romanian Epilobium sp. have never been chemically studied.

INTRODUCTION

In Romania's flora there are seventeen species, six of them being used in etnomedicine. From the total of six species, only three are studied from a chemical composition point of view. I studied five species which grow spontaneously in Romania. They are used for their therapeutic features, but haven't been phytochemically studied in Romania. Two of these species are unstudied.

The five studied species of *Epilobium are*: *E. collinum, E. hirsutum, E. montanum, E. parviflorum,E. montanum*. *Epilobium collinum* is chemically similar to the other species of E. , therefore I used the old name.

Phytochemically speaking, the *Epilobium* species include a variety of components: gums, flavonozides and flavonic aglycones, tannins, polyphenolic derivates of cafeic acid, pentacyclic triterpenic acids, coumarines, peptides derivative from serin and glicocol, essential oil.

From the total of five shown in this paper, only three species of *Epilobium* were studied, confirming some of the features that the popular medicine attributes them.

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Epilobium angustifolium in the most studied species of this genre and so there are many papers which verify or praise its therapeutic values: anti-infectious, anti-inflammatory (in prostatitis), antiproliferativ (benign prostatic hyperplasia), astringent, tonic, emollient, demulcent. *E.hirsutum* is used for its: anti-inflammatory, antihypertrofic (benign prostatic hyperplasia), depurative, choleretic - colagog, hepatoprotective, astringent, diuretic, dezinfectant effects.

E. parviflorum is used to treat: prostatitis, benign prostatic hyperplasia (adenom), kidney – diseases, urine path and bladder diseases, hepatitis, chronic hepatitis, cirrhosis, gastroduodenal ulcer.

The preliminary phytochemical analysis was a starting point for a more detailed analysis on identifying the chemical compounds unsignaled at these species, using CSS, HPLC, MS-GC[3,4,7,10].

MATERIALS AND METHOD

The analysed vegetal produces were harvested in July – August 2003-2007 as it follows: *E. hirsutum* and *E. parviflorum* were harvested from around Craiova; *E. collinum* was harvested from Lainici.

E. angustifolium and *E. montanum* were harvested from Rânca (Gorj)[1,2,8,9].

The vegetal produces were naturally aired in thin layer and then preserved, until extraction, in paper bags.

By successive and selective extraction with different polarity solvents I separated groups of active principles.

The vegetal produces were first extracted with an unpolar solvent (etilic ether), then with a polar one (methanol) and, finally, with water. There were obtained three extractive solutions: etheric, alcoholic, aqueous one[4,5,6,7].

The etheric extract analysis

Obtaining the etheric extract: one gram of vegetal produce pulverized using sieve V was extracted through manual, intermittent stir, after a three days rest with fifty millilitres etilic ether, until the etheric solution left no residue when evaporated.

After filtering the etheric extract I proceeded identifying the active substances: essential oil, fatty substances, sterols, carotenoids, triterpenes, fatty acids, resinic acids, alkaloids base, flavonic aglycones, emodols, coumarines.

The methanolic extract analysis

The vegetal produces left from the etheric extraction were put in a balloon with ascendent refrigerator. I added one hundred fifty milliliters of methanol and then extracted, using temperature, on the water bath, for 20-40 minutes.

The alcoholic extract was concentrated to fifty millilitres in a device called rotary evaporator.

Methanol extracts from the skimmed vegetal produce the following types of active substances: polyphenols and polyphenolic glicozidas (antracenozids, coumarines, flavonoids), demulsifing agents, alkaloids, amino acids, sterolic glicozids and triterpenic ones.

The active substances are identified through typical reactions made on the alcoholic extract as such or initially hidrolysated.

The aqueous extract analysis

The vegetal produces left from the methanolic extraction were dried and then extracted through boiling, with fifty-one hundred millilitres distilled water, for fifteen minutes.

The filtered aqueous solution was then concentrated to fifty millilitres.

In this solution there are identified the following active substances: glucids, heterozides, tannins, proteic substances, alkaloids sales.

RESULTS AND DISCUSSION

Tables 1-3 show the results of the preliminary phytochemical analysis of the etheric, methanolic and aqueous extracts from the aerial parts of *E. angustifolium, E. collinum, E. hirsutum, E. montanum, E. parviflorum.*

Table 1

The preliminary phytochemical analisys of the etheric extract of Epilobii herba

Crt. nr.	Active principles	Identification reactions	E1	E2	E3	E4	E5
1.	Essential oil	Method NeoClevenger	(+)	(+)	(+)	(+)	(+)
2.	Sterols, triterpenes	r. Liebermann-Burchard	(+)	(+)	(+)	(+)	(+)
3.	Carotenoids	r. Carr-Price	(+)	(+)	(+)	(+)	(+)
4.	Fatty acids	after soaping	(+)	(+)	(+)	(+)	(+)
5.	Resinic acids	r. Hirschsohn	(-)	(-)	(-)	(-)	(-)
6.	Alkaloids base	r. Mayer / Bertrand	(-)	(-)	(-)	(-)	(-)
7.	Flavonic aglycones	r. Shibata / intense yellow in alkalin medium	(+)	(+)	(+)	(+)	(+)
8.	Emodols	r. Bornträger	(-)	(-)	(-)	(-)	(-)
9.	Coumarines	Fluorescence UV / r. Feigl-Frehden-Anger	(+)	(+)	(+)	(+)	(+)

Table 2

The preliminary phytochemical analisys of the methanolic extract of Epilobii herba

Crt. Nr.	Active principles	Identification reactions	E1	E2	E3	E4	E5
1.	Cathetic tannins	r. cu $FeCl_3$ / r. Styassny	(+)	(+)	(+)	(+)	(+)
2.	Reducing compounds	r. Fehling	(+)	(+)	(+)	(+)	(+)
3.	Alkaloids base	r. Mayer / Bertrand	(-)	(-)	(-)	(-)	(-)
4.	Alkaloids cuaternary base of ammonia	r. Mayer / Bertrand	(-)	(-)	(-)	(-)	(-)
5.	Amino acids	r. Ninhidrină	(+)	(+)	(+)	(+)	(+)
6.	Anthracenes	r. Bornträger	(-)	(-)	(-)	(-)	(-)
7.	Coumarines	Fluorescence UV / r. Feigl-Frehden-Anger	(+)	(+)	(+)	(+)	(+)

8.	Cardiotonic heterozides	r. Kedde / r. Legal	(-)	(-)	(-)	(-)	(-)
9.	Saponosides, triterpenes	r. Liebermann-Burchard / foamy test	(+)	(+)	(+)	(+)	(+)
10.	Flavonoids	r. Shibata / intense yellow in alkalin medium	(+)	(+)	(+)	(+)	(+)
11.	Proantocianidols	r. with HCl conc., t^0 C	(-)	(-)	(-)	(-)	(-)
12.	Antocianosides	Red solution in acid medium, with turn of colour	(-)	(-)	(-)	(-)	(-)

Table 3

The preliminary phytochemical analisys of the aqueous extract of Epilobii herba

Crt. nr.	**Active principles**	**Identification reactions**	**E1**	**E2**	**E3**	**E4**	**E5**
1.	Polyuronids	Precipitate in acetone and colouring with methylen blue	(+)	(+)	(+)	(+)	(+)
2.	Reducing compounds	r. Fehling	(+)	(+)	(+)	(+)	(+)
3.	Oozes	r. timol	(+)	(+)	(+)	(+)	(+)
4.	Saponosides	r. Liebermann-Burchard / foamy test	(+)	(+)	(+)	(+)	(+)
5.	Cathetic tannins	r. cu $FeCl_3$ / r. Styassny	(+)	(+)	(+)	(+)	(+)
6.	Alkaloids salt	r. Mayer / Bertrand	(-)	(-)	(-)	(-)	(-)

E1- Epilobii angustifolii herba, E2 – Epilobii collini herba, E3 – Epilobii hirsuti herba, E4 – Epilobii montani herba, E5 – Epilobii parviflori herba.

CONCLUSIONS

It was made the preliminary phytochemical analysis of the aerial parts harvested from *E. angustifolium, E. collinum, E. hirsutum, E. montanum, E. parviflorum.*

In order to separate different groups of active principles it was used the successive and selective extraction with different polarity solvents: etilic ether (unpolar solvent), methanol (polar solvent) and, finally, water.

In the etheric extract the tests were positive for: essential oil, sterols, triterpenes, carotenoids, fatty acids, flavonic aglycones, coumarines.

The methanolic extract gave positive reactions for: tannins, demulsifing agents, oozes, flavonozides.

As a result of the preliminary analysis of the five phytoextracts the following active principles were identified: essential oil, sterols, triterpenes, carotenoids, lipids, coumarines, flavonosides, flavonic aglycones, polyuronids, oozes, reducing agents.

Sterols, carotenoids, lipids, oozes are not mentioned in different scientific investigator papers interested in knowing the chemical composition of *Epilobium* species.

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CONTRIBUTIONS TO THE KNOWLEDGE OF THE MEDICINAL PRODUCE *CATHARANTHI HERBA*

Ludovic Everard Bejenaru, Cornelia Bejenaru[1]

KEY WORDS: Catharanthus roseus, Catharanthi herba, stem, leaf

ABSTRACT

Objective: To characterize the histo-anatomical structure of the aerial part of the species Catharanthus roseus (L.) G. Don.

Materials and method: The vegetal produce harvested from the greenhouse of the Craiova University Botanical Garden was preserved using a 70º ethanol solution and sectioned with an anatomical razor through stem and leaf. Fucsine and methyl green were used as dyestuff for the sections and then photographied with a system Soligor SR 350 adapted to the microscope type Krüss (objectives x 10, x 40).

Results and discussions: The structure of the stem is a secondary type. The cortical parenchyma contains cellulose fibres from place to place, the conducting tissues are disposed on two concentric rings separated from the basted-ligneous cambiu. The medullary parenchyma is a meatic type. The leaf has a bifacial, amphystomatic structure with the mesophyl constituted of only one layer of palisadic tissue and 5-6 layers of lacunose tissue.

Conclusions:The stem has a secondary structure and the leaf presents bifacial, amphystomatic structure.

INTRODUCTION

The medicinal produce *Catharanthi herba* represents the aerial part of the species *Catharanthus roseus* (L.) G. Don, which is cultivated in Romania as an ornamental plant. Catharanthi herba is important in therapeutics because of its contents in indolic alkaloids, monomers and dimers with antitumoral properties [1, 2, 3]. Histo-anatomical data of the stem and leaf are presented in this work.

MATERIALS AND METHOD

The vegetal produce was harvested in April 2008 from the greenhouse of the Craiova University Botanical Garden. The vegetal material was preserved using a 70º ethanol solution. The transversal sections were effectuated with an anatomical razor through the stem and leaf. After washing with distilled water, these sections were clarified with 10% potassium hypoclorine solution (Javel water). This succesive wash of the sections was necessary for the elimination of the clarifying agent. Fucsine and methyl green were

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used as dyestuff for the sections. The coloured and fixed sections were studied using a binocular microscope type Krüss (objectives x 10, x 40) and then photographied with a system Soligor SR 350 adapted to the microscope [4, 5].

RESULTS AND DISCUSSIONS

The stem

There is a sinuous ellipsoidal outline and secondary structure. It can be observed the next succesion of the tissue, from the inside towards outside:

The unistratified epiderma containing isodiametrical cells, protected by a thin cuticula. The cortical parenchyma is well represented, it contains cellulose fibres from place to place. The conducting tissues are disposed on two concentric rings separated from the basted-ligneous cambiu. The basted tissue is constituted of riddled tubes, annexed cells and basted parenchyma.

The ligneous tissue is made of secondary big ligneous vessels which are spread in libriform tissue mass. The primary ligneous tissue is reduced to some small vessels.

The medulary beams are uniseriated, wide and cellulosed.

The medulary parenchyma fills the center of the steam. It is a meatic type [5, 6, 7, 8](fig.1,2).

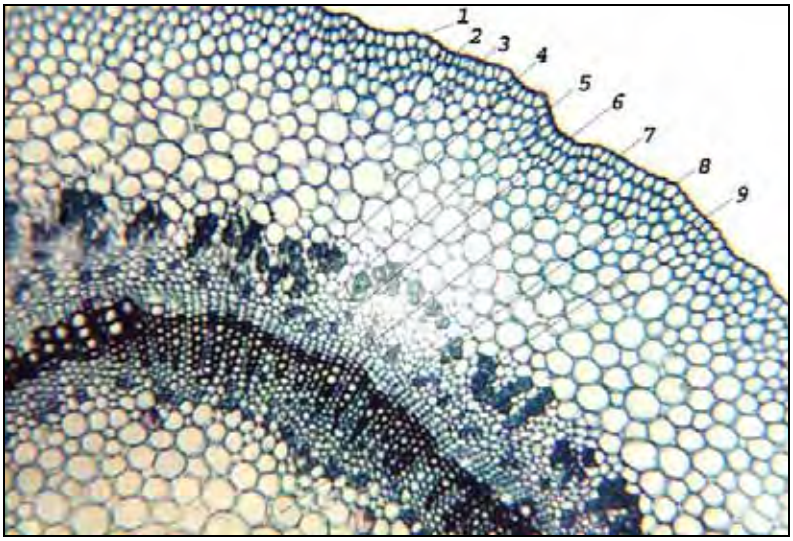

Figure 1. Transversal section through the stem of *Catharanthus roseus* L. (objective x40, methyl green and fucsine coloration)

The leaf

There is a bifacial, amphystomatic structure. The both epiderms are unicellular with long cells and stomates from place to place. The mesophyl is constituted by only one layer with palisadic tissue and long cells and 5-6 layers with lacunose tissue with small cells uniformly situated with small intercells spaces. There is only central semicircular bast-ligneous fascicle. The stalk of the leaf presents winged circulary contour. It can be observed from place to place, small unicellular tectors peri to the epiderma. There is only central half round bast-ligneous fascicle in the fundamental parenchyma mass [5, 6, 7, 8] (fig.3,4).

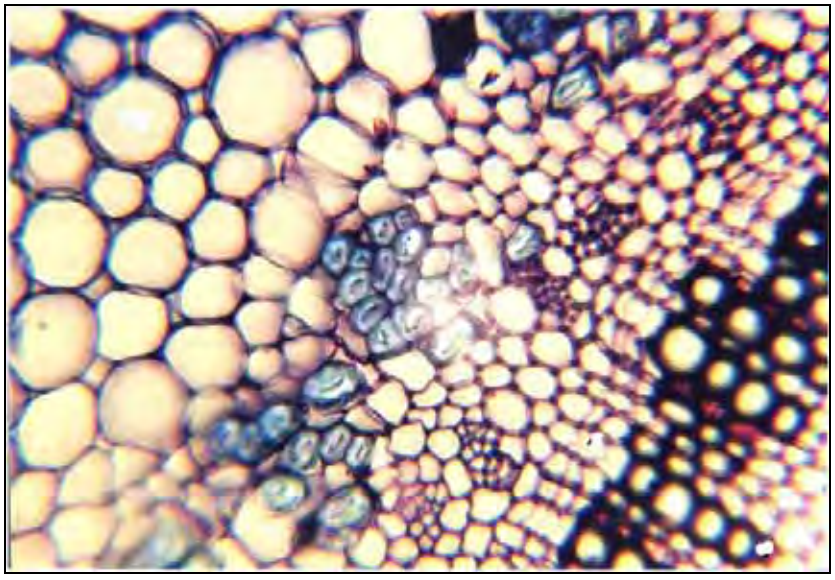

Figure 2. Transversal section through the stem of *Catharanthus roseus* L. (objective x40, methyl green and fucsine coloration)
1. - epiderma, 2. – clorenchim, 3. – cortical parenchym, 4. – packets with cellulosic fibres, 5. – bast, 6. – bast-ligneous cambiu, 7. – secondary wood, 8. – primary wood, medullary parenchyma.

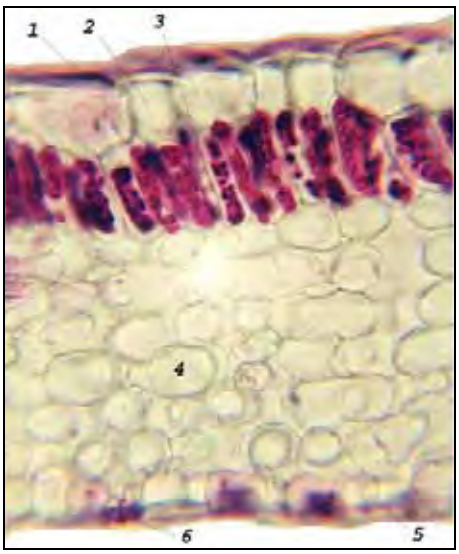

Figure 3. Transversal section through the leaf of *Catharanthus roseus* L. (objective x40, methyl green and fucsine coloration)

Figure 4. Transversal section through the leaf of *Catharanthus roseus* L. (objective x40, methyl green and fucsine coloration)
1. – cuticula, 2. – superior epiderma, 3. – palisadic parenchyma, 4. – lacunose parenchyma, 5. – inferior epiderma, 6. – stomates, 7. – bast-ligneous cambiu.

CONCLUSIONS

1. The stem presents the conducting tissues arranged on two concentric circles separated from the bast-ligneous cambiu.
2. The leaf presents bifacial, amphystomatic structure.

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SOME ASPECTS CONCERNING SOIL EROSION CONTROL BY USING SLOPES' TERRACING IN CORRELATION WITH GULLY EROSION CONTROL

Sevastel Mircea, Ioan Alexandru Calin[1]

KEY WORDS: soil erosion, terraces, orchards, alluvia, check dams

ABSTRACT

Soil erosion control on small torrential and agricultural watersheds represents – at least from theoretically point of view, the ideal solution for the sustainable soil resources conservation in the hilly areas. But, practically, as regard to what has been happening in our country, especially untill year 1990, not always in all Romania's territory has been paid a special attention to an adequate correlation between surface and gully erosion works. Thus, there are some situations where, unfortunately, gully erosion control did not always take into account the future soil erosion works on slopes, existing in this way some over designed works.

The paper presents a case study from the Curvature area of Sub-Carpathians, in Buzau County, this area being one of the most affected by water erosion region in the country, concerning the existence of such un-concordances related to surface and gully erosion control.

INTRODUCTION

Soil erosion control on small torrential and agricultural watersheds represents the most suitable solution for the sustainable soil resources conservation in the hilly areas. Human intervention in a torrential watershed by using soil erosion works has to fit to the different natural conditions of the studied area. In the meantime, an adequate correlation between the surface and gully erosion works has to be taken into account by the engineers in order to get the best practically results, as well as to avoid the high costs of the works, in terms of initial investment and maintenance.

It can be mentioned that out of the total amount of the soil erosion works in a watershed, gully erosion control represents about 20-40%, while in the watersheds where it has to protect important socio-economical objectives, gully erosion works, mainly check-dams, exceed in some cases about 50-60% of the total works.

There will be presented in the paper, in the form of synthetic and personal appreciations, some relevant examples and aspects, conclusions and recommendations for the designing activity, execution and exploitation, concerning the effects of the erosion works from a case study carried out in the Curvature region of Sub-Carpathians, Buzau County. It has to be mentioned that the most important part of the studied sub-watersheds have been completely equipped with erosion works in the period of years 1973-1987 and, unfortunately, no additional works were completed up to day.

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It has be said from the begining that the Slanic torrential watershed, as well as the majority of the water courses from the Curvature region of Sub-Carpathians, Buzau County, are ones of the most degraded areas from Romania by water erosion and landslides. This situation is being reflected in that region mainly in the very high values of the annual mean turbidity in the waters, of more than 5000 g/m^3 , as well as in the alluvia specific suspensional flow, exciding 25 t/ha/yr.

MATERIAL AND METHOD

A long time soil erosion survey (cartography) in the selected torrential watershed, Slanic River Basin - which is a tributary of the Buzau River, was carried out. There were taken into account, both uncontrolled and controlled watersheds, in term of soil erosion works. As regard to the watersheds morphometry, as well as soil erosion risk, there were used maps at the 1:25000 scale, as well as at 1:5000, 1:2000 and 1:1000 scales, realized in the years 1962, 1981 and 1989. Also, some aerial photographs of the selected area were used. In addition to that, the initial antierosion projects designs of the region were used, mainly the longitudinal and transversal profiles in the gullies.

The site research concerning runoff and soil erosion were carried out within the Laboratory of Soil Erosion of the University of Agricultural Sciences, Bucharest – Faculty of Land Reclamation and Environmental Engineering. This field laboratory is located in the Road Valley from the Aldeni village, Buzau County, and consists mainly in some 12 runoff plots located on different slopes and cultivated with different arable crops, as is presented below (Fig. 1).

Figure 1. General view of the Laboratory of Soil Erosion Control with the runoff plots

Regarding the surface soil erosion loss on some of the studied watersheds, there were used the prediction model established by professor M. Moțoc for the torrential watersheds with predominant agricultural land use. This model, with a similar structure like the well-known Wischmeier model, Universal Soil Los Equation (USLE, 1978), is based on the results obtained over a research period of 15 years in Romania, having the following equation (M. Motoc, 1979):

$$E_s = K_a \cdot L^m \cdot i^n \cdot S \cdot C \cdot C_s$$

where E_s is the mean annual soil loss, in t/ha/year; K_a is rainfall aggressivity correction

factor, having the values of 0.08 ÷ 0.16, which represents the ratio between soil loss on the standard runoff plots - having 100 m^2 (25 × 4 m), 15% slope and maintained bare soil - and I_p index (I_p index represents the product by the total amount of precipitation (H - in mm) times the maximum I_{15} intensity (I_{15} - in mm/min) for a given rainfall; L^m is the slope length factor with L in meters; i^n is the slope steepness factor with i in %; S is the soil erodibility factor, which have been determined by means of information from runoff plots under natural rain and findings; C is the crop management factor and C_s is the erosion-control practice factor.

For the gully erosion prediction, field experiments have been conducted by comparing aerial photographs taken at least at ten years intervals. As an indicator, the volume of annual eroded soil related to the gully active surface unit has been used. The computation of the total annual volume of the eroded soil by gulling is as follows (M. Motoc, 1979):

$$W_s = q \cdot S_{active}$$

where W_s is the total annual volume of eroded soil by gulling, in m^3/an; q is the volume of sediments, in cubic meters/ha/year and S_{active} is the gully active surface, in hectares.

RESULTS AND DISSCUSIONS

The main results on the soil erosion survey in the studied area can be summarized as regard to some topics, as follows:

AS REGARD TO THE SURFACE SOIL EROSION WORKS EFFECT

The effect of the surface soil erosion works performed in the torrential watersheds, consists mainly in the mitigation of the solid and liquid runoff, both on slopes an in the gullies. There are several cases in the region, where due to an unsatisfactory correlation between the design of the check dams on the gullies and the versants' management, the check dams are not completely siltated, mainly because of the lack of sufficient alluvia. There is the case of the 5.5.m concrete checkdam, from the Funduri Valley (a), that is currently uncompletely siltated on about 2.0 m high, after about 20 years time from its execution, as well as the 3.5 m checkdam, built up on the Tatarului Valley (b) in the same time (Fig. 2). Such situation can be seen, unfortunately, very often in the studied area, that implies waste money in the soil erosion control.

Figure 2. Checkdams on the gullies uncompletely siltated due to unsatisfactory correlation between the design of the checkdams and the versants' management

The main cause of uncompletely siltation of the chechdams' reservoirs is in close connection to the terasses execution on the slopes, in order to be cultivated with fruit trees. Despite the fact that several slopes have been remaining uncultivated after '90 th (Fig. 3a), the simply execution of the terraces on the slopes has determined the severe reduction of the surface soil loss, in some cases in the admissible limits, of maximum 6 t/ha/yr.

But, there are also several situation in the studied region where the agricultural works of th soils are completely unproper realized, the soil being plough out up to down (Fig. 3b),. In such a situation, the soil losses are quite big, exceeding 3-5 times the admissible limits. There is the case of the Baiesti Valley, which is very severe affected by water erosion.

Figure 3. General view of the terrased versants and land use and cultivation direction of the agricultural lands in the Slanic watershed

Using the above-mentioned USLE model, a computation of the soil loss for about 20 small torrential watersheds was performed, in the majority of them obtaining big soil loss. In the meantime, the soil loss due to gully erosion was calculated and measured in the field, the total amount of soil loss (total and efluent erosion) being then compared with the measured losses at some gauges from the national network.

CONCLUSIONS

As a main result of the long time soil erosion survey performed in the Slanic watershed, mainly regarding the effect of the surface and gully erosion works performed in the torrential watersheds, consists mainly in the mitigation of the solid and liquid runoff. There are several cases in the region, where due to an unsatisfactory correlation between the design of the check dams on the gullies and the versants' management, the check dams are not completely siltated, mainly because of the lack of sufficient alluvia. Also, the major cause of the big soil loss in the region is in a close connection with the lack maintenance of the soil erosion works. Thus, more attention has to be paid for their maintance.

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STRUCTURAL PECULIARITIES OF THE VEGETATIVE APPARATUS OF SPONTANEOUS AND CULTIVATED *ORIGANUM VULGARE* L. PLANTS

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KEY WORDS: Origanum vulgare L., anatomo-ecological characters, vegetative organs, secretory hairs

ABSTRACT

The authors investigate the structure of the subterranean and aerial vegetative organs in spontaneous (collected from different populations from Romania) and cultivated Origanum vulgare L. plants. The aim of this study is to determine the anatomo-ecological characters through which the analyzed individuals are distinguished from each other. Peculiar attention has been given to the distribution and structure of the secretory hairs, these characters being important in the estimation of the value of Origanum vulgare as aromatic plant.

INTRODUCTION

The *Origanum* L. genre consists of approximately 30 Mediteranean species, among which in the Romanian flora, only *Origanum vulgare* L. grows (Chifu et al., 2001). Popular named "şovârf", this species is a representative member of the aromatic flora, due to the essential oils it produces.

The literature on this aromatic species is quite rich, if considering the researches on the plant ontogenesis in order to determine the developmental stage at which the maximum amount of essential oil is produced, related with seasonal and altitudinal conditions (Basker and Putievsky, 1978; Putievsky et al., 1986; Müller-Riebau et al., 1997; Boira and Blanquer, 1998) (cf. Kofidis et al., 2003). However, there are a few studies referring to alterations of the structural features of higher plants in general, and particularly of aromatic plants, along seasonal and altitudinal gradients (Kofidis et al., 2003).

The present paper analyzes comparatively the structure of the vegetative apparatus of some *Origanum vulgare* L. plants in relation with plant origin (cultivated and native) as well as with the altitude of the region in which the spontaneous individuals grow.

MATERIAL AND METHODS

The research material is represented by cultivated and spontaneous flowering *Origanum vulgare* L. plants, the latest ones belonging to 5 different populations from Romania (grown in different altitude, climatic and soil conditions): 1. Măgura, Bacău

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Country (300m); 2. Bâtca Doamnei, Neamț Country (330m); 3. Gârcina, Neamț County (450m); 4. Dorna Arini, Suceava Country (600m); 5. Bicaz Chei, Neamț Country (700m).

The material was fixed and preserved in 70% ethylic alcohol. Cross-sections of the rhizome, aerial stem and leaf were performed using a manual microtome, coloured with iodine green and ruthenium red and embedded in glicero-gelatine. The superficial sections through the foliar limb were made by scouring and coloured with iodine green. The obtained permanent slides were analyzed on a Novex (Holland) microscope and photographed at the same microscope with a photo digital camera.

RESULTS AND DISCUSSIONS

In all analyzed *Origanum vulgare* L. (cultivated and spontaneous - belonging to 5 different populations) individuals, the **rhizome** (Fig. 1, 2) presents a constant secondary structure resulted from the activity of both lateral meristems, the cambium and the phellogen.

The rhizome is protected by epidermis; the cortical parenchyma is thin (5-6 layers); both tissues consist of tangentially elongated cells. The phellogen, differentiated from the endodermis, produces a small quantity of secondary protected tissues (1-3 cork layers consisted of big cells with thin and suberified walls and 3-4 mildly collenchymatous layers of phellodermis).

The secondary xylem is the most developed tissue of the rhizome; the vessels have different diameter and are irregularly dispersed in the libriform mass; the libriform fibres present thick and lignified secondary wall.

The phloem ring is thin and comprises two distinct zones: an internal one consisted of sieve-tubes and companion cells and an external one formed by parenchyma cells. Both secondary vascular tissues are traversed by uniserial medullary rays, composed by parenchymatous cellulosic cells (in the phloem) or cells with lignified walls (in the xylem).

At the inner part of the xylem ring, four islands of primary xylem (corresponding to the four vascular bundles from the primary structure of the rhizome) may be observed.

The pith is relatively thin, parenchymatous - cellulosic of meatic type, with big rounded cells in its central part. Towards the periphery of the pith, the diameter of the compounded cells decreases, their walls being lignified.

In all investigated *Origanum vulgare* L. plants, the **aerial stem** (Fig. 3, 4) presents quadratic contour in transverse section, with four (more or less prominent in its upper third and attenuated in the rest of its length) ribs which define (only in its upper third) four valleculae, among which two are more profound.

The aerial stem's cuticle is thick; the stomata are rarely, being situated above the external level of the epidermis.

The hairs are of two categories: 1. trichomes - long, multicellular, uniseriated, with obtuse tip, more numerous in the valleculae and 2. secretory hairs - short, multicellular, with uni- (Fig. 12) or octocellular gland (Fig. 11), the latest ones being located at the same level with the epidermis cells. The number of both categories of hairs decreases from the upper to the lower third of the aerial stem. Some trichomes are formed on a pedestal of the epidermis.

The mechanic tissue is represented by thick (in the upper third of the stem) or thin (in the middle third) cordons of angular or tangential (in the rest of the stem's length) collenchyma in the ribs and by a unistratified collenchymatous hypodermis in the rest.

The cortex (with or without aeriferous cavities of tangentially elongated shape) ends with a Casparyan endodermis with small tangentially elongated cells.

The stele with primary structure (in the upper third of the aerial stem) comprises four big vascular bundles of open - collateral type (in front of the ribs), separated by narrow parenchymatous-cellulosic rays; in the thickness of the latest ones, in the vicinity of the endodermis, four bundles of phloem, may be observed.

The secondary structure of the aerial stem (Fig. 5, 6) results only from cambium, which can have or not a constant activity on the entire circumference of the stem. In general, this secondary meristem produces more elements of phloem and xylem (especially vessels) in the four big vascular bundles from the primary structure than in the rest of the stele, where libriform fibres with very thick and slightly lignified walls are predominated. The secondary phloem ring is always thinner than the xylem one.

The pith of the aerial stem is very thick, in general, consisting of big cellulosic parenchymatous cells in its central part and small cells with lignified walls towards its periphery; some central cells are disorganized resulting more or less aeriferous cavities.

The **foliar limb** is amphistomatic with diacytic stomata (Fig. 9) and rare (long, multicellular, uniserial) trichomes (especially on the median nervure) and numerous secretory hairs with uni- or octocellular gland (Fig. 10), the latest ones being located in some excavations of the epidermises.

The mesophyll (Fig. 7) is differentiated in unistratified palisade tissue (at the adaxial face of the limb) and multistratified (3-4 layers) lacunous tissue (at the abaxial face of the limb).

The median nervure (Fig. 8) is prominent on the abaxial face of the limb and comprises, in the fundamental collenchymatous parenchyma, a single big vascular bundle, in which the vessels are disposed, in generally, in radiary columns separated by a few parenchymatous - cellulosic cells

Comparing the structure of the spontaneous *Origanum vulgare* L. individuals from different populations (grown at different altitude, in different climatic and soil conditions), we settled several histological characters of the aerial vegetative organs from which they are distinguished, e. g. *in the stem*: 1. the number of trichomes and secretory hairs per surface unit of the organ (more numerous trichomes at the individuals from Gârcina and Măgura population); 2. the presence and frecvency of the cortical aeriferous cavities (absence of the aeriferous cavities at the individuals from Bâtca Doamnei population; numerous aeriferous cavities at the individuals from Bicaz-Chei, Dorna Arini, and Măgura population;); 3. the degree of pith lignification (entire lignified pith at the individuals from Bicaz-Chei population); 4. *in the foliar limb* - the height of the palisade cells (very high palisade cells at the individuals from Bicaz-Chei (the palisade tissue representing 60% from the thickness of the mesophyll) and Măgura population (the palisade tissue representing 40% from the thickness of the mesophyll).

The combined effects of altitude and season on growth of oregano showed that the increasing elevation resulted in a progressive decrease of plant height, while, during the growing period, plants in autumn are somehow shorter than plants in summer (Kofidis et al., 2003). Our comparatively histo-anatomical observations at the spontaneous *Origanum vulgare* L. individuals dignify the fact that the thickness of the aerial stem (resulting especially from the development of the pith) progressively decrease at once with the increase of the altitude of which the plants grow.

In the same time, our comparatively study revealed some histo-anatomical differences of the aerial stem between the spontaneous and cultivated *Origanum vulgare* L. individuals, e. g. 1. the height of the ribs and the width of the valleculae in the upper third

of the stem (more higher ribs at the spontaneous individuals; more wider valleculae at the cultivated individuals); 2. the frequency of the secretory hairs with octocellular gland (more numerous secretory hairs with octocellular gland in cultivated individuals); 3. the ratio libriform fibres/vessels in the secondary xylem of the middle third of the stem (more libriform fibres in the spontaneous individuals)

CONCLUSIONS

Our histo-anatomical investigations reveal the fact that the structural differences between the *Origanum vulgare* L. plants of different origin (cultivated or native) are rather qualitative as the differences between the spontaneous (native) individuals belonging to populations situated at different altitude, in different climatic and soil conditions. The mentioned differences refer to several anatomo-ecological characters of the aerial stem and foliar limb, e. g. the frequency of the trichomes and secretory hairs per surface unit of the organ; the lignification degree of the stem's stele; the thickness of the stem and foliar limb; the development degree of aerenchyma.

To appreciate the value of a species as aromatic plant, the number of secretory hairs, producers of the essential oil, in all the aerial parts is very important as well as the number of cells which compose the secretory gland. Our researches dignifies the fact that both types of secretory hairs (with uni- and octocellular gland) are present in the aerial vegetative organs of spontaneous and cultivated *Origanum vulgare* L. plants; in the latest ones, the secretory hairs with octocellular gland are more numerous, especially in the aerial stem.

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Fig. 1. Cross-section through the rhizome of spontaneous *Origanum vulgare* plant – Bâtca Doamnei population (orig. photo) (x200)

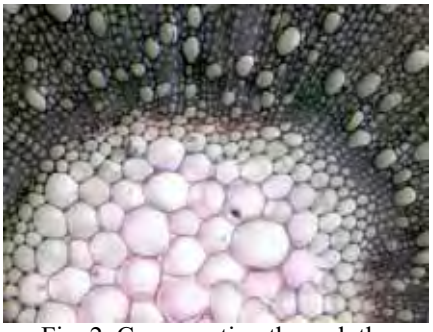

Fig. 2. Cross-section through the rhizome of spontaneous *Origanum vulgare* plant – Bâtca Doamnei population (orig. photo) (x200)

Fig. 3. Cross-section through the upper third of the aerial stem of spontaneous *Origanum vulgare* plant – Bâtca Doamnei population (orig. photo) (x40)

Fig. 4. Cross-section through the upper third of the aerial stem of cultivated *Origanum vulgare* plant (orig. photo) (x40)

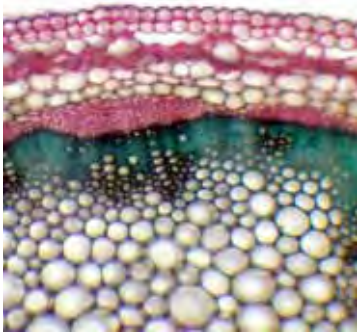

Fig. 5. Cross-section through the middle third of the aerial stem of spontaneous *Origanum vulgare* plant – Măgura population (orig. photo) (x200)

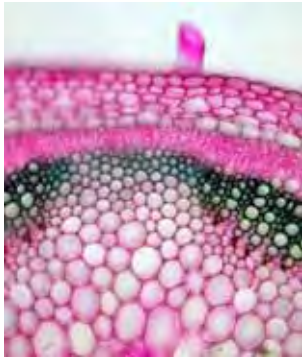

Fig. 6. Cross-section through the middle third of the aerial stem of cultivated *Origanum vulgare* plant (orig. photo) (x200)

Fig. 7. Cross-section through the mesophyll of the leave of spontaneous *Origanum vulgare* plant –Dorna Arini population (orig. photo) (x200)

Fig. 8. Cross-section through the median nervure of the leave of spontaneous *Origanum vulgare* plant – Măgura population (orig. photo) (x200)

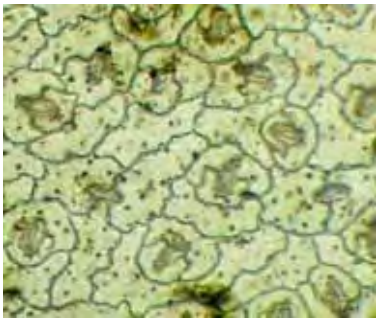

Fig. 9. Superficial section through the foliar limb (lower epidermis) of spontaneous *Origanum vulgare* plant – Bicaz population (orig. photo) (x400)

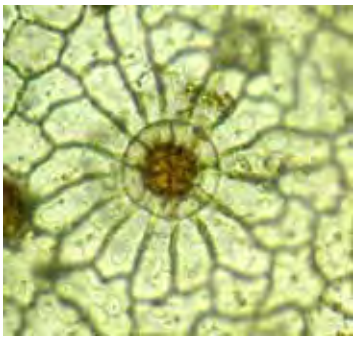

Fig. 10. Secretory hair with octocellular gland in superficial section through the foliar limb (upper epidermis) of spontaneous *Origanum vulgare* plant – Bicaz-Chei population (orig. photo)

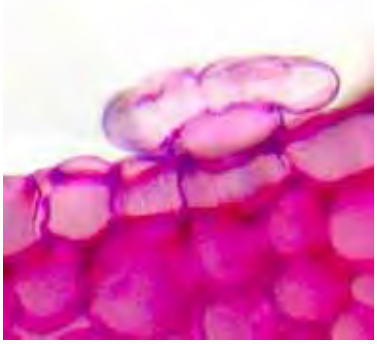

Fig. 11. Secretory hair with octocellular gland in cross-section through the aerial stem of cultivated *Origanum vulgare* plant (orig. photo) (x400)

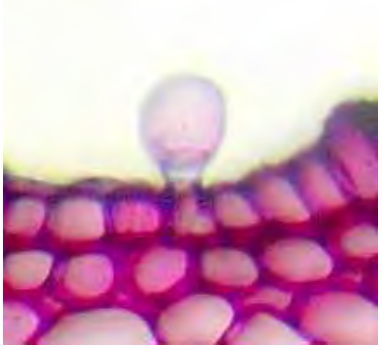

Fig. 12. Secretory hair with unicellular gland in cross-section through the aerial stem of spontaneous *Origanum vulgare* plant –Dorna Arini population (orig. photo) (x400)

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ASPECTS OF DIGITO-PALMAR DERMATOGLYPHS APPLIED TO CHILDREN OF AN APROPIATE AGE IN CRAIOVA

Grădinaru Mihaela[1]

KEY WORDS: dermatoglyphics, group of 40 students, simian line

ABSTRACT

The paper focus on the analysis of digito-palmar dermatoglyphs on a group of 40 children from the School no.30 in Craiova. In this study we followed some aspects of digito-palmar dermatoglyphs on the whole group of children and also a comparison between boys and girls from a digito-palmar dermatoglyphical point of view. It has been noticedt a slight resemblance of the appearance of dermatoglyphs but what is noteworthy is the fact that in this group of healthy children appears simian line which is correlated in literature with the presence of some genetic diseases such as heart diseases or deficiency intelligence although in this case this does not denote any kind of affection. This study of dermatoglyphics aspects on children of the same age can be useful for further researches in anthropology, genetics and medicine.

INTRODUCTION

Dermatoglyphs are also known as fingerprints, representing the totality of the papillary drawings from fingers, palms and soles of a person determinated by the location of the dermal increase and flexural creases. Their disposal is unique to each person, formed in the first few weeks of intrauterine life remaining completely unchanged after birth and whole life.

Dermatoglyphs are known for individual identification in legal medicine and also in studies for characterization from the dermatoglyphic point of some population from different regions and for identification of various hereditary diseases.

In our country the first research on the characterization of human populations from different regions, dermatoglyphs heredity and the aspects of the dermatoglyphs in some hereditary diseases were done by C-tin. Turai.

At the present moment, there are also many researches in the country and abroad, aiming to characterize the dermatoglyphs aspects of populations from different region and also the correlation of them with some diseases (Down syndrome, infantile autism, cancer, mental retardation).

Digital dermatoglyphs include aspects of papillary increase from phalanx. Are studied, in particular, papillary drawing from the distal phalanx that can be 3 types: arch (A), loop (L), circle (W) each of the 3 types are subdivided into several subtypes.

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Palmar dermatoglyphs are represented by flexura line in hand, palmar triradius, papilar drawings. Flexural lines are of 3 types; thenar, medium, superior, each being characterized by different forms and particularities. Palmar thriradius (the point where the dermal crests leaves in 3 different directions) are situated 4 in superior region: underlying index (a), underlying medium (b), underlying finger ring (c), and underlying auricular finger (d) and in thenar region there is the fifth thriradius, *axial thriradius* noted whit "t" (t, t', t''). Palmar drawings are represented by arch, loops and circles, sometimes appearing complex figures.

MATERIAL AND METHODS

The assay of fingerprints was done on a group of 40 scholars (20 girls and 20 boys) aged between 12-14 years from school no. 30 Craiova. It has been used method of impressing with printing ink: in the palm of a person will be situated a quantity of printing ink, depending on the size of the hand. The person will lie the printing ink on the internal surface of the palms ang fingers, afterwards, on a piece of paper will apply the fingerprint of each hand, each finger. In the right part of the paper will be noted person's initials and age. After drying fingerprints taken(about 24 h) fingerprints are analyzed with magnifying glass, emphasizing characteristic aspects of each person.

It was studied the type and frequency of papillary drawing from the fingers, palm design lines, the presence or absence of simian line, presence or absence of palmar thriradius, position of axilar thriradius, Penrose angle, papilar palmar drawings. Have been collected data on the origin of children, parents' origin, the health condition of the parents and children.

RESULTS AND DISCUSSION

The 40 scholars in the study come from families where father and mother are originary from the townships in the vicinity of Dolj district, the health condition of children being good.

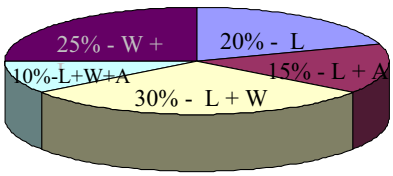
As a result of the examination of the digital and palmar dermatoglyphs at the 40 subjects, we found the following characteristics:

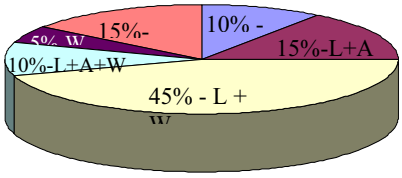
Characteristics of digital dermatogyphics:

- The constitutional type with predominants of arch are missing at both girls and boys;

- The constitutional tipe with predominats of the loop are meet like this: monomorphism in loop is meet in 20% of the boys and 10% at the girls; dimorphism (L+A) is in egal percentage at the boys and girls; dimorphism (L+W) is meet in a bigger procent at girls (45%) compared with the boys (30%); trimorphism (L+A+W) is meet only at girls in procent of 10% and absent at the boys; trmorphism (L+W+A) is meet only at boys (10%) and absent at girls. At the whole group of students it found a highr percentage of constitutional tipe (L+W) (37,5%) in comparation with the other tipes with predomination of the loop: (L+A)=15%, L=15%, (L+W+A)=5%, (L+W+A)=5%;

- The constitutional type with predominats of the circle; at the boys is meet only dimorphism (W+L) in percentage of 25%, and at the girls either dimorphism (W+L) in percentage of 15% and also monomorphism (W) in a percent of 5%.Looking the whole group it is remarked that W is present only like monomorphism (W) in percentage of 2,5%, and dimorphism (W+L) in percentage of 20%. Trimorphism in circle is absent at the whole group.





Frequency types costitutionale at the boys

Frequency types costitutionale at the girls

Characteristics of palmar dermatoglyphs

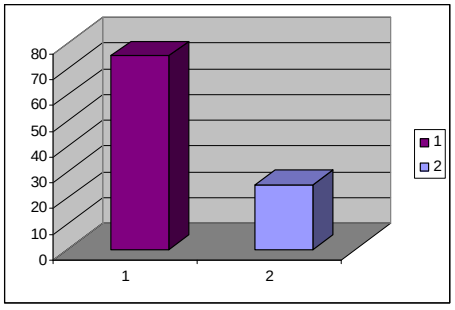
- Palmar lines: thenar linies apears in the majority of the cases, surrounding thenar region, in some cases forked or branched down; the median line appears in the majoriry of the cases linear horizontal, slightly arched towards the down side of the basis of ulnar region; Superior line is finished between index and medium finger, or liniar side by radial. The presence of simian line (a single flexion crease in the median palmar region) it was remarked at 2,5% children. This line was frequently encountered in all chromosomal diseases and correlated with somatic malformations obvious or hidden. It is encountered at normal population in procent of 2,6% in his typical form, and 5% in the form of transition. In this case the presence of simian line wasn't correlated with any affection, the person concerned is perfectly normal phenotypic. The parents of the child do not present Simian line, although this line is inherited father-son, mother-daughter. Certain dermatoglyphics malformations and the presence of simian line were correlated with diseases in origin, when have been studied aspects of parents dermatogyphs at children with Down syndrome or infantile autism (Ana Tarca, 2003, etc.)

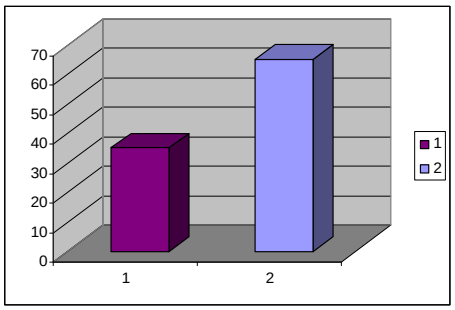
- In superior region of the palms dominate morphological papilar figures type loop or arch in interdigital space b-c

- The presence of superior thriradius is in a percentage of 100%

Axial thriradius is in t position at the boys in a percentage of 75%, and at the girls in a percentage of 35%. In "t' " position, at boys can be observed a percentage of 25%, at the girs being in a higher percentage (65%). Axial thriradius in "t'' " wasn't remarked.

the angle of Penrose is in most cases 45^0, smaller amounts recorded as insignificant.





Frequency thiraduius the boys: 1-t; 2-t'

Frequency thiraduius the girls: 1-t; 2-t'

CONCLUSIONS

The dermatoglyphics investigations perfomed on a group of 40 children from Craiova have put into evidence the following aspects:

- The most widespread type of digital papillary design was type loop (77,5%) which is found also like monomorphism and dimorphism (the predominant form L+W) and trimorphism. Papillary drawing type (A) is rarely met, not present constitutional types dimorph or trimorph with arch predomination. Arch is often met in dimorph or trimorph constitution of loop or circle, met in special at the index finger.
- Circle (W) is met often like dimofism (W+L) and in a little procent like monomorphism (5%)
- In superior region of the palm, in b-c interdigital spaces are frequently papillary drawings type arch or loop.
- Axial thriradius is often met at the boys in "t" position and at the girls is often met in "t'" position
- In the entire group of normal children investigated was remarked the presence of simian line, line which is frequently correlated with different diseases as a cause of concern for parents whose children have this line. Its presence in this case is accidental, not inherited by heredity from parents.
- The analogy concerning the aspect of dermatoglyphs at the group of 40 children can be a consequence of the parent's derivation in close localities
- The results can contribute in the future at the dermatoglyphic characterization of the population from Craiova.

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STUDY UPON THE INFLUENCE EXERTED BY THE TYPE OF RECIPIENTS USED FOR NUTRITIVE LAYERS IN CULTURE PLACE ON THE PRODUCTION OF FUNGI AGARICUS BISPORUS

Alexandra Becherescu, Horgoş A.[1]

KEY WORDS: compost, culture system, mound, flat layer, recipient

ABSTRACT

The introduction and layout of the nutritive layer in the culture place are performed with different methods, which have evolved along time and are in concordance with the culture system applied. For example, mound-type cultures are not a novelty anymore, and they are abandoned; on the contrary, flat-layer cultures or the ones in polyethylene sacks are used in all mushroom beds at the moment. This work presents the experimental results achieved in the study upon fungi Agaricus bisporus, where the layout of the incubated compost was made in parallelepiped recipients, in comparison with the polyethylene sacks.

INTRODUCTION

According to culture systems – classic, demi-intensive and industrialized intensive (monozonal, bizonal and plurizonal), there are: cultures in mound placed on the floor; cultures in mounds placed on racks; flat-layer cultures; cultures in polyethylene sacks or other types of recipients.

Until 1971, the method with polyethylene sacks had been unknown in Romania, and afterwards it was experimented on small areas, with perspectives of extinction especially for the classic culture, fact that has actually happened. The polyethylene sacks have been used more and more since 1981. Our country produces 0.1 mm- polyethylene sacks with dimensions of 50-70 cm length and 30-40 cm width (Horgoş A., 2002).

The sacks, filled with the compacted culture substratum, are placed in the culture place, according to its form, in longitudinal or transversal rows. The sacks may be also used like in the case of the ***rack-stepped culture***, with 1-3 parapets made of concrete prefabricated pieces, of metallic net or reinforcing iron. In the classic culture without pasteurization, sacks are for a single use because the viroses from the previous culture can be destroyed only with chemical disinfection based on copper sulphate and formalin. Their reutilization is possible only in the case of steam thermal treatment, at the temperature of 80-85^{o} C, for 4-5 hours, or with Basamid GR or Dazomet 98%, 10 g/m^2, during the warm period (15 June - 15 July). Fungi culture in polyethylene sacks has the following advantages: the nutritive layer in sacks is protected from the infestation with nematodes, compared to the mounds placed on soil; sack filling is made with a smaller intake of labor force than mound filling; the culture place get infested harder with the specific diseases and

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pests, even after several culture cycles; sacks introduction and evacuation from the culture place is made under more advantageous conditions than in the case of mound or flat layers; the nutritive layer may be transported at longer distances from the place where the compost is made. Under the intensive culture system, the layout of the nutritive substratum may be performed in two ways: on racks – for monozone culture; in boxes – for bizone and plurizone culture. At the moment, in the mushroom beds from Romania, the mostly used recipients are the PE sacks (Ø – 38.2 cm, l – 60 cm, h – 30 cm) and the parallelepiped recipients (L – 60 cm, l – 40 cm, h – 20 cm).

MATERIAL AND METHOD

The researches on the effect exerted by the utilization of PE-sack recipients and of parallelepiped sacks were carried out at S.C. Champignon S.R.L. Arad.

This mushroom farm, which was recently founded, meets all the qualities of a modern farm endowed with warming and cooling equipment (for winter and summer), the production cycle being performed continuously, with installation for mechanical ventilation and assurance of humidity in the microclimate of the culture rooms, all these being controlled by PCs with the help of a software set in concordance with crop technology.

The experience was tri-factorial by type and the experimental factors are:

Factor A –System of compost layout in the culture place: a_1 – in polyethylene recipients laid vertically on 3-level racks; a_2 – in rectangle polyethylene recipients laid horizontally on 3-level racks.

Factor B – Treatment of the culture nutritive substratum with the product Nemasys M: b_1 – nutritive culture substratum with no application of Nemasys M; b_2 – nutritive culture substratum with application of Nemasys M.

Factor C – Fungus strain cultivated: c_1 – Strain A-15; c_2 – Strain A-x.

The aim of our researches was to establish which of the two recipient types (PE sacks or parallelepiped sacks), laid differently on the racks in culture room, is the most indicated for utilization in production. The nutritive culture substratum (compost prepared in the 2nd and 3rd phase) was imported from specialized companies from Hungary, in the recipients mentioned above. The objectives of our researches are: the supervision of the production differently performed in the two recipient types; the supervision of the production generated by the two strains A-15 and A-x in both recipient types used; the establishment of the influence exerted by the product Nemasys M on yield, this product having effects of fighting against the pests specific to the nutritive culture substratum.

RESULTS AND DISCUSSIONS

In table 1, we present the experimental results of the researches on the cultivation of two fungi strains, A-15 and A-x, in different recipients (PE sacks and parallelepiped sacks); the product Nemasys M was applied on the nutritive culture substratum for phyto-sanitary protection. We may notice yield differentiation under the influence exerted by a_1 and b_1 and b_2 (PE sacks with cellulose nutritive substratum treated or not treated with Nemasys M) with up to 5.1 %, and under the influence exerted by a_2 and b_1 and b_2 (parallelepiped recipients with cellulose nutritive substratum treated or not treated with the same product) with up to 6.4 %. Under the influence of the factor B, in a_1 yield differentiation between b_1 and b_2 is 2.7 %, and in a_2 – 3.5 %. The table 2 presents the mean yields achieved for factors B and A during a production cycle and during a calendar year, for 8 culture cycles, with utilization of compost prepared in the 2nd phase, respectively incubated compost. The mean yield achieved in a_2 (parallelepiped recipients), during a cycle and a year as well, in a culture room, is 5.8% bigger than in a_1 (PE sacks), in the same periods of the year.

Table 1

Experimental results regarding the yield of fungi *Agaricus bisporus*, strains A-15 and A-x, cultivated in recipients of „polyethylene sacks" and of „parallelepiped" polyethylene sacks during a 46-day production cycle (nutritive cellulose layer prepared in the 3rd phase – incubated), 8 culture cycles per year

Factor A	Factor B	Factor C	No. of fungi / recipient (pieces)	Fungus mean weight (g/piece)	Yield achieved for factor C				Yield achieved for factor B				
					kg/ recipient	kg/100 kg n.c.l. or %	kg/culture room (240 m^2)	kg/m^2 culture area	No. of fungi / recipient (pieces)	kg/ recipient	kg/100 kg n.c.l. or %	kg/ culture room (240 m^2)	kg/m^2 culture area
a_1*	b_1	c_1	268,7	19,8	5,320	28,0	6384	26,60	262,6	5,558	29,3	6669	27,80
		c_2	256,4	22,6	5,795	30,5	6954	28,98					
	b_2	c_1	280,9	20,9	5,871	30,9	7045	29,35	267,3	6,080	32,0	7296	30,10
		c_2	253,6	24,8	6,289	33,1	7547	31,44					
a_2**	b_1	c_1	338,5	19,5	6,600	30,0	6653	27,72	336,3	6,941	31,6	6997	29,15
		c_2	334,0	21,8	7,282	33,1	7340	30,58					
	b_2	c_1	359,2	20,7	7,436	33,8	7495	31,23	345,8	7,722	35,1	7784	32,43
		c_2	332,3	24,1	8,008	36,4	8072	33,63					

* - 1200 sacks / culture room;

** - 1008 parallelepiped recipients / culture room.

Table 2

Synthesis of the experimental results regarding the yield of fungi *Agaricus bisporus* (strains A-15 and A-x) achieved in recipients of PE sacks and parallelepiped sacks with compost prepared in the 3rd phase (incubated), 8 culture cycles per year

Factor A	Factor B	Factor C	Yield achieved / cycle for factor C			Mean yield achieved for factor B					Mean yield achieved for factor A				
						per cycle			per year		per cycle			per year	
			kg/100 kg n.c.l. or %	kg/culture room (240 m^2)	kg/m^2 culture area	kg/100 kg n.c.l. or %	kg/culture room (240 m^2)	kg/m^2 culture area	kg/culture room (240 m^2)	kg/m^2 culture area	kg/100 kg n.c.l. or %	kg/culture room (240 m^2)	kg/m^2 culture area	kg/culture room (240 m^2)	kg/m^2 culture area
a_1	b_1	c_1	28,0	6384	26,60	29,3	6669 (100,0%)	27,80	53352 (100,0%)	222,3	30,6	6982,5 (100,0%)	29,1	55860 (100,0%)	232,8
		c_2	30,5	6954	28,98										
	b_2	c_1	30,9	7045	29,35	32,0	7296 (109,4%)	30,10	58368 (100,0%)	243,2					
		c_2	33,1	7547	31,44										
a_2	b_1	c_1	30,0	6653	27,72	31,6	6997 (100,0%)	29,15	55976 (103,8%)	233,2	33,3	7390,5 (105,8%)	30,8	59124 (105,8%)	246,4
		c_2	33,1	7340	30,58										
	b_2	c_1	33,8	7495	31,23	35,1	7784 (111,2%)	32,43	62272 (106,7%)	259,5					
		c_2	36,4	8072	33,63										

*According to the compost preparation phase, the period of a culture cycle may be:

- 60 days in the case of the compost prepared in the 2nd phase (seeded and not incubated), of which 54 days incubation and harvest and 6 days evacuation of the exhausted nutritive layer and the preparation of a new production cycle; 365 days/year: 60 days/cycle = 6 production cycles/year;

- 46 days in the case of the incubated compost (3rd phase), of which 40 days harvest and 6 days the preparation for a new production cycle; 365 days/year: 46 days/cycle = 7.9 ≈ 8 production cycles/year.

The yield achieved in a_1 during a cycle, in a culture room under the influence of b_2 (nutritive substratum treated with Nemasys M), is 9.4% bigger than under the influence of b_1 nutritive substratum not treated with Nemasys M). In a_2, during the same period, under the influence exerted by b_2, we recorded an extra yield of 11.2 %. In a_2b_2, the yield achieved is 6.7% bigger than in a_1b_2, and in a_2b_1 3.8 % bigger than in a_1b_1.

Table 3 presents, according to calculations of mathematical statistics, specific to the method of variance analysis, the significance of yield differences achieved in the comparisons made as effect of the interdependence between the experimental factors.

Table 3

Singular influences and influences exerted by the interactions between experimental factors on the production of fungi *Agaricus bisporus* under conditions provided by the utilization of PE sack recipients and of parallelepiped recipients

Variant	Mean yield (kg/ha)		Relative yield (%)	Difference (± t/ha)	Significance of difference
1. Singular influence exerted by PE recipients on fungi yield					
a2-a1	33,33	30,63	108,82	2,70	*
DL 5%= 2,62 DL 1%= 3,96 DL 0,1% = 6,37					
2. Singular influence exerted by the product Nemasys M on yield					
b2-b1	33,55	30,40	110,36	3,15	*
DL 5%= 2,32 DL 1%= 3,20 DL 0,1% = 4,41					
3. Singular influence exerted by fungi strains on yield					
c2-c1	33,28	30,68	108,48	2,60	-
DL 5% = 2,81 DL 1% = 3,81 DL 0,1% = 5,09					
4. Influence exerted by the interactions between different recipients and the same treatment or different treatments and Nemasys M					
a2b1-a1b1	31,55	29,25	107,86	2,30	-
a2b2-a1b2	35,10	32,00	109,69	3,10	-
a2b2-a1b1	35,10	29,25	120,00	5,85	**
DL 5% = 3,49 DL 1% = 5,06 DL 0,1% = 7,60					
5. Influence exerted by the interactions between the same recipient and different treatments with Nemasys M					
a1b2- a1b1	32,00	29,25	109,40	2,75	-
a2b2- a2b1	35,10	31,55	111,25	3,55	*
DL 5% = 3,29 DL 1% = 4,53 DL 0,1% = 6,23					
6. Influence exerted by the interactions between different fungi strains and the same recipient					
a1c2- a1c1	31,80	29,45	107,98	2,35	-
a2c2- a2c1	34,75	31,90	108,93	2,85	-
DL 5% = 3,97 DL 1% = 5,38 DL 0,1% = 7,20					
7. Influence exerted by the interactions between different fungi strains and the same treatment with Nemasys M					
b1c2- b1c1	31,80	29,00	109,66	2,80	-
b2c2- b2c1	34,75	32,35	107,42	2,40	-
DL 5% = 3,97 DL 1% = 5,38 DL 0,1% = 7,20					
8. Influence exerted by the interactions between different treatments with Nemasys M and the same strain or different fungi strains					
b2c1- b1c1	32,35	29,00	111,55	3,35	-
b2c2- b1c2	34,75	31,80	109,28	2,95	-
b2c2- b1c1	34,75	29,00	119,83	5,75	**
DL 5% = 3,65 DL 1% = 4,97 DL 0,1% = 6,73					

9. Influence exerted by the interactions between different recipients and the same or different fungi strains					
a2c1- a1c1	31,90	29,45	108,32	2,45	-
a2c2- a1c2	34,75	31,80	109,28	2,95	-
a2c2- a1c1	34,75	29,45	118,00	5,30	*
DL 5% = 3,83 DL 1% = 5,44 DL 0,1% = 7,93					

Successive to the unilateral analysis of the experimental factors, from points 1-3 table 3, we may conclude the following:

- the significances of yield differences between a_2 and a_1 (parallelepiped recipients – PE-sack recipients) and between b_2 and b_1 (nutritive substratum treated with Nemasys M and not treated) are positive, proving that the yields achieved under the influence exerted by a_2 and b_2 are statistically covered.

- the yield difference between c_2 and c_1 (strains A-x and A-15) does not have any significance; the yield achieved is not statistically covered.

- analyzing the points 4-9, we may notice that the significance of yield differences as effect of the interaction between experimental factors are significantly or distinctly significantly positive in four cases; in the other cases, it does not have any significance.

CONCLUSIONS AND RECOMMENDATIONS

1. The utilization of different types of recipients (PE sacks and parallelepiped recipients) for the layout of the nutritive culture substratum (compost prepared in the 3rd phase) has determined the achievement of differentiated yields, bigger in both strains in the case of the parallelepiped recipients (a_2) compared to the PE sacks (a_1).

2. The mean yield achieved under the influence of factor A is 30.6 % in a_1 – PE sacks and 33.3 % in a_2 – parallelepiped recipients, the yields being statistically assured.

3. The total yield achieved in one culture room, during a culture cycle and within one year, in the case of the utilization of parallelepiped recipients (a_2), is 5.8 % bigger than the one achieved in the case of PE sacks (a_1), during the same time periods.

4. The yields achieved under the influence of factor B is 29.3 % in a_1b_1 and 31.6 % in a_2b_1 (nutritive substratum in both recipient types with no application of Nemasys M) and 32.0 % in a_1b_2 and 35.1 % in a_2b_2 (nutritive substratum with application of Nemasys M).

5. The yields achieved under the influence of factor C (c_1 – A-15 and c_2 – A-x) do not have a statistical cover, so that we may draw the conclusion that both strains can be used in production with advantages and disadvantages in both situations, but their specification is not subject of this analysis.

6. We recommend the continuation of researches and the calculation of the economic efficiency to deepen the conclusions resulted.

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STUDIES REGARDING THE APPLICATION OF PROTECTION COMPLEXES WITH EFFECT OF INFLUENCE UPON YIELD IN ONION CROP

Alexandra Becherescu[1]

KEY WORDS: protection complexes, fighting against, yield

ABSTRACT

During the vegetation period, the attack caused by pathogens and pests may superpose, with synergic harming effects regarding yield decrease.

The information related to yield losses represents the best synthesis of the effects exerted by pathogens and pests on onion crop.

The information comprised within this work is taken from the notifications performed during 2003-2005 in the untreated control variant, compared to the variants in which we applied protection complexes and also from the comparisons between variants and the experimental mean ($M\overline{X}$) and the mean of protection complexes ($M\overline{X}_{cp}$), too.

INTRODUCTION

Onion is one of the vegetables that occupy an important position worldwide and in our country, as well, in terms of area and yield. Being one of the most gainful vegetable species, when all technological steps are applied, the onion may be also attacked by numerous pathogens and diseases that diminish yield significantly.

MATERIAL AND METHOD

Our studies were performed under the pedo-climatic conditions from Albina, Timis county, during three years (2003-2005). The onion crop was started with scallion.

The experimental plot was situated on a 4-year crop-rotation: 25% bulbous species, 25% *Solanaceae* species, 25% legumes and 25% straw cereals.

The experiment, repeated during the three consecutive years, was identically structured by using the Latin rectangle system, 8 variants in 4 replications. Each parcel was 20 m^2.

During the three experimental years, there were optimal conditions of development for pathogens (downy mildew - *Peronospora destructor* (Berk.) Casp and onion rot - *Botrytis allii* Munn.) and pests (onion fly – *Delia antiqua* Meig.).

In order to calculate the attack degree, we made observations on the attack frequency and intensity monthly.

Four treatments were applied at warning, and the following protection complexes were used in all 8 variants:

- V_1 – Trichodex 25 WP 0.2% (Tricoderma harzianum (T-39)/ l g dry product) + Victenon 50 WP 0.05% (Bensultap 50%);

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- V_2 – Bravo 500 SC 0.15 % (Clorotalonil 500 g/l) + Actara 25 WG 0.01% (Thiametoxam 25%);

- V_3 – Previcur 607 SL 0.15% (Propamocarb 607 g/l) + Confidor 70WG 0.02% (Imidacloprid 700 g/kg);

- V_4 – Folpan 80 WDG 0.15% (Folpet 80%) + Karate Zeon 0.02% (Lambda-cihalotrin 50 g/l);

- V_5 – Ridomil Gold MZ 68 0.25 % (Mefenoxam 4% + Mancozeb 64%) + Victenon 50 WP 0.075% (Bensultap 50%);

- V_6 – Ridomil Gold Plus 42.5 WP 0.3% (Mefenoxam 2.5%+ Iron Copper 40%) + Mospilan 20 SP 0.025% (Acetamiprid 20%);

- V_7 – Dithane M45 0.2% (Mancozeb 80%) + Fastac 10EC 0.02% (Alpha-cypermethrin 100 g/l);

- V_8 – Untreated control variant.

Data processing was performed in concordance with the method of variance analysis, applied in the case of the experiments placed in Latin rectangle, for each year separately, and with the monofactorial experiments performed for several years, in the same locality.

The production data obtained in the statistical calculation are supported by observations and determinations.

RESULTS AND DISCUSSIONS

From tables 1, 2, 3 and 4 (the centralizing synthesis table) we may conclude the cover degree, in terms of statistical calculation, of the yields achieved during 2003-2005, with the significances of yield differences to the untreated control variant (Mt. – table 1), to the experimental mean ($M\bar{x}$ – table 2) and to the mean of protection complexes ($M\bar{x}_{cp}$ – table 3).

Table 1

Mean yield achieved in bulb onion during 2003-2005

Var.	Protection complex	Conc. (%)	Dose (l/ha, kg/ha)	Mean yield achieved (t/ha)	Relative mean yield (%)	Difference to Mt (t/ha)	Significance
V_1	Trichodex 25 WP	0,2	1,20	22,60	251,11	13,60	***
	Victenon 50 WP	0,05	0,30				
V_2	Bravo 500 SC	0,15	0,90	22,93	254,81	13,93	***
	Actara 25 WG	0,01	0,06				
V_3	Previcur 607 SL	0,15	0,90	24,03	267,04	15,03	***
	Confidor 70 WG	0,02	0,12				
V_4	Folpan 80 WDG	0,15	0,90	21,10	234,44	12,10	***
	Karate Zeon	0,02	0,12				
V_5	Folpan 80 WDG	0,15	0,90	26,83	298,15	17,83	***
	Karate Zeon	0,02	0,12				
V_6	Ridomil Gold Plus 42,5 WP	0,3	1,8	25,73	285,93	16,73	***
	Mospilan 20 SP	0,025	0,15				
V_7	Dithane M 45	0,2	1,2	17,93	199,26	8,93	***
	Fastac 10 EC	0,02	0,12				
V_8	Control	-	-	9,00	100,00	-	Mt

DL 5%	DL 1%	DL 0,1 %
1,56	2,16	3,00

The yield differences compared to the control variant (Mt) are between 8.93-17.83 t/ha, the yield achieved in all seven variants being statistically assured, and the significances of yield differences are very significantly positive. Analyzing the significance of yield differences, by comparing them to the experimental mean ($M\bar{x}$), we may notice that there is an emphasized differentiation between variants in terms of significance degree, namely the very significantly positive signification is available in only three variants (V_5, V_6 and V_3). In V_2, the significance is just positive, while in the variants V_2 and V_4 there is no significance; in variants V_7 and V_8, the significance is very significantly negative.

Table 2

Significance of yield differences during 2003-2005
by reporting them to the experimental mean ($M\bar{x}$)

Var.	Protection complex	Conc. (%)	Dose (l/ha, kg/ha)	Mean yield achieved (t/ha)	Relative mean yield (%)	Difference to Mt (t/ha)	Significance
V_5	Ridomil Gold MZ 68 WPVictenon 50 WP	0,25 0,075	1,5 0,45	26,83	126,15	5,56	***
V_6	Ridomil Gold Plus 42,5 WPMospilan 20 SP	0,3 0,025	1,8 0,15	25,73	120,98	4,46	***
V_3	Previcur 607 SL Confidor 70 WG	0,15 0,02	0,9 0,12	24,03	112,99	2,76	***
V_2	Bravo 500 SC Actara 25 WG	0,15 0,01	0,9 0,06	22,93	107,82	1,66	*
V_1	Trichodex 25 WP Victenon 50 WP	0,2 0,05	1,2 0,3	22,60	106,25	1,33	-
$M\bar{x}$	Experimental mean	-	-	21,27	100,00	0,00	Mt
V_4	Folpan 80 WDG Karate Zeon	0,15 0,02	0,9 0,12	21,10	99,20	-0,17	-
V_7	Dithane M 45 Fastac 10 EC	0,2 0,02	1,2 0,12	17,93	84,31	-3,34	000
V_8	Control	-	-	9,00	42,31	-12,27	000

DL 5%	DL 1%	DL 0,1 %
1,44	1,98	2,72

When the comparison is made only between the significance of yield differences and the mean of the yield achieved under the influence exerted by protection complexes, excluding the yield achieved in V_8 – untreated Mt, the situation is dramatically different: the yield achieved is statistically covered only in two variants, the significance of yield differences being very significantly positive in V_5 (Ridomil Gold MZ 68 0.25 % + Victenon 50 WP 0.075%), and distinctly significantly positive in V_6 (Ridomil Gold Plus 42.5 WP 0.3% + Mospilan 20 SP 0.025%) (table 2). In variants V_3, V_2 and V_1, there is no significance, and in the variants V_7 and V_4, the significance of yield differences is very significantly negative, respectively distinctly significantly negative.

The analysis of the centralizing table 4 and fig. 4 leads to conclusions regarding the graduation of interferences exerted by protection complexes (V_1 – V_7) on the yield achieved, when the significances of yield differences are differently reported (to untreated Mt, to $M\bar{x}$ or $M\bar{x}$ cp).

Table 3

Significance of yield differences during 2003-2005

by reporting them to the mean of protection complexes ($M\bar{x}_{cp}$)

Var.	Protection complex	Conc. (%)	Dose (l/ha, kg/ha)	Mean yield achieved (t/ha)	Relative mean yield (%)	Difference to Mt (t/ha)	Significance
V_5	Ridomil Gold MZ 68 WPVictenon 50 WP	0,25 0,075	1,5 0,45	26,83	116,55	3,81	***
V_6	Ridomil Gold Plus 42,5 WPMospilan 20 SP	0,3 0,025	1,8 0,15	25,73	111,77	2,71	**
V_3	Previcur 607 SL Confidor 70 WG	0,15 0,02	0,9 0,12	24,03	104,38	1,01	-
$M\bar{x}_{cp}$	Mean of protection complexes	-	-	23,02	100,00	0,00	Mt
V_2	Bravo 500 SC Actara 25 WG	0,15 0,01	0,9 0,06	22,93	99,61	-0,09	-
V_1	Trichodex 25 WP Victenon 50 WP	0,2 0,05	1,2 0,3	22,60	98,16	-0,42	-
V_4	Folpan 80 WDG Karate Zeon	0,15 0,02	0,9 0,12	21,10	91,64	-1,92	00
V_7	Dithane M 45 Fastac 10 EC	0,2 0,02	1,2 0,12	17,93	77,89	-5,09	000

DL 5%	DL 1%	DL 0,1 %
1,55	2,15	2,99

By studying the centralizing table 4, we may get a general image, comparatively synthetic, concerning the influences exerted by treatments on the yield level achieved in the experimental variants; the effects on the two diseases studied and on the pest are made evident by the significance degree – positive or negative – of the yield differences to the triad Mt, $M\bar{x}$ and $M\bar{x}_{cp}$.

Among the seven variants that include different protection complexes, only in two of them, V_5 (Ridomil Gold MZ 68 0.25 % + Victenon 50 WP 0.075%) and V_6 (Ridomil Gold Plus 42.5 WP 0.3% + Mospilan 20 SP 0.025%) the significance of yield differences is positive in all comparisons. So, in V_5 (Ridomil Gold MZ 68 0.25 % + Victenon 50 WP 0.075%), the significance of yield differences is very significantly positive in all three different cases of comparisons (Mt, $M\bar{x}$ and $M\bar{x}_{cp}$), and in V_6 (Ridomil Gold Plus 42.5 WP 0.3% + Mospilan 20 SP 0.025%), in two cases (Mt and $M\bar{x}$), the significance is very significantly positive, and in one case (to $M\bar{x}_{cp}$), the significance is distinctly significantly positive.

Table 4

Mean yield achieved and the significances of yield differences compared to the untreated control variant Mt, mean of the experience $M\bar{x}$ and mean of the protection complexes $M\bar{x}_{cp}$ during 2003-2005

Analysis elements of statistical calculation \ Variant			V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
Mean yield (t/ha)			22,6	22,93	24,03	21,10	26,83	25,73	17,93	9,00
Relative yield (%)		Mt	251,11	254,81	267,04	234,44	298,15	285,93	199,26	100,00
		$M\bar{x}$	106,25	107,82	112,99	99,20	126,15	120,98	84,31	42,31
		$M\bar{x}_{cp}$	98,16	99,61	104,38	91,64	116,55	111,77	77,89	-
Yield difference (t/ha)	Mt		9,00	13,60	13,93	15,03	12,10	17,83	16,73	8,93	-
	$M\bar{x}$		21,27	1,33	1,66	2,76	-0,17	5,56	4,46	-3,34	-12,27
	$M\bar{x}_{cp}$		23,02	-0,42	-0,09	1,01	-1,92	3,81	2,71	-5,09	-
Significance of difference		Mt	***	***	***	***	***	***	***	Mt
		$M\bar{x}$	-	*	***	-	***	***	000	000
		$M\bar{x}_{cp}$	-	-	-	00	***	**	000	

	DL 5%	DL 1%	DL 0,1 %
DL Mt	1,56	2,16	3,00
DL $M\bar{x}$	1,44	1,98	2,72
DL $M\bar{x}_{cp}$	1,55	2,15	2,99

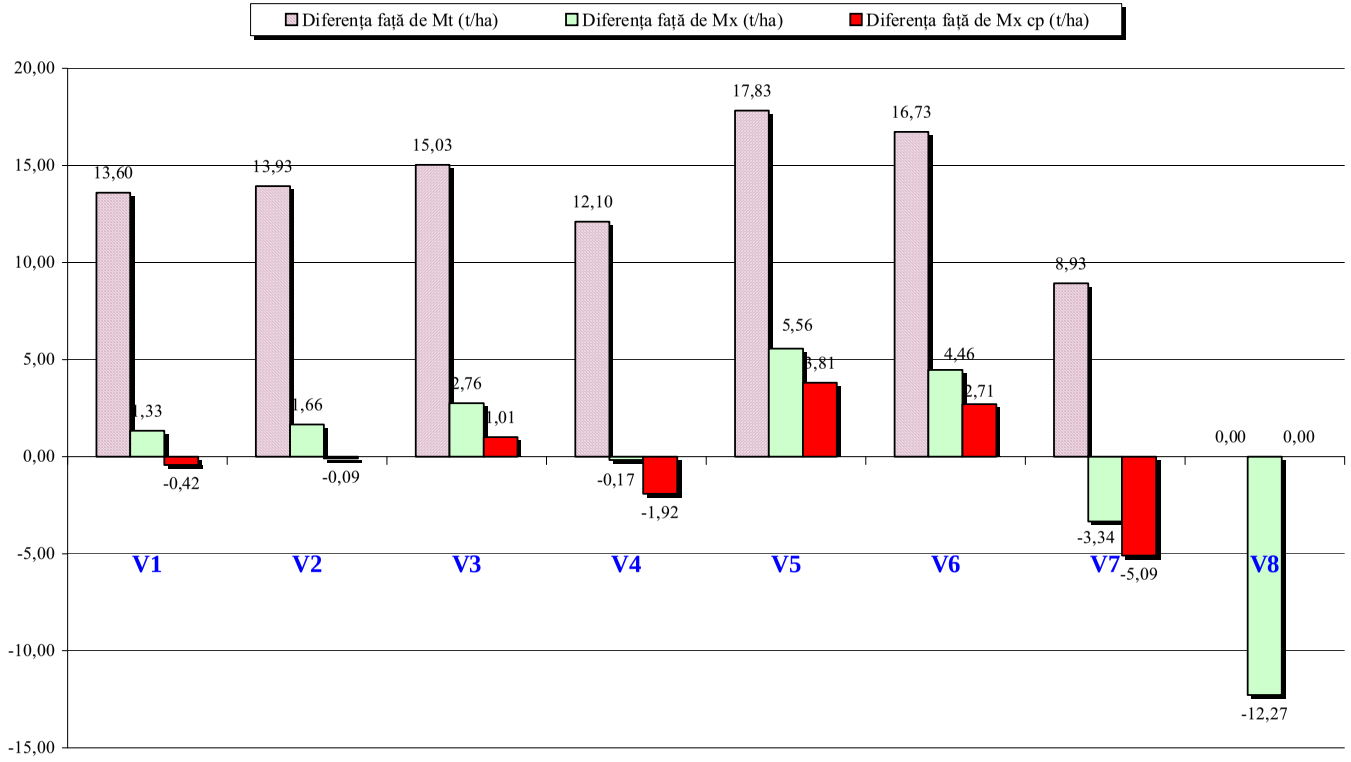

Fig.1. Differences to Mt, $M\bar{x}$ and $M\bar{x}_{cp}$ of the onion yield achieved during the experimental period 2003-2005

CONCLUSIONS

1. We may draw the conclusions that, for a real and correct assessment of the efficacy of protection complex-based treatments (V_1 – V_7), materialized in the effect exerted on the yield level achieved, it is necessary to perform a strict calculation of mathematical statistics. The appreciation of the significance of yield differences has to be done with multiple comparisons, not with a singular one, in our case to the untreated Mt.

2. The protection complexes from V_5 (Ridomil Gold MZ 68 0.25 % + Victenon 50 WP 0.075%) and V_6 (Ridomil Gold Plus 42.5 WP 0.3% + Mospilan 20 SP 0.025%) had the best effects in the fighting against the two diseases (downy mildew - *Peronospora destructor* (Berk.) Casp and onion rot - *Botrytis allii* Munn.) and the pest (onion fly – *Delia antiqua* Meig.); this fact is proved by the yields achieved, by the statistical cover and also by the highest degree of appreciation of the significances of yield differences.

3. The worst effect in the fighting against the two diseases and the pest was exerted by the protection complex used in V_7 (Dithane M45 0.2% + Fastac 10EC 0.02%), because of the fact that the significance of yield differences to $M_{\bar{x}}$ and $M_{\bar{x}_{cp}}$ is very significantly negative.

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STUDY ON THE ANTHOCYANIC COMPLEX IN BLACK GRAPES OF FRENCH ORIGIN, CULTIVATED IN THE SOUTH OF DRAGASANI VINEYARD

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KEY WORDS: *vineyard, breed, grapes, anthocyanins, chromatic structure*

ABSTRACT

The South of Dragasani vineyard, where there are famous areas, such as:Dobruşa, Eforie-Greaca, Dealul Banului etc., representing a high degree of favourability for the sustainable viticulture and extraordinary quality including the breeds destined to obtaining the red wines.

In the areas of the South of Dragasani Vineyard, found under the direct influence of some spread forestry massifs, of Olt River and Mamu, Dalga and Beica Creeks, there are the breeds: Burgund mare, Cabernet Sauvignon, Merlot and Pinot noir behave exceptionally under an oenological relation. For the grapes of these breeds, besides the important proportions of glucides and proper contents of acidity, considerable quantities of anthocyanins are also accumulated, the phenyl compounds conferring the base feature and specificity of the red wines.

INTRODUCTION

The geographical and climatic elements of the Dragasani Vineyard as a whole (table 1), which are very close to or even better than those specific to other famous vineyards for quality red wines have formed an objective and especially sound reason, which has been the basis in taking decisions regarding the enlargement in the great wine-growing area and breeds with black grapes.

Table 1

Geographical and climatic elements of Dragasani Vineyard compared to other vineyards for red wines (based on Teodorescu St. and coll., 1987).

Vineyard	N. Lat.	Alt. m	Average T° per year, °C	Precipitations mm	Enoclimatyc Ability
Drăgăşani	44°30′	182	10.8	684	4757
Valea Călugărească	44°58′	170	11.0	656	4724
Recaş	45°47′	140	10.6	636	4606

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In the last decade, some researches performed in the central areas and even the Southern ones on the theme of the technological potential of the brands, have greatly confirmed the superior behaviour of the breeds for red wines in the South of Dragasani Vineyard (Ionica Laura – 2006, Muntean Camelia, Ionica Laura – 2006, Nicolaescu C. – 2007).

Taking into account the importance the red wines have acquired, due to their superior value under a hygienic-alimentary ratio, the studies have been lately amplified concerning the anthocyanic colouring compound in the grapes of the black varieties of French origin, whose partial results are the aim of this work here.

MATERIAL AND METHODS

The study has pointed the investigation of the anthocyanic potential of the varieties: Burgund mare, Cabernet Sauvignon, Merlot and Pinot noir.

The researches performed in the wine-growing years of 2005, 2006 and 2007 have tried to: establish the contents of anthocyanins in grapes in full maturity, the phenyl anthocyanic maturity and technological maturity; the extractability of the anthocyanins in grapes and quantifying the proportion of extractable anthocyanins also known as the technological reserve; the percentage participation of the three pigment categories (yellow, red, blue); establishing the values of the chromatic attributes (colour intensity – I_c; colour tonality – T_c; Flaviliu cations – dA%), of the anthocyanic extracts, for the three phenophases of maturation.

In order to determine the chromatic components of the anthocyanic compound, advanced spectrophotometry methods were used, recommended by OIV. The participation of the pigments was established by determining the optical densities: at 420nm for the yellow component; at 520nm for the red component; at 620nm for the blue component. The quantification of the chromatic attributions was accomplished by applying the following formulae: Ic = Do 420 + Do 520 + Do 620 nm; Tc = Do 420/Do 520 nm;

$$dA\% = Do\ 520 - \left(\frac{Do420 + Do620}{2}\right) \times \left(\frac{1}{Do520} \times 100\right)$$

RESULTS AND DISCUSSIONS

In table 2, there are mentioned the values of the anthocyanin contents in grapes, the proportions of extractable anthocyanins and the technological reserves of colouring matter that can reach the wine.

All these elements of chromatic order differ, as levels depending on the breed, and for the same breed, there are also differences recorded from one wine-growing year to another. Among the breeds, the richest one in anthocyanins is Cabernet Sauvignon and the poorest one is Pinot noir and Merlot and Burgund mare occupy intermediate positions.

The proportions of extractable anthocyanins for all breeds are higher in the good wine-growing years (e.g.: 2007) compared to those years with some problems of climatic order (2005). The highest extractable anthocyanins proportions, in relation to the anthocyanins contents in grapes belong to the Pinot noir brand (56.9%), and the lowest ones belong to the Burgund mare (49.03%).

Depending on the anthocyanins quantities stocked in grapes and their extractability, the technological reserves order the breeds in a decreasing direction, thusly:

Cabernet Sauvignon (693,2 mg), Merlot (685,9 mg), Burgund mare (533,8 mg), Pinot noir (415,2 mg).

The chromatic structure of the anthocyanic compound in grapes, conferred by the participation of the various categories of pigments for the black breeds of foreign origin is presented in table 3.

Table 2

The contents of anthocyanins, the extractability of the anthocyanins and technological reserves of the grapes of French origin
(at technological maturity)

Features	Production years	Varieties			
		Burgund mare	Cabernet Sauvignon	Merlot	Pinot noir
Anthocyanins mg/kg grapes	2005	1065	1320	1208	671
	2006	1066	1395	1220	730
	2007	1134	1475	1321	786
	Average	1088.3	1396.7	1249.7	729.0
Extractability anthocyanins %	2005	48.5	49.0	54.1	56.4
	2006	48.7	49.2	54.9	57.1
	2007	49.9	50.6	55.6	57.3
	Average	49.03	49.6	54.9	56.9
Technological reserve	2005	516.5	646.8	653.5	378.4
	2006	519.1	686.3	669.8	416.8
	2007	565.9	746.4	734.5	450.4
	Average	533.8	693.2	685.9	415.2

The proportions of the various chromatic components differ depending on the variety of the grapes.

The yellow-orange component (extraction at 420nm) has indicated the lowest value (28.8%) for the Cabernet Sauvignon variety and the highest value (33.70%) for the Pinot noir variety. For Merlot and Burgund mare, the yellow-orange component occupies intermediate positions (30.10% and respectively 31.30%).

The red component (extraction at 520nm) was in proportions ranging between 59.1% for Pinot noir and 61.9% for Cabernet Sauvignon, realising a reverse situation compared to the yellow-orange component.

The blue component (absorption at 520nm) indicates as averages values situated within close limits, ranging from 7.10 % for Pinot noir and 9.20 % for Cabernet Sauvignon, proportions that cannot significantly influence the quality of the colouration even when the wines are very young.

The anthocyanic extracts, obtained from comparable quantities of grape skins, were subject to a rigorous analysis of spectrophotometry, which is a method recommended by OIV. The values of the colouring intensity resulted by summing up the optical densities at 420, 520 and 620nm, of the tonality representing the ratio between the optical densities at 420nm and 520nm and of the flavin cations determined by a special formula, where all categories of pigments are taken into consideration (yellow, red and blue) are presented in table 4.

Table 3

The elements of chromatic structure of the anthocyanic extracts in grapes of black varieties of French origin cultivated in the South of the Dragasani Vineyard (at technological maturity)

Variety	Production years	Participation of the pigments					
		Yellow pigments		Red pigments		Blue pigments	
		Do 420	%	Do 520	%	Do 620	%
Burgund mare	2005	0.648	31.6	1.225	59.8	0.176	8.6
	2006	0.658	31.3	1.257	59.9	0.184	8.8
	2007	0.681	31.1	1.305	59.6	0.203	9.3
	Average	0.662	31.3	1.262	59.7	0.187	8.9
Cabernet Sauvignon	2005	0.670	29.2	1.832	61.9	0.263	8.9
	2006	0.872	28.9	1.869	61.9	0.277	9.2
	2007	0.890	28.3	1.956	62.1	0.260	9.6
	Average	0.810	28.8	1.885	61.9	0.266	9.2
Merlot	2005	0.720	30.4	1.452	61.3	0.196	8.3
	2006	0.740	30.2	1.496	61.1	0.213	8.7
	2007	0.807	29.9	1.652	61.2	0.240	8.9
	Average	0.755	30.1	1.533	61.2	0.216	8.5
Pinot noir	2005	0.603	34.3	1.034	58.8	0.121	6.9
	2006	0.611	33.6	1.077	59.2	0.131	7.2
	2007	0.647	33.4	1.148	59.2	0.143	7.4
	Average	0.620	33.7	1.086	59.1	0.131	7.1

Cuvette 1 mm

Table 4

The chromatic attributions of the anthocyanic extracts in grapes of black varieties of French origin cultivated in the South of the Dragasani Vineyard (at technological maturity)

Variety	Production years	Intensity	Colour tonality	Flaviliu cations
		Ic	Tc	dA%
Burgund mare	2005	2.05	0.528	66.34
	2006	2.10	0.522	66.50
	2007	2.19	0.523	66.13
	Average	2.11	0.524	66.32
Cabernet Sauvignon	2005	2.96	0.471	74.56
	2006	3.02	0.466	69.28
	2007	3.15	0.455	70.60
	Average	3.04	0.464	71.48
Merlot	2005	2.37	0.495	68.45
	2006	2.45	0.494	68.18

	2007	2.70	0.488	68.34
	Average	2.50	0.492	68.32
Pinot noir	2005	1.78	0.583	64.07
	2006	1.82	0.567	65.55
	2007	1.94	0.564	65.59
	Average	1.84	0.571	65.07

The chromatic attributions of the colouring matter in the black breed grapes of foreign origin indicate, by their values, important enough differences between the varieties, regarding the quantitative and qualitative potential of the colouring phenyl compound.

From the point of view of colour richness, stated by the colouring intensity, the Cabernet Sauvignon breed is at the top of the group (I_c = 3.04). The last place is occupied by the Pinot noir breed (I_c = 1.84). The Merlot and Burgund mare varieties occupy intermediate positions (I_c = 2.50 and respectively I_c = 2.11). The sizes of the colouring intensity faithfully represent the values of the specific extinctions of the various categories of pigments.

From a visual point of view, the values of the tonality, with a reverse order, signify a more favourable ratio between the yellow-orange pigments and the red ones for the colouring matter in the Merlot and Burgund mare grapes.

The quality of the anthocyanic compound, stated by the flaviliu cations (dA%), where the red component (Do 520nm) plays the essential role, ordering the breeds thusly: Cabernet Sauvignon, Merlot, Burgund mare, Pinot noir.

CONCLUSIONS

In the Southern areas of the Dragasni Vineyard, the breeds for the red wines of French origin have highly favourable ecological conditions for growing and fruitage.

For grapes, besides the considerable glucide proportions, there are also accumulated important contents of anthocyanins, which can accomplish the colour parameter even for those wines, situated on the highest quality level. Under this ratio, there are distinguished the Cabernet Sauvignon and Merlot brands.

The highly balanced chromatic structure does not only allow the typical red wines to be obtained, but also elaborates the pink wines and the Clairet types, especially from the grapes of the Burgund mare and Pinot noir varieties.

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EFFECT OF STORAGE AND METHOD OF PROCESSING ON PHYSICOCHEMICAL COMPOSITION OF RASPBERRY FRUITS AND THEIR PRODUCTS

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KEY WORDS: fruits, ascorbic acid, anthocyans, nectars, purees.

ABSTRACT

In 2002-2003 at the Institute of Mountain Stockbreeding and Agriculture, Troyan a study was conducted on physicochemical composition of fruits from raspberry cultivars Shopska alena and Meeker. On the basis of their fruits two products, nectars and purees, were developed at the Institute of Canning Industry, Plovdiv. Content of ascorbic acid in the fruits reached to 31.86 mg% (Shopska alena – 2002) and that of anthocyans to 59.68 mg% (Meeker – 2002). After 1-year storage of the fruits the ascorbic acid decreased 1.5 times and the anthocyans about 4 times. The ascorbic acid and anthocyans were preserved better in the purees made from cv. Meeker.

INTRODUCTION

Raspberry is a widespread crop in fore-mountain and mountain regions. It is a major source of incomes for the greatest part of the population in them (Georgiev et al., 2005, 2007). In addition to the fact that it is a fruit with very good palatability and attractive appearance, the content of compounds with an antioxidant effect having attained broad popularity in the last years is also of great importance (Boycheva, 2001, Dale et al., 2001, Deighton et al., 2000, Stavroulakis et al., 1998, , McGhie et al., 2002). In many countries, as well as in Bulgaria the biochemical composition of raspberry fruits, as well as that of the products obtained after their processing is studied more and more profoundly (Boycheva, 1999, Rommel et al., 1992, 1993; Spanos et al., 1987; Wrolstad et al., 1993).

The objective of this study was to observe the variation in the physicochemical composition, including the biologically active substances, such as ascorbic acid and anthocyans of fresh and frozen fruits stored for one year and the products obtained from them.

MATERIAL AND METHODS

The study was conducted in 2002-2003. The cultivars Shopska alena and Meeker were studied. The physicochemical composition of fresh and frozen fruits and their products was determined in the chemical laboratory of IMSA, Troyan.

The following parameters were studied: Dry matter Re, total sugars, inverted sugar, sucrose – by the method of Schoorl Regenbogen, acids – titrimetrically, ascorbic acid – by the method of Fialkov, anthocyans – by the method of Fuleki and Francis.

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The mineral composition was determined by the method of atomic emission spectrophotometer, with inductively coupled plasma (ICP) in the chemical laboratory of ICI, Plovdiv.

The following elements were determined: Na, K, Ca, Mg, P, Fe, Cu, Zn.

In laboratory conditions the products of nectar and puree were obtained from the studied raspberry cultivars, presented in two variants:

I – products obtained from fresh fruits

II – products obtained from frozen raw material.

RESULTS AND DISCUSSION

Table 1 presents the results of the physicochemical composition of fresh and frozen raspberry fruits. The climatic conditions has a direct effect on biochemical composition of fruits. In 2002 the average values for May and June were: tº - 17 ºC and rainfall – 87 l/m^2. In 2003 they were tº - 18.8 ºC and rainfall 106.7 l/m^2. It is evident from the obtained data that in 2002 the Meeker fruits had higher content of dry matter - 10.76 %, sugars (total – 6.23 %, inverted – 4.74 %, sucrose – 1.4 %). With regard to the biologically active substances the content of ascorbic acid was higher in the fruits of Shopska alena - 31.86 mg% and that of anthocyans in those of Meeker – 59.68 mg%. After 1-year storage of the fruits there were no significant changes in the content of dry matter, sugars and acids. The differences were most significant with regard to the biologically active substances. The ascorbic acid decreased 1.5 times and the anthocyans about 4 times. In 2003 the Meeker fruits had higher values of dry matter and sugars. The ascorbic acid content was higher in the fruits of Shopska alena - 29.92 mg% and the anthocyans in those of Meeker – 50.00 mg%.

The results of the physicochemical composition of nectars and purees in the two variants are given in Table 2. The differences were significant with regard to the biologically active substances. Among the nectars of fresh fruits the ascorbic acid content was higher in those of Shopska alena - 11.44 mg% and the same in the two nectars from frozen raw material – 7.92 mg%. The Meeker nectar had higher content of anthocyans in both variants (from fresh fruits - 10.97 mg%; from frozen fruits - 6.05 mg%). The ascorbic acid decreased in the nectars of the two variants 2.3 to 2.9 times and the anthocyans 2.5 to 5 times, as against the initial raw material.The purees from fresh fruits of cv. Meeker had higher content of ascorbic acid and anthocyans: 16.72 mg% and 23.23 mg%. Among the purees from frozen raw amterial that of cv. Shopska alena had higher content of biologically active substances (ascorbic acid - 10.52 mg%; anthocyans – 10.16 mg%). The decrease of ascorbic acid in them was 1.5 to 2.3 times and that of anthocyans 1.2 to 2.6 times in contrast to the initial raw material.

The decrease of the biologically active substances in the nectars and purees was probably due to their thermolability during heat processing. The differences with regard to dry matter, sugars, acids were explained by the addition of sugar syrup during production of appropriate products.

Table 3 presents the mineral composition of the studied raspberry cultivars and their products. The results showed that the raspberry fruits has rich mineral composition. The fruits of Shopska alena had higher content of K, Ca, P. The values of Mg were almost the same in the two cultivars and with regard to Fe the Meeker raspberry fruits were superior to those of Shopska alena. The values of the elements remained high in the nectars in spite of dilution. The Meeker nectars and purees had higher content of K, Mg, Fe.

CONCLUSION

It was found from the obtained results that:

1. The fruits of cv. Meeker had higher content of dry matter, sugars and anthocyans.

2. After 1-year storage of frozen fruits of Shopska alena and Meeker the ascorbic acid decreased 1.5 times and the anthocyans about 4 times in both cultivars, as against the fresh fruits.

3. After fruit processing the ascorbic acid content of nectars decreased 2.3 to 2.9 times and that of purees 1.5 to 2.3 times. The anthocyan decrease was 2.5 to 5 times in the nectars and 1.2 to 2.6 times in the purees.

4. The fruits of cv. Shopska alena had higher content of K, Ca and P and the Meeker nectars and purees had higher values of K, Mg and Fe.

Table 1.

Physicochemical composition of fresh and frozen raspberry fruits

Characteristics / Raw material	Dry matter Re %	Total sugars %	Inverted sugar %	Sucrose %	Acids as malic %	Ascorbic acid mg %	Anthocyans mg %
Fresh fruits harvested in 2002							
Shopska alena	9.0	4.56	4.01	0.53	1.84	31.86	44.07
Meeker	10.76	6.23	4.74	1.4	1.54	25.96	59.68
Frozen fruits after 1-year storage							
Shopska alena	8.7	4.03	4.03	-	2.11	21.18	12.5
Meeker	10.4	5.88	5.88	-	1.52	17.82	14.84
Fresh fruits harvested in 2003							
Shopska alena	8.05	3.92	3.25	0.64	1.81	29.92	36.85
Meeker	11.75	6.15	4.70	1.35	1.54	25.17	50.00

Table 2.

Physicochemical composition of nectars and purees

Products	Dry matter Re %	Total sugars %	Inverted sugar %	Sucrose %	Acids as malic %	Ascorbic acid mg %	Anthocyans mg %
Nectars from fresh fruits							
Shopska alena	14.40	12.0	7.15	4.60	1.09	11.44	7.42
Meeker	14.20	12.45	9.03	3.25	0.87	8.80	10.97
Nectars from frozen raw material							
Shopska alena	14	11.10	7.70	4.70	1.37	7.92	3.39
Meeker	13.5	10.25	9.40	0.81	1.07	7.92	6.05
Purees from fresh fruits							
Shopska alena	13.70	7.70	7.35	0.33	2.19	13.20	14.35

Meeker	13.40	10.43	10.25	0.18	1.74	16.72	23.23
			Purees from frozen raw material				
Shopska alena	13.55	8.20	6.15	1.15	2.37	10.52	10.16
Meeker	18.25	12.80	6.24	6.23	1.99	9.15	8.87

Table 3.

Mineral composition of raspberry fruits and their products (mg/kg)

Cultivar	Na	K	Ca	Mg	P	Fe	Cu	Zn
				Raw material				
Shopska alena	75.6	720	243	172	144	6.66	0.58	5.10
Meeker	57.2	700	196	174	126	9.47	0.65	5.66
				Nectars				
Shopska alena	31	325	88.8	52.2	38.3	2.97	0.23	1.90
Meeker	28	380	84.6	57.0	26.8	3.69	0.25	2.14
				Purees				
Shopska alena	31	440	121	91.2	84.2	4.80	0.40	1.92
Meeker	28	480	114	110	62.9	5.97	0.37	2.24

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APPLICATION DU SYSTÈME D'ANALYSE DANGERS ET POINTS DE CONTRÔLE CRITIQUE (APPCC) DANS LA LIGNE D'ÉLABORATION DE VIN ROSÉ

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MOTS CLÉ: dangers, contrôle, surveillance, rosé, entreprises

RÉSUMÉ

La Directive Générale d'Hygiène des Aliments 93/43/CEE, établit que les entreprises du secteur alimentaire, dans lesquelles on inclut, évidemment, les entreprises, doivent mettre en marche un système d'auto-contrôle de leurs productions, basé le système d'Analyse Dangers et Points de Contrôle Critique (APPCC). Dans ce travail on décrit les dangers propres qui peuvent être trouvés dans la ligne d'élaboration de vin rosé, les mesures préventives qui peuvent être appliquées dans l'entreprise et les systèmes de surveillance à implanter, ainsi que les mesures correctrices prévues, en étant nécessaires, et les registres de contrôle qui devront rester dans l'industrie. La mise en pratique de ces connaissances permettra, à tout entreprise (indépendamment du type de vin élaboré, mais plus adapté à celles qui élaborent du vin rosé), un auto-contrôle de ses productions basé le système APPCC.

INTRODUCTION

La vérification la qualité et la salubrité de l'huile d'olive vierge s'est primordialement basée le contrôle du produit final. Ce critère est totalement interrogé de nos jours puisqu'il ne contribue pas à obtenir la sécurité alimentaire. Devant ceci les services d'inspection évoluent vers un plus grand contrôle en origine, en se basant des systèmes qui analysent les dangers qui peuvent être donnés dans l'activité industrielle et essayent de les éviter par la prise de mesures in situ. Pour cette raison, l'entreprise devra être insérée dans la réalisation des contrôles (BRUN, 1998). L'APPCC, défini comme un système préventif de contrôle des aliments dont l'objectif principal est la sécurité ou l'innocuité alimentaire, essaye d'identifier les dangers microbiologiques, chimiques et physiques existants dans un processus ou une pratique, pour identifier les points de contrôle critique (PCCs), dans lesquels peuvent être contrôlés de tels dangers, et établir des systèmes basés principalement des essais chimiques et physiques, et sur l'appréciation ou l'observation visuelle, au moyen de laquelle peut être monitorée ou être surveillée l'efficacité du contrôle (ICMSF, 1991 ; NÈGRE, 1996). L'objectif principal de ce travail consistera l'implantation du système APPCC dans la ligne d'élaboration de vin rosé.

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MATÉRIEL ET MÉTHODES

Pour la réalisation de ce travail il a été nécessaire de visiter différents entreprises qui avaient prévu l'implantation du système APPCC. Dans une première étape nous compilons une information sur les caractéristiques physico-chimiques et microbiologiques des vins et les matières premières auxiliaires. Postérieurement, nous élaborons le diagramme de flux du processus productif complet. Une fois révisé ce diagramme, nous révisons chacune des étapes à la recherche des possibles dangers (biologiques, physiques ou chimiques) pour le consommateur. Une fois identifié un danger, nous cherchons un ou plusieurs mesures préventives qui pourraient le diminuer ou l'annuler. Quand les mesures préventives ne seront pas suffisantes ou adéquates pour réduire un danger, on dépassera la limite critique établie, ce qui sera détecté grâce au système de surveillance établi. On appliquera alors une série de mesures correctrices qui, aussi, devront être prévues précédemment, dans le but d'éliminer, dans la mesure du possible, les causes des dangers détectés. Tous les pas donnés pour définir le système APPCC seront documentés et enregistrés.

RÉSULTATS ET DISCUSSIONS

Dans ce paragraphe on décrit le processus ou le diagramme de flux (Figure 1), depuis la réception des matières premières jusqu'à la mise en bouteilles du vin, en accord avec le cadre d'étude et toujours en nous basant ce qui est observé dans les différentes industries visitées. On inclut aussi un synoptique d'application (Tableau 1) où, pour chaque phase, on décrit les principaux dangers qui peuvent être prévus, ainsi que les mesures préventives à tenir compte pour diminuer ou éliminer ce danger. Ce qui est synoptique reflète la limite critique pour chaque mesure préventive et la surveillance nécessaire pour démontrer qu'un point critique est sous contrôle. Afin de corriger les déviations qui pourraient superficiellement se produire ou sous les limites critiques marquées, nous avons formulé toutes les mesures correctrices spécifiques pour chaque PCC du système. Finalement, on énumère les essais documentaires qui doivent être enregistrés (contrôle de température dans ce qui est fermentadores, analyse d'eaux, etc.), pour pouvoir savoir qu'il s'est produit dans notre industrie à un moment concret ; ceux-ci registres seront aussi très utiles au moment d'établir la traçabilité du produit fini.

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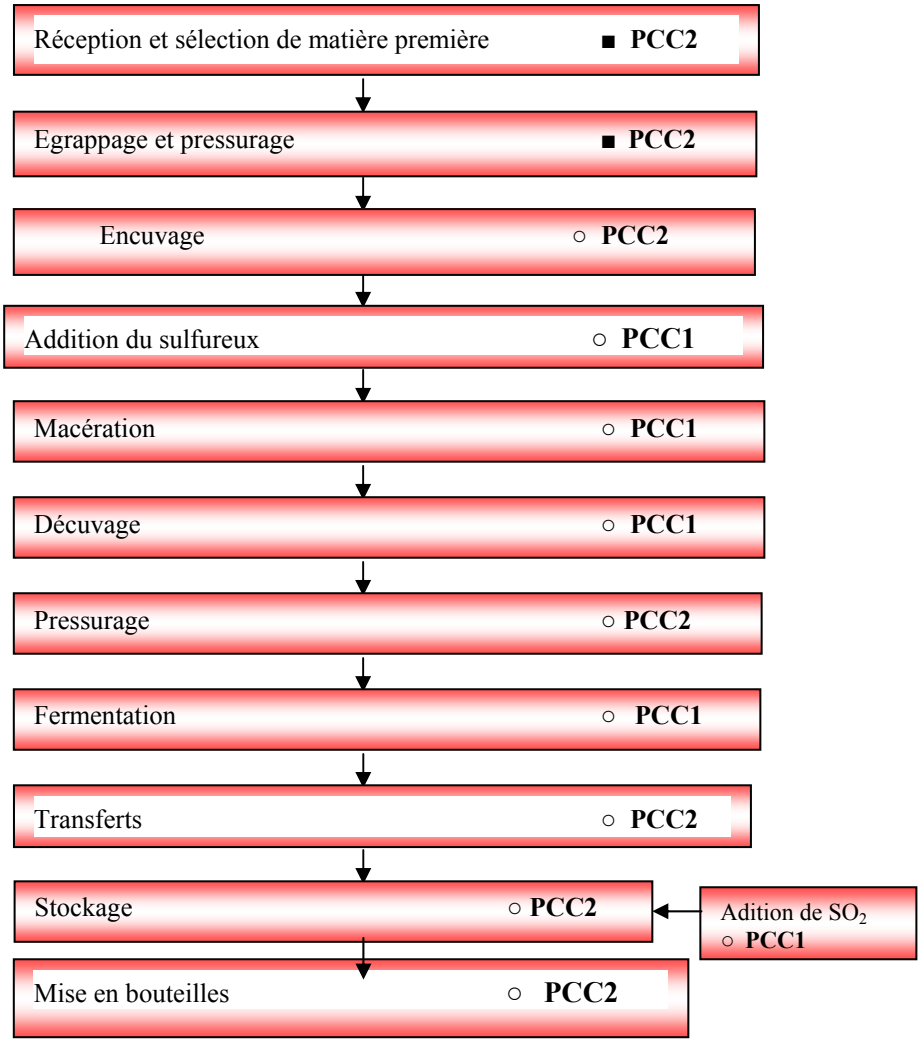

■ Importante contamination / ○ Baise contamination / PCC1 = Point de Contrôle Critique totalement efficace / PCC2 = Point de Contrôle Critique partiellement efficace

Figure 1. Diagramme de flux de la ligne d'élaboration de vin rosé

Tableau 1

Synoptique d'application de la ligne d'élaboration de vin rosé

PHASE	DANGERS	MESURES PRÉVENTIVES	PCC	LIMITE CRITIQUE	SURVEILLANCE	MESURES CORRECTRICES	REGISTRES
1. Réception et sélection de la matière première.	• État sanitaire déficient. • Matière première contaminée par funges. • Aplatissement prématuré des raisins et le début de la fermentation • Degré Baumé incorrect. • Pollution microbiologique des milieux de transport.	• Fixer le moment optimal de vendange. • Contrôle de l'état sanitaire du raisin. • Transfert adéquat à l'entreprise. • Conditions hygiéniques des trémies.	2	• Ne pas dépasser de limites de charge. • Bonnes pratiques hygiéniques. • Bonnes pratiques de transport. • Bonnes pratiques hygiéniques.	• Contrôle visuel des raisins. • Déterminations chimiques. • Conditions des moyens de transport. • Contrôle hygiénique des moyens de transport. • Propreté et désinfection de la trémie.	• Rejet de la matière première non apte. • Corriger des pratiques de transport. • Propreté adéquate du moyen transport et trémies.	• Entrée de départs. • Analyse en raisin. • Mesures correctrices. • Programme de propreté.
2. Égrappage et pressurage	• Laboure du rafle et des pépites • Pressurage très énergique. • Pollution microbiologique.	• Maintien adéquat des équipements. • Propreté et désinfection adéquates.	2	Bon fonctionnement des équipements. • Bonnes conditions hygiéniques.	• Correcte application des programmes de maintien préventif d'équipements, propreté et désinfection.	• Corriger les deux programmes, quand il est nécessaire.	• Programme propreté et désinfection. • Maintien d'équipements
3. Encuvage	• Pollution microbiologique.	• Programme propreté et désinfection adéquat.	2	• Conditions hygiéniques satisfaisantes.	• Inspection visuelle des réservoirs.	• Corriger programme propreté et désinfection. • Addition d'enzymes extractrices de couleur et tannin stabilisateur de couleur.	• Programme propreté et désinfection. • Mesures correctrices. • Contrôle des doses de produits oenologiques.

Tableau 1

Synoptique d'application de la ligne d'élaboration de vin rosé (continuation)

PHASE	DANGERS	MESURES PRÉVENTIVES	PCC	LIMITE CRITIQUE	SURVEILLANCE	MESURES CORRECTRICES	REGISTRES
4. Addition d'anhydride sulfureux.	• Doses incorrectes de sulfureux.	• Maintien préventif d'équipements. • Suivre des instructions de l'oenologue.	1	• Bon fonctionnement des équipements. • Dosage correct.	• Contrôle du programme de maintien. • Contrôle en laboratoire.	• Corriger des instructions de travail. • Corriger le programme de maintien.	• Contrôle du maintien. • Contrôle des doses de sulfureux. • Mesures correctrices.
5. Macération.	• Pollution microbiologique.	• Conditions hygiéniques adéquates.	1	• Temps de macération autour de 8 heures. • Conditions hygiéniques.	• Suivi du processus de macération. • Programmes d'hygiène.	• Correction du programme de maintien. • Restaurer programme d'hygiène.	• Programme de maintien préventif d'équipements. • Mesures correctrices.
6. Décuvage.	• Pollution microbienne. • Danger d'oxydations.	Déterminer moment optimal de décuvage. Suivre des instructions de l'oenologue.	1	• Bonnes pratiques de manipulation. • Bonne qualité organoleptique du vin.	• Contrôles physico-chimiques et sensoriels. • Nombre important de micro-organismes.	• Addition d'anhydride sulfureux en doses correctes, quand il sera nécessaire.	• Contrôles de laboratoire. • Mesures correctrices.
7. Pressurage	• Pressurage excessif (extraction de substances indésirables). • Oxydations des vins. • Pollution microbiologique.	• Adapter correctement la force du pressurage. Programme propreté et désinfection adéquat. • Programme de maintien préventif d'équipements. • Instructions de l'oenologue.	2	• Appliquer des pressions adéquates. • Conditions hygiéniques satisfaisantes. • Bon fonctionnement de la presse.	• □ Intensité de la force du pressurage. • Contrôle visuel du processus de pressurage. • Maintien préventif d'équipements.	• Correction des conditions de travail. • Correction des programmes propreté et désinfection et de maintien d'équipements.	• Extraction quotidienne de moût. • Pressions utilisées. • Contrôles de laboratoire. • Programme propreté et désinfection.

Tableau 1

Synoptique d'application de la ligne d'élaboration de vin rosé (continuation)

PHASE	DANGERS	MESURES PRÉVENTIVES	PCC	LIMITE CRITIQUE	SURVEILLANCE	MESURES CORRECTRICES	REGISTRES
8. Fermentation	• Montée ou baisse excessive de la température. • Pollution microbiologique.	• Maintien adéquat d'équipements de froid. • Addition de ferments lactiques. • Conditions hygiéniques appropriées.	1	• Bonnes pratiques de manipulation. • Températures adéquates (≈17º C) • Conditions hygiéniques satisfaisantes.	• Contrôle la température et la densité. • Analyse physico-chimique. • Contrôle du programme de maintien d'équipements.	• Refroidir dans le plus petit temps possible, en cas d'arrêt. • Addition de activateurs. • Correction du programme de maintien d'équipements.	• Programme de maintien d'équipements. • Contrôles analytiques. • Températures. • Mesures correctrices.
9. Transferts	• Production arômes et saveurs indésirables. • Pollution microbiologique. • Faillite oxydante. • Retard dans le transfert.	• Instructions de l'oenologue. • Accomplir le programme propreté et désinfection. • Maintien adéquat d'ustensiles. • Correct dosage de SO_2.	2	• Dose optimale de SO_2. • Conditions hygiéniques satisfaisantes. • Bonnes pratiques de manipulation .	Inspection visuelle des conditions travail et hygiène. • Doses de SO_2 ajoutées. • Essai faillite oxydante. • Goûte.	• Rejet de départs dans de mauvaises conditions. • Correction du programme propreté et désinfection. • Addition de SO_2 et éviter une aération. • Correction du plan de travail.	• Programme de travail. • Programme propreté et désinfection. • Maintien préventif d'équipements • Mesures correctrices.

Tableau 1

Synoptique d'application de la ligne d'élaboration de vin rosé (continuation)

PHASE	DANGERS	MESURES PRÉVENTIVES	PCC	LIMITE CRITIQUE	SURVEILLANCE	MESURES CORRECTRICES	REGISTRES
10. Stockage.	• Éviter des oxydations. • Modifications microbiologiques.	• Suivi du programme d'hygiène. • Maintien équipements et ustensiles. • Analyses physico-chimiques et organoleptiques.	2	• Bonnes conditions hygiéniques . • Maintien adéquat d'équipements . • Valeurs positives des paramètres analysés.	• État sanitaire et qualité du vin. • Programme propreté, de désinfection et de maintien préventif d'équipements.	• Addition d'anhydride sulfureux en doses correctes, quand il sera nécessaire. • Suivre des instructions de l'oenologue. • Rejet du départ . • Correction des programmes propreté, désinfection et maintien préventif d'équipements.	• Contrôles de laboratoire. • Contrôles du programme propreté et désinfection. • Contrôles du programme de maintien d'équipements.
11. Mise en bouteilles.	• Rempli incorrect des bouteilles. • Mauvais état des lièges. • Modifications physico-chimiques. • Pollution microbienne.	• Adapter le niveau de remplissage. • Maintien préventif équipements et ustensiles. • Bon état les lièges et les bouteilles. • Propreté.	2	• Rincé correct. • Maintien adéquat équipements et ustensiles.	• Contrôle visuel de l'opération. • Correcte application des programmes de propreté, désinfection et de maintien préventif des équipements.	• Rejeter des départs non aptes. • Correction du programme propreté, désinfection et maintien préventif d'équipements.	• Nombre de bouteilles empaquetées. • Programme propreté et désinfection. • Programme de maintien équipements et ustensiles. • Mesures correctrices.

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APPLICATION DU SYSTÈME D'ANALYSE DANGERS ET POINTS DE CONTRÔLE CRITIQUE (APPCC) DANS LA LIGNE D'ÉLABORATION DU VINAIGRE

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MOTS CLÉ: dangers, contrôle, vinaigre, qualité

RÉSUMÉ

L'Analyse Dangers et Points de Contrôle Critique (APPCC) est un système préventif qui essaye de garantir la sécurité et l'innocuité alimentaire, et qui permet la protection du produit et la correction de jugements, en améliorant les coûts de qualité par des défauts et en économisant presque le supercontrôle final. Dans ce travail on décrit les dangers propres qui peuvent être trouvés dans la ligne d'élaboration de vinaigre, les mesures préventives qui peuvent être appliquées dans l'entreprise et les systèmes de surveillance à implanter, ainsi que les mesures correctrices prévues, en étant nécessaires, et les registres de contrôle qui devront rester dans l'industrie. La mise en pratique de ces connaissances permettra, à toute industrie d'élaboration de vinaigre (indépendamment du type de vinaigre qui produit), un auto-contrôle de ses productions basé le système APPCC.

INTRODUCTION

La Directive Générale d'Hygiène des Aliments 43/93/CEE (transposée à l'ordre juridique espagnol à travers l'arrêté royal 2207/1995, de du 28 décembre), établit que les entreprises du secteur alimentaire, dans lesquelles on inclut, évidemment, les industries du vinaigre, elles doivent mettre en marche un système d'auto-contrôle de leurs productions, basé le système d'Analyse Dangers et Points de Contrôle Critique (APPCC) (DOUZE, 1993). L'APPCC, défini comme un système préventif de contrôle des aliments dont l'objectif principal est la sécurité ou l'innocuité alimentaire (ICMSF, 1991), il introduit comme première nouveauté le fait qui la responsabilité de la sécurité du consommateur est transféré depuis l'inspection officielle jusqu'au cadre du producteur, qui doit garantir cette sécurité avec la prévention (NOVOTEC, 1999). L'objectif principal de ce travail consistera l'implantation du système APPCC dans la ligne d'élaboration de vinaigre.

MATÉRIEL ET MÉTHODES

Pour la réalisation de ce travail il a été nécessaire de visiter différentes industries élaboratrices de vinaigre qui avaient prévu l'implantation du système APPCC. Dans une première étape nous compilons une information sur les caractéristiques physique-chimiques

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et microbiologiques des vinaigres et les matières premières auxiliaires. Postérieurement, nous élaborons le diagramme de flux du processus productif complet. Une fois révisé ce diagramme, nous révisons chacune des étapes à la recherche des possibles dangers (biologiques, physiques ou chimiques) pour le consommateur. Une fois identifié un danger, nous cherchons un ou plusieurs mesures préventives qui pourraient le diminuer ou l'annuler. Quand les mesures préventives ne seront pas suffisantes ou adéquates pour réduire un danger, on dépassera la limite critique établie, ce qui sera détecté grâce au système de surveillance établi. On appliquera alors une série de mesures correctrices qui, aussi, devront être prévues précédemment, dans le but d'éliminer, dans la mesure du possible, les causes des dangers détectés. Tous les pas donnés pour définir le système APPCC seront documentés et enregistrés.

RÉSULTATS ET DISCUSSIONS

Dans ce paragraphe on décrit le processus ou le diagramme de flux (Figure 1), depuis la réception des matières premières jusqu'au stockage du vinaigre, en accord avec le cadre d'étude et toujours en nous basant ce qui est observé dans les différentes industries visitées. On inclut aussi un synoptique d'application (Tableau 1) où, pour chaque phase, on décrit les principaux dangers qui peuvent être prévus, ainsi que les mesures préventives à tenir compte pour diminuer ou éliminer ce danger. Ce qui est synoptique reflète la limite critique pour chaque mesure préventive et la surveillance nécessaire pour démontrer qu'un point critique est sous contrôle. Afin de corriger les déviations qui pourraient superficiellement se produire ou sous les limites critiques marquées, nous avons formulé toutes les mesures correctrices spécifiques pour chaque PCC du système. Finalement, on énumère les essais documentaires qui doivent être enregistrés (contrôle de température dans ce qui est fermentadores, analyse d'eaux, etc.), pour pouvoir savoir qu'il s'est produit dans notre industrie à un moment concret; ceux-ci registres seront aussi très utiles au moment d'établir la traçabilité du produit fini.

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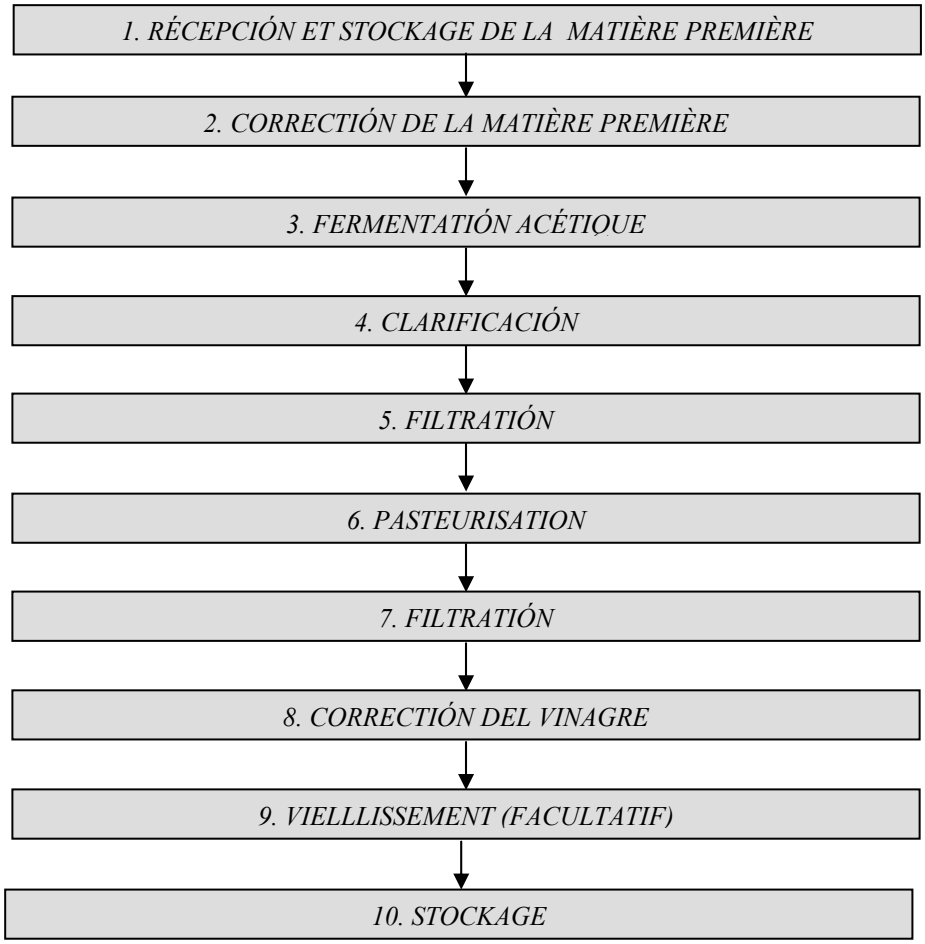

Figure 1. Diagramme de flux de la ligne de l'élaboration du vinaigre

Tableau 1

Synoptique d'application de la ligne d'élaboration de vinaigre

PHASE	DANGERS	MESURES PRÉVENTIVES	LIMITE CRITIQUE	SURVEILLANCE	MESURES CORRECTRICES	REGISTRES
1. Réception et stockage de la matière première. Approvisionnement d'eau.	• Réception des matières premières non aptes. • Pollution microbiologique des milieux de transport (réservoirs) et des réservoirs de réception de la matière première. • Eau non potable	• Rejeter des matières premières non aptes. • Signaler au proveedor . • Propreté et désinfection préalable des réservoirs et des réservoirs de réception de la matière première. • Approvisionnement d'eau potable	• Remplir les normes establies. • Propreté et désinfection adéquate des réservoirs et des réservoirs de réception de la matière première. • D'accomplir R.D. 140/2003.	• Analyse de matières premières réceptionnées. • Exiger le certificat propreté et désinfection préalable des cisternas. • Contrôle de l'état hygiénique des réservoirs de réception. • Analyse physico-chimique et microbiologique de l'eau.	• Retirer l'homologation aux proveedores. • Correction du programme propreté et désinfection des réservoirs et des réservoirs de réception des matières premières. • Chloration l'eau et le changement du point d'approvisionnement.	• Fiche de réception de chaque départ. • Résultés des analyses pratiquées à la matière première. • Programme propreté et de desinfección. • Résultés de l'analyse de l'eau. • Mesures correctrices.
2. Correction de la matière première.	• Pollution microbiologique.	• Fonctionnement correct du programme propreté et désinfection.	• Conditions hygiéniques satisfaisantes.	• Inspection visual. • Analyses microbiologiques.	• Répéter l'operación. • Correction du programme propreté et désinfection.	• Programme propreté et desinfección. • Contrôles analytiques realisés. • Mesures correctrices.
3. Fermentation acétique.	• Arrêtée fermentative. • Perte de viabilité des cultures acétiques choisies.	• Maintien adéquat de l'équipement de froid et de ce qui est doseur d'oxigène. • Addition d'activateurs • De suivre des instructions du fabricant des cultures acétiques.	• Fonctionnement correct de l'équipement de froid et de ce qui est doseur d'oxigène. • Température optimale de fermentation < 35ºC. • Débit optimal d'air : selon volume du fermentateur. • Doses adéquates et bon état des cultures acétiques.	• Fonctionnement de l'équipement de froid et de ce qui est doseur d'oxigène. • Analyse physico-chimique de la matière première. • Contrôle quotidien l'entrée et le débit d'oxigène. • Bonnes pratiques de dosage.	• Refroidir le fermentateur. • Corriger le programme de maintien préventif d'equipamentes. • Corriger des conditions de stockage des cultures acétiques ou les remplacer par d'autres.	• Programme de maintien préventif d'equipamentes. • Registre graphique quotidien de température. • Contrôles analitiques. • Débit d'oxigène. • Caractéristiques des cultures acétiques. • Mesures correctrices.

Tableau 1

Sinóptico de aplicación de la línea de elaboración de vinagre (continuation)

PHASE	DANGERS	MESURES PRÉVENTIVES	LIMITE CRITIQUE	SURVEILLANCE	MESURES CORRECTRICES	REGISTRES
4. Clarification	• Dosage inadequate. • Mauvais état des clarifiantes. • Addition de produits toxiques ou un certain clarifiante non autorisés. • Pollution microbienne.	• Suivre des instructions du técniques. • Bon état et identification correcte des clarifiantes. • Conditions hygiéniques des réservoirs adéquates.	• Ajouter des doses adéquates (charbon active = 2 g/l ; bentonite= 0.56 g/l). • Bon état de conservation des clarificantes. • Ne pas incorporer de produits toxiques ou clarifiantes non autorisés. • Réservoirs hygiénisés.	• Dosage et état des clarifiantes. • Accomplir programme propreté et désinfection dans les réservoirs.	• Nouveau clarification du vinagre. • Correction des conditions de stockage des clarifiantes. • Retrait de lots en mauvais état. • Corriger programme propreté et désinfection.	• Doses des clarifiantes. • Dossier clarifiantes d'autorisés. • Conditions de stockage. • Programa de Programme propreté et de desinfection. • Mesures correctrices.
5. Filtration.	• Filtration défectueuse. • Pollution microbienne.	• Réviser les filtres. • Conditions hygiéniques appropriées.	• Bon état des filtres. • Conditions hygiéniques satisfaisantes.	• État physique des filtres. • Limpidité du vinaigre après la filtration. • Propreté du filtre.	• Nouveau filtration. • Changer ou nettoyer le filtre. • Correction du programme de propreté des filtres.	• Volume vinaigre filtré et état des filtres. • Programme de propreté des filtres. • Mesures correctrices.
6. Pasteurisation.	• Ne pas atteindre ou dépasser la température de pasteurisation établie.	• Remplir le maintien préventif du pasteurisateur.	• Bon fonctionnement du pasteurisateur. • Température optimale pasteurisation = de 80ºC, pendant 30-40 sécundes.	• Température du vinaigre dans le pasteurisateur et dans le dépôt intermédaire. • Fonctionnement du pasteurisateur.	• Répéter l'operation. • Mise sur le point du pasteurisateur.	• Registres de la température du vinagre. • Programme de maintien preventif . • Mesures correctrices.

Tableau 1

Sinóptico de aplicación de la línea de elaboración de vinagre (continuation)

PHASE	DANGERS	MESURES PRÉVENTIVES	LIMITE CRITIQUE	SURVEILLANCE	MESURES CORRECTRICES	REGISTROS
7. Filtration.	• Filtration défectueuse. • Pollution microbienne.	• Réviser les filtres. • Analyses microbiologiques du vinaigre filtré. • Determination du niveau de turbidité. • Conditions hygiéniques appropriées.	• Bon état des filtres. • Conditions hygiéniques satisfaisantes.	• État des filtres. • Limpidité du vinaigre après la filtration • Propreté du filtre.	• Nouveau filtration. • Changer ou nettoyer le filtre. • Correction du programme de propreté des filtres.	• Volume vinaigre filtré, état des filtres et résultat des analyses practiqués. • Programme de propreté des filtres. • Mesures correctrices.
8. Correction du vinaigre.	• Pollution microbienne. • Eau non potable.	• Correct fonctionnement du programme propreté et de desinfection. • Approvisionnement d'eau potable.	• Conditions hygiénico-sanitaires satisfaisantes. • Accomplir R.D. 140/2003.	• Inspection visual. • Analyses physico-chimiques et microbiologiques pratiquées dans les réservoirs et à l'eau.	• Répéter l'operación. • Correction du programme propreté et de desinfección. •Chlorosation de l'eau et le changement du point d'approvisionnement.	• Programme propreté et desinfection. • Contrôles analytiques realisés. • Mesures correctrices.

Tableau 1

Sinóptico de aplicación de la línea de elaboración de vinagre (continuation)

PHASE	DANGERS	MESURES PRÉVENTIVES	LIMITE CRITIQUE	SURVEILLANCE	MESURES CORRECTRICES	REGISTROS
9. Vieillissement (facultatif)	• Conditions environnementales nonadequates. • Temps de permanence dans barriques nonadequates. • Remplie inadéquate des barriques. • Pollution microbiologique.	• Contrôle des conditions environnementales de vieillissement. • Suivre des instructions du téchniques. • Correct état des barriques. • Éviter la présence de bourses d'aire. • Conditions hygiéniques appropriées.	• Tª= 15ºC ; HR= 60-70%. • Permanence dans barrique selon les instructions téchnicques. • État adéquat des barriques. Bonnes pratiques de manipulation: remplissage adéquat, d'éviter des bourses d'air etc	• Contrôle température et de humidité. • Analyse périodique du vinaigre. • État physique et la propreté des barriques. • Opération de remplissage.	• Rétablissement des conditions température et humidité. • Prolonger la permanence du vinaigre dans le barrique. • Rejet de barriques en maavais état. • Rempli de barriques. • Corriger de programme propreté et désinfection.	• Température et humidité du navire de vieillissement. • Départ du vieillissement. • Analyse des vinaigres. • Programme propreté et de desinfection. • Mesures correctrices.
10. Stockage.	• Éviter les oxidations. • Modifications microbiologiques.	• Bonnes pratiques de manipulation. • De réviser l'état propreté et désinfection des réservoirs. • Analyses physico-chimiques et organoleptiques.	• Bonnes conditions de travail. • Propreté et désinfection adéquates des réservoirs. • Valeurs positives des paramètres analysés.	• Pratiques de manipulation. • État propreté et désinfection des réservoirs. • Interprétation des analyses.	• Suivre des instructions téchniques. • Rejet de départs non aptes. • Correction des programmes de bonnes pratiques de manipulation et de propreté et de désinfection des réservoirs.	• Résulté des anályses. • Programmes de bonnes pratiques de manipulation et de propreté et de désinfection des réservoirs. • Mesures correctrices.

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THE MALOLACTIC FERMENTATION AND THE SENSITIVE FEATURES OF RED WINES OBTAINED IN THE VINEYARDS OF OLTENIA COUNTY HILLS FROM ROMANIA

C. Baduca Cimpeanu[1], G. Beleniuc[2], D. Boboc[3], N. Istudor[4], J.E. Pardo Gonzalez[5]

KEY WORDS: malolactic fermentation, red wines, lactic bacteria

SUMMARY

It was studied the influence of the spontaneous MLF and the different ways of starting and stimulating of the controlling MLF on the organoleptic features of the obtained wines. When were used the selected lactic bacteria, the MLF have had a fast starting, the malic acid was more fast metabolized and finally the MLF period was shortened. This permitted an early biological stability of the obtained wines. Organoleptical, the obtained wines, were appreciated as balanced, with a very good smell and taste, with a lot of aromas, especially from the second categories, of fermentation, depending of the kind of lactic bacteria used and the moment and mode of inoculation.

INTRODUCTION

The malolactic fermentation like the alcoholic fermentation can be started in two ways: **spontaneous**, under the action of indigenous lactic bacteria and **induced**, through seeding of so called starter cultures of selected lactic bacteria. The spontaneous malolactic fermentation is often influenced by major treads, especially in the vines, which do not offer favorable growing conditions for lactic bacteria. Between the limitative factors that limit the multiplication of lactic bacteria is mentions the high alcoholic content, low pH, low temperatures (1), the high content of SO_2, the high content in nitrogen of the grape juice, which oblige the yeasts to produce more SO_2 (2) and a series of products of yeast metabolism, the most inhibitor proving to be dodecanoic acid (3).

Starting of the malolactic fermentation is conditioned by intrinsec factors of the vinification conditions (physical and chemical, microbiological and technological parameters, etc) that are why two different situations occur. The first refers to the too rapid malolactic fermentation, even before the end of alcoholic fermentation – that is the case of high pH wines. The second situation refers to the wines with difficulties in achieving malolactic fermentation (low pH, high alcohol content, low temperature, high SO_2 content, etc.). Both situations are not good and have negative consequences for wine quality. In the

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first case that is referring to the risk of premature stop of alcoholic fermentation, lactic souring, organoleptic deviations due to undesirable indigene bacterial florae (4). For the second case, during the prolonged latent phase there is the risk of oxidation and contamination with undesirable microorganisms of bacterial type – pediococus, lactobacillus, acetic bacteria – or yeasts – *Brettanomyces*, pellicular yeasts (1). For a rapid start of malolactic fermentation, the wines are kept in favorable conditions for lactic bacteria propagation but under these circumstances other contaminant species can multiply (5).

MATERIAL AND METHODS

The researches were effectuated between 2004–2007, in the main vineyards of Oltenia hilly areas, situated in South-West of Romania: Sâmbureşti, Drăgăşani şi Dealurile Craiovei. Foe this study we used 4 varieties of grapes for high quality red wines: 2 varieties very knowable in international plane Cabernet-Sauvignon and Merlot, and also 2 autochthonous varieties Fetească neagră and Novac.

The period of researches include the different years like climatic conditions. So, year 2005 was for Romanian viticulture the worst in the last 50 years, very rainy and cold. Those conditions influenced the grapes and wines production, quantitative and also qualitative. The years 2004 and 2006 was a good vine-growing years, the climatic conditions was favorable for viticulture. The year 2007 was excellent for vine-growing by point of view of climatic conditions during the vegetation period and also during the ripening and over ripening.

RESULTS AND DISCUSSIONS

The length of time of the malolactic fermentation

During the years have existed visible differences upon the length of time of the malolactic fermentation among the fermentated variant with lactic bacteria resulted from the indigene microflora of the grapes and the fermentated variant with selected lactic bacteria, but the differences where variable, depending on the chemical composition of the wine, as a result of the climate conditions of the viticultural year. In the good viticultural years (2004 and 2006), the length of time of the spontaneous malolactic fermentation was between 45 and 60 days while the variants with selected lactic bacteria, the duration was, on an average,10 days shorter, between 35 and 50 days. In drought-stricken years and very warm (2007), the length of time of the malolactic fermentation was between 35 and 45 days in case of the produces fermentation. On unfavorable climate conditions, in an rainy cold year (2005), the differences were big: 80–120 days in the case of spontaneous fermentation and 45-60 days in case of the produces fermentation, so with 1–2 months shorter. Also, it has to be mentioned that in 2005, some red wines stopped the malolactic fermentation after 90–120 days without finishing the conversion of the malic acid into lactic acid. This accidents happend in wines that presented totally contains of SO_2 over 100 mg/l (fig. 1).

A study made in 2006 on red wines from the vineyard "Dealurile Craiovei" pointed out the important differences concerning the length of time of the malolactic fermentation, depending on the moment of the inoculation of the lactic selected bacteria. Thus in all the cases in witch the inoculation was made in unfermented wine, the length of time of the malolactic fermentation was between 30 and 36 days. In the case of the inoculation of the lactic bacterium into wine, the length of time of the malolactic fermentation was between 36–48 days, in case of the spontaneous malolactic fermentation,

without lactic selected bacterium the length of time was between 42–60 days (fig. 2).

Therefore, the inoculation of the lactic bacterium into unfermented wine determined saves of time between 6 and 12 days confronted by de variant of the culture inoculation starter the lactic bacterium in wine and of 6 to 30 days unlike the cases when the malolactic fermentation occurred without selected lactic bacterium.

This economies in time are very important because they offer the real possibility of realizing malolactic fermentation on secure during the first weeks adder the end of the alcohol fermentation.

The sensorial characteristic of wine

The malolactic fermentation ameliorates significant the equilibrium olfactory-gustative of red wine, by decrease some tasty characters that are little agreeable-hardness, cruelty, grassy-and to ameliorate the suppleness, equilibrium, roundness. In the case of the taste, is was established that the malolactic fermentation determined a significant reducement of smells of vegetal nature, dominated by the raw wine, and intensification of the floral tastes, the fructoside and the expressiveness of red wine.

The ameliorate of the appropriation olfactory-gustative of red wines is influenced by the type of malolactic fermentation, by the way of hoi is optated for the use of the selected lactic bacteria resulted from the indigene microflora of grapes. In the good viticulture years, when the grapes come to maturity good, the harvesting is big, the amelioration of the olfactory-gustative characteristics of wines under the action of selected lactic bacteria was less then visible, comparative with the rainy cold viticulture years, when the use of selected lactic bacterium was particularly useful (fig. 3 and 4).

CONCLUSIONS

The malolactic fermentation is a biologically desacidification process of wine made by lactic bacterium that transforms the malic acid into lactic acid and CO_2. Normally, this change takes place of the alcoholic fermentation, therefore is called secondary fermentation. As a result of this transformation there are important changes in the chemical composition of the wine (the diminution of the total acid, increases of pH because the dicarboxilic acid is replaced by the monobarboxilic acid); the taste and the aroma of the wine (the malic acid, more aggressive for the gustative papilla, is replaced by one acid that stamps suppleness to the wine; the diminution of vegetal aroma, grassy and the appearance of new tastes, more agreeable); the biologically stability of the wine(the malic acid unstable biologically, is replaced by the lactic acid, biologically stable).

The moment of the sowing of the lactic bacteria is determinative agent for the success of the operation. Traditionally "he sowing" of the lactic bacterium is made at the final of the alcoholic fermentation, but in the last years is practiced more and more the sowing of lactic bacterium into unfermented wine, that represents the advantage of a shorter duration of the malolactic fermentation witch can not be neglected, because it allows the quicker biological stabilization of the wine and the entrance without lateness into the normal sequence of the evolution stages.

In exchange, the good viticultural years, on the devoted vineyards, the use of selected lactic bacterium is absolutely necessary, if the wines have a equilibrated composition, they are healthy and are coming from mature grapes. In this situations, by directing and controlling the alcoholic fermentation and using a temperate diet of sulfitation is possible a good derulation of the malolactic fermentation even with bacterium from the indigene flora of the grapes.

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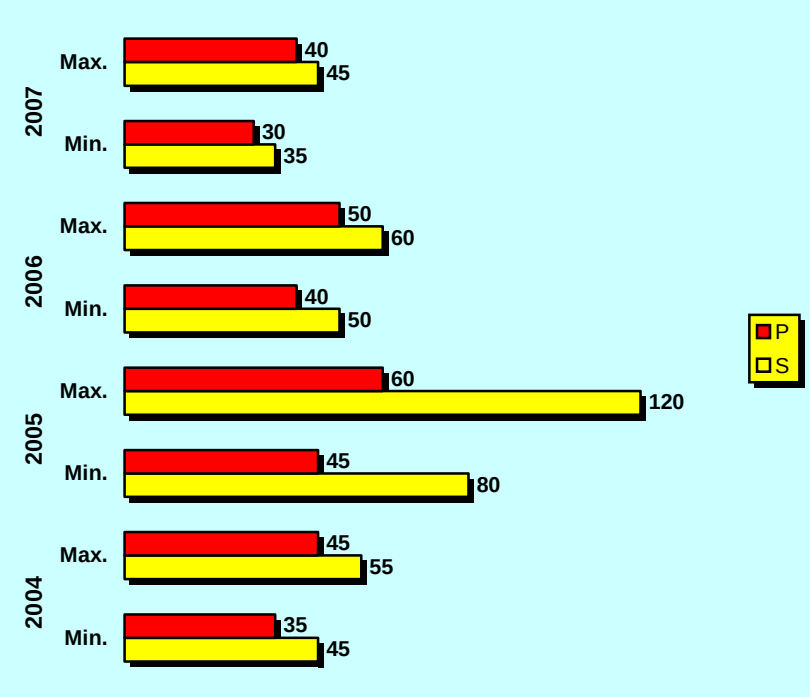

Figure 1. The length of time of the malolactic fermentation (days)
after the type of lactic bacteria
S – MLF spontaneous, with the bacteries from the indigene microflora of the grapes
P – MLF induced with selected lactic bacteria

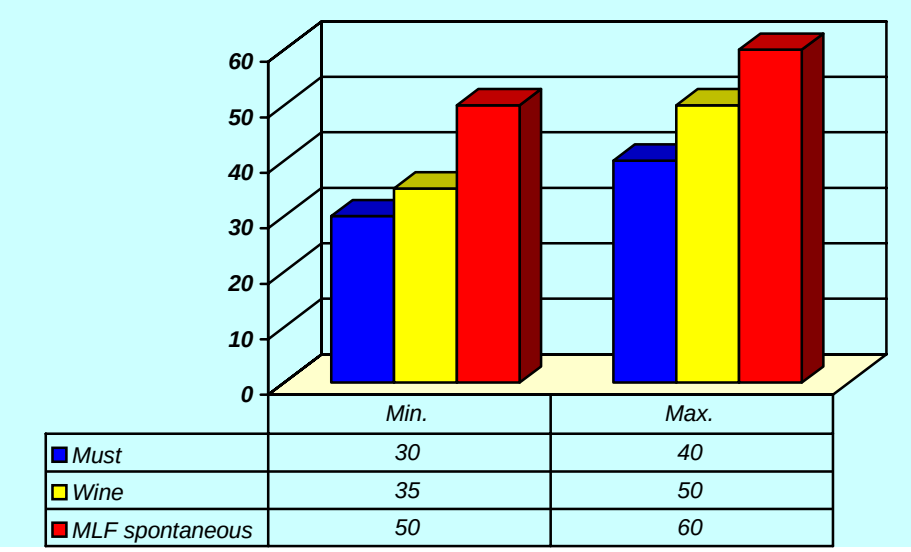

Figure 2. The length of time of the malolactic fermentation (days) after the inoculation time of lactic bacteria

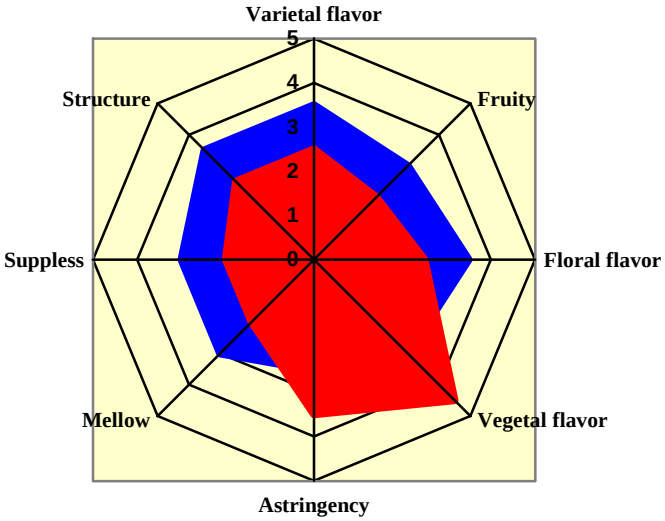

Figure 3. The influence of the malolactic fermentation on sensorial characteristic of red wines in unfavorable climate conditions
MLF induced with selected lactic bacteria
MLF spontaneous, with the bacteries from the indigene microflora of the grapes

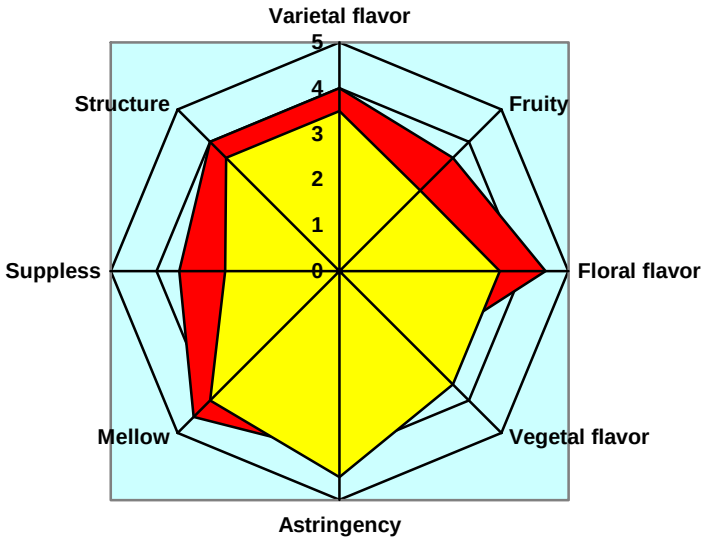

Figure 4. The influence of the malolactic fermentation on sensorial characteristic of red wines in favorable climate conditions

MLF induced with selected lactic bacteria

MLF spontaneous, with the bacteries from the indigene microflora of the grapes

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EVALUATION OF CONFORMITY FOR FOOD PRODUCTS- CONDITION OF FREE CIRCULATION WITHIN THE UNIQUE MARKET OF THE EUROPEAN UNION

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KEY WORDS: conformity, standardization, certification, accreditation, food

ABSTRACT

This paper presents the general frame of market surveillance concerning the food products, based on a global systems including the certification, evaluation and assurance of food products conformity to applicable requirements and, also, the role of accreditation and conformity evaluation, standardization and metrology in functioning of the unique market of the European Union.

The frame of legislation, principles and general requirements of food legislation and procedures concerning food security are presented.

GENERAL REMARKS

The unique market of the European Union came from the fusion of national markets of the member countries in a single economic area where the four main rights are guaranteed: free circulation of goods, of services, of persons and of assets.

The products circulate freely, without custom duties and without being controlled at the inner frontiers of the Union, excepting those products that are potentially dangerous for consumers, public health or environment.

The European Union has created systems of rapid alert for products presenting serious risks (RAPEX) and separately, systems for food and pharmaceutical products and thus it enacted the necessary laws to withdraw these products from the market.

A product is seen as safe and reliable, that is it presents no risks or acceptable minor risks, if it is in accordance with the communitary legislation or, if this is missing, in accordance with the national regulations specific to every member country.

The member countries must supervise the conformity of products, both by structures adequate for the evaluation of conformity and by the necessary actions in the case of dangerous products. Accordingly, the new Regulation (EC) no. 765/ 2008 of the European Parliament and Council from July, 9, 2008 [6], settles the general frame of market supervision concerning the products, based on a global systems including the certification, evaluation and the assurance of product conformity to the applicable requirements. In its turn, this system is based on standardization and metrology.

Due to the fact that the evaluation of conformity with certification, standardization and metrology are used by firms and governments in order to improve products and services in every field, they are seen as landmarks for a long term development, meanwhile facilitating the world trade.

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These landmarks are interdependent and have a crucial importance in every field of activity. The evaluation of conformity is made according to the specifications stipulated by standards and the exactness of measurements is achieved with the standards supplied by metrology.

CERTIFICATION

Certification is a fundamental element of quality infrastructure, both in the regulated and the voluntary field, both at the global and the European level.

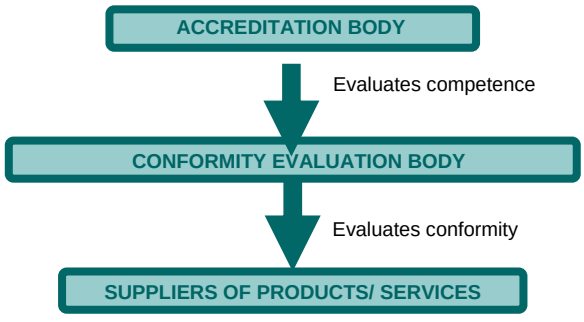
Since 1989, the Global Approach granted an important role to the certification of bodies for conformity evaluation, reinforced by the Council Decision 93/465/EEC[5] stipulating that the notified bodies that can prove their conformity to the harmonized standards (series EN 45000) by presenting an accreditation or by any other documented proof, are considered conform to the requirements of directions." Similarly, the directions of the New Approach consider that those bodies that satisfy the criteria of relevant harmonized standards are considered conform with the minimal criteria corresponding the directions.

Following the application of the new Regulation (EC) no. 765/2008 [6], the role of certification is increasing, covering both the regulated and non- regulated fields of activity.

The specific value of certification lies in the fact that it provides a declaration vested with authority concerning the technical competences of those bodies whose task is to evaluate that products are in accordance with the corresponding applicable requirements.

If the communitary harmonization legislation stipulates the selection of bodies for conformity evaluation, the transparent certification, as stipulated by the Regulation, guarantees the necessary reliability concerning the conformity certificates issued by the certified bodies and it is considered a special instrument for proving the technical competence of respective bodieS [2].

The accreditation system works according to compulsory norms and helps to reinforce the mutual reliability between the member countries regarding the bodies competence for conformity evaluation [1] and, implicitly, the certificates and test reports issued by these bodies; thus they strengthen the basis for the application of mutual recognition.



Accreditation System

Because it is the last level for controlling the activities of conformity evaluation, the certification represents a public interest service, organized under the responsibility of public authorities which, on one hand guarantees its impartiality and the lack of commercial motivations and, on the other hand avoids the competition between the certification services of the member countries.

The certification bodies have to be third party organizations, totally independent from their clients, the bodies for conformity evaluation, so that the certification decision could be taken by excluding any external intervention and must be in accordance with the criteria of standard EN/ISO/IEC 17021.

According to the definition from the same standard, the body for conformity evaluation is an organization dealing with services of conformity evaluation and can be the object of certification; these services are:

- tests/ analyses;
- stampings;
- certifications of management systems, product, persons;
- inspections.

The new Regulation 765/2008/EC states the activity of certification at the level of European Union, no matter if it is about regulated or non- regulated fields of activity. So, every member country should acknowledge a single national body of certification that is a member of the European certification infrastructure, acknowledged as such by the Regulation and it is EA-European cooperation for accreditation [1].

In order to ensure the equivalence of competence levels of the bodies for conformity evaluation, to facilitate the mutual acknowledgement and to promote the global acceptance of accreditation certificates and the results of conformity evaluations issued by the certified bodies, the regulation imposes that these national accreditation bodies should put into practice a strict and transparent system of evaluation at homologue level and thus could be directly evaluated.

The evaluation at homologous level represents the evaluation process of the national accreditation body by other national accreditation bodies, according to the requirements of the Regulation and, if the case, to any other supplementary technical specifications. In order to guarantee a coherent and equivalent application of communitary harmonization legislation, the Regulation introduces a supervision frame of the internal market, defining the minimal requirements against the objectives that have to be met by the member countries and a frame of administrative cooperation including the exchange of information between the member countries.

Whenever the economic agents hand in the authorities for market surveillance test reports or certificates of conformity, issued by a certified body for conformity evaluation, these should consider them accordingly, especially if they control the characteristics of the product, even if the communitary harmonization legislation does not require such reports or certificates. It is obvious that only those tests/ stampings/ analyses inside the certified laboratories make free way for products, no matter if regulated or non- regulated fields are concerned. Moreover, the standard ISO/IEC 17025 that is the referential for laboratory accreditation, imposes besides the requirements for a trustworthy management the technical requirements necessary for the correctness and reliability of tests and/or inspections.

EVALUATION OF CONFORMITY

Some products can be introduced on the internal market of the European Union only if they have the conformity EC mark, a symbol applied on the product, on its packing and/ or on the accompanying documents and signifies the conformity of product with every

appliable requirement, stipulated by the directions known as the directions of the New Approach.

These directions limit the settlement of products only to the essential requirememts of security and health , whose compliance with is compulsory. These essential requirements define only the expected result and allows the manufacturer to choose the technical solutions for obtaining it.

A harmonized standard is an European standard that stipulates the essential requirements for security and health of the European directions in technical specifications. That is why the compliance with the provisions of harmonized standards offers the products the assumption of conformity with the requirements of the directions associated to these standards.

The compliance with the harmonized standards is voluntary; the products can be manufactured by using other solutions than those stipulated by standards but the single compulsory condition is to comply with the essential requirements of the directions applicable to the product.

The EC conformity mark is mainly intended to the control authorities from the member countries and guarantees the consumer the conformity with the essential requirements of security and health included in the directions. It is applied by the manufacturer or by its authorized representative before the product is introduced on the market and it states practically that:

- the product is conform with every provision of the communitary directions;
- this conformity was settled by the adequate evaluation procedures achieved by the manufacturer or a body notified by a member country of the European Commission.

Similar to the passport that ensures the free movement of a person, the EC conformity mark ensures the free circulation inside the unique European market of many industrial products: electric and electronic equipment, cars, toys, medical instruments and equipment, radio sets, telephones, elevators, non-automatic weighing equipment, under pressure equipment, etc., because the products bearing the EC conformity mark can be sold both in every member country of the European Union and in EEA countries without authorizations, changes or supplementary examinations [4].

Whenever non- conforming products are found, the authorities can undertake an official action in order to withdraw the product from the market, a warning or the interdiction to be sold.

All directions of communitary harmonization based on the New Approach and the Global Approach contain provisions regarding:

- the procedures for conformity evaluation of products with the requirements of the direction;
- the notification of implied bodies concerning these procedures;
- the criteria of competence to be met by the bodies that are to be notified.

The procedures for conformity evaluation are settled taking into account the level of complexity and the estimated risk at the utilization of the product; the manufacturers have the opportunity to choose from many variants the combination they consider adequate for their product and production.

As a general rule, these procedures concern the design and production stages and are based on the 8 modules stipulated by European Directive 93/465/EC:

a) module A- internal control of production;
b) module B- EC examination of type;
c) module C- conformity with the type;

d) module D- assurance of production quality;
e) module E- assurance of product quality;
f) module F- product testing;
g) module G- testing of product unit;
h) module H- total assurance of quality.

The directions also stipulate the responsibility of the member countries for:

- notification (political/ administrative document);
- competence evaluation of the notified bodies (technical document);
- the ongoing maintenance of the competence of these bodies.

The directions do not offer practical solutions concerning the settled principles; they depend on the national authorities. In many member countries, the accreditation is the basis for the appointment and notification, no matter if this is proclaimed or just recommended.

In Romania, the accreditation of the bodies concerning the appointment and the notification is compulsory [4], on the ground of art.16 of Law 608/2001 regarding the conformity evaluation of products and it is achieved by RENAR. RENAR is the national accreditation body founded according to a specific frame of accreditation, mutually agreed with the national authorities and grafted on the standards EN 45000 (mentioned by the Global Approach and Decision 465 of the Council), respectively standards ISO/CEI 17000 that replaced the previously mentioned ones.

The European strategy concerning the food security refers to an integrated approach for an increased level of food safety, of vegetal and animal health all over the EU, aiming at:

- rules regarding the security of food and food for animals;
- necessary measures so that rules must be obeyed;
- scientific references for food control, independent and available for the public;
- the right of the consumer to choose food fully aware of its origin and content.

The frame legislation is Regulation (EC) no. 178/2002 of the European Parliament and Council from January, 28, 2002 that settles the principles and general requirements of food legislation, founds the European Authority for Food Security and states the procedures concerning food security [3]:

- settles the principles governing the food security;
- introduces the term of “traceability” so that food and its constituents could be found along their way “from the farm to the table”;
- creates the European authority for food security;
- reinforces the rapid system of alert used by the Commission and the member countries in the case of sanitary alert concerning a food product intended for the human or animal consumption.

The implementation of legislation is based on an efficient control and the evaluation of the way in which the communitary norms were respected, both inside the Union and by the third countries exporting to EU.

There are compulsory harmonized norms for the pre-packed food products referring to labelling and advertising; labelling must contain the name of the product, the list and quality of ingredients, the possible allergens, the minimal durability and the preservation conditions.

Other information can refer to supplementary elements such as the origin of the product or the manufacturing method.

The labelling of the pre-packed products must indicate their mass and the volume, taking into account certain weather conditions. If these requirements are met and the metrologic control is made, the products are marked “EEC”.

There are specific provisions for certain food products, such as the genetic modified organisms, allergenic food, food intended to the new born or different beverages.

The packaging itself must comply with certain requirements of manufacturing, so that the products should not be contaminated.

A farming product intended for the human consumption or a food product that has a traditional make-up or way of manufacturing can be registered at the European Commission as a guaranteed traditional speciality. The control of these products can be performed either by authorities designated by the member countries or by bodies for product certification, compulsorily certified according to standard EN 45011 after May, 1, 2010.

If there is a relation between the characteristics of a product and its geographic origin, this can be labelled under the mention of either "denomination of protected origin" (the manufacturing, the transformation and the elaboration of the product take place in a certain geographic area, according to acknowledged and established methods) or " protected geographic indication" (at least one of the manufacturing stages takes place in a certain geographic area). Also, in the case of these products the control can be made by authorities designated by member countries or by bodies for product certification, compulsorily certified according to standard EN 45011, after May, 1, 2010.

Nevertheless, we have to mention that the product certification, irrespective of its nature, is based on conformity tests, complying with the provisions of the same competence standard: ISO/CEI/17025. The tests must be made with standardized equipment so that the traceability of measurements at SI can be proved. And thus, we can see once again the close relationship between the conformity evaluation and metrology whose role is to ensure the above mentioned traceability.

CONCLUSIONS

We may say that the European approaches aiming at ensuring that market products, settled by the communitary legislation, meet requirements that offer a high protection of health and security, as well as other public interests; they also guarantee the functioning of the internal market, based on the three landmarks of long-lasting development: accreditation and conformity evaluation, standardization and metrology.

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ASPECTS REGARDING VALIDATION OF MEASUREMENT METHODS USED FOR THE CONTROL AND EXPERTISE OF FOOD PRODUCTS

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KEY WORDS: validation, measurement, performance, analyte, contaminants

ABSTRACT

The present work focuses on the principles and practical methods of validating the measurements methods used for the control and expertise of food products.

The influences of each characteristic parameters of these methods are analysed, based on the practical results and technical regulations.

We have taken into account the specific requirements stipulated by the European Union directions.

A special chapter, containing concrete examples, deals with the confirmation methods for organic residues and contaminants.

GENERAL REMARKS

Method validation is a process of:

- establishing the method's performance characteristics and limitations;
- identifying the influences that can modify these characteristics and their extent;
- establishing the analyte that shall be determined, in what matrix and in the presence of which interferences;
- verifying whether a method is in accordance to the purpose it will be used in (how can the analytical problem be solved by that method).

In accordance to the recommendations of the Eurachem Guide [3], method validation is necessary when there shall be:

- developed a new method for a specific analysis;
- established the modifications necessary for a method to be extended for another application;
- corrected a method that during the quality control proved that it changes in time;
- extended the method utilization in another laboratory, or by another operator, or with other instruments than those used for creating it;
- demonstrated the equivalence of two methods.

Method validation is performed by the laboratory that applies the method, or by a group of laboratories that agree to study a method with a large applicability, which has potential to be adopted as a standard.

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CHARACTERISTIC PARAMETERS

The studies for method validation are based on the determination of the performance parameters of the global method. These are realized during the method development, during the inter-laboratories [4] study or respecting the validation protocols used within the unit. The individual sources of uncertainty are investigated only when they are significant in comparison to the exactness indicators in use, the final purpose being the identification and elimination of the significant effects, less than their correction. This fact leads to the situation when the majority of the influence factors, potentially significant in comparison to the global exactness, were identified and there was demonstrated that they are negligible.

The validation studies for the qualitative analytical methods determine some or all of the following parameters [3]:

Accuracy - The main measures for the accuracy include the standard deviation of repeatability s_r, the standard deviation of reproducibility s_R (ISO 3534-1) and the intermediary accuracy, sometimes denoted s_{zi}, where i indicates the number of the variable factors (ISO 5725-3:1994). The repeatability s_r indicates the variability observed within a laboratory, during a short period of time, using a single operator, equipment etc. s_r can be estimated using a study within a laboratory or by an inter-laboratories study.

The standard deviation of the reproducibility for a particular method can be estimated only directly by an inter-laboratories study [5]; it shows the variability obtained when different laboratories analyze the same sample. The intermediary accuracy is related to the variation of the results observed when one or more factors (type of equipment, the operator etc.) are varied in a laboratory; there are obtained different values that depend on those factors which are kept constant. Frequently, the estimates of the intermediary accuracy are determined within the laboratory, but they can also be determined by inter-laboratories studies. The observed accuracy of an analytical procedure is an essential component of the global uncertainty, if it is determined by combining the individual variances or by the study of the complete method in operation.

Bias - The bias of an analytical method is usually determined by study on relevant reference materials or by marking studies. The determination of the overall bias with respect to the appropriate reference value is important in establishing traceability to recognised standards. Bias may be expressed as analytical recovery (value observed divided by value expected). Bias should be shown to be negligible or corrected for, but in either case the uncertainty associated with the determination of the bias remains an essential component of the overall uncertainty.

Linearity - Linearity is an important property of methods used to make measurements at a range of concentrations. The linearity to the response to pure standards and to realistic samples may be determined. Generally, the linearity cannot be quantified, but it is checked for by inspection or using some significance tests for non-linearity. Any remaining deviations from linearity are normally sufficiently accounted for by overall precision estimates covering several concentrations, or within any uncertainties associated with calibration.

Detection limit - During method validation, normally, the detection limit is determined only for establishing the lower limit of the method practical application interval. Although the measurement uncertainties in the proximity of the detection limit may require special attention, and separate treatment, the determined detection limit has no direct relevance on uncertainty estimation.

Robustness - Many developed methods or validation protocols require that the sensitivity towards a certain parameter to be directly investigated. Usually, this is realized by a Robustness test, where there is observed the effect of changing one or several parameters. If it is significant (as compared to the test accuracy), there is performed a more detailed study for measuring the extent of the effect, and correspondingly, there is chosen an allowed operating interval.

Selectivity/specificity - Both terms are related to the degree in which a method responds uniquely to a required analyte. Typically, selectivity studies investigate the effects of the apparent interferences usually by adding the interferential potential in blank and also in the sample and by observing the response. Normally, the results are used to demonstrate that the practical effects are not significant. Nevertheless, as the changes are measured directly from the response, it is possible to use the data for estimating the uncertainty associated to the potential interferences, providing knowledge related to the interval of interferential concentrations.

EVALUATION OF PERFORMANCE CHARACTERISTICS OF VALIDATION

For leading the experimental studies of evaluating the method performance [2], the representativeness' sample is essential. This means that, as much as possible, the studies shall be lead in order to assure a realistic follow-up of the number and interval of effects that manifest during the normal use of the method and which covers in the same time the concentration intervals and the types of samples according to the method's purpose. If, for example, a factor was varied representatively during a accuracy experiment, that factor's effects would appear directly in the observed variation and does not require additional studies, excepting the situation when there is desired the following optimization if the method.

In this context, the representatively variation means that a parameter of influence shall take a distribution of values according to the uncertainty of the considered parameter. For continuous parameters, this can be an allowed interval or a declared uncertainty; for the discontinuous factors, as the sample's matrix, this interval corresponds to the variety of types allowed or counted in the normal utilization of the method. There is to be observed that the representativeness is extended not only to the interval of values but also to their distribution.

In selecting the factors for variation, it is important to assure that as much as possible, there are varied the widest effects. For example, where the variation from a day to another (probably due to the re-standardization effects) is substantial as compared to the repeatability, two determinations at each of the five days shall assure a better estimation of the intermediary accuracy than five determinations every two days. Ten singular determinations in separate days make better the object of a sufficient control although this one shall not assure additional information regarding the repeatability during the day.

Generally, it is simpler to treat the data obtained from random selection than from the systematic variation. For example, experiments performed at random times on a sufficient period usually include the representative effects of the ambient temperature, while experiments performed systematically at intervals of 24 hours can be affected by bias due to the regulated variation of the ambient temperature during a working day. The first experiment shall evaluate only the global standard deviation, in the last experiment there is required the systematic variation of the ambient temperature, followed by adjustment in order to allow the real temperature distribution.

The random variation is, still, less efficient. A small number of systematic studies can rapidly establish the dimension of an effect while typically there are required 30 determinations for establishing a contribution of the uncertainty to more than approximately 20% relative exactness. Therefore, where there is possible, is often preferred to investigate few major systematic effects.

Where the factors are known or are suspected to interact, it is important to be sure that the interaction effect is taken into consideration. This can be realized either by assuring the random selection from different levels of the parameters that interact, or by systematic and careful projecting for obtaining both information regarding the variance and covariance.

When performing the studies for global displacement, it is important that the reference materials (RM) and their value to be relevant for the materials usually tested.

Any study made to investigate and test the signification of an effect shall have sufficient power to detect such effects before they become practically significant.

PRACTICAL ASPECTS REGARDING VALIDATION OF METHODS

Examples of how the evaluation of such method performance characteristics is performed are illustrated in table 1. On the left there is mentioned what it is analyzed, and on the right what it is computed.

Table 1

Manners of realizing the validation of the main performance characteristics

Detection limit	
10 individual independent measurements on the blank solution	Mean and experimental standard deviation multiplied by three
Quantification limit	
10 individual independent measurements on the blank solution	Mean and experimental standard deviation multiplied by five, six or ten
Method correctness	
10 individual independent measurements on the blank solution and on the reference material	The blank mean is subtracted from the analyte mean determined on the RM and is compared with the accepted value
Method accuracy	
10 individual independent measurements on the blank solution and on the reference material by a) the same analyst, operator, laboratory, on a short period of time b) analysts, different equipments, the same laboratory on a longer period of time c) analysts, equipments, different laboratories on a longer period of time	The experimental standard deviation of repeatability at each concentration The experimental standard deviation of reproducibility in the laboratory at each concentration The experimental standard deviation of inter-laboratories reproducibility at each concentration
Recovery	
6 measurements repeated on a matrix blank, marked sample/ enriched, CRM	Analyte recovery (%) = (concentration of the marked/enriched sample – concentration of the non-marked sample/ not enriched/ marcator concentration/ added substance ·100)

Because the validation [1] starts from the performance criteria, there will be presented representative aspects of the Directive in force, in the following.

The decision of the Committee 2002/657/CE for the implementation of the Directive 96/23/EC regarding the performance of the analytical methods and the interpretation of the results, notified by the document no.C(2002) 3044 provides the rules for the analytical methods that can be used in testing the official samples taken according the directive 96/23/EC and specifies the usual criteria for interpreting the analytical results of the control official laboratories.

The member states have the obligation to assure taking the official samples and their analysis by methods that [5]:

- are documented in instructions for testing according to ISO 78-2;
- were validated according to the regulated procedures;
- comply with the legal performances (RMLP).

RESULTS INTERPRETATION

1. The results of an analysis shall be considered non-conform if the decision limit of the confirmation method for the analyte is crossed.
2. If there was established an admitted limit for a substance, the decision limit is the concentration above which it can be decided with a statistic certitude of 1-α that the allowed limit was really crossed.
3. If for a substance there was not established any allowed limit, the decision limit is the lower lever of concentration at which a method can discriminate with a statistical uncertainty of 1-α that a certain analyte is present.
4. For the substances listed in the annex 1 of the directive 96/23/EC, the error can be 1% or less. For all the other substances the error α shall be 5% or less.

GENERAL REQUIREMENTS

Samples handling

The samples shall be obtained, handled and processed so that to maintain a maximum chance of detection. The handling procedures for the sample shall prevent the possibility of accidental contamination or of analyte loss.

Performance of tests

Recovery: during the samples analysis, if there is used a fixed correction factor, the recovery shall be determined for each set of samples. If the recovery is within the limits, the fixed correction factor can be used. If there will not be used the recovery factor obtained for that set of samples. If there shall be applied the recovery factor of the analyte in the sample, there shall be used the standards addition procedure or an internal standard for determining the analyte fro a sample.

Specificity: a method shall be capable to distinguish between the analyte and other substances in the experimental conditions and shall provide an estimation of the extent to which this is possible.

TESTING METHODS

There shall be used for testing purposes according to the directive 96/23/EC, only those analytical techniques for which there can be demonstrated in documented manner that they are validated and that they have false compliance rate < 5% (error α) at the interest

level. In the case of some results suspected as non-conform, these shall be confirmed by a confirmation method.

CONCLUSIONS

The confirmation methods for organic residues and contaminants shall provide information on the organic structure of the analyte. As a consequence, the methods based only on chromatographic analyses without using the spectrometric detection are not convenient for using them as confirmation methods.

If a single technique has not sufficient specificity this can be reached by analytical procedures consisting in convenient combinations of purifications, chromatographic separations and spectrometric detection. The convenient confirmation methods for the organic residues and contaminants are synthesized in table 2.

Table 5.

Indicated confirmation methods for organic residues and contaminants

Measurement technique	Substances according to the annex 1 96/23/EC	Limitations
LC or GC with MS detection	Groups A and B	Only if there follows either a on-line chromatographic separation or an off-line one. Only if there are used complete scan techniques using 3 (group B) or 4 (group A) identification points for those techniques which do not register the complete mass spectrum
LC or GC with IR spectrometric detection	Groups A and B	There shall be fulfilled specific requirements for the IR absorption spectrometry
LC complete DAD scan	Group B	There shall be fulfilled the requirements specific to the UV absorption spectrometry
LC with fluorescence	Group B	Only for molecules which present native fluorescence and molecules which present fluorescence after transformation
2-D TLC complete UV/VIS scan	Group B	HPTLC double dimensioned and co-chromatography are compulsory
GC with ECD	Group B	Only if there are used two columns with different polarities
LC-immunogram	Group B	Only if there are used at least two different chromatographic systems or two independent detection methods
LC-UV/VIS (o single wavelength)	Group B	Only if there are used at least two different chromatographic systems or two independent detection methods

PERFORMANCE CRITERIA AND USUAL REQUIREMENTS

When the method uses a convenient internal standard, this shall be added in the sample tested at the beginning of the extraction period.

When there is no convenient internal standard, the identification of the analyte can be confirmed by co-chromatography. In this case, there shall be obtained only a little, the increase in height (or area) being equivalent to the quantity of added analyte. In gas and in

liquid chromatography, the width of the peak at the half of the maximum height shall be in the domain of 90-110% as compared to the original width and the retention times shall be identical within an interval of ± 5%. For thin layer chromatography, the spot supposed to be due to the analyte shall be intensified.

PERFORMANCE CRITERIA AND ADDITIONAL REQUIREMENTS FOR QUANTITATIVE ANALYSIS METHODS

Correctness of the quantitative methods

In the case of some analyses repeated on certified reference material (RMC), the domain of the deviation of the mean mass fraction deviation corrected with the recovery determined experimentally, towards the certified value is resented in table 3.

Table 3

Minimum correctness of the quantitative methods

Mass fraction	Domain
≤ 1 µg/kg	-50% - +20%
> 1 µg/kg – 10 µg/kg	-30% - +10%
≥ 10 µg/kg	-20% - +10%

When there are not available RMC there is accepted the same correctness.

The accuracy of the qualitative methods

The variation coefficient (VC) inter-laboratories for the repeated analysis of a reference material or fortified in reproducibility conditions shall not cross the level computed with Horwitz equation. The equation is:

$VC = 2^{(1-0,5\log C)}$

Where: C is the mass fraction expressed as a power (exponent) of 10.

Table 4

Examples of variation coefficients of reproductibility for quantitative methods

Mass fraction	VC of reproducibility (%)
1 µg/kg	(*)
10 µg/kg	(*)
100 µg/kg	23
1000 µg/kg	16

(*) – for fractions smaller than 100 µg/kg the application of Horwitz equation leads to unacceptable high values. Therefore, the variations coefficients for concentrations lower than 100 µg/kg shall be as small as possible.

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THE CHEMICAL AND MICROBIOLOGICAL EVALUATION OF LACTIC ACID-FERMENTED MIXED VEGETABLE JUICES

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KEY WORDS: lactic acid fermented juice, spoilage

ABSTRACT

In the present work were realised experiments oriented to preparation of lactic acid fermented juices using carrots, red peppers and cabbage in proportions established through sensorial analysis. The processes were realised at 25 degrees Celsius for 96 hours, using lactic acid bacteria isolated from the microbiota of spontaneous fermentation of vegetables.

During the lactic acid fermentation of cocktails the evolution of pH values, lactic acid content and reducing sugars content were followed. After 96 hours of fermentation, the stability of cocktails was higher, due of their lactic acid content, which was increased from 0,15 to 1,06g/100g. A strain of mould involved in the lactic acid-fermented juice spoilage was isolated as pure culture and analyzed with a view to establish its capacity to assimilate the lactic acid as carbon source.

INTRODUCTION

The reasons for using lactic acid bacteria are to make food durable, to improve its taste and to maintain the nutritive, physiological and hygienic value of the fermentation products (Karovičová, 1999). Their selection depends on desired qualities of an end product, raw material properties and applied technological process (Rakin, 2004).

In recent years, great interest has been dedicated to vegetable juices processed by lactic acid fermentation because they contain high amount of beneficial substances such as vitamins, mineral compounds, dietary fibre and anticancer compounds (Karovičová, 2005). Lacto-juices processed by lactic acid fermentation bring about a change in the beverage assortment (Panda, 2007).

For an optimal course of lactic acid fermentation, the content of sugars in raw materials must be sufficiently high (Kopec, 2000, quoted by Karovičová, 2003). Also, a rapid decrease in pH at the beginning of fermentation is of great importance for the quality of the end product (Viander, 2003). A rapid increase in acidity minimises the influence of spoilage bacteria. By fermentation, the juices obtain pleasant acid taste and characteristic aroma (Karovičová, 2003).

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MATERIALS AND METHODS

Fresh vegetables - carrots, cabbage and red peppers - were shopped from the local free vegetable market of the Dambovita district (Romania) in September. There are specifically conditioned by washing, scrubbing (carrots) and non-edible pieces elimination. The outer leaves of cabbage were removed and it was chopped into small slices. Also the seeds of the peppers were removed.

Fresh juices were obtained by pressing with a home juice-maker. After the sensorial analysis the individual juices were mixing in proportions by 2:1:1 - v/v/v (carrots : red peppers : cabbage) and 1,5% salt was added. With a view to destroy the undesirable microorganisms, the cocktail was thermal treated (for 20 minutes at 70^0C).

After cooling at 28 degrees Celsius, the cocktail was supplied with a brine inoculum with $2,83x10^3$ cells/ml (50,37% streptococcus, 42,03% pediococcus, 7,6% lactobacilli, without yeasts), isolated from epiphytic microbiota of vegetables.

50ml juice was distributed in sterile tubes and the anaerobiosis was created by covering the cotton stopper of the tube by a metal folia. The carbon dioxide formed during the fermentation process ensured anaerobic conditions over the surface of the juices. Each tube represented a single sample and the experiments were performed in double.

The lactic acid fermentation was performed in a thermostat at 25 degrees Celsius and the samples were daily followed through sensorial and chemical analysis: reducing sugars according Schoorl, total acidity expressed as g lactic acid, pH values.

The spoilage microorganisms of lactic acid fermented juices were also analyzed for emphasize its capacity of metabolization the lactic acid – the principal factor involved in the preservation of the final products. In this purpose moulds involved in the adulteration of cocktails were isolated and obtained as pure cultures.

As culture medium was used one artificial with peptone (1%) as nitrogen source and with different quantities of lactic acid. The samples were aseptic inoculated with standard quantities of mould and kept at 22 degrees Celsius. The assimilation of the organic acid was followed at different intervals of time, until 4 weeks, by determination the total acidity. The purity of the biologic material, respectively the development of the microorganisms cultures due of the metabolization of the organic acid, were followed through microscopic exams.

RESULTS AND DISCUSSIONS

From the sensorial point of view the cocktail was slightly turbid in the first 24 hours of fermentation. The colour of the samples was lightly changed during lactic acid fermentation, in the sense of the loss of specified intensity shade of vegetables. However, it was kept the specified flavour which represents a characteristic from raw materials used at the cocktail processing. It was enriched with a gentle odour proceeded from lactic acid and fragrance substances production by lactic acid bacteria.

Concerning the dynamics of the most important biochemical parameters, it can be noticed that some factors such as fermentation temperature, the quantity and quality of lactic acid bacteria inoculum, respectively the substratum biodisponibility seem to be favourable for the pH decreasing with 2,12 unities in 48 hours (*figure 1*). Simultaneously, the acidity of the cocktail increases with 4,66g/kg, this one being a guarantee for the microbiological stability of the final products. In the first 24 hours the diminishing of the pH, respectively the increasing of the total acidity aren't so significant, probably as a result of the lactic acid bacteria necessity for accommodation at environment.

After 4 days from the beginning of the fermentation (when the fermented juice became stable), the production of lactic acid due of lactic acid bacteria activity was important: 9,09g/kg. The final acidity of 10,68g/kg (corresponding at the pH value of 3,96) wasn't excessive, diminishing the sweetness of the red pepper juice.

A relative constant decrease was found regarding the reducing sugars (calculated as glucose). According Kopec (2000), quoted by Karovičová (2003), from the viewpoint of optimal lactic acid fermentation course, the content of sugars in raw materials must be sufficient (i.e. at least 40g/dm^3). The initial content of the juice obtained from carrots, red peppers and cabbage was close from this value (3,97g/100g).

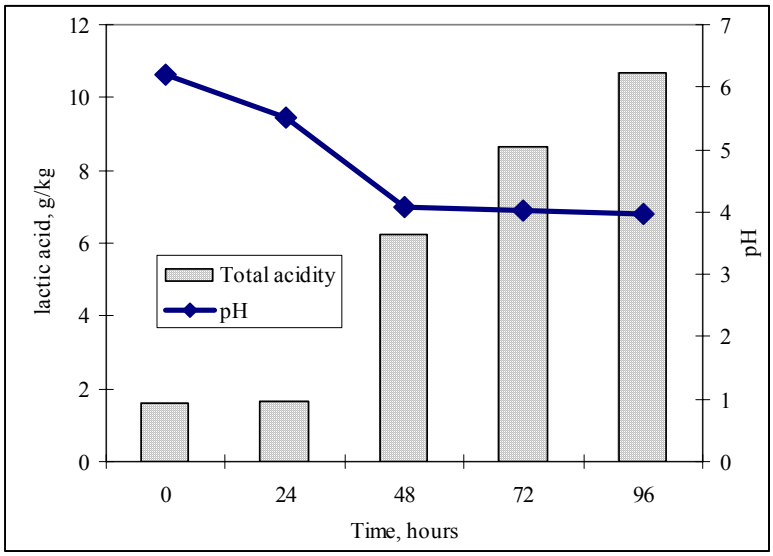

Figure1. The dynamics of some chemical parameters of lactic acid fermented cocktail

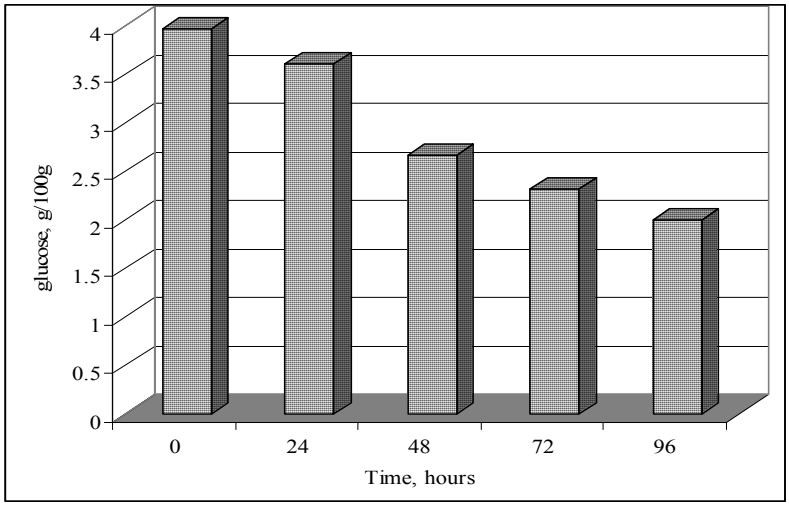

Figure 2. The dynamics of the reducing sugars during lactic acid fermentation of cocktail

The diminishing of this parameter represents 49,62% after four days of fermentation (*figure 2)*. It can observe that there is a relative proportionality between the reducing sugar consumption and the lactic acid accumulation. Thus, from 1,97 g glucose metabolized, the lactic acid produced by desirable bacteria was about 1,06g. It is obvious that in medium were present different kinds of lactic acid bacteria species, especially heterofermentative, which were produced secondary compounds by lactic acid.

Practical the acidity of the cocktail at the initial moment was due to the malic acid content of vegetables although all the values are expressed as lactic acid. It's noticed that also the malic acid can be transformed in lactic acid with the aid of enzymatic equipment of desirable bacteria. This is the possible explanation for the moment of time when the decreasing of reducing sugars isn't directly proportional with the increases of lactic acid content. Also, in the course of the fermentation the utilisation of citric acid (for acetoine and diacetil production) is recorded.

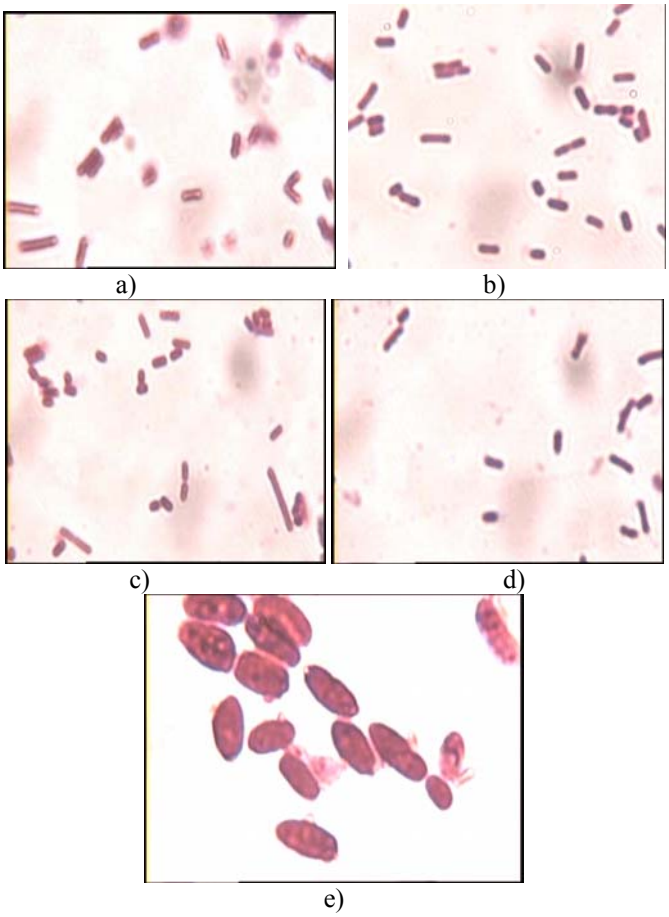

Figure 3**.** The microbiota evolution during the lactic acid fermentation of mixing juice obtained from carrots, red peppers and cabbage (2:1:1)
a) after 24h of fermentation; b) after 48h of fermentation; c) after 72h of fermentation; d) after 96h of fermentation; e) development of adulteration microbiota after 2 weeks of keeping at 25 degrees Celsius

In *figure 3* are showed as photo images a few moments from the changes happened in the microorganisms evolution during lactic acid fermentation of cocktail.

The useful microbiota development, respectively the growth of lactobacilli proportion in the interval 24-48 hours was correlated with the total acidity change (expressed as lactic acid) from 1,66 to 6,25 g/kg.

At the end of the analyzed period of 96 hours the lactic acid content of cocktail was growing by 6,71 times, it beginning to inhibit the lactic acid bacteria.

With a view to establish some possibilities for extension the preservation time of lactic acid fermented vegetable juices, moulds involved in the adulteration of them were isolated and obtained as pure cultures. *Geotrichum candidum* was studied for emphasize its capacity of metabolization the lactic acid, in the lack of the others carbon sources.

Six variants of lactic acid in medium were choice, from 0,5 to 3g/100ml (*table 1*). Normally, between this limits it is found the lactic acid resulted as a result of vigorous lactic acid fermentation of vegetables in brine.

Table 1

The evolution of the total acidy in medium with lactic acid, in presence of *Geotrichum candidum*

Lactic acid in medium (initial), g/100ml	*Lactic acid (g/100ml), after:*			
	1 week	2 weeks	3 weeks	*4 weeks*
0,5	0,12	0,045	0,02	*0*
1,0	0,3	0,04	0,018	*0,016*
1,5	0,44	0,08	0,02	*0,016*
2,0	1,02	0,32	0,04	*0,02*
2,5	1,3	1	0,75	*0,035*
3,0	*1,8*	*1,58*	*1,5*	*1,4*

In the samples with 0,5-2,5% lactic acid, this substratum was consumed by *Geotrichum candidum* in proportion of 100% to 98,6% during 28 days. It's obvious the stimulant effect of the smaller initial content of organic acid on the biomass production.

At the initial concentration of 3%, the rate of consumption was only of 53,33% at the end of the interval. The influence of the lactic acid concentration on the capacity of mould assimilation is more evidently in this variant. Even in the conditions of a higher initial concentration of lactic acid in medium, the decreasing of the total acidity was fast: 40% after one week.

This way is evidently that the mould studied has the enzymatic equipment necessary for the organic acids metabolization, with practical importance in the decreasing of the preservation of lactic acid fermented vegetable juices.

CONCLUSIONS

- ✓ The undesirable sensorial characteristics of vegetables as cabbage, respectively the excessive sweetness of red peppers can be successfully lessened through lactic acid fermentation of mixing juices;
- ✓ The sufficient initial content of vegetables in reducing sugars and the rapidly pH decreasing are essential for the lactofermented juices stability;

- ✓ *Geotrichum candidum* present a higher capacity of lactic acid assimilation, which is proved through the acidity decreasing and the biomass production in all the experimental variants of lactic acid concentration, from 0,5 to 3%;
- ✓ The lactic acid metabolization by the microorganisms involved in the spoilage of lactic acid fermented juices explains the pH increasing and the diminishing of the preservation period of time. A rapidly increasing of the juice acidity, associated with a smaller temperatures of storage after the product stabilization (4 or 5 days) represent a guarantee for the quality of lactofermented juices, without to be necessary the addition of preservatives.

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Note. The researches were made in the frame of the CNCSIS Project nr. A 1086/2005, „Researches Concerning the Improvement of the Biotechnological Parameters of Obtaining Lactic-Fermented Juices from Vegetables".

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Vol. XIII (XLIX) - 2008

STUDY CONCERNING THE CONDITIONS OF THE BEETROOT JUICE LACTO-FERMENTATION

Iuliana Manea, Lavinia Buruleanu[1]

KEYWORDS: Red beet juice, lacto fermentation, chemical factors

ABSTRACT

The aim of this study is to emphasize the importance of some chemical and physical factors on the lactic acid fermentation of the red beet juice.

Due of the higher content of sugar, the juice obtained from the red beet can be an excellent substratum for the lactic acid fermentation. As a result of juice lacto fermentation it is obtain a salubrious and stable product due of the lactic acid which removes the microorganisms of contamination.

In this study it was followed the evolution of lactic acid fermentation of the red beet juice in different conditions. Supplements of NaCl and honey were used and the fermentation was realised at darkness, at lightness and at the different temperatures.

The lactic acid fermentation of red beet juice in certain conditions, with a view to obtain the optimum final products concerning the quality and the time of processing, was followed. Finally were established the best conditions for the lactic acid fermentation of the red beet juice.

INTRODUCTION

Red beet (*Beta vulgaris*) presents a higher nutritive value, but also valuable therapeutic properties. With a view to improve the nutritive qualities and to obtain a maximum sanitary security, the red beet juice was submitted at lactic acid fermentation [5].

Through the acidification produced by the lactic acid bacteria the pathogenic germs and the adulteration germs were removed. In a number of countries, the consumption of the lactic acid fermented vegetable juices increases [4].

The vegetable juices processed by lactic acid fermentation introduced a change in the beverage assortment due to their high nutritive value and high contents of vitamins and mineral compounds. Fermented foods are often more easily digestible than unfermented foods [2,3].

Micro-organisms contain certain enzymes, such as cellulases, which are incapable of being synthesised by humans. Microbial cellulases hydrolyse cellulose into sugars which are then readily digestible by humans. Similarly pectinases soften the texture of foods and liberates sugars for digestion.

The lactic acid fermented vegetable juices can be produced by two procedures: the vegetable is first fermented in a usual way and then it is processed by pressing out the juice (manufacture of sauerkraut), or the vegetable is at first processed to mash or raw juice and

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it is consecutively fermented [1, 7]. By the fermentation, the juices obtain, a pleasant acid taste and, a characteristic aroma.

MATHERIALS AND METHODS

In this study the optimization of lactic acid fermentation of red beet juice through the change of some physical factors (temperature, light) and some chemical factors (the addition of carbohydrates, NaCl, citric acid and celery juice) was followed.

The juices where then supplied with a brine inoculums with 10^5 cells/ml lactic bacteria mixture. The brine was obtained by a 3 day lactic acid fermentation of a mixture of chopped vegetables (carrots/cabbage) prepared with 2,5% NaCl.

In the next study we did the following notes:

M- red beetroot juice (the approval sample);
B2-- red beetroot juice with honey added
B3- - red beetroot juice without NaCl
B4- the sample kept at the light
B5- - red beetroot juice with lemon juice added
B6- - red beetroot juice diluted with celery juice

The samples volume was about 100ml and the lactic acid fermentation was realised in fermentation bottles equipped with valve. The saccharides addition as honey was by 4%, that one of citric acid as lemon juice by 1% and celery juice by 20%. The fermentation was followed 168 hours at 22 degrees Celsius, respectively at 30 degrees Celsius through the determination of the carbon dioxide formed.

The pH determination was realized with the help of the electronic pH-meter-the HACH type. The acidity has been determined by the tri-trimetric method in the presence of the bromothymol blue.

RESULTS AND DISCUSSIONS

The experiment was conducted in two variants: at 22deg.C and 30 deg.C. The results of the lactic acid fermentation of red beet juice at 22deg. C can be followed in Fig1.

Concerning the carbon dioxide formed it's noticed that the lactic acid fermentation was started after 48 hours at the sample without NaCl, and its intensity was the smaller (CO_2 formed being 2,91 g/100ml). All the others were fermented faster because of the presence of some growth factors of lactic acid bacteria liberated from the vegetable cells through the plasmolyse induced by NaCl.

The lactic acid fermentation was going on with higher speed in the case of the samples with supplement of honey, respectively diluted with celery juice. In the sample exposed at the light the fermentation was slowly in comparison with the approval sample, giving off 4,36 gCO_2/100ml.

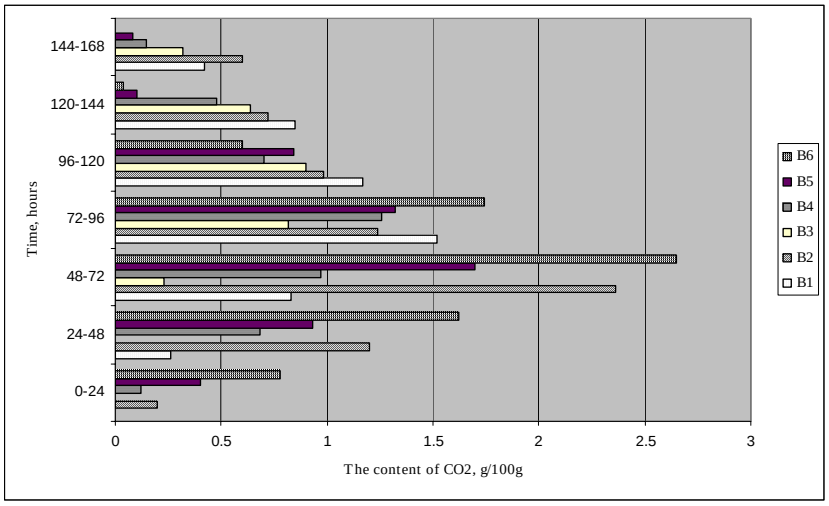

Fig. 1. The content of carbon dioxide formed in during the lactic acid fermentation al 22 deg.C

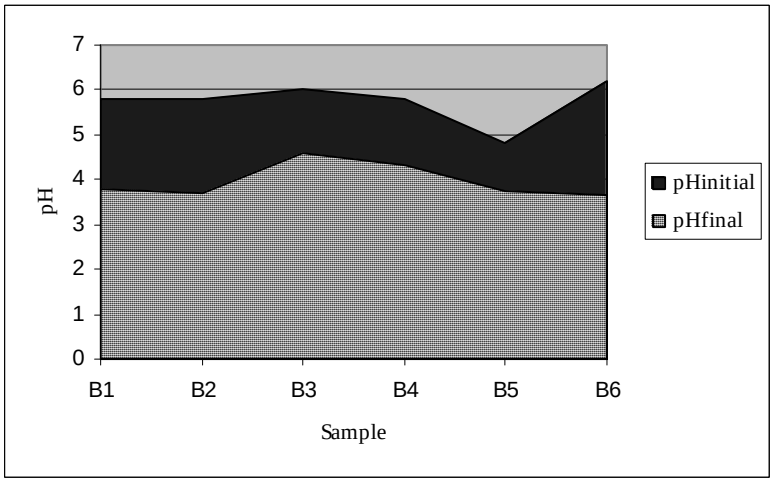

Fig. 2. The pH values of the sample for 22 deg. C and at 30 deg. C

In the case of the sample B5 acidified with lemon juice, the lactic acid fermentation was started faster because of the optimum pH for the multiplication and the activity of the lactic acid bacteria (pH=5,1).From distinguish by the others samples, in this one the lactic acid fermentation was realised with an almost constant speed in the first three days and afterwards was decreased.

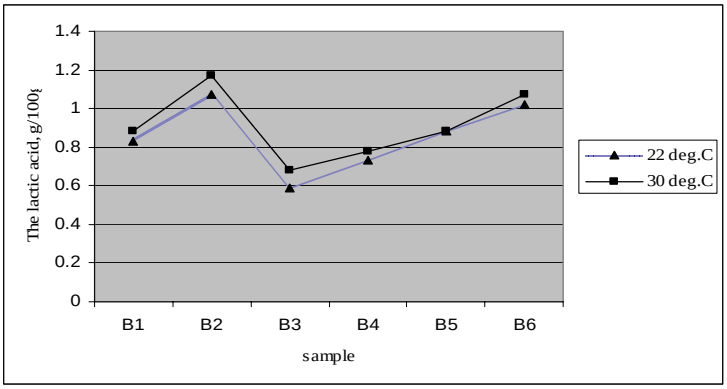

Fig. 3 The content of lactic acid for 22 deg. C and at 30 deg. C

In Fig.2 are presented the pH values of the samples at the initial moment and after 148 hours both at 22 deg. C and at 30 deg. C.It can be observed that at the initial values of pH the growth of lactic acid bacteria is difficult, excepting the sample B5.

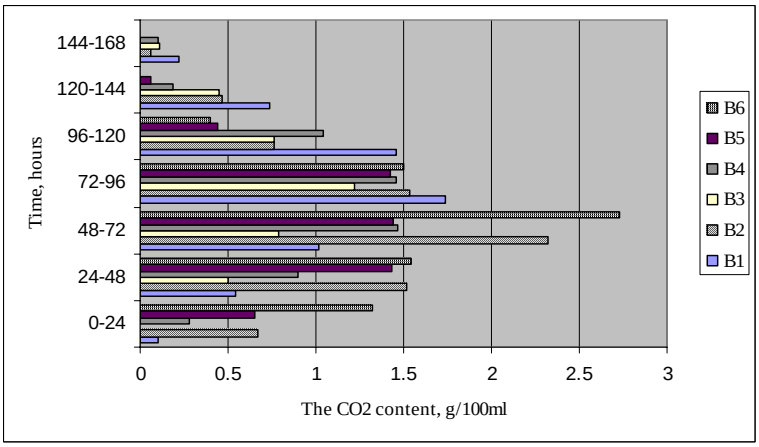

Fig. 4 The content of carbon dioxide formed in during the lactic acid fermentation al 30 deg.C

After the selection of the bacteria in the medium the lactic acid bacteria start to produce lactic acid and the pH decrease. This tendency was maintain also in the case of lactic acid fermentation at 30 deg. C (Fig.2), but the decreasing is more obvious.

The dynamics of lactic acid formed is presented in Fig.3. The samples with supplement of fermentescible saccharides (B2) and celery juice have accumulated the higher quantities of lactic acid. Through the change of the fermentation temperature at 30 deg. C it was observed the quickly starting of the fermentation, the carbon dioxide casual being more intense than in the case of the fermentation at 22 deg. C (Fig.4).

The fermentation dynamics was similar in this case too and the samples B2 and B6 have registered the greatest losses in CO2.

CONCLUSIONS

- Through addition of honey at the fermentescible glucides content of red beet juice, the speed and the intensity of lactic acid fermentation increase;
- The salt has a favourable role at the providing of growth factors for lactic acid bacteria;
- The temperature influences direct proportionally the fermentation speed, causing the diminution of the time of fermentation. However, it is possible the negative change at the taste and aroma;
- The dilution of the red beet juice with celery juice influences positively the lactic acid fermentation;
- In the presence of the light and of the sun the lactic acid fermentation was unfolded with a small speed;
- The initial acidification of the red beet juice permit the quickly development of lactic acid bacteria; through the metabolisation of the citric acid it can be obtain aroma compounds that influence positively the sensorial characteristics of the final product.

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RESEARCHES ON THE CONTENT IN PECTIC SUBSTANCES OF SOME FRUITS WITH A VIEW TO OBTAIN THE PRODUCTS ENRICHED IN PECTIN

Manea Iuliana, Buruleanu Lavinia[1]

KEY WORDS: Pectin, fruits, pectic extracts, calcium pectat

ABSTRACT

The purpose of this study was consisted in the analysis of the pectin content from apples, quinces, strawberries and citric fruits with a view to isolate the pectin and to obtain afterwards the products enriched in pectin. Because the pectin is a biologic active compound with a large spectrum of action on the human body, the products enriched in pectin were selected for the study. Firstly the pectic extracts were obtained and the test for identification the pectic substances and their degree of decomposition were made. In the second stage the pectic substances from the analyzed fruits were quantitative established. At the worldwide dairy products and vegetable products enriched with pectin are obtained because his numerous useful effects on the human health.

INTRODUCTION

The problem of the health keeping and the increasing of the life time represent one of the most important and up-to-date problem of the society. Its solution supposes the inclusion of food supplements in the food ration or the enrichment of the products with different constituents such as pectin. [5]

Pectin is a long chain of pectic acid and pectinic acid molecules. Because these acids are sugars, pectin is a *polysaccharide*. [1, 2] The structure of pectin is very difficult to determine because pectin can change during isolation from plants, storage, and processing of plant material [4]. Pectins are structurally complex plant cell-wall polysaccharides that contain 1,4-linked α-d-galactopyranosyluronic acid residues. Galacturonic acid (GalA) is the most abundant glycosyl residue in the three types of pectin present in all plant primary walls: homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II) [3]. Too, the pectin is a non-digestible food fibre.

Fruit fibres are by-products of the fruit processing industry, left behind when the fruit is de-juiced. Despite the benefits of fibre for human health – including boosting satiety, slowing glucose absorption, and functioning as prebiotics – fruit fibres often end up being thrown away or used as animal feed. The most important actions are: anticancer, the immunity increasing, the gastric mucous membrane protection, the intestinal transit stimulant, the prophylaxis of the intoxications with heavy metals and detoxifying in the case of chronic intoxications with heavy metals, antioxidant action, decrease the lipids absorption from intestines, regulate the gall bladder function.

The most important use of pectin is based on its ability to form gels. High metoxy-pectin forms gels with sugar and acid. [4]

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Dairy acid products with pectin, fruits and vegetables juices with pectin, fruits cream and thick soups with pectin are consumed. With a view to find some raw materials rich in pectin, the fruits and after the new combinations of fruits and vegetables creams enriched with pectin are studied.[5]

MATHERIALS AND METHODS

A lot of fruits - apples, pears, strawberries, oranges, lemons, peaches - were analyzed. The pectic extracts were obtained and their pectin content was established.

The pectic extract was obtained from marc of apples, pears, strawberries, respectively from the orange peels.

The raw material was washed with water at 40 degrees Celsius and then the insoluble protopectin was hydrolyzed, its being passed in solution.

The pectin identification test was made in the pectic extract. The test is used both for the pectic substances presence identification and their degradation degree. It permit the quickly study of the depectinization process unfolding with enzymatic products.

Then the pectin dosage under calcium pectat form was realised. The method principle consists in the protopectin hydrolysis with sodium hydroxide, acidification with acetic acid in excess and treatment with calcium chloride.

In the next study we did the following notes:

PE1- the pectic extract from marc of apples;

PE2 - the pectic extract from marc of pears;

PE3 - the pectic extract of orange peels;

PE4 - the pectic extract of lemons peels;

PE5 - the pectic extract from marc of peaches;

PE6 - the pectic extract from marc of strawberries.

RESULTS AND DISCUTIONS

After the performing the test of pectic substances identification, the following were observed:

The sample with pectic extract obtained from the apples and orange peels marc, treated with acidified alcohol, was presented after 20 minutes of homogenisation an intense precipitate. Concerning the pectic extract obtained from pears and peaches, in the presence of the acidified alcohol, not any modification was observed after 1 hour. After 24 hours a small precipitate (from quantitative point of view) became visible, being slightly dispersed at the lowest part of the test tube.

In the sample of pectic extract obtained from strawberries don’t appear any change of aspect after 24 hours from the ethylic alcohol and hydrochloric acid addition.

Because the identification test of pectins was rapidly, but orientated, the quantitative dosage of them through the Carre method was realised. In this purpose the same samples of extract obtained in the first stage were analyzed. The results can be observed in figure 1.

The higher quantity of calcium pectat was determined in the peels lemon extract (3,4 g/100). The oranges were characterized with a smaller quantity of pectic substances in peel comparatively with the apples. The lowest level of calcium pectat was established in the case of peaches and strawberries.

Due of the numerous benefits for the human health of pectin, in the world-wide alternative sources for its obtaining are look for.

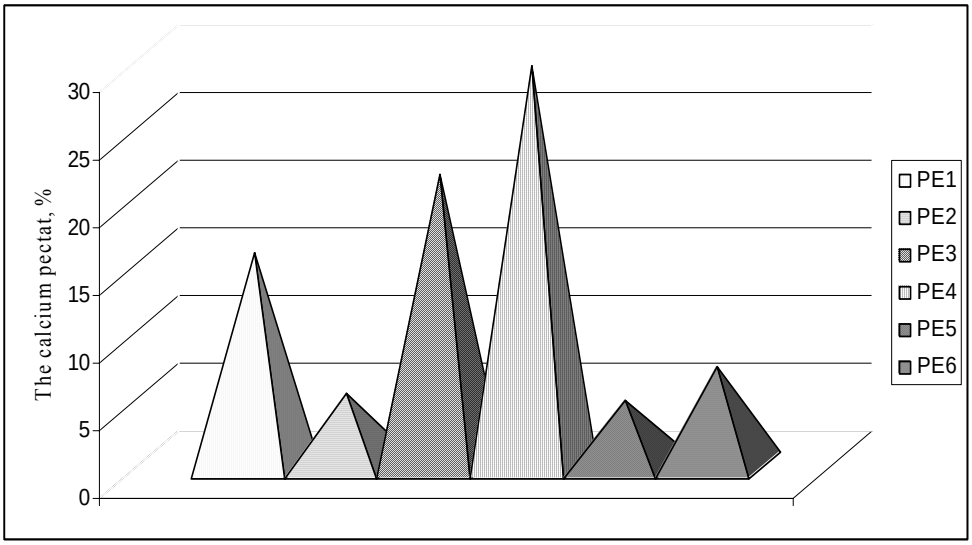

Fig. 1 The content of calcium pectat from difence sources

CONCLUSIONS

- The citric fruits present a higher content of pectic substances;
- Even the oranges has a smaller quantity of pectin, they are used with predilection due of the great production;
- In our country the apple marc is the source for the pectin production;
- The strawberries and the peaches marc are not very used, both at the reduced content of pectin and at the smaller production in Romania.

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THE MINERALS AND VITAMINS CONTENT OF DIFFERENT VEGETABLES - RAW MATERIALS FOR JUICES

Magda Gabriela Bratu, Daniela Avram, Lavinia Buruleanu[1]

KEYWORDS: vegetables, mineral substances, vitamins

ABSTRACT

The vegetables represent a category of protection food products. The juices obtained from fruits and vegetables are very easy assimilated in the human body, comparatively as such vegetables.

The study presents the variation of the minerals and vitamins content from some vegetables (carrots, cabbage, red pepper) in function of the race, respectively the compounds dynamic in the vegetables juices obtained from each type or using different combinations from theirs.

The mineral salt: calcium, magnesium, potassium, phosphorus were analyzed. Also the ascorbic acid and the carotenoids were analyzed. In all the cases the analyzed parameters were presented in tables and it was establish that their values were integrated in the limited values presented from the specialty literature.

INTRODUCTION

Vegetable belong to the group of protective foods. The most important reason for their use in human nutrition are as follows: attainment of good health condition, prevention of a series of diseases, attainment of balanced nutrition, rich and inexpensive source of vitamins, minerals and carbohydrates [3].

Vegetable juices are more easily assimilated in an organism than fresh vegetables. As the squeezing process destroys the fibre structure and releases bound phytonutrients[4]. Due their nutritive and biological potential, the fruits and vegetables juices are food products with numerous implications in the body equilibrium. They can be obtaining in different combinations, assuring in the same time the complex therapeutic effects-thus they are recommended in the diet of the hart, liver, kidney patients for the prevention of asthenia and the gastric-intestinal inflammations. The increasing consumption of vegetables and vegetable juices prevents the atherosclerosis, the hypertension, the obesity [2].

Because the alimentary value of vegetables and vegetable juices is given by their chemical composition and the proportion of its different compounds, this study was made analyse these parameters as following: vegetables and vegetable juices represented valuable source of β carotene, vitamin C, phosphorus, calcium, potassium, magnesium.

These parameters were analyzed from different species of vegetables and juices, them selves or in different combinations.

[1] Valahia Univesity from Targoviste

MATERIALS AND METHODS

Two races of carrots respectively two races of cabbages (early race and belated race) were analyzed. A single race of red pepper was analyzed. The fresh vegetables were analyzed after a preliminary conditioning by washing and mechanical elimination of non-edible parts.

The juices were obtained by conditioned vegetables pressing with a home made apparatus.

The analyzed samples were noted like this:

M1 – early carrots
M2 – autumn carrots
V1 – early cabbage
V2 – autumn cabbage
A – red pepper
SM1 – juice obtained from early carrots
SM2 - juice obtained from autumn carrots
SV1 – juice obtained from early cabbage
SV2 - juice obtained from autumn cabbage
SA - juice obtained from red pepper
SM1V1 - juice obtained from mixing M1 : V1 (1:1; v/v)
SM2V2 - juice obtained from mixing M2 : V2 (1:1; v/v)
SM2V2A - juice obtained from mixing M2 : V2: A (2:1:1; v/v/v)
SM2A- juice obtained from mixing M2 : A(1:1; v/v)

The chemical analysis was made using the following methods:

1. minerals content:
   - for calcium and magnesium by complexometric method
   - for potassium by qualitative method (reactive Kalignost)
   - for phosphorus by spectrophotometric method
2. vitamin content:
   - vitamin C- iodometric method
   - β caroten - spectrophotometric method

RESULTS AND DISCUSSIONS

The conditioning operation of vegetables had as result the inedible parts elimination in proportion of 7,5 – 10%. The variation of mineral salt content in fresh vegetables and juices will be showed in table 1 and figures 1-4. The results were expressed by mg/100g sample.

Table 1

Sample	Ca	Mg	K	P	ascorbic acid	β caroten
0	1	2	3	4	5	6
M1	min 50	min 37	min 280	75,7-84,3	7,74-8	7,58-10,3
M2	max 41	max 18	max 200	35-37,78	3,16-4,57	4,6-6,8
V1	min 48	min 20	min 225	44,2-56,68	45-45,8	-
V2	max 46	max 16	max 185	31-36,04	13,5-19	-
A	9,5	11,5	190	29-38,36	80-139	-
SM1				93-101,7	5,28-5,63	6,7-8,8

0	1	2	3	4	5	6
SV1				65,98-68,5	35,2-39,5	-
SM1V1				72,3	21-22,5	3,3-4
SM2				39,24-41,5	4,57-4,8	3,9-5,98
SV2				37,78-39,2	20,1-25,69	-
SM2V2				39,4	12,67-14,8	2-2,5
SA				40,69-41,2	147,48-150	-
SM2A				41,27	70,4-75	1,9-2
SM2V2A				43,6	31,6-40	1,8-2,2

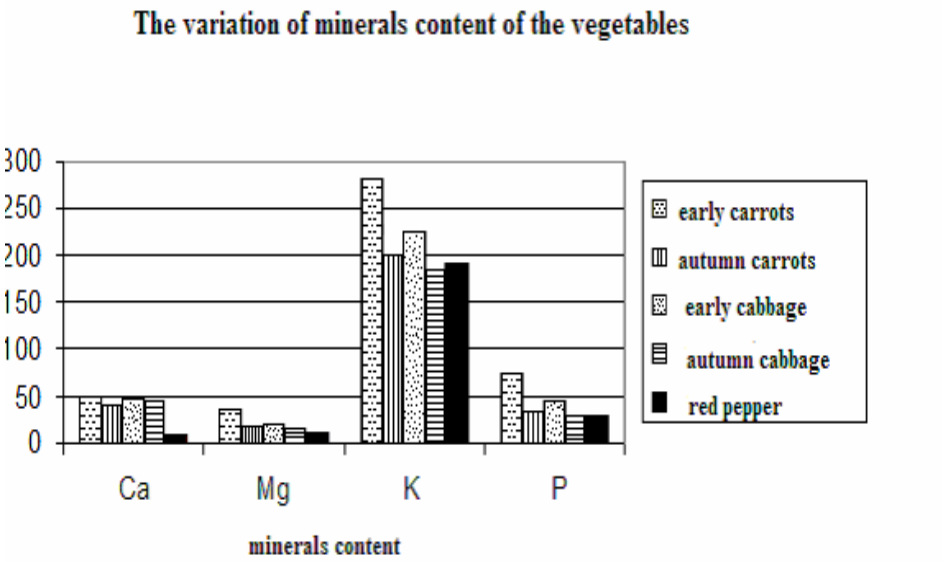

Figure 1. The content of mineral substances of vegetables

The mechanical conditioning and the pressing of vegetables caused an important lost of minerals, because the peel and the immediately adjacent stratum contain greatest quantities of minerals in comparison with the pulp. [1]

The calcium content of all the analyzed vegetables was very closed, excepting the red pepper. Its concentration represents, for example, a five part comparatively with the calcium content of early carrots. A similar situation was established also analyzing the others mineral compounds. Thus, it can be concluded that the red pepper, at the moment of analysis, was characterized by a smaller quantity of minerals.

Analyzing the vitamin content of vegetables (figure 2), the red pepper was remarked by the more higher quantity of ascorbic acid. This is for 40 times greatest comparatively with the autumn carrots, respectively for 1,77 times greatest comparatively with early cabbage.

The content of vitamins varied in function of the provenience and the species, ascertaining a diminishing at the autumn species in comparison of the early species.

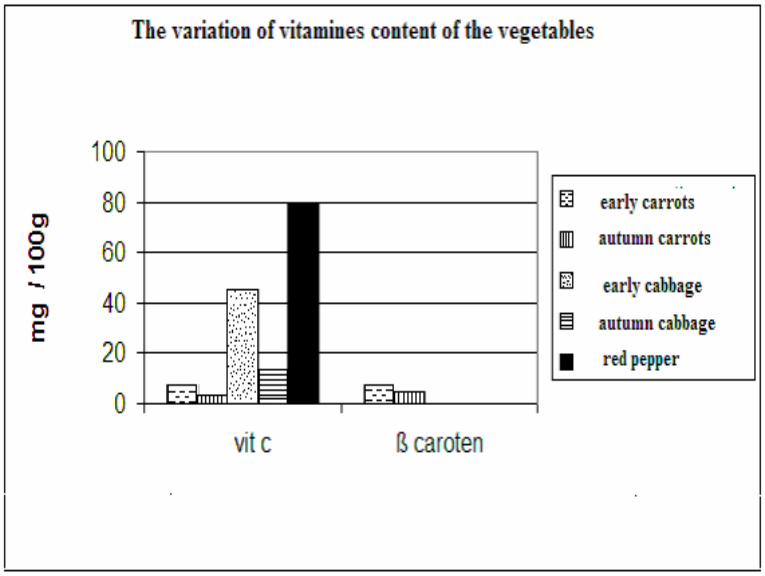

Figure 2. The variation of the vitamins content in fresh vegetables

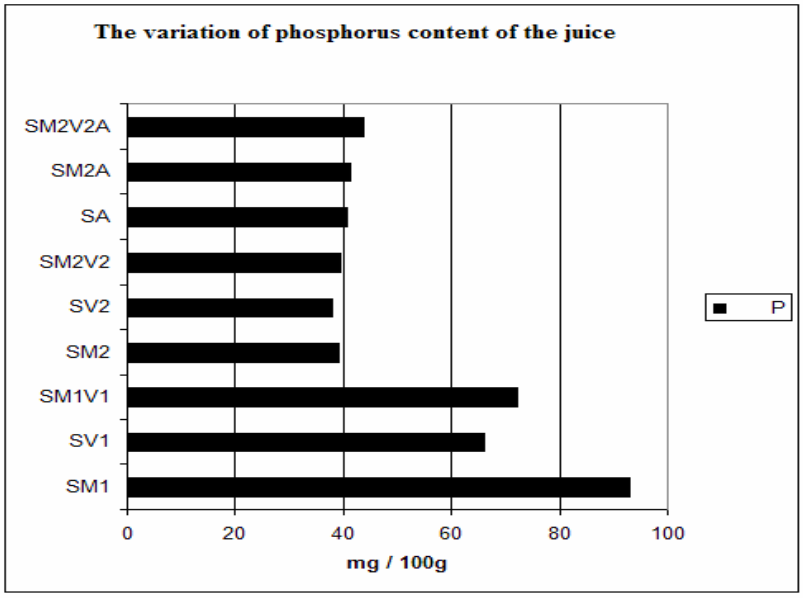

Figure 3. The variation of the phosphorus content in vegetables juices

The content of phosphorus expresses as dry material is diminish through the vegetables processing, keeping the variation limits in function of the species.

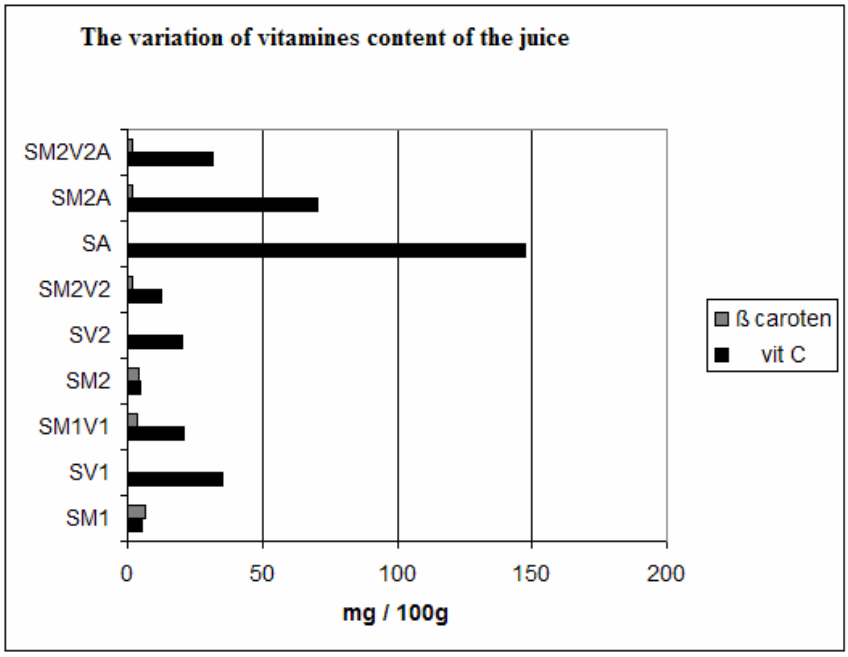

Figure 4. The variation of vitamins content in vegetables juices

Concerning the vegetables juices cocktails, the correlation is the same, in function of the percentage of each vegetable in mixture.

In the juices obtained from the early species of carrots and cabbage the content of vitamin C diminish, while these parameter increase in the juices obtained from autumn species, due of the different contribution of dry matter.

In the juice obtained from peppers, due of the pressing operation what was realized with a high efficiency in comparison of the others vegetables analyzed, the vitamin C content expressed as dry matter increase considerable.

Concerning the β-caroten content, it was determined in all the mixtures with carrots. It can observe a β-caroten content diminishing in juices in comparison with the vegetables, due of the oxidizing process in the time of juice processing.

CONCLUSIONS

After the analysis makes were found the largest variations of the minerals and vitamins content, function of the race, in the case of the same type of vegetable, while in the juices resulted it can't be establish a directly correlation between the initial content of vegetable and the juice content (due at the different efficiency at the conditioning and pressing, respectively at the transformations from the stages before the time of the analysis).

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NONCONVENTIONAL CONSERVATION OF MEDICINAL AND AROMATIC PLANTS BY DEHYDRATING PROCES WITH NON-IONIZED RADIATIONS

Emil Popa, Adriana Muscalu, Mihai Marin[1]

KEY WORDS: alternate methods, microwave, medicinal and aromatic plants,continous flow

ABSTRACT

The traditional technologies of herbs conservation are not sufficient for maintaining the active principles, the colour, the flavour and the vitamins content. Therefore, there have been developed alternate methods - also named - non-conventional, designed to reduce the effects that the time and the temperature had on medicinal and aromatic plants. The drying process of organic materials with nonionizing radiations (microwaves) is more efficient and advantageous in many cases in comparison with the conventional desiccation, as the processed material heating is more rapid and the standard temperature is reached more quickly. The paper contains a review of some nonconventional methods for plant desiccation and, at the same time, presents the achievements obtained by the team involved in the field of technologies and microwave drying installations, designed to process medicinal and aromatic plants under a continous technological flow.

INTRODUCTION

Among the nonconventional conservative methods for foods we remind: treatments with ionizing electromagnetic radiations (γ and X radiations) and nonionizing (microwaves and radio waves, UV radiation), ultrasounds, high presure processing, magnetic oscillatory fields, close packing in modified atmosphere or combination of these with conventional conservative methods.

MATERIALS AND METHODES
1. INACTIVATING METHODS

The absorbtion of the ionizated radiation by a material leads to the ionizated of the substance atoms with electrons emission. The free expelled electrons are reactive entities that can:

a) recombine with the formed cations, determining the returning of the molecule to its original shape;

b) react with other material compounds, generating free radicals;

The water is the molecular predominant species in all the living systems and the main primary reaction induced by the irradiation is that with the water, free or bound. The setting up of the primary radicals takes place, in accordance with the following reactions:

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$$H_2O \rightarrow H_2O^* \quad (1)$$
$$H_2O \rightarrow H_2O^+ + e^-_{aq} \quad (1)$$
$$H_2O \longrightarrow OH\bullet + H\bullet \quad (2)$$
$$H_2O^+ + H_2O \longrightarrow H_2O^+ + OH\bullet \quad (3)$$

The moleculary hydrogen dissolved into water can react with the electrons:

$$O_2 + e^-_{aq} \longrightarrow O_2^{-\bullet} \quad (4)$$
$$2O_2^{-\bullet} + 2H^+ \longrightarrow H_2O_2 + O_2 \quad (5)$$

The forming primary radicals are extremely reactive and react rapidly with other compounds of the material, creating secondary radicals.

Table 1

The main effects determined by the ionizated radiations at the interaction with the structures endowed with an essential biological role

Ionizations and excitation			
water radiolysis radical of pentodes	cleavage of chemical links	hydroxylation	oxidation of nitrates base
superoxides	disulphides, reticulates	cleavege of peptidic links	cleavage of chain molecule ADN, ARN
Transformation of tertiary structures	Genetics recombinations	retardation of ADN synthesis	blocking of phase
celullar effects, genetical transformations, chromozome anomalies, lethal effects			

2. MICROWAVES AND RADIOWAVES

The microwaves and the radiowaves can be employed at industrial level for the pasteurization and sterilization of food products. Unlike the irradiation, their action is mainly a thermical one. Therefore, the heating process with microwaves and radio-waves is reported to the use of electromagnetic waves of different frequences in order to generate the heat into a particular material.

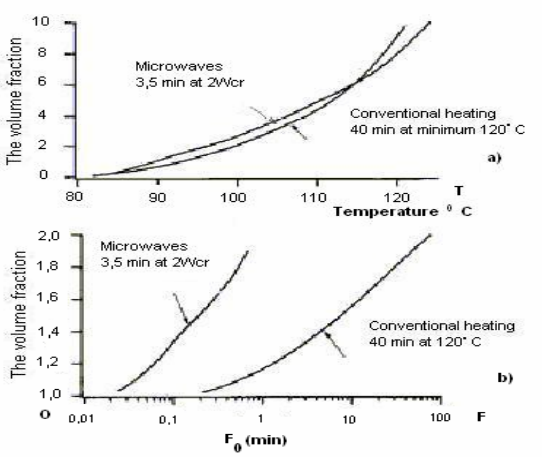

Fig. 1. The difference between the F_0 values for the conventional processing, respectively with microwaves (b) although the temperature domain remains the same (a).

3. THE FACTORS INFLUENCING THE PROCESS

Factors depending on the process. The dependence time temperature at the coldest point determines the microbiological security of the process, so as in the thermical conventional processing. If the temperature of the coldest point as time function is known, then the lethality can be calculated according to:

$$F_0 = \int_0^{t_f} 10^{(T-250)/z} \cdot dt \tag{6}$$

where: T - temperature at the coldest point at any moment of time t;

t_f - total period of heating;

z = the value of **z** on the abscissa OF

There are relevant differencies between the conventional heating and that with microwaves, due to the localization of the coldest point and the processing factors that influence the dependence time-temperature. Both the amplitude of the curve of time-temperature dependence and the localization of the coldest point (ionic content, density, specific heat) depend on the shape and size of the food product and of the microwave frequency and the equipment design.

Another important factor is represented by the time, because alongside with the food heating, its absorbtion properties can be modified and also the coldest point changes its position. The polarization phenomenon generates a capacitance current as:

$$J_D = \frac{\partial D}{\partial t} \tag{7}$$

In which D is the vector of electric induction.

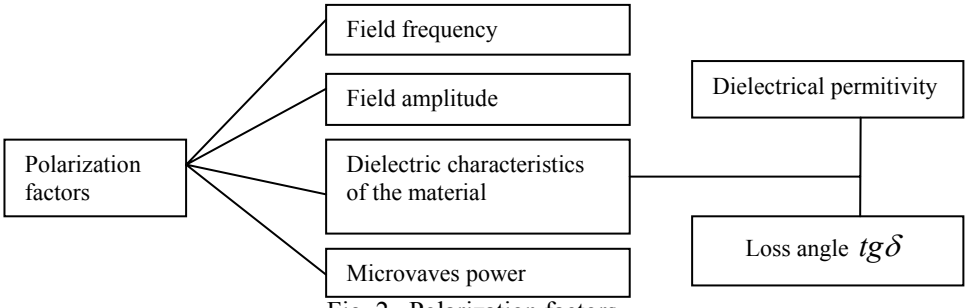

Fig. 2. Polarization factors

Following the experimental researches, there has been established that inductivity ε and the tangent of the loss angle $tg\delta$, necessary to the definition of the complete inductivity ε depend on the temperature T and the humidity H. This dependence can be the balanced average of the water mass of the load, because reporting to the humidity the water can be in a free or combined state and its involving in ε value differs very much. Therefore, the solution of the electromagnetic field is:

$$rotvrotE + \left(j\omega\sigma - \omega^2\varepsilon \right) E = 0 \tag{8}$$

With the condition of the standard calibration:

$$div\left(j\omega\sigma - \omega^2 \underline{\varepsilon} \right) \underline{\mathbf{E}} = 0 \tag{9}$$

This depends on the temperature and humidity, therefore the volume density of the dielectric losses due to the microwaves takes over this dependence:

$$P = Re(\underline{\varepsilon})\omega E^2 = \omega\varepsilon_i E^2 = \omega\varepsilon E^2 tg\delta \tag{10}$$

RESULTS AND DEBATES

The theoretical researches have encouraged the development or some dehydration technologies for medicinal and aromatic herbs under a microwave controlled regime using two types of treatment installations - of continuous flow and of stationary flow. The wave directories of a rectangular shape represent the entrance port and are placed at a distance λ (h = 122,4 mm). between them and the wall of the applicator. The work frequency of the applicator is f = 2450 MHz.

The installations which have been performed are equipped with multi-mode applicators of cavity type. A multi-mode applicator is a close metallic cavity connected to a microwave generator. The cavity dimensions are bigger than the wavelength of microwaves propagated through the guide to the respective cavity, propagation modalities $E_{l,m,n}$ and $H_{l,m,n}$, each mode corresponding to the relation:

$$\left(\frac{l}{a}\right)^2+\left(\frac{m}{b}\right)^2+\left(\frac{n}{d}\right)^2=\left(\frac{f}{c}\right)^2 \qquad (11)$$

where *a, b, d* are the dimensions of parallelipipedical cavity *l, m, n,* are the full numbers which correspond to the number of halves of wavelength belonging to quasi-sinusoidal field variation at the level of main coordinate axis, ***f*** is the resonance frequency and ***c*** is the light speed. The most frequent modes are TE_{101} and TE_{303} by which position it is obtained a homogeneous heating of cavity product, figure 3.

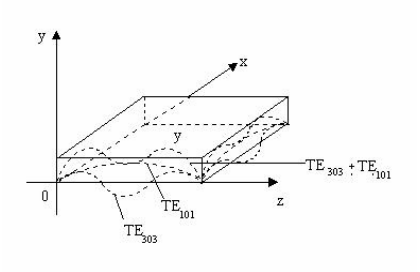

Fig. 3. Position of Modes TE_{101} and TE_{303}

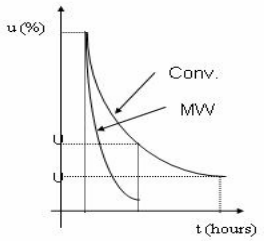

Fig. 4. Diagram of dessication.

The field distribution has as a result multiple mode reflections on the metallic cavity walls and on the product meant to be heated.

The analysis of the thermical diffusion problem has been performed for two situations:

a) the case of the static load;

b) the case of the dinamic load.

The efficiency of heating process, due to the simultaneous effect of microwaves wich penetrate in the material depth is graphically represented in figure 4 where is shown the diagram of dessication by conventional methods and by means of microwaves.

The firm survey of the temperature regime during the dessication process of the medicinal and aromatic plants herb is of the highest importance for the quality of the procesed material. For this porpose the installation is endowed with a system for temperature measurement on the surface of the material which is subjected to the dessicative process, with temperature transducers without contact, in infra red, which operating principle is based on the materials property to make effuzion of energy in infrared.

The state parameters which definite the dessication processus are as follow: the temperature (°C); humidity (%); dessication speed (% humidity/min.). The development of the dessication process of the herbe of medicinal and aromatic plants is donne in three successive stages as follow:

a) the preheating periode during which the heat is consumed almost in totality for the heating of the material until the establishment of the regime temperature, at which there is established an equilibrium beetwin the transmited heat quantity and that which is consumed for the water evaporation.

b) the dessication periode with constant speed wnich represent the dehydration periode for that matter;

c) the dessication periode with decreasing speed (the final periode), in which the dessication speed is gradualy reduced;

The microwaves power regime can be continually adjusted starting from zero to the maximal power.The dessication processus result in decreasing of the humidity from max. 90 % to aproximately 12 %, according with the material type.

Taking into consideration the significant phisico-chemical properties of the medicinal and aromatic plants species, it can disriminate two main groups:

a) plants containing volatile oils (culture thyme, hyssop, mint, etc.), which maximal dessication temperature must not be greater than 35 °C;

Table 2

Results: maintenance of the active principles

Species / **Dessication mode**	**Active principles of volatile oils (ml %)**				
	HYSSOP	SALVIA OFFICINALIS	MINT	BASIL	THYME
Naturale	0,60	0,67	3,12	0,47	0,51
Artificiale (micrwave)	0,74	0,92	3,91	0,65	0,64

b) plants containing alkaloids and glycoids (artichoke, bay, etc.), which optimal dessication temperature is content between 50 and 80 °C.

Table 2

Results of bioactive substances

Dessication mode	Polifenoli (cinarină) (%)	Flavone (rutin) (%)
Natural dry	0,56	0,74
Microwaves	0,74 – 1,04	0,85 – 1,35

The using domain of the installation can be extended also to the forest fruits, which have a special situation. Like this, the eglantine and hawthorn fruits have the optimal dessication temperature over 90 °C.

In fig.5, is presented an image of the continous flow installation and the conservated food aspect through the application of the microwave technonology of medicinal and aromatic plants herbs.

Fig. 5. The continous flow installation and the conservated plants aspect

CONCLUSION

Prospects generated by the new prospects technology:

- Orientating the production according to the foreseen market tendencies and encouraging the development of a new technology for taking over the agricultural products.
- Improving the processed products quality and the control of the technological procedures, with respecting the load security requirements, rclaimed by EU.
- Issuing and strengthening of some economic private and competitive operators.
- Increasing the business opportunities by diversifying the range of products designed to the food consumption.
- Developing the new ecological technologies of conservation, used in food industry which allon a high automation level, ensure the performance of a finite product of a high quality and with an improved commercial aspect.
- Extending the processing degree of the primary products, exploiting them in the most appropiate way and obtaining the added value, in compliance with EU standards.
- Promoting the new investments in an important economic field.

The drying process of organic materials with microwaves is more efficient and advantageous in many cases in comparison with the convetional desiccation, as the processed material heating is more rapid and the standard temperature is reached more quickly. This advantage is capitalized within the microwave drying installations, where the rapid processing has not only the effect of destroying the microorganisms but also that of reducing the damages and losses of active matters, as a consequence of conventional drying thermal processes.

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THE COMPARATIVE STUDY OF SOME EVALUATIVE METHODS OF THE ASTRINGENT FEATURE OF THE RED WINES

Delia Statescu[1], Maria Avramescu[1], Valentina Danisor[2]

KEY WORDS: astringency, red wine, tannin

ABSTRACT

One of the most important sensory attributes of a red wine is the astringent feature, that in most of the cases is estimated by tasting the product. The esteem has to be done by a group of expert tasters and it is not always objective. There is the possibility to make some esteems,by determining the "Index of the gelatin", (Glories, 1978) or by using an other new method, elaborated by a group of scientists from Spain (Llaudy and the collaborators 2003). In this work we present by comparison, the results obtained by using three methods of evaluation of the astringent characteristics of some red wines from the 2002 harvest, obtained by the treatment with enzymes at I.C.D.V.V., Valea Calugareasca.

1. INTRODUCTION

The present oenolog has well defined technologies and modern practices, which allow him to elaborate wines with organolepctical features wanted by him or by the consumer. The astringency of the young red wines is not always a well-appreciated feature, specially by the wine consumer who doesn't know very well the changes that take place during wine maturing and getting older process.

To evaluate the astringency of the red wines obtained by the experiments with enzymes preparations from the autumn 2002 at I.C.D.V.V. Valea Calugureasca, we have made a comparative study of the results obtained through sensory analysis and through two chemical methods.

In order to evaluate the level of their astringency after the methods described by Glories ("the index of gelatin", 1978) and Llaudy and the collaborators (2003), there have been chosen and analyzed twelve red wines from the twenty-eight experimental varieties obtained with different enzymes preparations at different work conditions. At the same time with the lab analysis, a group of five expert authorized tasters have done the organoleptical evaluation of the wines. The total polyphenol concentration of the studied wines was determined as the Index of the total polyphenol (Ipf) by measuring the absorbing waves of the diluted wines 1/100 at 280 nm.

[1] I.C.D.V.V. Valea Calugareasca
[2] SC. Doljchim SA Isalnita

2. MATERIAL AND METHODS

The twelve red analyzed wines were that from Cabernet Sauvignon variety and were obtained through the classical process of fermentation of the grapes in metal rotative vessels by the help of the enzyme preparations, with different contact time depending on the temperature. The analyzed wine samples were the following:

1. Basic wine – without SO_2 and without pectolitical enzymes
2. Wine – 60mg/l SO_2 without pectolitical enzymes
3. Wine – 60 mg/l SO_2 with pectolitical enzymes (Vinozym)
4. Wine – 60 mg/l SO_2 with pectolitical enzymes (Ultrazym)
5. Wine – 90 mg/l SO_2 with pectolitical enzymes (Vinozym)
6. Wine – 90 mg/l SO_2 with pectolitical enzymes (Ultrazym)
7. Wine – 90 mg/l SO_2 without pectolitical enzymes
8. Wine - 120 mg/l SO_2 with pectolitical enzymes (Vinozym)
9. Wine – 120 mg/l SO_2 with pectolitical enzymes (Ultrazym)
10. Wine – lower ph (Ca carbonate added) with pectolitical enzymes (Vinozym)
11. Wine – 60 mg/l with pectolitical enzymes (Vinozym) with short time fermentation
12. Wine – 60 mg/l with pectolitical enzymes (Vinozym) long term fermentation

The obtained wine have been analyzed and submitted to some conditioning and stabilizing operations and surveyed during the maturation process for 1 year.

2.1. THE DETERMINATION OF THE "INDEX OF GELATIN"

As it is known the tannins have the property to react with the proteins forming stable combinations; Glories (1978) applied this property for determining the "index of gelatin ".

Work method: in 50 ml of wine there have been added 5 ml from a solution of "solved gelatin" of 70 g/l; this corresponds to a concentration of 7 g gelatin in a litre of wine. It has been kept for three days at 10^0 C for completing the tannin reaction with the gelatin and then used with the centrifuge for 10 min at 3500 rpm and it was determined the content of tannins. The tannins were determined in the diluted wine 1/50 through the acid dipolymeric reaction and by measuring the red color at wave length of 550 mm obtained due to the formation of the composed (Vivas and the collaborators, 2003). There have been determined the tannins from the basic wine (to 50 ml of wine there have been added 5 ml of distilled water and was diluted 1/50).

The aggressive tannins have been calculated by the difference from the total concentration in the tannins and the tannins remained after deposing the gelatin.

So the index of the deposed tannins with gelatin or the "index of gelatin" is given by:

$I = [(C_0 – C)/ C_0]*100$ where C_0 represents the concentration (g/l) of the tannins from the basic wine and C represents the concentration (g/l) of the tannins after the reaction with gelatin.

The "index of gelatin" can be seen as a reflection of the astringent feature of the wine and when it is higher than 50, the wine can be considered rough, astringent.

2.2. THE METHOD WITH THE OVALBUMIN (Llaudy and the collaborators 2003)

In the method elaborated by Llaudy and the collaborators there have been used ovalbumin solutions (with concentration between 0,0 and 8,0 g/l) and tannic acid (with

concentration between 0,0 and 1,0 g/l) which had been prepared in a synthetic wine solution (4 g/l of tartric acid. 95 g/l ethanol, adjusted at ph 3,5 with NaOH). The solutions of tannic acid (0; 0,2; 0,4; 0,6; 0,8 and 1 g/l) have been used as standard. As protein for deposing the astringent tannins, it has been used the ovalbumin solution of different concentrations 0; 0,4; 0,8; 1,6; 2,4; 3,2; 4,0; 4,8; 5,6; 6,4; 7,2 and 8,0. There have been taken twelve test tubes where have been put the ovalbumin solution (0,0 and 8,0 g/l). In each test tube has been added the tannic acid solution or the analyzed wine. After shaking and letting it for 10 min, it has been used the centrifuge and the solution was diluted 1/50 with distilled water. Then it was measured the absorbing wave length at 280 nm in a quartz bath with the optical stratum width of 1 cm.

2.3. THE SENSORY ANALYSIS

As a result of the sensory analysis made by the five experts in testing the wines from I.C.D.V.V. Valea Calugareasca, the astringency of each wine was marked with values from 1 to 100 points. All the data were statistically expressed by an average of three repeated tests.

3. THE RESULTS AND DISCUSSIONS

The obtained results by the two chemical methods were compared to those resulted after the organoleptical determination.

3.1 THE METHOD OF DETERMINING THE "INDEX OF GELATIN" "GLORIES"

From fig.1 it can be seen that the sample with the most astringent feature of the wine, regarding the value of the "index of gelatin" and the organoleptic determination, was sample No.12 and the sample with the less important astringency of the wine was sample No.1. There have been noticed significant differences in evaluating the astringency of the wines using the two methods with the samples No. 7, 10 and 11. So the sample No.7 seems less astringent than it is indicated by the "index of gelatin" and sample No. 11seems to be the most astringent sample.

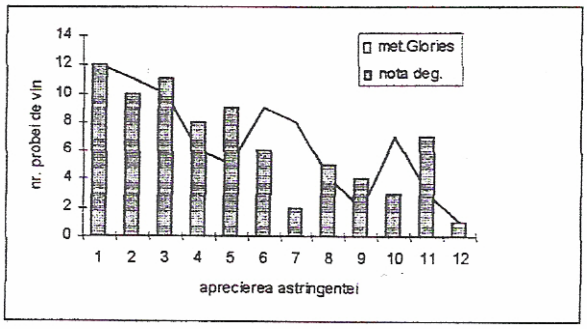

Fig. 1. The estimation of the astringency using the method of the "index of gelatin" (Glories, 1978) and the mark after tasting

3.2. THE METHOD WITH OVALBUMIN

Following the results presented in fig. 2, it has been noticed as above, that the sample No. 12 took the first place, as being the most astringent sample, and the sample No

1 took the last position, as being the less astringent sample. This time the differences between the organoleptical determination and the determination of the tannins after ovalbumin deposing were less significant than presented above. A close difference has been noticed with the sample No. 11 between the organoleptical determination and the tannin determination after gelatin or ovalbumin deposing.

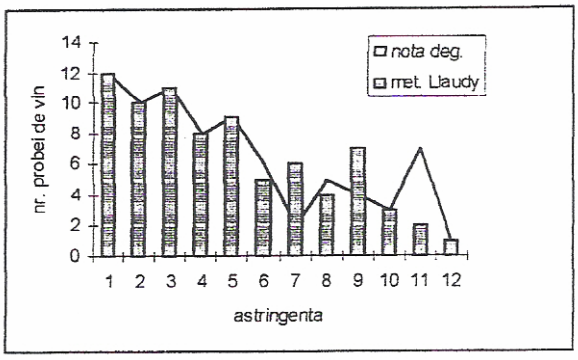

Fig. 2. The determination of the astringent feature using the method with ovalbumin and the mark after tasting

3.3. THE SENSORY ANALYSIS

The marks obtained after the organoleptical determination of the twelve samples varied from 25,2 (the wine No. 1 the less astringent wine) and 75,1 (the wine No.12 the most astringent wine) (table 1)

The marks obtained at tasting the wines for samples No. 1 and 12 depended on the total polyphenol content expressed through Ipf as well as the content in tannins remained after deposing the gelatin and ovalbumin.

Table 1

The results in evaluating the astringency of the analyzed wines

Sample No.	Ipf	Igelat	The ovalbumin method (g/l – acid tanic)	Tasting mark
1	21,87	29,4	0,129	25,2
2	36,89	42,5	0,144	44,5
3	31,26	34,8	0,156	38,3
4	35,46	45,7	0,192	39,7
5	37,56	53,5	0,294	42,1
6	41,02	57,9	0,289	51,4
7	29,08	39,2	0,172	35,3
8	37,04	49,6	0,329	58,6
9	38,29	51,2	0,318	56,8
10	63,26	61,5	0,381	68,2
11	69,07	64,3	0,338	66,5
12	66,64	65,4	0,571	75,1

The differences among the three methods of evaluating the astringency feature of the wines have been evidenced in fig. 3.

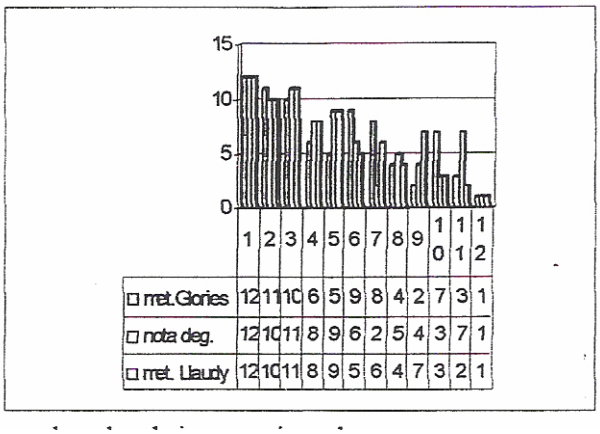

Fig. 3. The evaluation of the astringency feature of the wines by the three methods

It has been noticed (fig. 3) that the most astringent wine was sample No.12 and the less astringent one was sample No. 1. The 2nd and the 3rd places were taken by the samples No. 11 and 12, with little differences between them, significant differences between the astringentcy appeared in the samples No. 4 – 7 and No. 9 – 11. So, after the Glories method, sample No. 2 took the 9th place comparing to the 7th place according to the tasting mark and according to Llaudy method took the 11th place. As a result, this sample of wine was considered by the expert testers as being more astringent than it was been considered after using the other two methods.

The sample No. 8 took the 4th place after the organoleptical determination (tasting mark = 58,6) and after the Llaudy method, and according to the Glories method it was situated on the 7th place. The sample No. 5, after tasting mark was situated on the 8th place, as well as the sample No. 4th but after using the other methods took the 5th place (Glories) and 6th (Llaudy).

If we compare the top of the wines in their astringent decreasing order, obtained through each of the two chemical methods comparing to the tasting mark, we notice that by using the methods Llaudy, the positions 1-5, 10 and 11 were identical to those from the organoleptical determination; after using the Glories method only the positions 1 and 12 have been the same with the results after tasting the wines.

CONCLUSIONS

- For improving the technologies of processing the black grapes in order to obtain red wines of high quality it is necessary using a more precise method to evaluate the astringency of the wines.
- In general there is a well defined relation between the sensory analysis and the astringency of the red wines.
- If we compare the tasting mark to the results obtained through the other two methods, we can say that sometimes the evaluation of the astringency using the sensory analysis can be subjective.

- To firmly pronounce upon the precision and results of this new method, of deposing the astringent tannins with ovalbumin, there have to be always made more determinations comparing to the “Index of gelatin” and sensory analysis in more labs for studying the chemistry of the wine in our country.

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FORWARD OSMOSIS: THEORETICAL BACKGROUND

George Moise[1], Vasile Jascanu[2]

KEY WORDS: Osmosis; Forward osmosis; Direct osmosis; Desalination; Reverse osmosis; Pressure-retarded osmosis

ABSTRACT

Osmosis is a physical phenomenon that has been extensively studied by scientists in various disciplines of science and engineering. Early researchers studied the mechanism of osmosis through natural materials, and from the 1960s, special attention has been given to osmosis through synthetic materials. Following the progress in membrane science in the last few decades, especially for reverse osmosis applications, the interests in engineered applications of osmosis has been spurred. This paper provides dates of the physical principles and applications of forward osmosis as well as their strengths and limitations.

1. INTRODUCTION

Osmosis is a physical phenomenon that has been exploited by human beings since the early days of mankind. Early cultures realized that salt could be used to desiccate foods for long-term preservation. In saline environments, most bacteria, fungi, and other potentially pathogenic organisms become dehydrated and die or become temporarily inactivated because of osmosis. Conventionally, osmosis is defined as the net movement of water across a selectively permeable membrane driven by a difference in osmotic pressure across the membrane. A selectively permeable membrane allows passage of water, but rejects solute molecules or ions. Osmotic pressure is the driving force for many of the applications.

Present-day applications of the osmosis phenomenon extend from water treatment and food processing to power generation and novel methods for controlled drug release. In the field of water treatment, reverse osmosis is generally a more familiar process than osmosis. Because of this, a brief description of the RO process is given prior to further discussion of osmosis. RO uses hydraulic pressure to oppose, and exceed, the osmotic pressure of an aqueous feed solution to produce purified water.

In RO, the applied pressure is the driving force for mass transport through the membrane; in osmosis, the osmotic pressure itself is the driving force for mass transport. Numerous publications on the use of RO for water treatment and wastewater reclamation appear in the literature.

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Fewer publications on the use of osmosis – or forward osmosis (FO), or direct osmosis (DO) – for water treatment/engineering applications appear in the literature.

This paper presents a review of FO and closely related membrane processes. The review begins with an introduction of the basic principles of the FO process, including comparison to other closely related processes. Special aspects of mass transport in the process as well as the membranes used for the process are described.

2. OSMOTIC PROCESSES

2.1. CLASSIFICATION OF OSMOTIC PROCESSES

Osmosis is the transport of water across a selectively permeable membrane from a region of higher water chemical potential to a region of lower water chemical potential. It is driven by a difference in solute concentrations across the membrane that allows passage of water, but rejects most solute molecules or ions. Osmotic pressure (π) is the pressure which, if applied to the more concentrated solution, would prevent transport of water across the membrane. FO uses the osmotic pressure differential ($\Delta\pi$) across the membrane, rather than hydraulic pressure differential (as in RO), as the driving force for transport of water through the membrane. The FO process results in concentration of a feed stream and dilution of a highly concentrated stream (referred to as the draw solution).

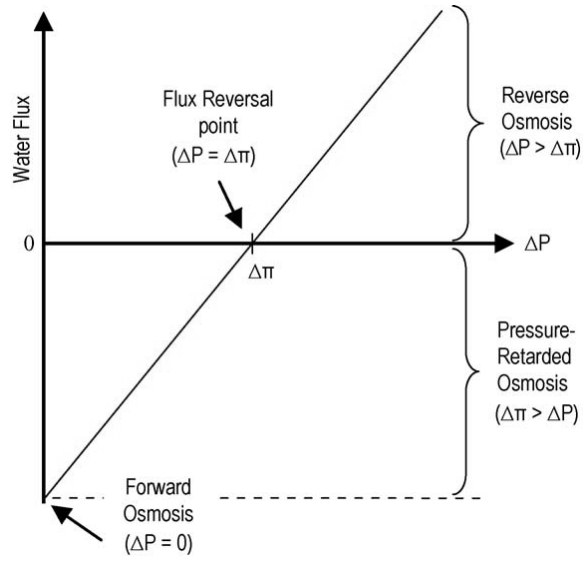

Fig. 1 Direction and magnitude of water flux as a function of applied pressure in FO, PRO, and RO.

PRO can be viewed as an intermediate process between FO and RO, where hydraulic pressure is applied in the opposite direction of the osmotic pressure gradient (similar to RO). However, the net water flux is still in the direction of the concentrated draw solution (similar to FO). The general equation describing water transport in FO, RO, and PRO is

$$J_w = A(\sigma\Delta\pi - \Delta P) \quad (1)$$

where J_w is the water flux, A the water permeability constant of the membrane, σ the reflection coefficient, and ΔP is the applied pressure. For FO, ΔP is zero; for RO, $\Delta P > \Delta\pi$; and for PRO, $\Delta\pi > \Delta P$. The FO point, PRO zone, and RO zone, along with the flux reversal point, are illustrated in Fig. 1.

2.2. DRAW SOLUTIONS

The concentrated solution on the permeate side of the membrane is the source of the driving force in the FO process. Different terms are used in the literature to name this solution including draw solution, osmotic agent, osmotic media, driving solution, osmotic engine, sample solution, or just brine. For clarity, the term draw solution is being used exclusively in this paper. When selecting a draw solution, the main criterion is that it has a

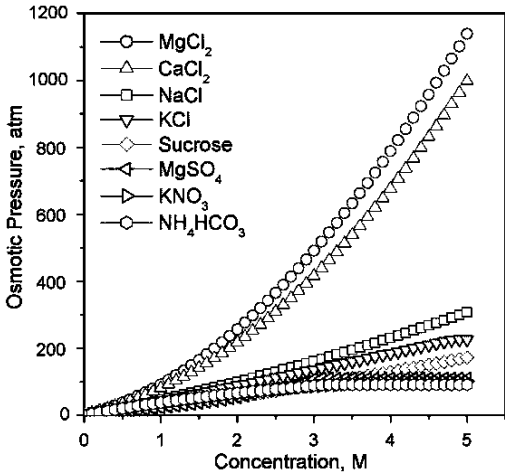

Fig. 2 Osmotic pressure as a function of solution concentration at 25 ◦C for various potential draw solutions. Data were calculated using OLI Stream Analyzer 2.0

higher osmotic pressure than the feed solution. The osmotic pressures of several solutions being considered for use as draw solutions were calculated using OLI Stream Analyzer 2.0 and are presented in Fig. 2 as a function of molarity. This software uses thermodynamic modeling based on published experimental data to predict the properties of solutions over a wide range of concentrations and temperatures.

3. CONCENTRATION POLARIZATION IN OSMOTIC PROCESSES

3.1. EXTERNAL CONCENTRATION POLARIZATION

In pressure-driven membrane processes, convective permeate flow causes a buildup of solute at the membrane active layer surface. Referred to as concentration polarization (CP), this phenomenon reduces permeate water flux due to increased osmotic pressure that must be overcome with hydraulic pressure. CP due to water permeation is not limited to pressure-driven membrane processes and also occurs during osmotic-driven membrane processes, on both the feed and permeate sides of the membrane.

When the feed solution flows on the active layer of the membrane (like in RO), solutes build up at the active layer. This may be called concentrative external CP and is similar to CP in pressure-driven membrane processes. Simultaneously, the draw solution in contact with the permeate side of the membrane is being diluted at the permeate–membrane interface by the permeating water.

This is called dilutive external CP. Both concentrative and dilutive external CP phenomena reduce the effective osmotic driving force. The adverse effect of external CP on osmotic-driven membrane processes can be minimized by increasing flow velocity and turbulence at the membrane surface or by manipulating the water flux. However, because water flux in FO is already low, the ability to diminish external CP by reducing flux is limited. For modeling external CP phenomena in FO, equations similar to those developed for CP of pressure-driven membranes can be used.

Due to the low hydraulic pressure used in FO, membrane fouling induced by external CP has milder effects on water flux compared to the effects in pressure-driven membrane processes. It has been shown that external CP plays a minor role in osmotic driven membrane processes and is not the main cause for the lower-than-expected water flux in such processes.

3.2. INTERNAL CONCENTRATION POLARIZATION

When an osmotic pressure gradient is established across a completely rejecting dense symmetric membrane, as depicted in Fig. 3a, the driving force is the difference in osmotic pressures of the bulk solutions in the absence of external CP. However, FO membranes are asymmetric, adding more complexity to the CP phenomena.

When a composite or asymmetric membrane consisting of a dense separating layer and a porous support layer is used in FO, two phenomena can occur depending on the membrane orientation. If the porous support layer of an asymmetric membrane faces the feed solution, as in PRO, a polarized layer is established along the inside of the dense active layer as water and solute propagate the porous layer (Fig. 3b).

Referred to as concentrative internal CP, this phenomenon is similar to concentrative external CP, except that it takes place within the porous layer, and therefore, cannot be minimized by cross-flow. As water permeates the active layer, the draw solution within the porous substructure becomes diluted. This is referred to as dilutive internal CP (Fig. 3c).

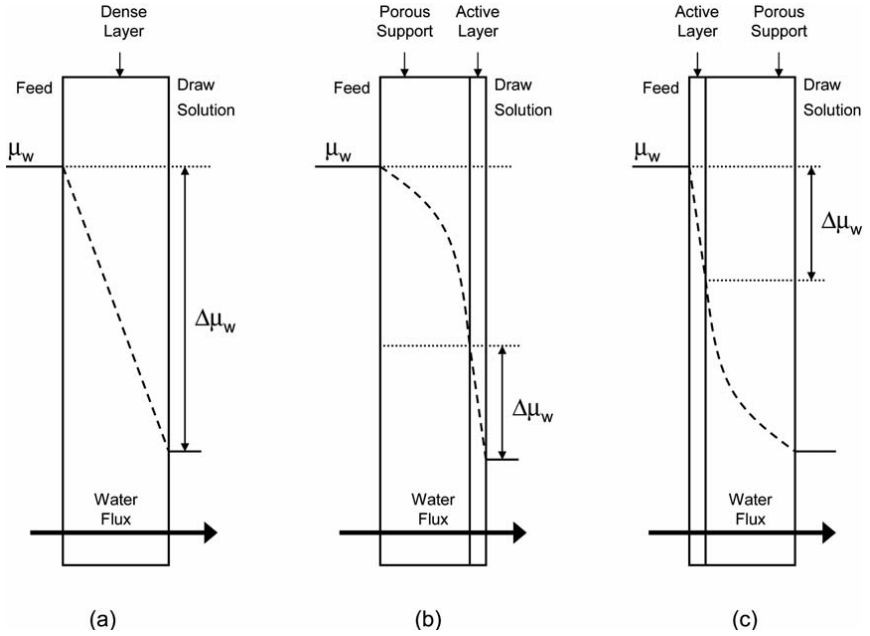

Fig. 3. Illustrations of driving force profiles, expressed as water chemical potential, μw, for osmosis through several membrane types and orientations. (a) A symmetric dense membrane. (b) An asymmetric membrane with the porous support layer facing the feed solution; the profile illustrates concentrative internal CP. (c) An asymmetric membrane with the dense active layer facing the feed solution; the profile illustrates dilutive internal CP. The actual (effective) driving force is represented by $\Delta\mu$w. External CP effects on the driving force are assumed to be negligible in this diagram.

3.2.1. MODELING CONCENTRATIVE INTERNAL CONCENTRATION POLARIZATION

Unlike RO, for which water transport through the membrane is mostly affected by the hydraulic resistance created by the membrane structure, in FO, internal CP in the porous support layer also substantially affects mass transfer of water across the membrane.

Adopting the models that were developed by Lee et al.. Loeb et al. introduced a simplified equation to describe the water flux during FO without consideration for membrane orientation:

$$J_w = \frac{1}{K} \ln \frac{\pi_{Hi}}{\pi_{Low}} \quad (2)$$

where$K$is the resistance to solute diffusion within the membrane porous support layer, and π_{Hi} and π_{Low} are the osmotic pressures of the bulk draw solution and feed solution, respectively, neglecting external polarization effects. K is defined as

$$K = \frac{t\tau}{\varepsilon D_s} \quad (3)$$

where t, τ, and ε are the membrane thickness, tortuosity, and porosity, respectively, and D_s is the diffusion coefficient of the solute. However, it has been recently demonstrated that Eq. (2) is valid only for very lowwater fluxes. Further development of this equation has led to a more general governing equation for concentrative internal CP:

$$K = \left(\frac{1}{J_w}\right) \ln \left(\frac{B + A\pi_{Hi} - J_w}{B + A\pi_{Low}}\right) \quad (4)$$

where B is the solute permeability coefficient of the active layer of the membrane, which can be determined from an RO-type experiment using

$$B = \frac{(1 - R)A(\Delta P - \Delta\pi)}{R} \quad (5)$$

where R is the salt rejection. Eq. (4) can be used to quantify the severity of internal CP; larger values of K are associated with more severe internal CP.

3.3. INFLUENCE OF INTERNAL CONCENTRATION POLARIZATION ON WATER FLUX

Mehta and Loeb studied the effect of the porous support layer on internal CP and the effect of high draw solution concentrations on the overall permeability coefficient of the membrane. Results show that upon swapping the working fluids on the two sides of the membrane, flux (as indicated by the permeability coefficient) sharply declines due to internal CP (Fig. 4).

Experimenting with DuPont B-9 (flat sheet) and B-10 (hollow fiber) Permasep RO membranes, Mehta and Loeb pointed out that A (the membrane permeability constant from Eq. (1)) is not constant in FO and PRO; it declines with increasing osmotic pressure (i.e., increasing concentration) of the draw solution. \

The decline of A was explained by partial drying or osmotic dehydration of the membrane at high osmotic pressures. Such partial drying can be accompanied by pore contraction, known as “osmotic deswelling”, and hence increased resistance to water transport. Results from recent studies confirmed that internal CP is actually the cause of the substantial flux decline.

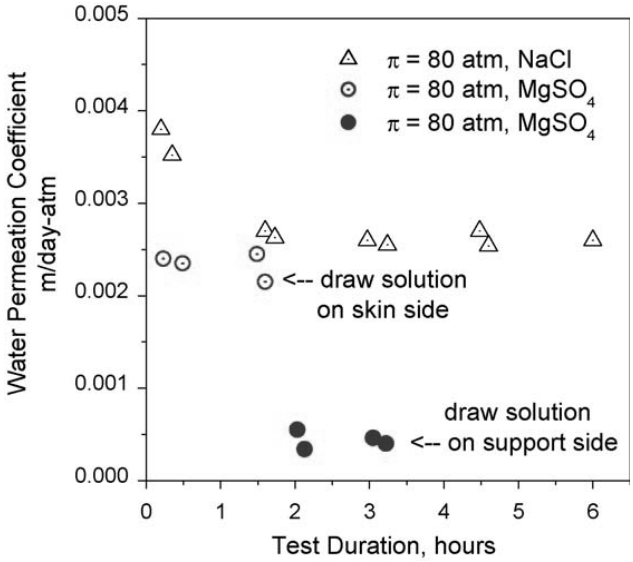

Fig. 4. FO tests with NaCl and MgSO4 solutions as draw solutions. The effect of internal CP can be seen after 2 h when the support side of the membrane is exposed to the draw solution instead of DI water. The water permeation rate sharply declines after dilutive internal CP starts

4. CONCLUSIONS

Theoretical background presented above open a horizon for applications of forward osmosis in many fields of industries. It can make real the usage of this process because of its advantages given by low energy consumption (low pressures) and not many parts involved in industrial devices.

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FORWARD OSMOSIS: RECENT DEVELOPMENTS

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KEY WORDS: Forward osmosis; Wastewater treatment; Desalination; Direct potable reuse; Osmotic pumps

ABSTRACT

Osmosis, or as it is currently referred to as forward osmosis, has new applications in separation processes for wastewater treatment, food processing, and seawater/brackish water desalination. Other unique areas of forward osmosis research include pressure-retarded osmosis for generation of electricity from saline and fresh water and implantable osmotic pumps for controlled drug release. This paper provides informations about recent developments of forward osmosis.

1. INTRODUCTION

Forward osmosis has been studied for a range of applications. Commercial applications, though still limited, are emerging in the water purification field (e.g., extraction bags) and in the pharmaceutical industry (e.g., osmotic pumps). The following section summarizes past and present applications of forward osmosis (FO) in wastewater treatment and water purification, seawater desalination, food processing, pharmaceutical applications, and power generation.

2. WASTEWATER TREATMENT AND WATER PURIFICATION

Several modern applications of FO in the field of wastewater treatment have been published in the literature. These include an early study on concentration of dilute industrial wastewater, an investigation on treatment of landfill leachate, a study on direct potable reuse of wastewater in advanced life support systems for space applications, and an investigation on concentration of liquids from anaerobic sludge digestion at a domestic wastewater treatment facility. It is worth noting that in most wastewater treatment applications FO is not the ultimate process, but rather a high-level pretreatment step before an ultimate desalination process.

2.1. CONCENTRATION OF DILUTE INDUSTRIAL WASTEWATER

One of the first feasibility studies of FO in an industrial application for wastewater treatment was published in 1974 and 1977. The objective was to use a low energy process to treat industrial wastewater containing very low concentrations of heavy metals for possible reuse. A bench-scale system was used to study the feasibility of using newly commercialized cellulose reverse osmosis (RO) membranes to concentrate dilute real or

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synthetic wastewater streams containing copper or chromium. Not aware of the effects of internal concentration polarization in RO membranes, the authors observed water fluxes ranging from zero to approximately 4.5 l/m2 h—much lower than the calculated fluxes of 10–17 l/m2 h from the mass transfer equation and manufacturer data for the membranes tested in RO mode under equivalent conditions. Attempts to investigate the effects of external CP on flux by varying the feed and draw solution flow rates yielded inconclusive results.

Simulated seawater was used as the draw solution because it is a potentially inexpensive source available in coastal areas. Passage of sodium chloride from the artificial seawater and diffusion of feed contaminants towards the draw solution occurred at a higher rate than expected. Relative salt passage was 1 g NaCl for every 11.5–688 g water passage in the opposite direction. Different approaches to enhance salt rejection were investigated including chemical treatment of the membrane with polyvinyl methyl ether and thermal treatment (tempering) by immersing the membranes in hot water (60–93 °C) for up to 4 min. While chemical treatment showed no effect on flux or rejection, thermal treatment resulted in elevated salt rejection but decreased water flux. Due to the poor performance of the RO membranes, further pilot-scale testing of the process was called off. The authors concluded that membranes must be improved and specifically 'tailored' for the FO process to be feasible for water treatment.

2.2. CONCENTRATION OF LANDFILL LEACHATE

Landfill leachate is a highly variable feed solution that presents a particularly difficult treatment challenge, especially when high effluent quality is required. It is a complex solution consisting of four general types of pollutants: organic compounds, dissolved heavy metals, organic and inorganic nitrogen, and total dissolved solids (TDS). The simplest treatment for landfill leachate is to process it in a wastewater treatment facility. However, wastewater treatment facilities generally focus on treating organics, heavy metals, and nitrogen. They often have no treatment for TDS, and in some cases, treatment facilities even increase TDS concentration. Two commercially available treatment processes that are able to efficiently remove TDS from wastewater are mechanical evaporation (e.g., vapor compression, vertical tube falling film, horizontal tube spray film, forced circulation) and membrane processes. A comprehensive evaluation of vapor recompression mechanical evaporation, RO, and FO revealed that FO can be very effective in treating landfill leachate. In 1998, Osmotek constructed a pilot-scale FO system to test the concentration of landfill leachate at the Coffin Butte Landfill in Corvallis, Oregon . Because this landfill is located in an area that receives more than 1400mm per year of rainfall, approximately 20,000–40,000m^3 of leachate is generated annually. In order to meet the National Pollutant Discharge Elimination System (NPDES) total maximum daily load (TMDL), the leachate must be treated to a TDS level lower than 100 mg/l prior to land application.

2.3. DIRECT POTABLE REUSE FOR ADVANCED LIFE SUPPORT SYSTEMS

Long-term human missions in space require a continuous and self-sufficient supply of fresh water for consumption, hygiene, and maintenance. Long-range/long-duration missions, like lunar missions or a human Mars exploration mission, depend on a water treatment system that recovers potable water from wastewater generated on board a spacecraft or in the planetary habitat. The three main sources of wastewater that can be reclaimed and reused in long-term space missions are hygiene wastewater, urine, and

humidity condensate. The system to treat these wastewaters must be reliable, durable, capable of recovering a high percentage of the wastewater, and lightweight. Additionally, the system should operate autonomously with low maintenance, minimal power consumption, and minimal consumables.

A pilot-scale FO system, referred to as the direct osmotic concentration (DOC) system, was developed for direct potable water reuse in space. DOC is one of several technologies that are being evaluated by the U.S. National Aeronautics and Space

Administration (NASA) for water reuse in space. The NASA DOC test unit originally consisted of a permeate-staged RO cascade and two pretreatment subsystems. The first subsystem utilized an FO process only and the second utilized a unique combination of FO and osmotic distillation (OD). The OD process targeted the rejection of small compounds, like urea, that easily diffuse through semi-permeable membranes.

Power consumption was measured during operation under variable operating conditions. The parameters that were found to be important in the operation of the process (e.g., draw solution flow rate, RO pump capacity, and the amount of NaCl loaded in the draw solution loop) were varied. The results indicate that under variable operating conditions, specific power consumption is almost always less than 30 kWh for every 1m^3 of purified water produced. Further optimization of the process is currently under investigation.

2.4. FORWARD OSMOSIS FOR SOURCE WATER PURIFICATION—HYDRATION BAGS

The concept of hydration bags was developed for military, recreational, and emergency relief situations when reliable drinking water is scarce or not available. Hydration bags are one of the few commercial applications of FO. Although slower than other water purification devices, FO hydration bags require no power and only foul minimally, even when used with muddy water. The high selectivity of an FO membrane ensures that in most situations and for most sources of water, the permeating water is free of microorganisms, most macromolecules, and most ions.

In the hydration bags, an edible draw solution (e.g., a sugar or beverage powder) is packed in a sealed bag made of a semi-permeable FO membrane. Upon immersion of the bag in an aqueous solution, water diffuses into the bag due to the osmotic pressure difference and slowly dilutes the initially solid draw solution. At the end of the process the diluted draw solution can be consumed as a sweet drink containing nutrients and minerals. In this regard, hydration bags represent an ultimate treatment process; not a pretreatment process.

For small, personal devices, the process can take 3–4 h to completely hydrate a 12 oz beverage. The extraction bags can be placed directly in the source water or they can be suspended in another sealed plastic bag that holds the source water—providing better mobility and autonomy to the user. In recent years, the military procured hydration bags for emergency and relief efforts around the world. Yet, there is debate among experts whether hydration bags provide water treatment per se because the product is not pure water but a sweet drink that can only be used for specific applications.

3. SEAWATER DESALINATION

Several patents have been awarded for different methods and systems for water desalination by FO; however, most of them have not matured or proven feasible. Very few peer-reviewed publications on FO desalination could be found. Kravath and Davis (1975) investigated desalination of Atlantic Ocean seawater by FO using cellulose acetate flat

sheet and hollow fiber membranes and glucose solution as a draw solution. Kessler and Moody (1976) and Moody and Kessler (1976) modeled and tested similar applications of FO for desalination. Their objective was to develop a batch desalination process for emergency water supply on lifeboats, not as a continuous process for seawater desalination.

In recent bench-scale studies, it was demonstrated that when using a suitable FO membrane (e.g., the FOCTA membrane) and a strong draw solution (highly soluble ammonia and carbon dioxide gases), seawater can be efficiently desalinated with FO. The draw solution was formed by mixing together ammonium carbonate and ammonium hydroxide in specific proportions. The salt species formed include ammonium bicarbonate, ammonium carbonate, and ammonium carbamate. Analysis of the process has shown that an osmotic pressure driving force as high as 238 bar for a feed water with a salt concentration of 0.05M NaCl, and as high as 127 bar for a feed water with a salt concentration of 2M NaCl, can be achieved with the ammonia/carbon dioxide draw solution. This is a rather high driving force considering that 2MNaCl is equivalent to brine from seawater desalination at approximately 70% water recovery. Water is extracted from seawater and dilutes the ammonia–carbon dioxide draw solution. Upon moderate heating (near 60 ◦C), the draw solution decomposes to ammonia and carbon dioxide. Separation of the fresh product water from the diluted draw solution can be achieved by several separation methods (e.g., column distillation or membrane distillation (MD)). The degasified solution left behind is pure product water and the distillate is a reconcentrated draw solution available for reuse in the FO desalination process.

Bench-scale FO data demonstrates that the ammonia–carbon dioxide FO process is a viable desalination process. Salt rejections greater than 95% and fluxes as high as 25 l/m2 h were achieved with the FO CTA membrane with a calculated driving force of more than 200 bar. Although this is a relatively high flux, much greater flux is actually expected for such a high driving force. Further analysis of the results has indicated that the performance ratio (defined as experimental water flux divided by theoretical water flux) of the FO CTA membrane used was at most 20%, and on average between 5% and 10%. This lower-than-expected flux is attributed to internal CP.

All of the previous, yet limited, work on FO as an alternative desalination process has exposed the two major limitations of FO—lack of high-performance membranes and the necessity for an easily separable draw solution. Moreover, when considering seawater desalination, and especially when high water recovery is desired, FO can be utilized only if the drawsolution can induce a high osmotic pressure.

4. FOOD PROCESSING

Although osmotic treatment of food products (e.g., preserved, fruits and meats) is very common in the food industry, FO treatment for concentration of beverages and liquid foods has been studied at laboratory-scale only. FO has several advantages as a process for concentrating beverages and liquid foods, including operation at low temperatures and low pressures that promote high retention of sensory (e.g., taste, aroma, color) and nutritional (e.g., vitamin) value, high rejection, and potentially low membrane fouling compared to pressure-driven membrane processes. Several patents have been awarded for inventions of FO devices and methods for the concentration of liquid foods. Short summaries of the main studies are provided below in chronological order.

Two detailed summaries were published on the potential use of FO in the processing of liquid foods. Petrotos and Lazarides (2001) reviewed low pressure membrane processes – FO, OD, and MD – for concentration of liquid foods. Jiao et al. provided a

more comprehensive summary on membrane processes (including FO (DOC)) for concentration of fruit juices.

Popper et al. (1966) were the first to use modern techniques and first generation polymeric RO membranes to concentrate fruit juices. They used both tubular and flat sheet cellulose acetate membranes and achieved an average water flux of 2.5 kg/m2 h using saturated NaCl as the draw solution. Highly concentrated fruit juices were produced but salt was found to diffuse through the membrane into the grape concentrate. Based on the development of a new plate-and-frame membrane module for FO by Osmotek Inc., Beaudry and Lampi (1990) published a short summary on the use of the DOC process for concentration of fruit juices. They recommended that 72◦ Brix sugar solution be used as the draw solution. Herron et al. (1994) were awarded a patent on a membrane module and a method to concentrate fruit juices and wines. Laboratory test results showed water fluxes as high as 4 and 6 l/m2 h for orange juice and coffee, respectively. These relatively high fluxes are partially due to the high turbulence achieved in their FO module. In the summary of the invention, the inventors recommended the use of 50–85 wt.% sugar solution as the draw solution.

Adopting the methods of Herron et al., Petrotos et al. further explored the optimal conditions by which FO can be used in the concentration of tomato juice—one of the most concentrated vegetable juices. Petrotos and co-workers used custom-made tubular thin-film composite aromatic polyamide membranes and different draw solutions including sodium chloride, calcium chloride, calcium nitrate, glucose, sucrose, and polyethylene glycol 400 (PEG400). Results showed that membrane thickness and draw solution viscosity are the main factors controlling water flux in the FO concentration of tomato juice. Thinner membranes and lower viscosity draw solutions yielded higher water fluxes, and therefore, faster juice concentration. In addition, it was shown that pretreatment in the form of filtration, especially ultrafiltration, enhances the efficiency of the FO process. In further studies, Dova et al. investigated and modeled the impact of process parameters (e.g., membrane characteristics, feed and draw solution concentrations, and flow rates) on process performance using thin-film composite aromatic polyamide RO membranes.

From these studies, the FO process appears to have a number of advantages over evaporation and pressure-driven membrane processes for concentration of liquid foods. Low energy use, low operating temperatures and pressures, and high product concentrations are the main advantages.

5. OTHER APPLICATIONS

In drug industry are currently used osmotic pumps. Controlled- or modified-release of drugs is possible through the use of osmotic pumps witch can release drugs in blood stream usually from 3 months to 1 year. Another field of FO usage is power generation. Renewable energy can be extracted wherever two streams of different salinity or different chemical potential meet.

7. CONCLUSIONS

The increased attention to FO from various disciplines arises from the fact that FO can be employed in many fields of science and engineering including water and wastewater treatment, seawater/brackish water desalination, food processing, drug delivery, and electric power production. Despite the lack of robust membranes and membrane modules for FO, basic research on FO and the development of new applications of FO are steadily growing. Currently, the most important measure to be taken in order to advance the field of FO is the

development of new membranes in both flat-sheet and hollow fiber configurations. The membranes need to provide high water permeability, high rejection of solutes, substantially reduced internal concentration polarization, high chemical stability, and high mechanical strength.

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THE APPLICATION OF OSMOSIS AS INTERMEDIATE STAGE IN THE ANALYSIS OF VOLATILE COMPOUNDS FROM AROMATIC OILS.

Simonati Claudiu-Nicolae[1], George Moise[2]

KEY WORDS: Forward osmosis; Wastewater treatment; Desalination; Direct potable reuse; Osmotic pumps

ABSTRACT

The forward osmosis appeared in the first plan of engineered applications relatively recently. Recent researches in the field of membrane science have demonstrated the advantage of use osmosis in various disciplines of science and engineering. This paper provides informations about using forward osmosis for concentration of solutions with volatile content, without to affect volatile and thermosensible components from this.

INTRODUCTION

An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. They are also known as volatile or ethereal oils, or simply as the "oil of" the plant material from which they were extracted, such as *oil of clove*. An oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant. Essential oils do not as a group need to have any specific chemical properties in common, beyond conveying characteristic fragrances. They are not to be confused with essential fatty acids.

Essential oils are generally extracted by distillation. Other processes include expression, or solvent extraction. They are used in perfumes, cosmetics and bath products, for flavoring food and drink, and for scenting incense and household cleaning products.

Various essential oils have been used medicinally at different periods in history. Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer, and are often based on historical use of these oils for these purposes. Such claims are now subject to regulation in most countries, and have grown correspondingly more vague, to stay within these regulations.

Interest in essential oils has revived in recent decades, with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by essential oils have curative effects. Oils are volatilized or diluted in a carrier oil and used in massage, or burned as incense, for example.

MATERIALS AND METHODS

Many of analytical procedures like chromatography need samples with high level of integrity. But many of preparation procedures involve manipulation of the samples to

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open air. In this case, when are used samples with volatile compounds content and also very diluted, need sometimes to work at low temperatures and concentrate them.

The solution prepared for osmotic concentration is made from macerated cinnamon. Chemical components of the essential oil include ethyl cinnamate, eugenol, cinnamaldehyde, beta-caryophyllene, linalool, and methyl chavicol. The properties of cinnamon volatile oils are presented in table 1. The main factor for developing of this method is the melting point of these oils.

Table 1

The properties of cinnamon essential oils

Essential oil	**Molecular formula**	**Molar mass g/mol**	**Density g/cm^3**	**Melting point °C**	**Boiling point °C**
Ethyl cinnamate	$C_{11}H_{12}O_2$	176.21	1.046	6.5-8	271
Eugenol	$C_{10}H_{12}O_2$	164.20	1.06	-9	256
Cinnamaldehyde	C_9H_8O	132.16	1.05	-7.5	248
β-Caryophyllene	$C_{15}H_{24}$	204.36	0.9052	< 10	262-264
Linalool	$C_{10}H_{18}O$	154.25	0.858	< 20	198 – 199
Estragole	$C_{10}H_{12}O$	148.20	0.946	<10	216

The pilot-scale installation used to concentrate the solutions with volatile compounds content is illustrated in figure 1.

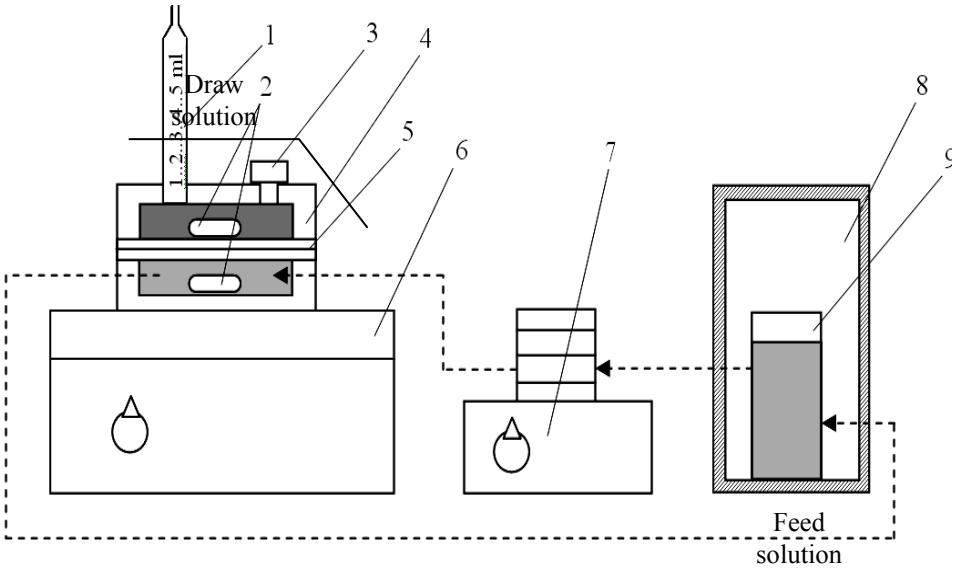

Fig. 1. Pilot-scale installation used to concentrate the solutions with volatile compounds content. 1. Pipette; 2. Magnetic tablet; 3. Coupling for feed solution; 4. Membrane module; 5. Membrane; 6. Magnetic agitating apparatus; 7. Peristaltic pump; 8. Refrigerator, 9. Draw solution tank.

The feed solution (solution proposed for concentration process) is introduced in the tank located in refrigerator. This is pumped and recirculated with a peristaltic pump through membrane module on feed solution side of membrane. The process of water

transfer through membrane is amplified by magnetic tablet what reduce the concentration polarization of membrane. The water who passed the membrane will increase the draw solution volume. This volume can be read on pipette.

RESULTS AND DISCUSSIONS

First step was to determine the best draw solution for this type of process.

The concentrated solution on the permeate side of the membrane is the source of the driving force in the FO process. When selecting a draw solution, the main criterion is that it has a higher osmotic pressure than the feed solution. The osmotic pressures of several solutions being considered for use as draw solutions were calculated using OLI Stream Analyzer 2.0 and are presented in Fig. 2 as a function of molarity. This software uses thermodynamic modeling based on published experimental data to predict the properties of solutions over a wide range of concentrations and temperatures.

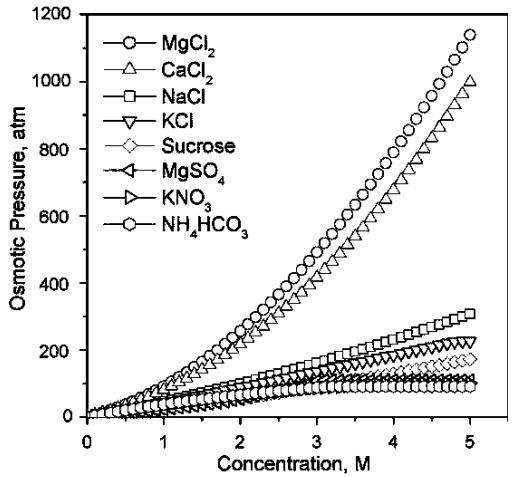

Fig. 2 Osmotic pressure as a function of solution concentration at 25 ◦C for various potential draw solutions. Data were calculated using OLI Stream Analyzer 2.0

Table 2

Properties of sucrose, CaCl2 and NaCl

Parametri	**Zaharoză**				**$CaCl_2$**	**NaCl**
Concentration [^{o} brix]	30	40	50	60	-	-
Concentration [%]	30	40	50	60	50	24,60
Dynamic viscosity [mPa x s] 35^{o}C	2,1	3,8	8,4	26,3	43,02	1,3
Osmotic pressure[bar] 25^{o}C	32	51	82	131	2121	356
Water activity 25^{o}C	0,971	0,955	0,936	0,898	0,214	0,772

The membrane used by us was Desal5-DK.

The concentration procedure was made 3 times with different draw solutions.

The experiments lasted 3 h and water flux was measured throughout the entire run. During the first two hours of the experiments, water fluxes were measured continuously and during the last hour they were measured every 20 minutes.

$CaCl_2$ and NaCl were chosen as stripping solutions. The initial concentration of the stripping solutions was 50 and 24.6 % (w/w) for $CaCl_2$ and NaCl, respectively. The chosen concentrations guaranteed a high driving force. At 50 % (w/w) concentration, the $CaCl_2$ solution presented very low water activity. At higher concentrations the decrease in water activity was negligible and the viscosity increased significantly . The NaCl concentration was close to the saturation point (26.4 % w/w), its viscosity is very low, but water activity is much higher than the water activity of $CaCl_2$.

The best results are given by $CaCl_2$ for concentration process.

After every experiment was measured quantitative the concentration in cinnamon essential oils on beginning of process and to end of process.

Was made a classical concentration by distillation and was repeated the quantitative method for determining concentrations.

The results ware similar for both processes.

CONCLUSIONS

As we seen, the results above are very good and this extraction process of water at low temperatures can be used as intermediate process in many type of analysis. This experiment open a new horizon for forward osmosis through applying of this process in analytical methods of analysis.

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THE MALOLACTIC FERMENTATION INFLUENCE ON THE ETHYL ACETATE CONTENT FROM RED WINES

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KEY WORDS: malolactic fermentation, malolactic bacteria, ethyl acetate, flavors, esterification.

ABSTRACT

Some researches have demonstrated that the malolactic fermentation doesn't significantly modify the sensorial characteristics of the wine but other results showed that the malolactic fermentation produces substantial modifications in the flavor of the wine and these modifications depend on the bacterial stem that leaded the fermentation and on the type of the wine. (Rosi and colab., 1998).

In this paper, the content of the ethyl acetate was determined before and after the malolactic fermentation, for the wine samples inoculated with commercial preparation Inoflore R (which contains the Oenococcus oeni variety) and for the wine samples that were not inoculated, at which the malolactic fermentation was developed spontaneously, on the basis of the indigene microflora. The results of this paper show that, the increasing contents of the ethyl acetate take place during the malolactic fermentation, especially at wine samples that were not inoculated with selected malolactic bacteria, in which the malolactic fermentation took place spontaneously, on the basis of indigene microflora.

INTRODUCTION

In present, there are controversial opinions in what concerns the malolactic fermentation contribution on the aroma and sensorial profile of wine. This controversy results also from diverse terms used to describe the wine flavors malolacticly fermented. These terms can be grouped in *positive* terms and *negative* terms. The positive terms used to describe the flavor are: nuts, yeast, fruity, vegetal, earthy, soft, round and persistent and the negative terms used to describe the unwanted flavors are: lactic, animal, butyraceous, acetate, bitter and viscous. **(Henick-Kling T. and colab, 1994).**

The negative qualities such as acetate, bitter and viscous are usually attributed to an uncontrolled malolactic fermentation, that is to a malolactic fermentation realized with unwanted bacteria or to a bacteria growth over the established level for decombustion. **(Davis C.R., and colab., 1985).**

The typical flavors of the malolactic fermentation ("of butter", "of dried leaves:, "of animal") can affect negatively the primary qualities of the wine if they are not equilibrated from the primary flavors of the fruit. **(Mc Daniel and colab., 1987).**

The malolactic fermentation reforms the gustative equilibrium of wines by disappearing the crude character in white wines and by reducing the tartness and asperity in

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red wines, where the excess tannins-acidity combination gives the taste an acerbate character, scanty liked. The acidity and aggressiveness decrease is accompanied by an increase of volume sensation in mouth, of silkiness due the liberation from the lactic bacteria in wine of some polysaccharides, aminoacids and nucleic acids.

The malolactic fermentation influences the olfactory qualities of the wine, by a better prominence of fruitiness or by hardening the secondary flavor of fermentation.

The main awe concerning the malolactic fermentation is that it could lead to loosing the fruitiness character of the wine, but a malolactic fermentation developed in optimal conditions sets solid this character, by significant reduction of tumble and vegetal smells, that masks other olfactory characters including the floral and fruity flavors, very appreciated in tender wines. **(Gheorghiță M. and colab, 2006)**.

Thus, some fruity flavors are better accentuated after malolactic fermentation. So, in a Chardonnay wine, the damascenone content increases under lactic bacteria action which possess β-glucosidase and β-galactosidase activities. **(Rosi I. and colab., 1998)**.

Of high importance is the fact that red wines produced in cold climatic areas, which have strong vegetal flavors, the malolactic fermentation reduces the vegetal flavors and intensifies the fruity ones. For example, a Cabernet Sauvignon wine can have intense herbal flavors, of green pepper or peas. After the malolactic fermentation, these flavors disappear and are intensified those of blue berries or raspberry, beside of black pepper or mint flavors. **(Henick-Kling T. and colab, 1994).**

During the malolactic fermentation many substances are formed that contribute to the taste and flavor of the wine. The increase of diacetyl concentration represents one of the most important contribution of lactic bacteria to the wine's flavor **(Laurent M.H. and colab., 1994)**. In large concentrations, it gives wine a stronger butter flavor, while in smaller concentrations the diacetyl contributes to nuts, yeast or caramel flavor. Diacetyl is produced in the second half of the malolactic fermentation and can be metabolized in acetoine by the same lactic bacteria. This last compound does not lead to any wine flavor. An alternative to producing acetoine in natural way by lactic bacteria metabolism is adding at the end of malolactic fermentation of a quantity of active yeast capable to reduce diacetyl to acetoine. **(Henick-Kling T.,. Acree T.E, 1988)**

During the malolactic fermentation there is a growing tendency of volatile acidity, being given by the increase of esters content, acetic acid and lactic acid. From esters, the ethyl acetate registers a substantial increase, especially when the malolactic fermentation is developed spontaneously on indigene micro flora basis.

Esters result from organic acids and alcohols combination, by eliminating a water molecule. Esters from wine have three ways of provenance. **(Gheorghiță M. and colab., 2006)**:

- come from wine (those esters that give wine the variety flavor);
- are formed in biological processes determined by microorganisms (biological esterification);
- are formed by chemical processes and reactions in maturation and obsolescence phases (chemical esterification).

The biological esterification is made by yeasts, lactic and acetic bacteria the reactions being catalyzed by enzymes from esterases group forming neutral and volatile esters. The esterification process takes place inside yeasts and bacteria cells. **(Țârdea C. and colab., 2001).**

The esters forming amount and rhythm depend on fermentation conditions and on yeasts and bacteria type concerned in fermentation processes.

Yeasts, depending on the amount of esters that they form are grouped as follows **(Pomohaci N., 1990)**:

- yeasts low esterogene (*Saccharomyces* and *Torulopsis* genders);
- yeasts moderate esterogene (*Hanseniaspora* and *Brettanomyces* genders);
- yeasts strong esterogene (*Kloeckera* gender).

From bacteria, powerful esterogene characters present acetic bacteria belonging to *Acetobacter* gender (*Acetobacter ascendes, Acetobacter xylinum)* and lactic bacteria belonging to *Lactobacillus* and *Pediococcus* genders.

The most important ester formed by biological way is ethyl acetate. The normal contents are considered to be to 150 mg/l. To proportion over 180 mg/l, ethyl acetate negatively influences the wine quality. Having olfactory characteristics more intense that acetic acid is the first to indicate the souring danger. **(Gheorghiță M. and colab., 2006)**.

In this paper, the ethyl acetate was determined before and after the malolactic fermentation, on wine samples inoculated with commercial preparation Inoflore R (which contains the *Oenococcus oeni* species) and on uninoculated wine samples in which the malolactic fermentation was developed spontaneously on the indigene micro flora basis.

MATERIAL AND METHODS

Principle of method

Ethyl acetate is separated by distillation from wine adjusted to pH 6.5. Next is saponification in alkaline medium and transformation in acetic acid. The resulted acetic acid is separated by water steams and is dosed with solution of NaOH of known molarity.

Used reagents:

- NaOH 1M;
- buffer solution of pH 6.5 which is prepared from KH_2PO_4 – 5 g, solution NaOH 1M – 50 ml and water until 1l.
- crystallized tartaric acid
- NaOH solution 0.02 M;
- phenolphthalein neutral solution 1% in alcohol 96% (v/v)

Working way

In a 500 ml flask are introduced 100 ml uncarbonated and neutralized wine with n ml NaOH solution 1 M, n being the NaOH 0.1 M solution volume used to measure the total acidity of 10 ml wine. 50 ml buffer solution with pH 6.5 is added and then begins the distillation. The distillate must be collected through a thin tube, which is introduced in a round bottom flack of 500 ml which contains 5 ml of NaOH 1 M and on which is made a sign that shows a volume of nearly 35 ml. 30 ml of distillate is collected.

The flask is covered and is let to rest 1 hour. The distillate is concentrated to 10 ml by putting the flask in a boiling water bathe injecting inside the flask a powerful air current. The flask content is cooled and 3 g of tartaric acid are added and CO_2 is eliminated by attaching the flask to the vacuum trunk. The liquid from the flask is quantitatively moved in the bubble flask of a distilling apparatus carried away with water steams, washing off the flask twice with 5 ml distilled water. At least 250 ml of distilled is collected. From the collected distilled the acetic acid is titrated with NaOH 0.02 M in presence of phenolphthalein as indicator.

Calculus

n – number of ml of NaOH solution 0.02 M used for titration. 1 ml corresponds to 1.76 mg ethyl acetate.

The ethyl acetate concentration in milligrams is given by 17.6 x n.

RESULTS AND DISSCUSIONS

Before the start of the malolactic fermentation the content of ethyl acetate was determined from wines submitted to malolactic fermentation. The analyses were completed also by total and volatile acidity determinations.

Table 1

The ethyl acetate content from wine samples before the malolactic fermentation

Wine samples	Ethyl acetate concentration (mg/l)	Total acidity (g/l)	Volatile acidity (g/l)
1. Oporto	4,15	3,21	0,28
2. Merlot	5,46	5,35	0,29
3. Pinot noir	5,12	4,40	0,29
4. Burgund	5,67	5,25	0,29
5. Cadarcă	7,21	6,28	0,35
6. Sangiovese	3,61	4,15	0,29
7. Blauerzweigelt	3,92	4,90	0,39
8.Cabernet Sauvignon	5,27	5,85	0,31

Table 2

The ethyl acetate content from wine samples after the malolactic fermentation finalization

Wine samples uninoculated (mark)	Ethyl acetate concentration (mg/l)	Total acidity (g/l)	Volatile acidity (g/l)
1. Oporto	35,81	3,15	0,65
2. Merlot	35,79	4,9	0,41
3. Pinot noir	35,88	4,05	0,47
4. Burgund	36,12	4,8	0,55
5. Cadarcă *)	37,15	6,0	0,45
6. Sangiovese	35,91	3,95	0,48
7. Blauerzweigelt	36,72	4,65	0,57
8.Cabernet Sauvignon	37,56	4,95	0,51
Wine samples inoculated with BMS			
1. Oporto	24,12	3,12	0,51
2. Merlot	20,31	4,8	0,4
3. Pinot noir	20,57	4,0	0,4
4. Burgund	23,62	4,8	0,43
5. Cadarcă	32, 67	4,5	0,55
6. Sangiovese	24,66	3,8	0,41
7. Blauerzweigelt	23,95	4,6	0,5
8.Cabernet Sauvignon	30,16	4,85	0,45

*) in Cadarca wine the malolactic fermentation wasn't finished in spontaneous conditions

From the data presented in table 1 it can be noticed that wines, before malolactic fermentation present contents quite modest in ethyl acetate, between 4.15 – 7.21 mg/l. These contents in ethyl acetate are due yeasts with estherasic activity which developed their activity during the alcoholic fermentation. It is worth to mark that there is a direct correlation between the ethyl acetate concentration and total and volatile acidity levels in

the way that wines that present high values of volatile acidity, present higher concentrations in ethyl acetate.

From the data presented in table 2 is noticed that after the malolactic fermentation development wines present more consistent concentrations in ethyl acetate between 20.31 – 37.56 mg/l. These increases of ethyl acetate concentration is due lactic bacteria from *Lactobacillus* and *Pediococcus* genders, which are present in the spontaneous microflora and possible in the accidental presence of acetic bacteria.

Still, in wine samples that had the malolactic fermentations there is a difference in what concerns the ethyl acetate content. So, for wine samples uninoculated (mark), at which the malolactic fermentation took place spontaneously on the indigene microflora basis, the ethyl acetate contents are quite high, being between 35.79 mg/l to Merlot and 37.56 mg/l to Cabernet Sauvignon. It must be said the high value (37.15 mg/l) of Cadarca wine, in spite of a smaller volatile acidity (0.45 g/l). It must be said that Cadarca wine did not succeed to have malolactic fermentation with the help of lactic bacteria from indigene microflora, probably because of a too high total acidity (6 g/l) and of a too low pH (2.9). By using the INOFLORE R preparation (which contains the Oenococcus oeni species), at this type of wine takes place the total degradation of malic acid, concomitant with the lactic acid accumulation. Although, according to the biotechnological characteristics, the Inoflore R preparation has as minimal pH tolerance 3.2 value, it succeeds to start the malolactic fermentation and to finish it at 2.9 value.

It is noticed that the volatile acidity values are higher with 0.1-0.2 g/l in spontaneous malolactic fermentation case, comparative with the conducted malolactic fermentation and the ethyl acetate contents are also higher (with 5-12 mg/l), in the spontaneous malolactic fermentation case.

On the other hand, for the wine samples inoculated with commercial preparation Inoflore R (which contains the bacterial species Oenococcus oeni), the contents in ethyl acetate are quite modest being between 20.31 mg/l to Merlot and 32.67 mg/l to Cadarca. This difference of the ethyl acetate content from wine samples inoculated and uninoculated (mark) leads us to the conclusion that the selected malolactic bacteria have a much more reduced capacity of forming volatile esters and implicitly of ethyl acetate.

Finally, it is worth to notice that at inoculated and uninoculated wine samples that had malolactic fermentation, the ethyl acetate concentration is at a secure level (under 150 mg/l).

CONCLUSIONS

The ethyl acetate dosage represents an important study from the volatile esters determination point of view that significantly influence the wine bouquet and from the wine souring point of view.

The results of this experiment show that the increases of ethyl acetate contents are during the malolactic fermentation, especially to uninoculated wine samples with malolactic bacteria, at which the malolactic fermentation took place spontaneously on the indigene microflora basis.

The ethyl acetate concentration from wines malolacticly fermented is significantly influenced by microflora (indigene or selected), but has a connection with the volatile acidity values registered to wines before the malolactic fermentation. So, wines with high concentrations in ethyl acetate after the maloalctic fermentation finalization are those who present a higher volatile acidity before starting the malolactic fermentation.

In conclusion, it is worth to mention that to inoculated and unionoculated wine samples that had a malolactic fermentation, the ethyl acetate concentration is in normal limits, improving the bouquet and sensorial profile of wines.

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RESEARCHES CONCERNING THE INFLUENCE OF CLIMATIC CONDITIONS ON DYNAMICS DEVELOPMENT OF THE MALOLACTIC FERMENTATION AT RED WINES

Ionel Popescu-Mitroi[1]

KEY WORDS: climatic conditions, malolactic fermentation, malic acid, harvest year

ABSTRACT

The climatic conditions and dynamic of sugars and organic acids accumulation in grapes present a high importance for the development of the malolactic fermentation.

In conditions of the harvest year 2004 (high real thermal balance, moderate quantity of precipitations in the vegetation period, high number of insolation hours in the vegetation period), at maturity, grapes have equilibrated contents in sugars and acidity. In these conditions, are obtained wines with equilibrated contents in alcohol and acidity, creating the premises of an easy development of the spontaneous malolactic fermentation.

In conditions of the harvest years 2005 and 2006 (low real thermal balance, high amount of precipitations in the vegetation period, low number of insolation hours in the vegetation period), at maturity grapes have low contents in sugars and high contents in acidity. In these conditions, are obtained wines with lower concentrations in alcohol, with a high total acidity and low pH, negatively influencing the start and development of the spontaneous malolactic fermentation.

INTRODUCTION

In a synthetic definition concerning the relation between natural conditions and grape vine, Pusais J (1975) said: „Wine is an ecological reflection and not a standardized product". Actually, the real sense of the problem made by the author expreses by the question – is wine an ecological refelction or is a standardized product?

The entire set of scientific arguments brought by Pusais J (1975) offers a clear answer to this question, which makes obvious justifying the fact that in wine, the natural factors assembly is fideliously reflected. (**Camelia Muntean, 2001).**

In our country's vineyards, the climatic conditions are different from one year to another. So, the grapes maturation registers significant differences for different climatic years. On the other hand, the climatic conditions and the sugars and organic acids accumulation dynamics in grapes present a high importance for de malolactic fermentation development.

The predominant acids in grapes, at maturity are tartaric and malic acids. From the studies made in our country, results that at the beginning of maturation in grapes is found more malic acid than tartaric acid. After acidity reductions made until the end of the

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maturation, grapes contain at full maturity 2-7 g/l tartaric acid and 2-3 g/l malic acid. **(Țârdea C, 1964)**.

The malic acid has as principal formation way the respiratory combustion process of sugars. The organic acids formation represents in fact intermediary steps of the respiratory process eliberating a part of stored energy in the sugars molecules. **(Camelia Muntean, 2001).**

In the vinification process, the malic acid comtent of grapes is reduced, as is well known due the malolactic bacteria activity, which act at the end of the alcoholic fermentation, transforming the malic acid in lactic acid.

The organic acids evolution during the grapes maturation is made by a descendent curve.

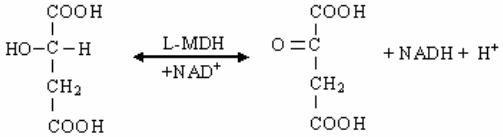
Grapes have at ripeness a high acidity (30-40 g/l), expressed in tartaric acid), which along with the maturation phase is continuously decreasing, achieving maturity at values lower than 10 g/l tartaric acid. This evolution is due metabolizing the acids during the respiration, water input and cations migration into the bean which neutralize o part of acids as well reconverting the malic acid into sugars. From the numerous acids which are accumulated in grapes, three are more representative (approximately 90% from titrable acidity): malic acid, tartaric acid and citric acid.

Malic acid is present in grapes or in must in amounts of 2-4 g/l, under levogyrate form, in free state or under acid or neutral salts form. The must content in malic acid vary according as variety and climatic conditions of the year. In years with chilly and more wet summers, the grapes and resulted must contain more malic acid than warm and dry years, when the malic acid is intense metabolized. (**Pomohaci N. and colab., 2005**).

In our country, the grapes and wine study under the malic acid content aspect was made for Moldova region by dr. ing. **Țârdea C. (1966),** in the doctoral paper "The malolactic fermentation study of wines from Moldova vineyard". Also studies concerning the malic acid evolution in the maturation period of grapes were made by Alexandra Alexiu and colab. (1966) the content in malic acid is determined chromatographicly by Michaud method (paper chromatography).

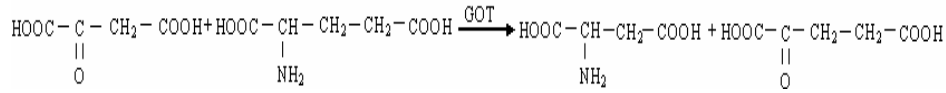
MATERIAL AND METHODS

The L-malic acid determination from grapes, must and wine was made by enzymatic method. The determining method principle consists in the following mechanism: L-malic acid is oxidized to oxalilacetate acid by NAD (nicotine – adenine – dinucleotide), in presence of L – mallate dehidrogenase (L-MDH).



L-malic acid oxalilacetate acid

This reaction's equilibrium is on the L-malic acid side. Eliminating the oxalacetate from the reaction system leads to the movement of the equilibrium toward oxaliacetate acid. In the reaction catalyzed by glutamate-oxalacetate-transaminase (GOT), the oxalilacetate acid is transformed in L-aspartic acid, in presence of L-glutamic acid.



oxalilacetate acid L-glutamic acid L-aspartic acid α-keto-glutaric acid

RESULTS AND DISCUSSIONS

Table 1

Climatic conditions in the vegetation period (1 IV – 30 IX), years 2004, 2005 and 2006 in Minis-Maderat Vineyard

Month	Real thermal balance (ºC)			Ilumination (insolation hours)			Precipitations (mm. col. Hg.)		
	2004	2005	2006	2004	2005	2006	2004	2005	2006
April	458,7	175,9	417,3	199,9	178,3	192,7	78,5	159,3	89,2
May	532,1	397,8	501,8	211,1	235,2	236,7	39,5	37,7	94,1
June	621,0	572,1	577,5	244,6	280,5	244,6	53,5	63,4	139,0
July	745,8	688,0	797,8	268,1	244,7	317,0	53,1	92,2	59,8
August	759,1	669,5	692,1	257,4	169,3	202,3	47,9	114,5	142,4
September	625,0	600,7	522,6	192,3	165,4	187,8	76,7	86,5	17,9
Total	3741,7	3104	3509,1	1373,4	1273,4	1381,1	349,2	553,6	542,4

Table 2

Total acidity (g/l sulphuric acid) and sugars (g/l) at full maturity of black grapes, Minis-Maderat vineyard, harves years 2004, 2005 and 2006

Variety	Sugars(g/l)			Total acidity (g/l sulphuric acid)		
	2004	2005	2006	2004	2005	2006
Burgund mare	204	188	191	5,78	6,44	6,81
Cadarcă	158	142	165	6,07	5,88	6,9
Cabernet Sauvignon	176	152	176	6,22	6,75	7,74
Merlot	204	172	185	4,31	5,83	6,12
Pinot noir	195	202	195	5,48	5,39	5,25
Oporto	215	184	206	4,90	4,95	3,92
Blauerzweigelt	198	174	193	5,14	5,29	5,88

The centralized data in table 1 and 2 shows that in harvest years 2004, 2005 and 2006, years very different from the climatic conditions point of wiew, the sugars and acidity content is significantly modified. The sugars content for the same grapes varieties is higher in the conditions of harvest year 2004, when the real thermal balance crosses 3700°C, the number of insolation hours crosses 1370 hours and the precipitations level is under 350 mm Hg col., comparative with harvest year conditions 2005 when the real thermal balance hardly reaches 3100°C, number of insolation period does not cross 1300 hours and the precipitations level is way cross 500 mm Hg col. The acidity content for the same varieties of grapes is lower in harvest year conditions 2004, when the real thermal balance and insolation hours is higher comparative with the same parameters registered in harvest years 2005 and 2006. From the 7 black grapes varieties analized only and Pinot noir and cadarca varieties are noticed higher total acidity contents in harvest year 2004 conditions comparative with harvest year 2005. All other varieties present higher total acidity values in harvest year 2005 comparative with harvest year 2004.

Table 3

Acid malic variation free content in maturation period of grapes and wine, Minis-Maderat vineyard, harvest 2004

Variety	Determination*)	Analisys dates						
		Grapes			Wine			
		16 VIII	23 VIII	30 VIII	6 IX	13 IX	23 IX	5 XI
1. Burgund mare	Acidity	13,23	9,11	7,44	6,96	5,78	4,96	4,55
	Malic acid	8,2	5,1	3,53	2,85	1,5	1,0	traces
2. Merlot	Acidity	14,40	9,41	6,46	6,02	4,31	4,22	3,95
	Malic acid	7,1	4,85	3,27	2,5	1,8	0,5	traces
3. Cabernet Saugvignon**	Acidity	19,79	13,2	11,0	9,59	6,22	5,65	4,20
	Malic acid	11,0	8,4	6,3	5,15	4,36	3,5	3,0
4. Oporto	Acidity	6,27	5,39	4,36	4,11	4,90	4,15	3,89
	Malic acid	3,7	2,26	1,7	1,3	1,0	0,5	traces
5. Pinot noir	Acidity	12,74	6,76	6,56	5,48	5,48	4,9	4,6
	Malic acid	6,3	3,86	3,05	1,75	1,5	1,0	traces
6. Cadarcă**	Acidity	18,03	11,2	8,53	8,52	6,07	6,0	5,97
	Malic acid	10,5	7,15	6,2	5,15	4,0	3,2	2,65
7.Blauerzweigelt	Acidity	11,27	7,79	6,66	6,12	5,14	4,45	3,90
	Malic acid	5,62	3,95	3,35	2,90	2,0	1,2	traces

*) Acidity is expressed in g/l sulphuric acid, and malic acid in g/l

**) Wines with lack of malolactic fermentation

From the analized varieties in the fall of 2004, in Minis-Maderat vineyard, the highest content in malic acid at the beginning of maturation have the grapes from Cabernet Sauvignon variety (11.0 g/l), which presents a high total acidity (19.79 g/l).

The lowest content in malic acid, at the beginning of maturation have the grapes from Oporto variety (3.7 g/l), which registers a low total acidity in the moment of analysis (6.27). In what concerns the bond between total acidity and malic acid content of wines after vinification, is noticed that wines that present a high total acidity at the beginning of vinification (Cabernet Sauvignon, Cadarca) present the highest contents of malic acid at the end of vinification, this fact being explained because high total acidity determins a low pH, inhibiting the malic acid degradation through malolactic fermentation.

From the analized varieties in fall of 2005 in Minis vineyard, the highest content in malic acid at the beginning of maturation is found in grapes from Cabernet Sauvignon variety (12.1 g/l) which presents a high total acidity (18.13 g/l).

The lowest content in malic acid at the beginning of maturation have the grapes from Oporto variety (4.8 g/l) which registers a low total acidity at the determination moment (9.70 g/l) At the end of maturation, the malic acid content decreases to value 2 g/l while after vinification, can hardly find traces of malic acid, fact being explained by malolactic fermentation completion.

On the other hand, wines obtained from Burgund mare, Cabernet Sauvignon and Cadarca varieties, register an the end of vinification important malic acid contents (3.1-4.5 g/l) due the fact that these wines did not have malolactic fermentation.

Table 4

Free malic acid variation content in maturation period of grapes and in wine, Minis vineyard, harvest 2005

Variety	Determination*)	Analisys dates						
		Grapes		Wine				
		22 VIII	29 VIII	5 IX	12 IX	21 IX	1 X	15 XI
1. Burgund mare**	Acidity	14,21	9,1	8,13	7,44	6,44	5,95	5,20
	Malic acid	9,5	6,2	4,55	3,90	3,5	3,5	3,1
2. Merlot	Acidity	13,47	11,3	9,21	8,42	5,83	4,60	4,20
	Malic acid	8,2	5,95	4,15	3,5	2,8	1	0,5
3. Cabernet** Saugvignon	Acidity	18,13	14,5	12,25	9,1	6,75	6,25	6,15
	Malic acid	12,1	9,2	7,1	6,25	5,0	4,75	4,5
4. Oporto	Acidity	9,70	8,23	6,46	5,14	4,95	4,10	3,90
	Malic acid	4,8	3,25	2,9	2,3	2,0	0,5	traces
5. Pinot noir	Acidity	12,44	9,31	7,54	7,05	5,39	4,55	4,00
	Malic acid	7,1	4,95	4,00	2,85	2,5	1	traces
6. Cadarcă**	Acidity	16,26	12,2	10,29	8,91	5,88	5,70	5,50
	Malic acid	11,5	8,12	6,7	5,25	4,2	4	3,75
7.Blauerzweigelt	Acidity	10,29	8,5	7,35	6,75	5,29	4,80	3,90
	Malic acid	6,32	4,90	4,45	4,11	3,75	2,1	traces

*) Acidity is expressed in g/l sulphuric acid, and malic acid in g/l

**) Wines with lack of malolactic fermentation

Table 5

Free malic acid variation content in maturation period of grapes and in wine, Minis vineyard, harvest 2006

Variety	Determination *)	Analisys dates						
		Grapes		Wine				
		21 VIII	28 VIII	4 IX	11 IX	25 IX	6 X	15 XI
1. Burgund mare**	Acidity	11,1	10,0	9,21	7,54	6,81	6,23	5,78
	Malic acid	8,3	6,15	4,92	3,15	3,0	1,77	1,07
2. Merlot**	Acidity	12,7	9,41	9,71	7,84	6,12	5,87	5,29
	Malic acid	7,7	5,57	4,63	3,32	2,92	1,55	0,86
3. Cabernet** Saugvignon	Acidity	16,1	13,0	12,1	10,7	7,74	6,27	5,58
	Malic acid	11,3	9,8	7,63	6,39	5,15	3,25	2,5
4. Oporto	Acidity	7,15	6,27	5,83	5,78	3,92	3,7	3,7
	Malic acid	3,92	3,21	2,76	2,22	1,86	0,63	traces
5. Pinot noir**	Acidity	11,7	9,80	7,84	7,64	5,25	5,20	5,19
	Malic acid	6,42	4,57	3,25	2,73	2,12	1,55	1,0
6. Cadarcă**	Acidity	14,7	11,2	10,3	9,80	6,9	6,12	5,39
	Malic acid	10,5	7,57	6,12	5,77	4,13	3,37	2,78
7.Blauerzweigelt**	Acidity	8,35	7,54	7,33	6,17	5,88	5,62	5,48
	Malic acid	5,27	4,63	4,12	2,86	2,17	1,55	1,0

*) Acidity is expressed in g/l sulphuric acid, and malic acid in g/l

**) Wines with lack of malolactic fermentation

In fall of 2006, in Minis vineyard we notice that all wines except Oporto cannot succeed to have spontaneous malolactic fermentation until middle November, having large contents in total acidity and malic acid. The highes content in malic acid at the beginning of maturation is found at grapes from Cabernet Sauvignon variety (11.3 g/l) that also present a high total acidity (16.1 g/l).

All obtained wines, except Oporto register at the end of vinification important contents in malic acid (0.86-2.78 g/l) due the fact that these wines did not have a malolactic fermentation.

CONCLUSIONS

From the made experiments to follow the malic acid evolution in grapes and wines obtained in Minis vineyard we can conclude:

- Climatic conditions influence the malic acid accumulation in grapes and it's evolution during maturation; in harvest years 2005 and 2006 (rich in precipitations, with a real thermal balance relatively low, low number of insolation hours during vegetation period) a large amount of malic acid is accumulated at all studied varieties comparative with harvest year 2004;
- The massive accumulation of malic acid in grapes , in conditions of high acidity and low pH, determines an obstruction of malic acid degradation through malolactic fermentation, during vinification and conservation period of wines; Undecayed quantities may present risk factor to wine stability;
- There is a direct correlation between variety and content in malic acid of grapes; grapes from Oporto and Pinot noir variety accumulate at maturity reduced quantities of malic acid, that can be degraded easily during the malolactic fermentation, while grapes from Cabernet Sauvignon and Burgund mare varieties are the first in what concerns the malic acid content of grapes in maturity phase, the malic acid reduction being insignificant not even during conservation period.
- The most important reduction of the malic acid content takes place at the end of August in the case of grapes maturation until end of October, in case that the malolactic fermentation has initiated in wines immediately after alcoholic fermentation.

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INFLUENCE OF PLUM ADDITION ON RHEOLOGICAL PROPERTIES OF BREAD AND BREAD CRUMB

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KEYWORDS: compression, relaxation, Young modulus, viscoelastic properties, bread.

ABSTRACT

The Young modulus for bread with different contents of plum and nuts was determined from compression tests. A sigmoid compressive stress-strain relationship is a characteristic of the bread. A linear decrease with duration of fermentation for dough Young modulus was established. Also, the influence of plum and nuts content addition on viscoelastic characteristics of bread was studied with relaxation tests. The relaxation data could be fitted by equations that derived from generalized Maxwell model and by normalization and linearization of the experimental force relaxation curves.

INTRODUCTION

Nowadays, the use of additives has become a common practice in the baking industry. The objectives of their use are to improve dough-handling properties, to increase the quality of fresh bread and extend the shelf life of stored bread.

With this objective, a large extent of additives of different chemical structure are used, and lately, the enzymes due to be clean label are preferred by the baking market *(Dogaru et. al., 2004).* A method of improving the properties of dough and the quality of bread is adding to the dough, dough ingredients, ingredient mixture or dough additives or additive mixture.

In bread baking, addition produces an effect that can result in many desirable benefits including increased extensibility, increased product volume and improved crumb softness. The hemicellulose undergoes a hydrolysis reaction which improves the availability of moisture in the dough *(Caballero-Briones et. al., 2000).*

The recently determined three-dimensional structure of the widely applied amylase for anti staling provided further insight into the mechanism of enzyme action *(Dauter et. al., 1999).* Plums are rich in vitamins A, B, C, B_1, B_2, PP, in sulphouros, phosphoros, Mn, Mg, Na and K (300 mg k/ 100 g fresh fruits) *(Terry et. al., 2001)*.

These fruits bring the appetite to normal, lighten the intestinal transit, stimulate the liver, determine the umoral detoxification and refresh the organism. Have antifeverish, antihelmitic, depurative, laxative, nourishing, nervous stimulating, detoxifiating and

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decongestive properties, producing the tissues relaxation and diminishing the inflammations *(Kirk et. al., 2002)*.

The influence of plum content on rheological properties of bread crumb was studied. Rheological characterization was made by compressive loading tests and relaxation tests. To obtain the Young modulus of dough at low values of Cauchy strain the compressive test was used *(Dogaru et. al., 2004)*. Also, relaxation test was used *(Gamero et. al., 1993; Steffe et. al., 1996)*.

MATERIAL AND METHODS

Bread-making procedure. A straight dough process was carried out for preparing the bread samples. A basic bread formula, based on flour weight, was used: 450 g flour, 56% water, 1.6% yeast, 2% salt, and different plum addition: 5% (22g), 10% (44g) and 15% (66g). Likewise, a witness bread sample without plum addition was obtained. ALASKA BM 2000, a device for whole bread making process was used.

The optimal parameters for this device are: mixing – 30 minutes, fermentation – 130 minutes, backing – 50 minutes.

Evaluation of bread crumb quality. Parallel to bread bottom a medium slice of about 3 cm was cut from bread, and 3 flat, cylindrical specimens were prepared from them using a cork borer, avoid the crust. The slices were cut from bread after 2 hours of room temperature storage. The specimens had a diameter of 20 mm and their height was adjusted at 15-25 mm. Compression and relaxation tests were conducted as above. Calculus and graphical representation were realized with ORIGIN computer program.

Two replicates were analyzed and averaged.

RESULTS AND DISCUSSIONS

Compression curves obtained, $\boldsymbol{\tau} = f(\varepsilon)$, express the dependence of compression stress $\boldsymbol{\tau}$ by Cauchy strain $\boldsymbol{\varepsilon}$ *(Steffe et. al., 1996)*. A compression curve for bread crumb with 0.20% hemicellulose is presented in figure 1. As it can be observed there is a sigmoid dependence, being similarly with compressive stress-strain curves obtained for rye bread *(Swyngedau et. al., 1991)*.

From the slope of the first part of the experimental curve ($\boldsymbol{\varepsilon_c} < 0.2$) the compression modulus or Young modulus (E) was calculated. It was ascertained that the bread crumb is not uniform.

That is why, the rheological characteristics obtained from the compression curve (compression modulus and the mechanical work needed for 12 mm compression) are very different. Graphically, the compression curve for the witness sample is showed in figure 1.

The obtained values for E and L for all three samples, and the average values too, are showed in table 1. The force relaxation curve is graphically showed in figure 1.

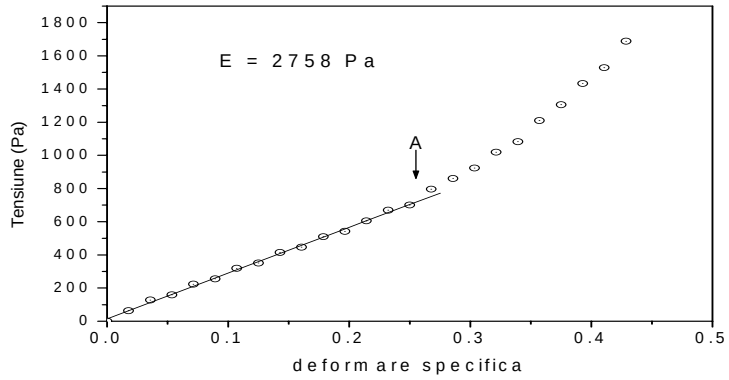

Figure 1. Compression curve for bread crumb for withness sample (o)

Table 1.

Rheological characteristics of the bread crumb from the witness sample, calculated from the compression and relaxation curves, respectively

Withness	**E**	**L (J/kg)**	**y_o**	**A_1**	**λ**
Sample 1	2758	0.771	0.777	0.215	18.41
Sample 2	9125	1.610	0.741	0.248	38.76
Sample 3	4490	1.135	0.798	0.183	13.29
Medium values	5458 ± 1900	1.172±0.243	0.772 ± 0.016	0.215 ± 0.018	23.5 ± 7.7

The relaxation test is a static procedure used to characterize viscolelastic properties of the studied bread crumb. The obtained relaxation data could be excellent fitted by equations that were derived from generalized Maxwell model, consisting of two parallel Maxwell element connected in parallel with a spring (*Steffe et. al., 1996*):

$$F(t) = F_e + A_1 \cdot \exp\left(-\frac{t}{\lambda_1}\right) + A_2 \cdot \exp\left(-\frac{t}{\lambda_2}\right) \qquad (1)$$

In this relation Fe (equilibrium force) represents the value of relaxation force at high values of time (t), A1 and A2 are the initial values of force on Maxwell elements, λ_1 and λ_2 are relaxation times for dough. A way to overcome some of the difficulties of the Maxwellian models is to normalize and linearize the experimental force relaxation curves using an empirical equation proposed by Peleg *(Steffe et. al., 1996)*:

$$\frac{F_{(o)} \cdot t}{F_{(o)} - F_{(t)}} = k_1 + k_2 \cdot t \qquad (2)$$

where F(o) is the force at time zero, F(t) the force after time t, 1/k1 is related to the initial stress decay rate, and1/k2 to a hypothetical asymptotic level of stress not relaxed at long time constants *(Gamero et. al., 1993).*

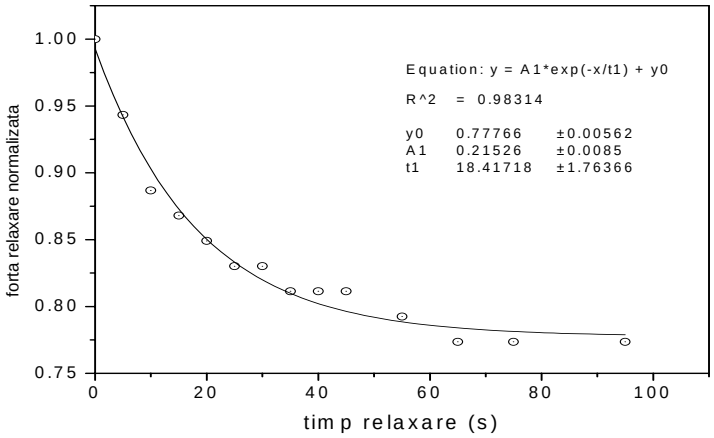

Figure 2. Force relaxation curve, for the bread witness sample(o)

The calculated data by first order experimental fitting for the three witness samples, are showed in table 1, too. It can be observed from the table that the values for E and L are very different due to bread crumb unhomogeneity. In exchange, the obtained values for the normalized equilibrium force (Fe) are closed to each other and represent about 77% of the initial force. On the other side, the relaxation time (λ) and the difference between the initial force and the equilibrium force (A_1) are different.

In table 2, the average values of all rheological characteristics obtained at different concentrations of the plums content in bread are showed. It can be seen the influence that the plum content has on all studied rheological characteristics. Also, it can be ascertained the same evolution type for E, L, Fe and A_1 .In exchange, the relaxation time increases with the plums concentration increase.

Table 2.

The influence of the plums content on the studied rheological characteristics for the crumb of plums content bread.

Plums content (%)	**E (Pa)**	**L (J/kg)**	**y_o**	**A_1**	**λ (s)**
0	5458 ± 1900	1.172±0.243	0.772 ± 0.016	0.215 ± 0.018	23.52 ± 7.70
5	3590 ± 953	0.800±0.160	0.766 ± 0.007	0.223 ± 0.005	26.88 ± 2.88
10	2744 ± 584	0.764±0.079	0.748 ± 0.019	0.234 ± 0.019	28.59 ± 5.02
15	4876 ± 588	0.801±0.096	0.763 ± 0.006	0.219 ± 0.009	29.84 ± 0.93

The graphical dependence between the compression modulus and the plums content from figure 3, emphasyse that a 10% plums content, ensures a better elasticity for the bread crumb. Also, from table 2 it can be ascertained that this 10% plums content is the most appropriate for obtaining a plums content bread with good rheological characteristics.

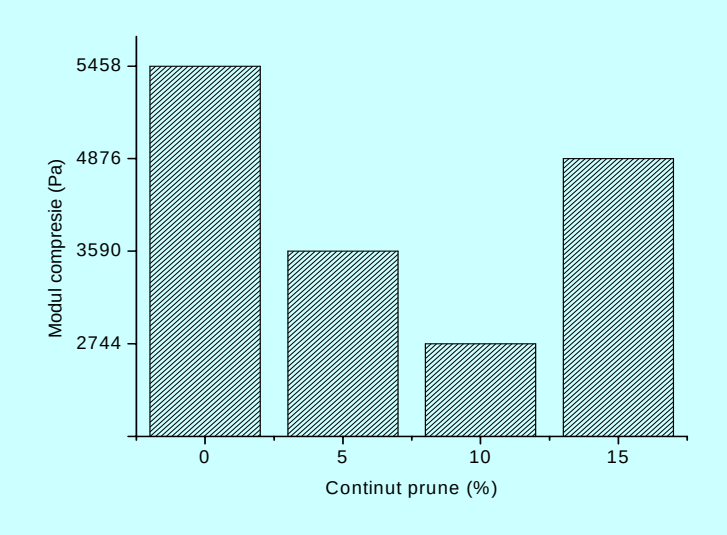

Figure 3. The plums content influence on the compression modulus of the bread crumb

Due to the fact that the compression modulus and mechanical work evolution is the same (see table 2), it was graphically presented in figure 4 the linear correlation between these two measures. From the gigure it can be ascertained a relative good linear correlation, the linear correlation factor being R=0,887.

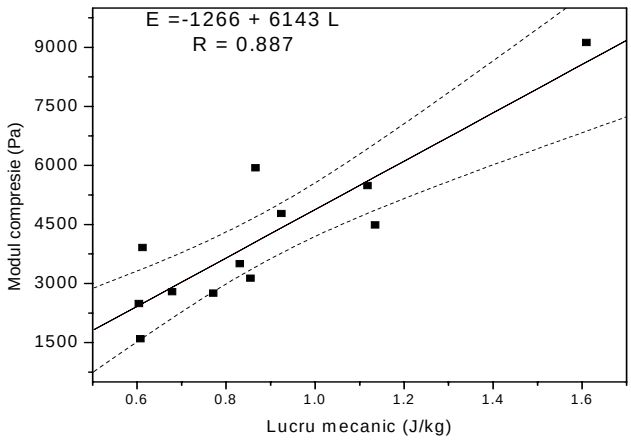

Figure 4. Correlation between compression modulus and mechanical work necessary for 12 mm bread crumb compression

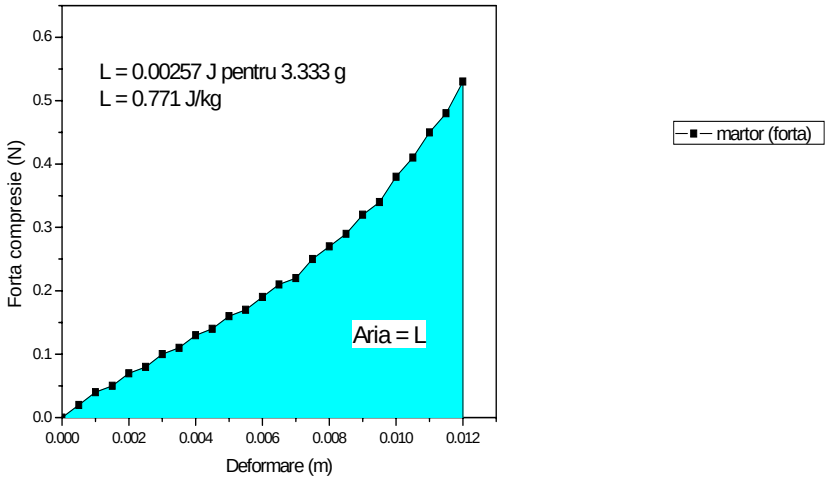

Figure 5. The calculus way of the mechanical work needed for the 12 mm deformation of the witness bread sample.

CONCLUSIONS

The plums addition to bread improves it's rheological and nutritional qualities, acting not only on the content, but also on the bread texture.

Also, upon plums addition to bread, the A,B,C vitamins, sulphure, manganese, magnesium, sodium and especially potassium content increases (300 mg potassium per 100 g fresh fruit).Thus, a new range of bread with high energy and vitamin value is obtained.

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RESEARCH REGARDING THE VIRGINIA TOBACCO FERMENTATED WITH AND WITHOUT VEINS

Giurgiulescu Liviu[1], Stoica Felicia, Savescu Petre[2]

KEY WORDS : vein, strips, leaves, fermentation process, carbohydrates, purity index

ABSTRACT

The big differences between the thickness and the cellular structure of the leaves and veins have as result a different behavior of these during in the technological operation make for drying and moistening. Through separately processing of these (laminating, cutting and drying). So, it is obtaining a homogeneous mass of tobacco with higher, physical and chemical proprieties and higher efficiency in production.

INTRODUCTION

Because of the physical, chemical and technological different proprieties of the tobacco leafs with veins, in the process of cigarette making it is necessary to eliminate the parts with veins applying different treatments.

In our country the cigarettes are made using the tobacco leaves technology with veins and without veins too.

The economical and technological results of the strips are in a real evacuumence when this operation is realized before of fermentation, eliminating in this way the suplimentary costs appeared in the technological process with use the vein peeling after fermentation. The eliminating of veins is usefull for the tobaccos with big leafs like Virginia and Burley and is not recommend for Oriental tobaccos with short leafs.

This paper wants to present the physical, chemical and organoleptic differences of the tobaccos obtaining from these both technologies of fermentation.

MATHERIAL AND METHOD

It used the Virginia tobacco leafs drying at the sun (sun cured) and strips through next methods:

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- the moisture in vacuum – the cutting of top leafs – strips (V1)
- the moisture in vacuum – without cutting of top leafs - strips (V2)
- the moisture in tambour - strips (V3).

At Virginia tobacco leafs drying at indirect fire (fire cured) it was applied the method : moisture in vacuum – strips.

The tobacco obtaining after the elimination of the veins (the strips) it was introduced in a fermentation room as package (baluri) with 29-47 kg weight. It was applied similar fermentation method at both tobacco types Virginia sun cured and Virginia fire cured. The fermentation period it was 5 days at 50 ^{0}C and 65-70 % relative humidity in the same fermentation room.

After fermentation process were measured the next parameters: humidity, purity index, oxygen index, total reduced sugars, carbohydrates, total substances with nitrogen.

Oxygen index represents oxygen quantity absorbed by 1 gram tobacco/1 hour. The purity index represents the oxygen quantity emitted 1 gram tobacco/1 hour. The Virginia tobacco are pure in the end of fermentation when oxygen index didn't exceeded 1,0 cm^3 and purity index didn't exceeded 10 cm^3.

The purity index put in evidence the moldy quantity and microorganisms existed on tobacco leafs. These all microorganisms in contact with peroxides of oxygen go on decompose of leaf with oxygen liberation.

The oxygen index put in evidence the activity of oxidaze enzymes, which shows the tobacco capacity for adsorb the oxygen from fermentation environment. This capacity diminished for the dry tobacco at the fermentation tobacco.

RESULTS AND DISCUSSION

Table 1

Variation of chemical composition at Virginia tobacco leafs before and after fermentation

Analyze	Virginia sun cured strips						Virginia fire cured strips	
	Variant 1 (V1)		Variant 2 (V2)		Variant 3 (V3)		Before	After
	Before	After	Before	After	Before	After		
Oxygen index	0.602	0.286	0.630	0.272	1.552	0.641	0.605	0.210
Purity index	8.5	3.5	22.5	1.2	11.2	7.5	1.2	1.1
Total reduced sugars	17.21	15.72	17.65	19.21	11.51	15.57	11.42	17.76
Carbohydrates	15.11	9.02	14.31	11.09	10.12	10.65	7.05	12.94
Total nitrogen	2.78	2.60	2.48	2.21	3.08	2.31	2.81	2.51

The results obtaining for the fermentation of tobaccos leafs with or without veins are present in the table 1 and 2.

The chemical composition of the tobaccos fermenting in strips had a real increase comparative with the tobaccos fermenting in leafs.

Soluble carbohydrates (glucose, fructose, saccharose) record a best results at Virginia strips sun cured bigger than Virginia fire cured.

In function of eliminating veins method at Virginia sun cured it is possible to observe next remarks:

- the second variant (V2) has a higher contents of carbohydrates the next classification is the third variant (V3) and the last is the first variant (V1);

- at V3 variant we record an increase of the carbohydrates after fermentation comparative with the other variants, which register a decreasing;

- the total nitrogen registers at all of the three variants after fermentation. This thing explains clearly the necessity of fermentation process who reduced the detrimental substances through enzymatic hydrolysis process and their elimination as ammonia. ;

- the oxygen index registered the decreasing at all three variants. The first and the second variants had better results than the last one;

- the purity index (wich determine the end of fermentation and the catalase activity) registered the higher decreasing at the second variants (V2) from 22.5 in the beginning at 1.2 in the end af the fermentation. All of these denote a higher capacity of fermentation of these tobaccos and the possibility of their preservation for a longer time than the others too.

Doing a comparison between Virginia indirect fire strips and the media of all of three variants of Virginia sun cured before and after fermentation, it observe the superior quality of the tobaccos drying at artificial warmth comparative with the variants drying at sun.

Table 2

Variation of chemical composition at Virginia tobacco strips before and after fermentation

Analyze	Virginia dry tobacco				Virginia fermentation tobacco			
	Virginia fire cured		Virginia sun cured		Virginia fire cured		Virginia sun cured	
	Leaf	Vein	Leaf	Vein	Leaf	Vein	Leaf	Vein
Oxygen index	1.686	1.217	1.615	0.823	0.981	1.215	0.193	0.238
Purity index	10.2	10.1	10.2	10.1	6.2	7.2	4.1	5.1
Total reduced sugars	18.65	7.72	11.51	5.32	29.73	22.12	10.82	10.13
Carbohydrates	10.58	2.46	10.12	9.96	16.01	14.01	4.13	7.59
Total nitrogen	0.36	1.82	0.58	2.16	1.60	1.96	0.46	1.96

A comparative study realized at Virginia tobaccos fermenting with veins and Virginia tobacco fermenting without veins shows a higher chemical composition at strips variants. The fermentation index (oxygen purity) are lower at tobaccos fermenting in strips comparative with the tobacco fermenting with veins at the Virginia sun and Virginia fire cured too.

Carbohydrates registered higher values at the variants without veins comparative with the variants with veins, the total reduced sugars and the substances with nitrogen

registered the variation which confirm the advantage of the veins elimination before fermentation.

The chemical compounds at the level of the leaf surfaces at the veins variants evolve in accordance with the percent of substances on the leaves. The higher values were registered in the nitrogen substances in veins and higher values of reduced substances record on the surfaces of leafs. These substances kept the proportion before and after fermentation.

CONCLUSIONS

Begin the researches results we observe that it is possible to pass from the tobaccos fermentation with veins at the tobaccos fermentation in strips, in special for to reduced the technological loss and for to increase the quality of the products.

The temperatures during the fermentation in stabilization phases are approximate the same for the both variants (with or without veins).

For the strips fermentation is necessarily to a rigorous control of humidity comparative with the veins fermentation. It is recommended that the humidity to be under 15-16% in the moment of tobacco pressure in strips for to eliminate the possible loss made by the stick together of the leafs without veins.

The chemical composition of the tobaccos fermenting in strips is better than the leafs with veins tobaccos fermentation for all the quality classes and for all the analyzed tobaccos variants.

In the next study will be follow the evolution of the humidity, of the chemical and organoleptic composition during the fermentation of the veins result from the tobacco leafs. It will be follow the possibility of reconstruction of the strips tobaccos with these fermenting veins too.

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RESEARCHES REGARDING THE INFLUENCE OF LEVURAJE AND THE RELATION OF MUST PHASES ON THE RED WINES CHEMICAL COMPOSITION

Giurgiulescu Liviu[1], Stoica Felicia, Savescu Petre[2]

KEY WORDS: yeasts, pomace phases, must, berry, alcohol

ABSTRACT

The knowledge and the control of the biological transformation - provoke by the microorganisms and enzymes catalysis - constitute a major preoccupation in modern oenology.

The actual vine making use different biotechnological preparations which participate for a better fermentation-maceration process.

INTRODUCTION

In the technology of red wines obtaining an important place have the relation between grape juice phases which strive to separate during the fermentation – maceration under the action of CO_2 obtaining through the metabolism of the sugars by yeasts.

The maintaining of the separation for a longer time gives unpleasant results from the compositional, microbiological and organoleptic point of view. The present paper propose to realize a study on the influence of the indigene and selected yeasts in combination with the relation between grape juice phases for the chemical compounds of the red wines (Merlot variety – Banu Mărăcine vineyard)

MATHERIAL AND METHOD

For to realize this experiment it was followed the influence of the indigene yeasts derive from Banu Mărăcine vineyard and the influence of the selected yeasts from Saccharomyces Bayanus sp. on the grape juice phases relations.

It was used four phases of the grape juice pomace: with sink pomace, with remount of the liquid phases by 5 times/ day, with the mechanic homogenization of phases by 5 times/day.

For to obtaining of the grape juice it used the Merlot grape with next proprieties: sugars 218 g/l; acidity 4,76 g/l H2SO4, anthocyans 1326 mg/kg berries .

The technological conditions for to experiment the primary wine making: maceration period 96 hour; fermentation temperature 27-28 0C, sulfur addition 60 mg/l ; Indigene yeasts, Selected yeasts Saccharomyces Bayanus variety.

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[2] University of Craiova, Horticulture Faculty

RESULTS AND DISCUSSION

The results of the experimental part are present in table 1.

For the un-yeasts variants (fermentation with Indigene Yeasts) the principal chemical constituents have different levels which depend by the solid phases of the grape juice in rapport with liquid phases.

When the agglomeration of solid parts keep all the time on the surface of the static recipient (floating pomace) there are the lowers contents in alcohol, unreduced extract, ash and glycerol, the highest proportion in volatile acidity and residual sugar.

In the agglomeration for the surface of the liquid phases an aerobe metabolism and that`s the reason why it consume a higher quantity of sugars for 1% alcohol.

Another results of the strong aeration an of the temperature from the agglomeration bigger that in the liquid phases because a part of sugars are consume on respiratory way – acetic bacterium find a good medium for activity which giving the volatile acidity.

The non-existence of the mechanic action actions and of the "washing" phenomenon for solid parts, explain why the contents in unreduced extract and ash are lower than the contents of these parameters at the other variants of the group with fermentation made by indigene yeasts.

At the variants with sink stationary registered insignificant increasing at alcoholic grad, extract, ash and a diminution of volatile acidity with 0,10 g/l H_2SO_4 . The higher increasing for alcohol, extract and ash it obtained when these are 5 remount by day through the pass of the half of liquid phases over the solid part agglomeration.

The best results, even at the fermentation with local yeasts, are register for the apply of 5 homogenization by day of grape juice phases. In these situations there are higher increasing for the alcohol content, because of the metabolism favor by fermentative type with a limited quantity of air, extract contents and ash as a result of the mechanic action on the solid phases as physic factor for to accelerate the maceration.

The same yeasts variants (selected yeasts) present the evidence increasing at all composition characteristics. For the same using raw material, the alcohol degree doesn`t descend under 12 volume even at floating pomace variant. The other constituents doesn`t register significant differences for te same variants fermentation with indigene yeasts.

Better results are register for the variant with 5 remount by day and best result for the variant with the homogenize of grape juice phases.

At this last yeast variants (Selected yeasts) and with mechanic homogenization of must phases by 5 times/day it registered the highest level of alcoholic degree (12,52 % vol.), unreduced extract (26,88 g/l), ash (2,54 g/l), glycerol (10,16 g/l) and the lowest at volatile acidity (0,42 g/l) and for residual sugar (1,62 g/l).

For the anthocyans and chromatic characteristics (I,T, dA%) it discern the yeasts variant (Selected yeasts) and with the both phases of the grape juice through remount or through mechanic homogeneity. At these variants in the moment of the separation phases the anthocyans have the highest contents (817 and 832 mg/l),the same for the colorant intensity (1,336 and 1,402) and dA% (72,11 and 72,20).

But between the both variants with moving of must phases, the variants with mechanic homogenization has the priority.

CONCLUSIONS

1. Using selected yeasts it observe an increase of the concentration in alcohol for all the variants doesn`t matter about the existing rapport between the must phases.
2. The best results were register for the variants with mechanic homogenization 5 times/day, realized a better contact between the both phases.
3. The maceration period permits the adjust al poliphenols quantity but in equal measure it influences the structure of the molecule, too; a maceration with a short duration gives operate the obtaining of light wines which have a lower quantity of poliphenols.
4. For the stationary sink pomace variant it realize the insignificant increasing for alcohol degree, extract, ash and a decreasing of volatile acidity.
5. When the agglomeration of solid parts is keeping all the time on the surfaces of static recipient (floating pomace) it register the lowest contents in alcohol, unreduced extract, ash and glycerol and the highest proportion for volatile acidity and residual sugar.

Table 1

Impact of yeasts type and relation between grape juice phases on basis chemical composition of red wine
(Merlot, Banu Mărăcine) – micro wine making

Yeasts Type	Technological variants	Alcohol % vol.	Total acidity H_2SO_4 g/l	Volatile acidity H_2SO_4 g/l	Unreduced extract g/l	Dash g/l	Glycerol g/l	Residual sugars g/l
Indigene Yeasts	Floating pomace	11.60	4.63	0.64	23.48	2.19	8.53	3.61
	Douse pomace	11.82	4.58	0.54	24.16	2.29	8.54	3.42
	With liquid phase remount 5 time by day	11.98	4.51	0.50	25.09	2.34	8.66	2.05
	With homogeneous of mechanic phase 5 time by day	12.06	4.51	0.48	25.95	2.39	8.66	2.00
Selected Yeasts	Floating pomace	12.00	4.62	0.59	23.69	2.20	9.05	2.00
	Douse pomace	12.05	4.57	0.47	25.32	2.31	9.16	1.85
	With liquid phase remount 5 time by day	12.43	4.52	0.44	26.10	2.48	10.07	1.77
	With homogeneous of mechanic phase 5 time by day	12.52	4.50	0.42	26.88	2.54	10.16	1.62

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CONSIDERATIONS CONCERNING THE INFLUENCE OF HIGH PRESSURE AND LOW VACUUM PROCESSING ON CANDY FRUITS PRESERVATION

Rosca Adrian, Rosca Daniela[1]

KEY WORDS: equipment, fruits, infusion, syrup

ABSTRACT

The paper presents experimental equipment special designed and made for interdisciplinary research concerning the influence of high pressure and low vacuum process on candy fruits preservation technologies. The research studies concern in infusion speed and infusion quality of the fruits utilizing non-thermal preservation process: high pressure up to 500 bar, alternant with low vacuum up to 0,01 bar processing.The paper presents experimental results proving that the possibility of increasing the infusion speed and the fruit infusion quality depend, beside the fruits characteristics (fruits' size, epidermis' thickness, fruits' texture), on the range values of the high pressure and low vacuum, and on the duration and succession of the process.

INTRODUCTION

In the last 20 years, High pressure processing (HPP) has been explored extensively in food industry and related research institutions due to the increased demand by consumers for improved nutritional and sensory characteristics of food without loss of "fresh" taste. The European "Novel Foods" Directive (1997) has introduced regulatory conditions and slowed the introduction of new pressurized products. After year 2004, the European "Novel Foods" Directive has been reviewed, remaining few limitations for HPP products research development. [1, 2, 3]

In recent years, HPP are extensively used in Japan, US and Europe and a variety of food products like jams and fruit-juices have been processed. Nevertheless, interest in HPP derives from its ability to deliver foods with fresh-like tastes without additives and non thermal processing. After year 2004 in Europe there are more then 120 types of commercial pressurized products (*fruit juice* by Pernod Richard Company, France; *sliced fruits* in France, Spain and Holland; *acidified avocado puree* in Spain, Denmark, Sweden and Germany). [2, 3]

In order to begin high pressure food process research, in University of Craiova, in the Unconventional Technologies and Equipment for Agro-Food Industry Laboratory within Faculty of Horticulture in Craiova, a high pressure equipment was made. This high pressure equipment consist in 0,5 liter vessel capacity; the maximum pressure up to 800bar is reached using a test strength tensile machine, and low pressure up to 500bar can be obtain using a special screw mechanism. The vessel is made in 10TiNiCr180 stainless steel, and consists in a single cylinder sealed by special stainless steel and PTFE gaskets. [5]

In the experimental equipment the high pressure is obtained using the hydrostatic method (isostatic process). The food product is immersed in a quasi-incompressible liquid, which transmits the pressure uniformly without direct contact with the food product.[1,2, 4]

[1] University of Craiova

Using this experimental equipment there were made tests on Ionathan variety apples and cherries with ripening in May from the Oltenia region. The fruits were infused in sugar syrup with 70 % dry soluble mater content using the following work options: fruits infused at atmospheric pressure for 2 hours; fruits infused at atmospheric pressure for 20 hours; fruits infused at a pressure of 600 bar for 10 min; fruits infused at a pressure of 600 bar for 20 min; fruits infused at a pressure of 200 bar for 2 minutes, at 400 bar for 2 minutes and then at 600 bar for 6 minutes After infusion, for each variant it was determined the dry soluble mater content of fruits and syrups. [4]

At 600 bar pressure for 10 min apple processing during it was observed dry soluble mater increasing (about 27%), larger than the increase obtained due to the infusion at the atmospheric pressure for 2 hours (22,2 %). Maintaining the high pressure level and increasing the infusion duration up to 20 min, it was observed the increase of the dry soluble mater content of the apples cubes (about 28%), accompanied by the decreasing of dry soluble mater content of the syrup. An interesting behavior was found at the gradual application of the pressure, in three steps, with maintaining on each step, where, although the total infusion time is of only 10 min, the increase of the dry soluble mater content recorded by the fruits was larger (about 29%) even than the increase obtained at 600 bar for 20 minutes. Apples cubes infused using high pressure were translucent, without meaningful browning, with a good maintaining of the shape and with the typical properties of the candied fruits. [4]

The cherries were high pressure processed in the same variants methods; it was observed important increases of the dry soluble mater content at the high pressure process infusion, but not as important as the apples. Also, at the atmospheric pressure it was observed a lower velocity of cherries infusion than for apples, due to fruits texture, but especially the presence of the epidermis which represents an important barrier for the processes of diffusion and osmosis. [4]

The fruits infusion at high pressure process is based on the duration of the diffusion and osmosis process; between fruits and syrups is realized an osmotic exchange, due to the fruits increase gradually with sugar, in the same time the juice of the fruits dilutes the syrup. The process continues until the syrup and the fruit until the equilibrium state is realized depending on processing parameters. [1, 2, 3, 4]

MATERIAL AND METHOD

In order to determine the influence on fruits infusion under lower pressure process followed by vacuum processing were performed experimental research using strawberry fruits produced in a small farm in Oltenia region. For the strawberries with red pulp, middle firmness, juicy, sweet pleasant taste, dry soluble mater content was determined (12,5 %).

The strawberries fruits have to be infused in sugar syrup with 70 % dry soluble mater content, in a report of 60 g fruits at 150 ml syrup, packed in a plastic bag.

Taking into account the strawberry fruits properties, the plastic bags containing the fruits and the syrup were processed 10min duration under 100,…,500bar in the experimental equipment presented in figure 1. According MAP principles, in order to preserve the initial colour of the strawberry, a 5bar CO_2 infusion in strawberries was obtained connecting the high pressure cylinder with a CO_2 high pressure vessel.

Experimental vacuum equipment was made to determine the syrup infusion in strawberry (figure 2 and 3).This experimental equipment, in main, is composed in a middle vacuum pump, condensed gases dryer with molecular sieve device, and a special stainless steel vessel in which the vacuum process is performed. To observe the inlet process, the stainless steel vessel is covered with a high resistant transparent polycarbonate flange/plate.

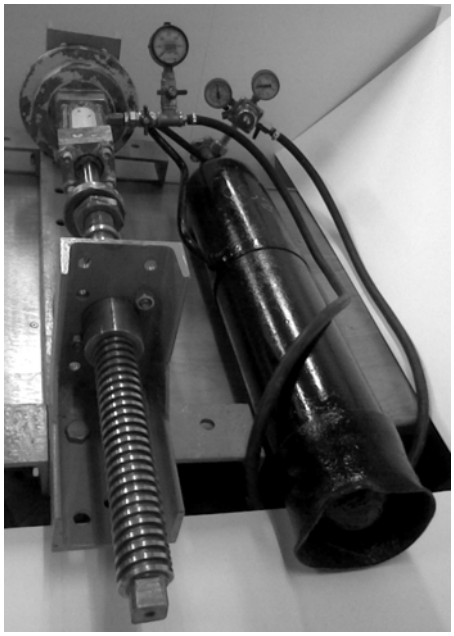

Figure 1. Experimental equipment for high pressure and MAP processing

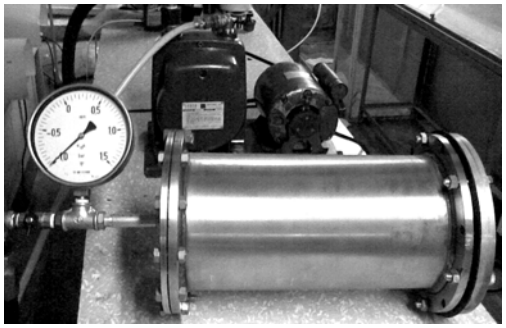

Figure 2. Experimental equipment for low vacuum processing

Figure 3. Stainless steel vessel covered with high resistant transparent polycarbonate flange/plate

In figure 2 is observed the vacuum value (-0,99bar) during the maximum vacuum processing step. In figure 3 is observed the sugar syrup infusion in strawberry due to the osmosis process (fruit cold boiling). The experimental research starts introducing each plastic bag in the experimental equipment presented in figure 1, pressuring 5min at low pressure 5 bar CO_2 (to obtain MAP conditions). Then the plastic bag containing strawberry and sugar syrup is pressured during 5min up to 50bar for a initial syrup infusion under low pressure.

The experimental research continues introducing the plastic bag into the stainless steel vessel vacuum equipment. Then the plastic bag containing strawberry and sugar syrup is gradually vacuum processed during 5min, up to -0,7; -0,8; -0,9; -0,95; -0,99bar vacuum.

In order to put in evidence the quantitative comparisons between the high pressure processing method, and the low pressure processing combined with middle vacuum processing method, respectively, for each experimental variant there were determined the strawberry dry soluble mater after infusion, and the syrup dry soluble mater after infusion.

RESULTS AND DISCUTIONS

The strawberry dry soluble mater and the syrup dry soluble mater before and after the infusion under different high pressure are presented in table 1.

Table 1

Strawberry dry soluble mater and the syrup dry soluble mater before and after the infusion under different high pressure process

High pressure [bar]	Strawberry dry soluble mater, [%]		Dry soluble mater increase [%]	Syrup dry soluble mater, [%]	
	before infusion	after infusion		before infusion	after infusion
100	12,5	23,4	10,9	70	65,1
200		25,5	13,0		64,5
300		28,2	15,7		63,2
400		32,7	20,2		61,4
500		37,3	24,8		59,1

The strawberry dry soluble mater and the syrup dry soluble mater before and after the infusion under different middle vacuum process are presented in table 2.

Table 2

Strawberry dry soluble mater and the syrup dry soluble mater before and after the infusion under different middle vacuum process

Vacuum [bar]	Strawberry dry soluble mater, [%]		Dry soluble mater increase [%]	Syrup dry soluble mater, [%]	
	before infusion	after infusion		before infusion	after infusion
- 0,70	12,5	24,8	12,3	70	65,1
- 0,80		26,9	14,5		64,5
- 0,90		30,3	17,8		63,2
- 0,95		34,2	21,7		61,4
- 0,99		39,7	27,2		59,1

CONCLUSIONS

The syrup infusion in strawberry process under middle vacuum is a faster and economically method then the high pressure processing, and the dry soluble mater has more important values due to vacuum process up to -0,99bar, comparatively with the high pressure up to 500bar process. The strawberry obtained using low vacuum process have red initial colour, freshness test, with translucent clear syrup.

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RESEARCHES REGARDING THE CHANGES OF THE REDOX STATE OF LEMON JUICE AFTER SWEETENING TASK

Petre Savescu[1], Liviu Giurgiulescu[2], Maria Dinu[1]

KEYWORDS: Lemon juice, sweetener, $NAD^+/NADH+H^+$ ratio

ABSTRACT

The work paper is a side of complex study regarding the effects of natural and synthetic edulcorants on the lot of liquid foods. Follow the increased consume for the lemon juice in present time it is necessary to knowing the effects of sweetening task on the consumers' human bodies for prove and promote the best edulcorant. The lemon juice experimental variants were prepared and sweetenered with most used edulcorants for Romania and the changes of the redox state of juice were monitorised. The monitoring can be use for promote the healthy edulcorant and for establish the best time of preserve for this juice.

INTRODUCTION

The exact origin of the lemon has remained a mystery, though it is widely presumed that lemons are wildly grown in both India and China. It is also speculated that lemons were first grown on Mediterranean bushes, coined lemon bushes, but they have evolved and modern-day lemons grow on trees. In South and South East Asia, it was known for its antiseptic properties and it was used as antidote for various poisons. The lemon was later introduced to Iraq and Egypt around 700 A.D. [Wright, A.C.]

The popular drink lemonade may have originated in medieval Egypt. It was distributed widely throughout the Arab world and the Mediterranean region between 1150 A.D. and 1000 A.D. At this time, the lemon was first recorded in literatures to a tenth century Arabic treatise on farming and was used as an ornamental plant in early Islamic gardens. Lemon juice, fresh, canned, concentrated and frozen, or dehydrated and powdered, is primarily used for lemonade, in carbonated beverages, or other drinks [www.limmi.it].

Lemon juice is widely known as a diuretic, antiscorbutic, astringent, and febrifuge. In Italy, the sweetened juice is given to relieve gingivitis, stomatitis, and inflammation of the tongue. [www.middlepath.com.au].Citrus juices (especially lemons juice) are heat-treated to inactivate the endogenous pectin esterase, which would otherwise provide pectic acid which can aggregate and flocculate in the presence of Ca^{2+} ions. However, since heat treatment damages fruit aroma, the use of polygalacturonase is preferred. This enzyme degrades the pectic acid to such an extent that flocculation does not occur in the presence of divalent cations [Belitz E].

Nicotinamide adenine dinucleotide, abbreviated NAD^+, is a coenzyme found in all living cells. The compound is a dinucleotide, since it consists of two nucleotides joined

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through their phosphate groups: with one nucleotide containing an adenosine ring, and the other containing nicotinamide.

In metabolism, NAD^+ is involved in redox reactions, carrying electrons from one reaction to another. The coenzyme is therefore found in two forms in cells: NAD^+ is an oxidizing agent – it accepts electrons from other molecules and becomes reduced, this reaction forms NADH, which can then be used as a reducing agent to donate electrons. These electron transfer reactions are the main function of NAD^+[Dawson MC].

The midpoint potential of the NAD^+/NADH redox pair is −0.32 volts, which makes NADH a strong reducing agent. The reaction is easily reversible, when NADH reduces another molecule and is re-oxidized to NAD^+. This means the coenzyme can continuously cycle between the NAD^+ and NADH forms without being consumed.

After use the natural or synthesis edulcorants the lemon juice composition will be changed and this change can be proved using the UV-Vis spectroscopy (like as cheapest analysis method). The new effects create by the new lemon sweetened juice are today not descript but we can considered that the best edulcorant for lemon juice is the edulcorants that induce the minimally effects than unsweetened lemon juice (prove through the changes of molecular spectra at maximal points for the sweetening variants pertain to simile molecular spectra for unsweetening variant).

MATERIAL AND METHODS

For to quantify the changed juice content of NAD^+ and $NADH+H^+$ on sweating task with natural and synthetic's edulcorants it is constituted nine experimental variants.

From unsweetened variant of natural lemon juice V_0 it is obtained through sweetness task the follow experimental variants:

-V_0 – the unsweetened variants of natural lemon juice,
-V_1 – the sugar sweetened variant of natural lemon juice,
-V_2 – the honey sweetened variant of natural lemon juice,
-V_3 – the saccharine sweetened variant of natural lemon juice,
-V_4 - the natural glucose sweetened variant of natural lemon juice,
-V_5 – the variant of natural lemon juice that has been sweetened with Flix,
-V_6 – the variant of natural lemon juice that has been sweetened with Equal,
-V_7 – the variant of natural lemon juice that has been sweetened with Clio,
-V_8 – the variant of natural lemon juice that has been sweetened with Edulciclam,
-V_9 – the variant of synthesis lemon juice.

The used sugar for V_1 have proved a concentration of 2.50g/50mL natural lemon juice concentration. The sodium saccharine has proved in to V_3 a 25 mg/50mL natural lemon juice and the longer solvated time. The used honey for the V_2 has 4g/50mL and it is from acacia honey type. For the V_4 it was used the pharmaceutical natural glucose (5%) obtained though the separation- concentration task. For to obtain the V_5 was used Flix (one pills was composed by lactose 1mg, saccharine 8mg, aspartame 3mg, excipients E468 and E641).

Equal was a synthetic edulcorants (with aspartame) and was used for V_6. Edulciclam was a synthetic sweetener (sodium cyclamate) and was proved a 25mg/50mL natural lemon juice into V_8.

The synthesis lemon juice was constituted by: citric acid E330, maltodextrine E140, sodium cyclamate E952, sodium saccharine E954, aspartame E951, ascorbic acid E300, tartrazine E102, the lemon flavour, and was constituted the V_9. The „Clio" contain the sodium cyclamate (57.8%), saccharine (15.5%), sodium bicarbonate (13.7%), mono-sodium citric acid (13%) and was used for V_7.The samples were cleaned (for the

interference substances) and were spectrophotometered in the nearly UV ranges. The variations of molecular absorption spectra were recording in report by the wave-length. Then, these molecular absorption spectra were analysed, help by the statistical soft „SPSS for Windows 11.0", the deviation from the base variant, the analysis of the mean square for the obtain data and establish mathematic what is the best sweetening variant for the natural lemon juice.Before the spectrometry task the samples were prepared in the same conditions of temperature, pressure and for spectrometry task it used an digital spectrophotometer UNICAM 2 UV-Vis, with 1cm cuvette broad and the automatically change of deuterium lamp with tungsten lamp at 325nm (this mechanism was set up before analysis).

Both NAD^+ and NADH absorb strongly in the ultraviolet due to the adenine base. The peak absorption of NAD^+ is at a wavelength of 259 nanometers (nm), with an extinction coefficient of 16,900 $M^{-1}cm^{-1}$. NADH also absorbs at higher wavelengths, with a second peak in UV absorption at 339 nm with an extinction coefficient of 6,220 $M^{-1}cm^{-1}$. This difference in the ultraviolet absorption spectra between the oxidized and reduced forms of the coenzymes at higher wavelengths makes it simple to measure the conversion of one to another in enzyme assays – by measuring the amount of UV absorption at 340 nm using a spectrophotometer.[Dawson MC]

For decreasing the limits of errors, the obtained results were replayed in to auto-retracking and save in to files .qnt format and convert with the soft Visio ver.2.0.

RESULTS AND DISCUSSIONS

Result as the analysis were obtained the concentration for NAD^+ and $NADH+H^+$ from lemon juice - experimental variants like as the figures 1 and 2.

The greatest NAD^+ content were registered at the experimental variants of sweetenered lemon juice that use the saccharine (only or in the combination like as Flix and Clio). The experimental variant that use the Clio (saccharine with cyclamate and Natrium bicarbonate) are registered the greatest value of NAD^+ content. The saccharine added in to V3 are influenced the $NADH+H^+$ greatest content (figure1).

The synthetic lemon juice are unrecommended for consummers, the $NAD^+/NADH+H^+$ ratio was greatest, the oxidise status can be installed and the consummer metabolism can be affected (figure 2)[F.N.B.I.M].

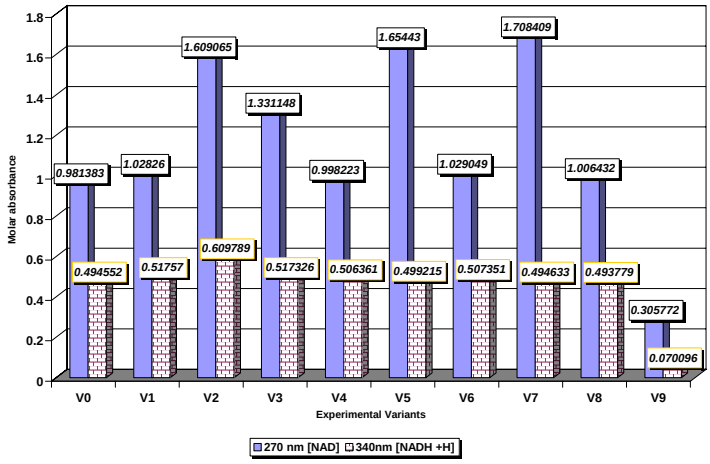

Fig. 1. – The NAD^+ and $NAD+H^+$ content in the experimental variants

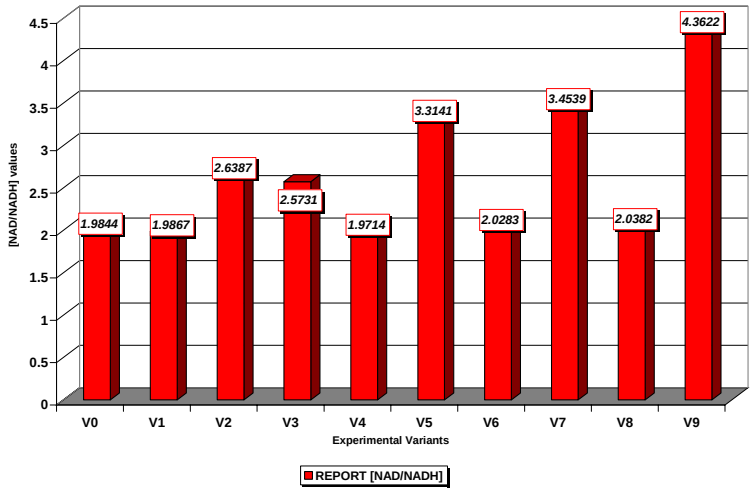

Fig. 2. – The $NAD^+/NADH+H^+$ ratio content in the experimental variants

CONCLUSIONS

- The method of analysis that use the UV VIS spectrometry can be a good and cheaper method of analyse than HPLC methods for determinate the concentration and effect of sweeteners, the UV- VIS optical methods can be used for to determinate the best edulcorants for the natural lemon juice and can be complete with FTIR spectrometry (for analysis the any isomers derivate from compounds of base);

- For the natural lemon juice and for the any thermal solvated conditions *the natural glucose* (V_4) was the best natural edulcorant, the curve of the molecular absorption spectra (especially in to near UV range) for this sweetener has showed the minimum changes from the simile basis curve of the unsweetened natural lemon juice;

- For the people that have some digestive aches or cardiac problems, who cannot use sugar in there consumption, have an alternative with *Edulciclame* (*sodium cyclamate*).

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KEY WORDS: Grapefruits juice, sweetener, $NAD^+/NADH+H^+$ ratio

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INTRODUCTION

The grapefruit juice is very important for the variety chemical composition: lot of hydro soluble vitamins, potassium, phosphorus, 1-p-menthene-8-thiol, nootkatone. Trace of hydrogen sulphide and dimetyl sulphide are present in grapefruit juice and also contribute to their aromas. Grapefruit juice is more different like another citrus juice through the action across the antibiotic compounds, this juice is able to inactivate the active principles of antibiotic and another drugs.

In 1989, a group of Canadian researchers studying a blood pressure drug were astonished to discover that drinking a glass of grapefruit juice dangerously increased the drug's potency [Bakalar, Nicholas].Grapefruit juice, and grapefruit in general, is a potent inhibitor of the Cytochrome-P450 enzyme CYP3A4, which can impact the metabolism of a variety of drugs, increasing their bioavailability[He K; Iyer KR and col.]. In some cases, this can lead to a fatal interaction with drugs like Astemizole. The effect of grapefruit juice with regard to drug absorption was originally discovered in 1989. However, the effect became well-publicized after being responsible for a number of deaths due to overdosing on medication.[Bailey DG and col.]

Recently some researchers have shown that furanocoumarins rather than flavonoids may be the ingredients causing the various drug interactions [Florea T].

Citrus juices are heat-treated to inactivate the endogenous pectin esterase, which would otherwise provide pectic acid which can aggregate and flocculate in the presence of Ca^{2+} ions. However, since heat treatment damages fruit aroma, the use of polygalacturonase is preferred. This enzyme degrades the pectic acid to such an extent that flocculation does not occur in the presence of divalent cations [Belitz E].

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Result as the analysis were obtained the concentration for NAD^+ and $NADH+H^+$ from grapefruit juice - experimental variants like as the figures 1 and 2.

The greatest NAD^+ content were registered at the experimental variants of sweetenered grapefruit juice that use the glucose and the variant that use the saccharine (only or in the combination like as Flix and Clio). The experimental variant that use the Clio (saccharine with cyclamate and Natrium bicarbonate) are registered the greatest value of NAD^+ content. The saccharine added in to V3 are influenced the $NADH+H^+$ greatest content (figure1). The sweetenered grapefruit juice with glucose are unrecommended for consummers, the $NAD^+/NADH+H^+$ ratio was greatest, the oxidise status can be installed and the consummer metabolism can be affected (figure 2).

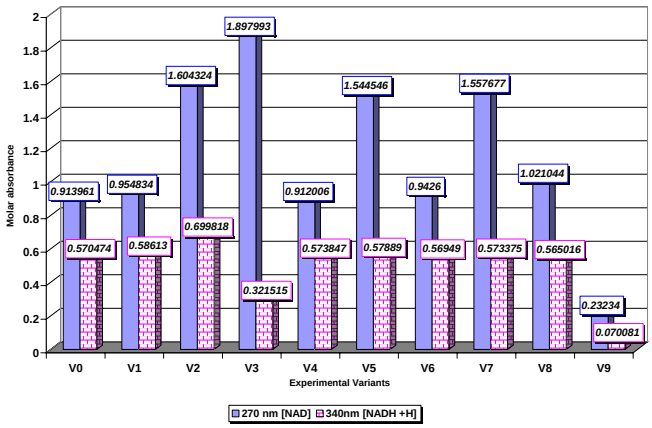
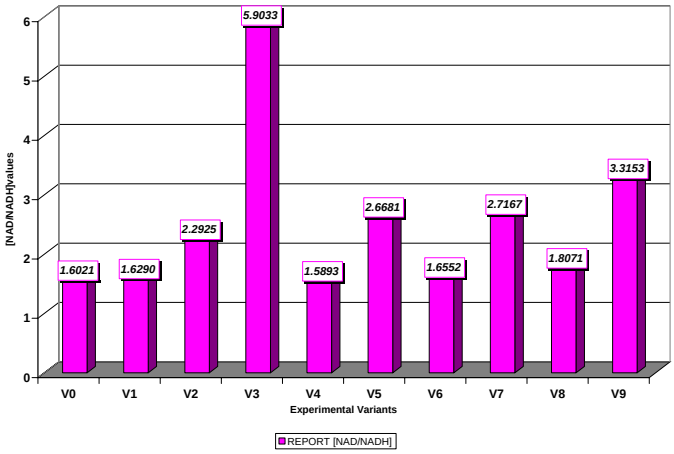


Fig. 1. – The NAD^+ and $NAD+H^+$ content in the experimental variants



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CONCLUSIONS

- The method of analysis that use the UV VIS spectrometry can be a good and cheaper method of analyse than HPLC methods for determinate the concentration and effect of sweeteners, the UV- VIS optical methods can be used for to determinate the best edulcorants for the natural grapefruit juice and can be complete with FTIR spectrometry (for analysis the any isomers derivate from compounds of base);

- For the natural grapefruit juice and for the any thermal solvated conditions *the sugar* (V_2) was the best natural edulcorant, the curve of the molecular absorption spectra (especially in to near UV range) for this sweetener has showed the minimum changes from the simile basis curve of the unsweetened natural grapefruit juice;

- For the people that have some digestive aches or cardiac problems, who cannot use sugar in there consumption, have an alternative with *Edulciclame* (*sodium cyclamate*).

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STUDY MAIN PARAMETERS OF COMPOSITION OF WINE – RAW MATERIAL USE IN VINEGAR INDUSTRY SHOW IN THE QUALITY OF THE FINITE PRODUCT

Felicia Stoica[1], L. Giurgiulescu[2]

KEY WORDS: wine-raw material, quality parameters, vinegar

ABSTRACT

In the context in which consumers are starting to appreciate naturalness and tipicity food should be necessarily a study following scientific objectives: specification in detail the technology to produce the fermentation of vinegar, study wine - the raw material to produce vinegar, optimizing raw materials used in production of vinegar, study aspects of microbiology, biochemical and technological leadership necessary to fermentative process.

INTRODUCTION

The vinegar is defined as a solution of the acetic acid in water. The touring sour of the wines as a process of deterioration is known from the antique. The vinegar or the sour wine is natural products which can be obtained by the action of the acetic bacteria on the wine. In a larger use of the term they are named as *vinegar* all products obtain by the acetic fermentation of drinks or low alcoholic liquids (apple vinegar from cider, malt or beer vinegar, fruit vinegar, rise or sake vinegar) (Stoica Felicia, 2007).

The fermentation/oxidation agents develop quickly a veil on the surface of low alcoholic mediums in not fully barrel. The veil can be more or less compact. Also, they oxidant the ethanol from the medium to acetic acid. To notice that the fermentation is an improper term because the formation of the acetic acid from ethanol is a simple biological process of oxidation. Therefore, the appearance of the vinegar is contemporary with wine's appearance, because soon as the man want to keep the wine, he realize that he turns to sour, the process being main aerobe disorder. They are called as vinegars all products obtained by acetic fermentation of drinks and low alcoholic liquids (apple vinegar, malt vinegar, beer vinegar fruit vinegar) (Lonvaud-Funel A., 1995; Garcia P., Carmen M., 1997).

The making of vinegar is as old as wine technology. The making wine is knew sine 10 centuries ago, so, it can be imagined that the making of the vinegar as a result of acetic fermentation is equally old.

In the Antiquity, was not only a pleasant spice or a conservation agent but it was used as a refreshing, hygienic and very cheep drinks in agriculture, in military campaigns and in see travels (Colțescu H.I. şi colab., 1943).

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In modern ages, the vinegar kept his multiple usages. It remains an excellent conservation for vegetables and fish. The vinegar will be always use as a delicious and nourishing spice for all kind of salads, sauces and different foods.

His acid character and solubility of his salts in water, made possible the removing of fruits spots from fingers, the removing of luster of old stuffs, revigoration of fine texture, the cleaning-up of water stone from glass or porcelain. It can be also used cold in order to clean-up the silver objects (Colțescu H.I. citat de Felicia Stoica, 2007).

MATERIAL AND METHODS

As the consumers began to appreciate the naturalness and the typical of the feeding products it is necessary a study regarding the wine like raw-material for making vinegar and also a study for some micro-biological, biochemical and technological aspects useful for directing of fermentation process.

The experiences were done in the Enology laboratory in 2007-2008. The variants used for this experiment was:

V – wine

V_1 – wine + vinegar

V_2 – wine + ethylic alcohol

V_3 – ethylic alcohol + vinegar

V_4 – wine + ethylic alcohol + vinegar

There were studied physical-chemical characteristics of wines used as raw-material for making vinegar, wines providing Dragasani vineyard.

In order to establish the physical and chemical properties of wine as raw-material, it has been performed next determination: total acidity, volatile acidity, extract, ash, alcoholic concentration and the iron and phenols contents.

RESULTS AND DISCUSSIONS

Results regarding the analyses of wines used for making the vinegar

The results regarding the analyses of wines used for making the vinegar are present in Table 1.

Table 1

The compositional characteristics of the wines used in making vinegar

Sample	The characteristics of compositions						
	Alcohol vol %	Tot. acidity g/l tartaric acid	Volatile acidity g/l ac. acetic	SO_2 free mg/l	SO_2 total mg/l	Extract g/l	Iron mg/l
1.	8,5	4,5	0,97	10,2	30,7	13,8	4,1
2.	9,0	4,3	0,88	8,9	28,2	12,9	4,2
3.	8,9	4,2	1,03	8,9	25,6	13,0	4,1
4.	9,5	4,1	1,10	10,2	29,4	14,5	3,9
5.	8,2	4,4	1,15	7,7	26,9	13,2	4,2
6.	9,3	4,0	1,20	7,6	24,3	14,1	4,1

The six variants used as prime material for making vinegar are white wines obtained from mixtures of white grape varieties for current wines.

It can be noticed that all the wines had low alcohol contents about 8,5 % vol. to 9,5 % vol. (figure 1.); so, they are low alcoholic.

From figure 2 it can be noticed that the total acidity is about 4,1 g/l tartaric acid and volatile acidity is at the maximal limit even over it like in sample 6.

Figure 1

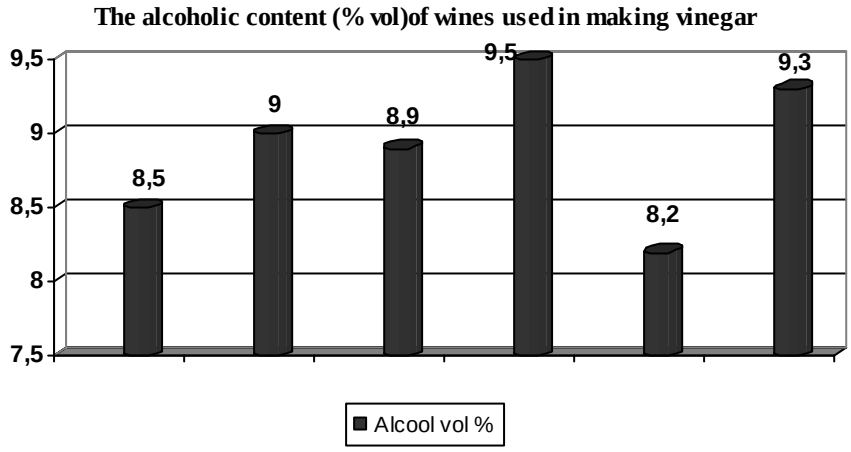
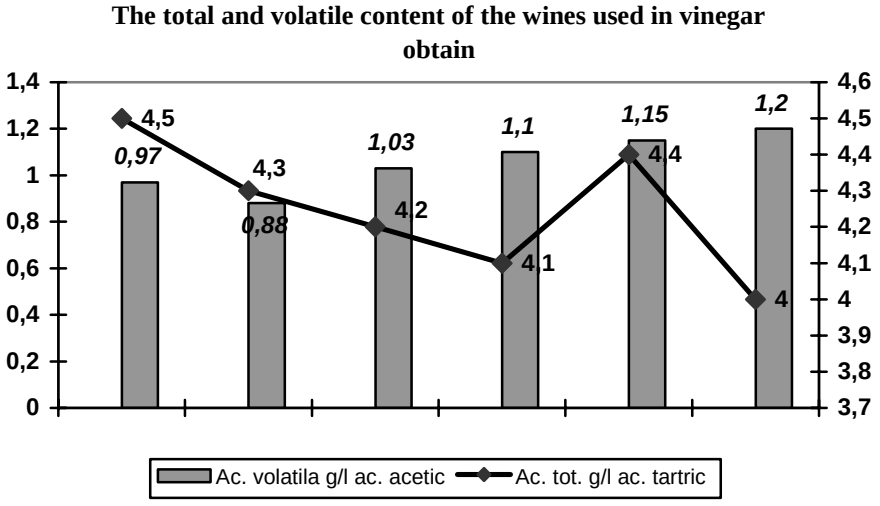
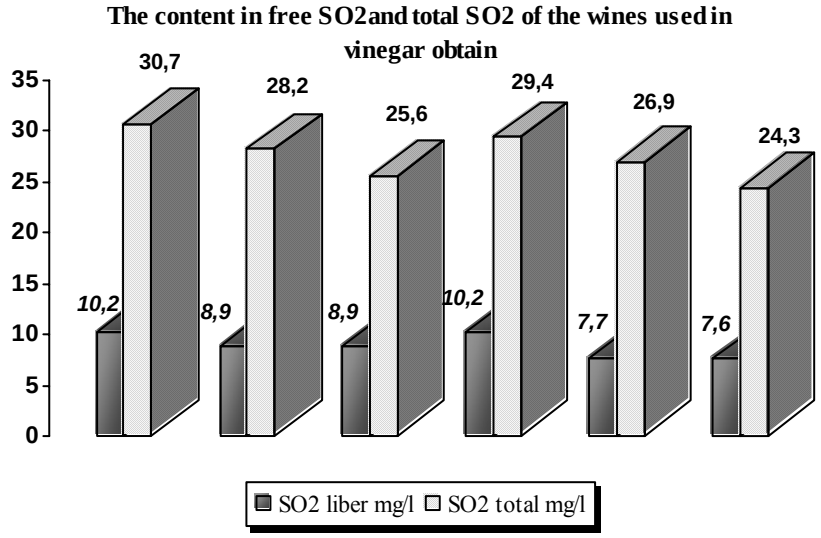


Figure 2



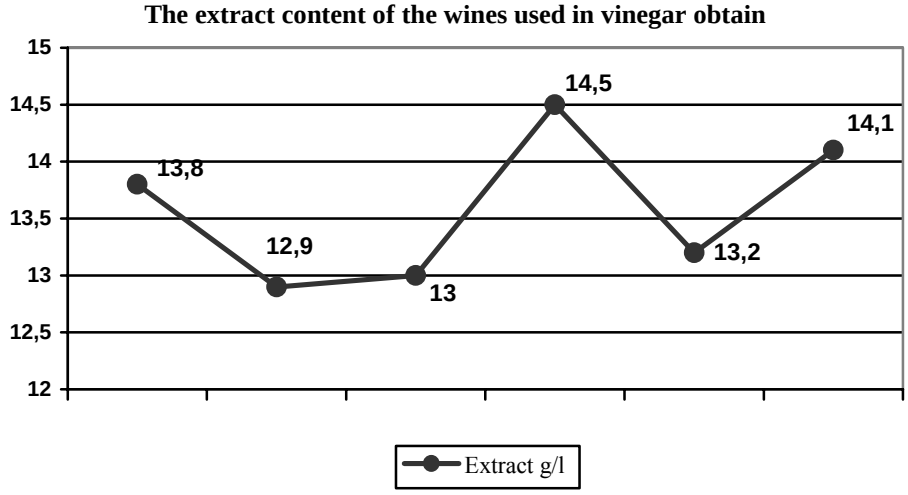
Regarding the SO_2 content, free and total, it can be observed from figure no. 3 that all samples are low, which means that the development of acetic bacteria is not affected.

Figure 3



From point of view of extractivity (figure 4) it can be all samples with low extract (12,9-14,5 g/l) and iron content is in admissible limits.

Figure 4



As a result of those determinations, it can be noticed that all samples match regarding the quality of the wines as raw-material for making the vinegar.

Results regarding the analyze of the vinegar

The experimental samples was 5, one of them is control samples (V). The samples are different according the contained fermentation medium. The experiment made

in equal samples as 1 liter volume. Therefore, the sample V contained 1 liter wine; sample V_1 contained ½ l wine + ½ l vinegar; V_2 = ½ l wine + ½ l alcohol; V_3 = ½ l alcohol + ½ l vinegar and sample V_4 = ⅓ l wine + ⅓ l alcohol + ⅓ l vinegar.

The results regarding the main characteristic of the vinegar obtained in this experiment are in Table 2.

Table 2

The dynamic of ethanol oxidation in different mediums of oxidation

Sample	Acidity, acetic degree						
	initial	3 days	8 days	11 days	14 days	17 days	20 days
V	3,0	3,04	4,82	5,26	6,18	6,92	7,6
V_1	2,80	3,06	5,4	6,72	7,68	7,8	8,08
V_2	2,80	3,10	3,90	4,43	5,80	6,74	7,2
V_3	3,0	3,2	3,96	5,52	6,80	7,38	8,22
V_4	2,82	3,12	5,74	7,56	8,82	8,82	9,02

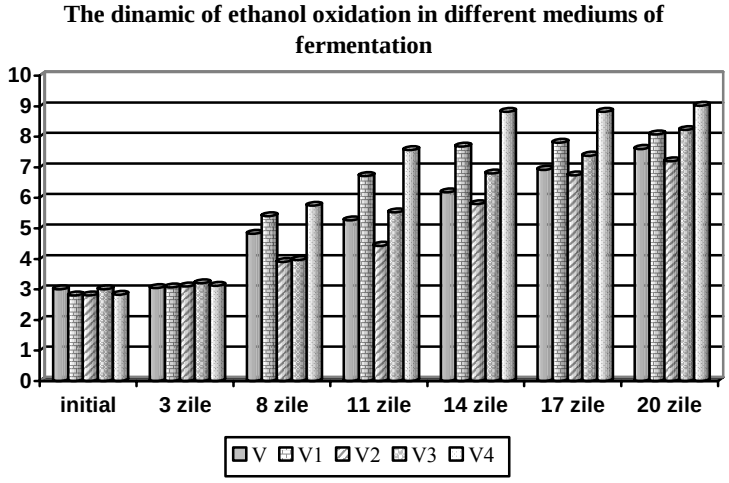
By studying those dates, it can be told that the sample which contained in their fermentation medium ethylic alcohol, except V_4, the evolution of alcohol transformation by acetic bacteria is slower. It increase to the end of the period till 7,2-7,8 g/l.

The lower acidity content was obtained at the sample V_2 with wine and ethylic alcohol followed by the control variant with only wine which acidity was over 8 acetic degrees.

Between variants V_1 and V_3, both with vinegar are not big differences.

The best acidity, comparing the approved values was the last variant, by 9,02 acetic degrees.

Figure 5



CONCLUSIONS

After this study it can be concluded:

- The physical-chemical parameters of composition which characterized the wines prime material correspond in totality to the requirements of the technology for making of good quality vinegar.
- The best results from the point of view of the principal quality parameter of the vinegar – acidity – have been revealed at the variants which have in their fermentative medium vinegar.
- The vinegar obtained from wine, wine and vinegar is clear, with no shine, no colored or lightly yellow to brown, with nice smell, lightly esterificated with a characteristic sour taste.
- The vinegar obtained from wine and ethylic alcohol is clear or opalescent, yellow, with a nice smell, aromatic, with a sour prickling taste.
- The vinegar obtained from ethylic alcohol is clear, no shined, no colored or lightly yellow, with a prickling, characteristic smell, not aromatic.

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THE STUDY OF THE TECHNOLOGICAL QUALITY OF THE BREED OF TOBACCO DJEBEL GROWN IN OLTENIA REGION

Capruciu Ramona[1]

KEY WORDS: tobacco, strips, physical and chemical characteristics

SUMMARY

In Romania there are soil and climate conditions appropriate for the grow of the tobacco and a rather long tradition in growing some breeds of tobacco: oriental, semioriental, Virginia and Burley in particular used as raw material for the manufacture of the cigarettes.

The Djebel breed used for an experiment in this study belongs to the type of oriental tobacco, with good results for the crop from the central area of Oltenia, upon the less fertile breeds (alluvions, sands) from Jiu Valley and in the hills area from western Olt being chosen for an experiment due to his particular resistance to drought, the manna of the tobacco (Peronospora tabacina) and the black rottening of the roots (Thillaariopsis basicola).

The crop was first grown during the year 2007, on a slanting land, with semipermeable soil, in Şimnicul de Sus region.

INTRODUCTION

The tobbaco crops in Oltenia are of high quality, representing a real alternative for the diversification of the agricultural crops in the regions with low-nutrient soils and also can cover in a high percentage the necessary of raw material for the tobacco industry in Romania.

The results of the scientific reasearch conducted in time in Romania (L.S.Muntean and colab.- 2001) and in the Oltenia region (I.Matei şi col.-1983, Aniţia N. şi col-1974, Giurgiulescu L.- 2002, etc) is the most frequently planted type of tobacco plants in the pedo-climatic conditions of the area is Virginia and Djebel.

Besides the manufacturing of the cigarettes, from tobacco leaves it is extracted the nicotinic acid (provitamin PP) used in the pharmaceutical industry, as well as the acetic acid. Also, the tobacco seeds contain 35-40 % oil used in food industry and the industry of paint manufacture.

In spite of the campaign against smoking, the production of tobacco on the global scale increased in the last years and important raises are foreseen also in the following period, both due to the growth of the surfaces that are going to be cultivated with this plant, and most of all by increasing the production for the surface unit (Aniţia N. and col. 1974).

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MATERIAL AND METHOD

The vegetal material (the strips) submitted to analysis was obtained from the seeds of the Djebel breed, germinated in specific conditions of humidity and temperature with biological purity of 97 %. Leaves taken as samples have been aligned and submitted to the fermentation process and then to drying by natural method – sun drying. The stripping process consists of removing the tobacco ribs from the foliar limb by obtaining the parts of the leaves that were submitted to the analysis.

From the strips there were determined within the Research Base with Multiple Users - University of Craiova, the physical and chemical characteristics of the analysed soil according to the effective methodology, as well as Smuck quality index.

The determination of the soluble hydrates of carbon was made by the manganometric method (Bertrand) with the aid of Fehling II solution.

In order to easier express the qualitative value of Djebel breed, we counted the Smuck quality index as beeing the rapport between the soluble hydrates of carbon and the albuminoid substances.

OBSERVATIONS AND DETERMINATIONS

The physical and chemical characteristics of tobacco are influenced a lot by the growing conditions of the plant, the fertilization level and chemical structure of the soil, the humidity conditions, the position of the leaflets on the plant (the leaflets floor) and the breed, type and quality of the tobacco.

During the graining period of the seedling, there were noticed three phases and namely:

- the germination phase under the previously mentioned conditions (the seeds have germinated in 4 days). It must be mentioned that the germination percent of the seeds was of 90 %.
- the seedling phase with the appearance of the plantlets. After the germination, the seeds were seeded in hotbeds, for which we used a high quality nutritive mixture (manure, garden soil and sand in proportion of 1:2:1).

Following the insurance of the corresponding nutritive layer according to the humidity conditions and temperature between 25-29 ºC, the plantletes appeared after 5 days.

- the hotbed phase, when the plantlet has 5-6 leaves on average, a length of the stem of 8-9 cm, the radicular system well developed, in fascicle.

When the first leaves appeared (the cross phase – according to Aniția N. -1974) in the hotbeds we made measurements upon the roots in order to determine the rooting level.

It must be mentioned that for a better rooting we used the chemical complex NPK.

For the plantation it was used on average, seedling with stem of 7,5 cm lenght, root of 10,9 cm and the majority of the plants with 6 formed leaves (table 1).

The field used for starting the crop is a slanting field with a southern panorama, semipermeable, provided with an irrigation system with water dropping.

The plantation was made manually on a field preliminarily prepared (tilled, leveled, preemergent extinction of verminous plants with Goal 3,5 l/ha), using as planting sizes 25 cm between the plants and 50 cm between the lines. For the plantation, there were used 5 l water/plant. By making this system, we followed up the formation of a larger density for the plantation in order to increase the quality of the tobacco.

Table 1

The forming of the roots for the Djebel breed

Characteristics	**Sizes (cm)**
The emergence of the plantlet	3,6
The forming of the cross	6,8
The formed cross	7,6
The emergence of the first leaves over the cross	8,3
Semi-formed hotbed	9,6
Formed hotbed	10,9

Due to the restrictive minimum temperatures of the tobacco plantation registered in March 2007 (0,0ºC on the 09th of March 2007, with the maintenance of the low level for several days) the plantation was postponed until April (15th of March 2007) on the background of a minimum temperatures of over 5,5º C and with a maximum monthly temperature of 21ºC. Although the maximum and minimum temperatures registered were optimal for the seedling process, the relative lower humidity of the air (46 %) correlated with the total absence of rainfalls led to a percent of 80% in the seedling process. It must be mentioned that in this period it was used the irrigation system with water dropping.

The maintenance works consisted in making the hoeing (3), preemergent extinction of verminous plants with Galant Super 1,5 l/ha, cutting the top of young sprouts, pulling out the shoots and irrigating (2 times/week with 300 m^3 water/ha after the plantation and 400 m^3 water/ha after the blooming).

The tests were made on strips obtained from the leaves of the Djebel breed positioned on the middle floor, reaching at the same time the technological maturity. We made the qualitative analysis of the soil by physical and chemical determinations of the middle leaves sampled during July and August. (table 2 and 3).

Table 2

Physical characteristics of the Djebel breed

Breed	Characteristics	01.VII.07	15.VII.07	01.VIII.07	15.VIII.07
Djebel	Foliar surface (cm)	22	26	30	30
	Color	Intense green	Green	Green yellow	Yellow green
	Main rib %	20,8	16,6	13,4	12,5
	The weight of the strips (g)	1,43	0,91	0,41	0,32
	Volumetric weight (g/cm^3)	0,9914	0,8163	0,2432	0,2011

Chemical composition of the tobacco is very much influenced by the growth conditions of the plant, degree of fertility and chemical composition of the soil, humidity conditions, the position of the leaves on the plant (leaves level), by the soil, tobacco type and quality.

Table 3

Chemical characteristics of the Djebel breed

Breed	Characteristics		01.VII.07	15.VII.07	01.VIII.07	15.VIII.07
Djebel	Soluble hydrates of carbon (%)		18,5	16,3	11,4	9,3
	Nicotine (%)	Base	1,20	1,28	0,96	0,48
		Middle	1,82	1,65	0,86	0,36
		Top	0,71	0,70	0,68	0,63
	Albumen (%)		7,21	6,91	6,28	5,16
	N total (%)		2,53	2,48	1,77	1,20
	Smuck coefficient		-	-	-	1,8

By burning, sugars in normal quantities form acid products which soften the smoke and make it more pleasant (sweetish).

During the growing phase, the content in the soluble hydrates of carbon is high (18,5 %) - table 3, and when reaching the technological maturity it was of 9,3 %, being able to form by burning acid products which make the smoke smoother and likable (sweetish).

The nicotine registered a lighter growing for the lower positioned leaves, until the middle of July, after which it suddenly dropped until the middle of August (0,48 %). The middle leaves registered a steady drop from 82 % on the 01 VII 2007 to 0,36 % on the 15 VII 2007, being in accordance with the regulations from the point of view of the content in the nicotine. (diagram 1).

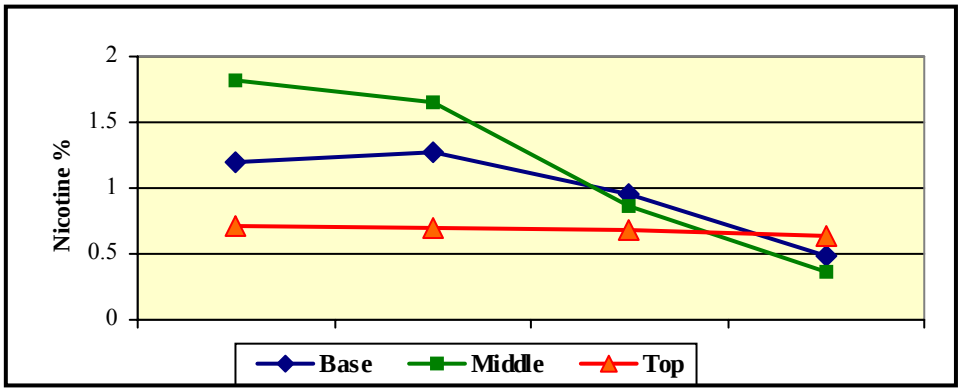

Diagram 1. The evolution of the nicotine (%) during the brooming process of the Djebel breed

The negative action of the albumens upon the quality of tobacco consists in a spicy and sour taste and a displeasing smell of burned feathers. This aspect wasn't registered in this study, the content of albumen decreasing constantly during the period of physiological brooming.

Following the determination of the Smuck coefficient, it is noticed the achievement of an unfermented end product of medium quality.

The chemical characteristics of the analyzed tobacco leaves were largely influenced by the climatic conditions of the year 2007. Therefore, from the climatic point of view, May and June were favorable for the development of tobacco plants through the maximum registered temperatures (< 34,5 ºC, with an absolute maximum temperature on the 26th VII of 38,6º C and a glowing period of the sun of 304,3 hours/month) and restrictives from the minimum registered temperatures point of view (monthly average of 16,24 ºC), lower quantitative rainfalls and a relative humidity of the air with values below 50 % in the second part of June.

The maximum temperatures registered in July (42,6ºC – the absolute temperature), the higher minimum temperatures, the relative humidity of the air, the lower rainfalls and an impressive glowing period of the sun (347,1 hours/month) lead to the early brooming of the leaves with significant diminution of some chemical compounds (soluble hydrates of carbon – table 3). These restrictions imposed the irrigation, the process maintaining the ordinary limits of the high class tobacco from the point of view of the content in nicotine and albumen.

CONCLUSIONS

The determination of the rooting level of the plantlets in the hotbeds during different stages of evolution led to the establishment of the optimum planting moment of 80 % in the seedling process of the plants.

The plantation in a slanting field with a southern panorama, the selection of a breed resistant to drought, to a disease and verminous attack, the use of the irrigation system, of the fertilizers and providing on time the maintenance works led to the achievement of an appreciable quality in conditions of stress imposed by the clime.

The accomplishment of a larger density on planting led to the achievement of a lighter color of the tobacco leaves, with a fine, silky tissue, and a normal content in nicotine and albumen. Djebel tobacco dried in the sun have a high content of carbon hidrates (18,5%).

The vegetation period diminished and the ripeness process of the leaves was made evenly, allowing the echeloned reaping.

Under the conditions of thermical and hydrical stress of the year 2007, the tobacco leaves harvested at the technological maturity were introduced in the IInd class of quality after the careful analysis of the state standard parameters (foliar surface, weight, color, consistency, flavor) being able to give good results in making mixtures for different brands.

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THE INFLUENCE OF THE OXID-REDUCING POTENTIAL OF THE OLD RED WINES UPON THEIR QUALITY

Valentina Danisor[1], Maria Avramescu[2]

KEY WORDS: oxid-reducing potential, red wine, color, total polyphenols

ABSTRACT

A great interest in knowing the oxidation processes that take place and directly influence their polyphenol content, and as a consequence their quality, is represented by determining the oxid-reducing potential of the red wines kept to get older into wood vessels of different volumes. The phenol composition of the wines influences the polymer pigments formation which contribute at maintaining the pleasant color of the red wines kept to get older.

In this work we present the way in which develops the redox potential of wines kept to get older in vessels made of oak wood, of different volumes, as well as the wines kept in vessels made of glass.

INTRODUCTION

Making wine is itself a biotechnological process where the enzymes play a determinant role and where using the industrial preparations based on enzymes allow substituting the absence or lack of the natural enzymes activity. The preparations based on enzymes represent a new performant way which use is based on a technology well adapted to the noble prime material that is the grape.

At the moment, the use of enzymes in oenology (pectolitical enzymes) contributes to improve the quality of the red wines by a reduced content of aggressive tannin (astringent) and a more stable color.

In the process of making wine the oxygen and the oxid-reducing potential are two important elements both in conducting the prefermentation and fermentation step and in

In this work we present the results obtained by analyzing some red wines kept for three years in vessels made of oak wood, of different volumes, in the wine-cellars of I.C.D.V.V. Valea Calugareasca, as well as some red wines that have been obtained by using pectolitical enzymes and kept for one year in bottles at the same temperature and humidity conditions.

[1] SNP Petrom Doljchim Plant Craiova
[2] I.C.D.V.V. Valea Calugareasca

MATERIAL AND METHODS

In order to establish the relation between the level of wine oxidation and the changes that take place during the wine being kept, they have been studied twelve red old wines, kept in
vessels made of oak wood for three years, of different volumes (table 1) and twelve red wines kept in bottles for one year. All the wines belonged to Merlot variety and had been obtained by the classical process of making wine, in wood baths, with or without using the preparations based on enzymes.

The determination of the features of the physic-chemical composition have been made after the standard methods STAS (SR) and the analysis of the phenol composition has been made using the methods described by Glories (1978). The redox potential has been measured by combined electrodes (Pt electrode – the measure electrode) and a reference electrode (Ag electrode) and it was expressed by the physic unit E_H (mV). Of the natural constituents of the wines, the phenols are responsible for the chromatic and organoleptical features.

The chromatic features of the wines are defined by intensity and nuance.

● **The intensity** represents the sum of the absorbing at 420, 520 and 620 nm. The readings are made at these wave lengths because the color of a wine is determined by the red (520 nm), yellow (420 nm) and violet composed (620 nm). These absorbing are also an index of the age of the wine. Thus a young wine is characterized by the richness in red and violet composed; while getting older the color becomes red-brick-colored. The very old wines are rich in yellow and brown pigments.

● **The nuance** represents the division of the absorbing to 420 and 520 nm.

While getting mature and older, the phenol composed of the wines develop complex degradation processes, the oxidation and the condense having an influence upon the quality of the wines.

Table 1

No. of the wine tasting	No. vessel/volume (l)
1	H4-600/2431
2	H4-601/2655
3	H4-602/2461
4	H4-604/2300
5	H4-605/2241
6	H4-592/2840
7	H4-589/2190
8	H4-584/1815
9	H5-551/2040
10	H5-554/2110
11	H6-523/2780
12	H6-521/2900

The volume of the vessels made of oak wood where the wine have been kept to getting older

The twelve wines kept in bottles for one year, at the same conditions of temperature and humidity as the wines kept in wood vessels were the following:

1. Basic wine - without SO_2 and without pectolitical enzymes

2. Wine - 60 mg/l SO_2, without pectolitical enzymes
3. Wine – 60 mg/l SO_2 + pectolitical enzymes
4. Wine – 60 mg/l + pectolitical enzymes (Ultrazim)
5. Wine – 90 mg/l SO_2 + pectolitical enzymes (Vinozym)
6. Wine – 90 mg/l SO_2 + pectolitical enzymes (Ultrazym)
7. Wine – 90 mg/l SO_2 without pectolitical enzymes
8. Wine – 120 mg/l SO_2+ pectolitical enzymes (Vinozym)
9. Wine – 120 mg/l SO_2 + pectolitical enzymes (Ultrazym)
10. Wine - higher ph (tartric acid added) + pectolitical enzymes (Vinozym)
11. Wine - 60 mg/l + pectolitical enzymes (Vinozym), less time to process
12. Wine – 60 mg/l + pectolitical enzymes (Vinozym), high time to process

RESULTS AND DISCUSSIONS

The oxid-reducing potential of the wines kept to get older in wood vessels

The evolution of the wines kept in wood vessels was first of all influenced by their initial composition but also by the volume of the vessel. In small volume vessels the oxygen got in through the wood stave and easier got into the wine mass comparing to the high volume vessels.

From the fig. 1 it results that regarding the initial determinations, the values EH after three years were smaller for all the wines kept in wood vessels. The highest value of EH (305mV) was registered for the wine sample No. 8 which was kept in the vessel with the smallest volume (1815 l). The greatest potential values were registered for the wine samples No.7 and No. 9 (282 mV and 285mV), which were kept in vessels of volumes close to that of the vessel where the wine for the sample No. 8 was kept. With the other vessels of higher volumes (the samples No.1-5) there haven't been registered big differences of the value EH.

The evolution of the tannin from these wines was underlined by the changes that determined the decrease of the color intensity of the wines and the increase of their nuance (due to the formation of some new more stable pigments) as well as the decrease of the astringent feature of the tannin, being organoleptically underlined.

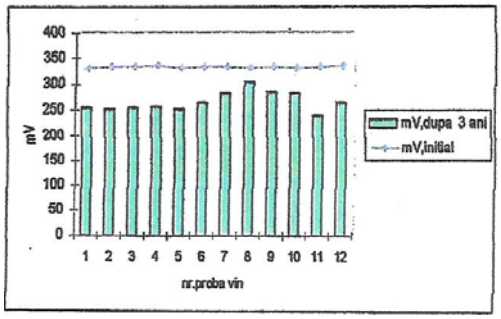

Fig.1. The evolution of the oxid-reducing potential of the wines kept in wood vessels

The higher increase of the nuance (by 0,199 comparing to the initial moment) as it can also be seen in fig. 2 was registered in the wine sample No. 8 which also had the highest oxid-reducing potential.

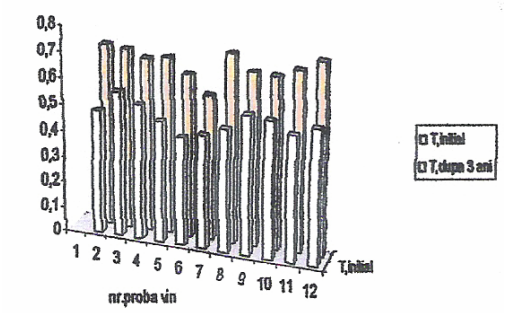

Fig. 2. The increase of the nuance of the wines kept in wood vessels

The total polyphenol content got also lower comparing to the initial content of the wine, even if the wood vessel brought some polyphenol content. The decrease registered content was different from a wine to an other, as it could be noticed from fig. 3. The wines that had a lower total polyphenol content (sample No. 8) have also had a higher oxid-reducing potential.

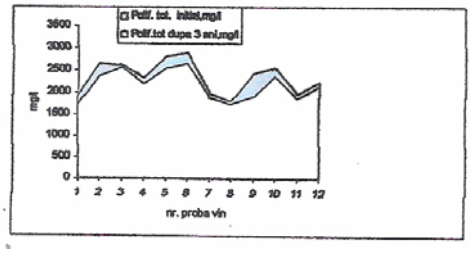

Fig. 3. The evolution of the total polyphenol content of the wines kept to get older in wood vessels for 3 years

About the tannin polyphenol, it has been noticed the same decrease of their content after keeping the wine in the vessel for 3 years (fig.4)

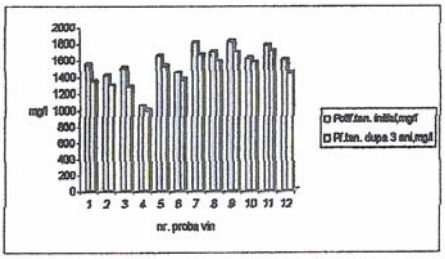

Fig. 4. The tannin polyphenol content of the wines kept to get older in wood vessels for three years

The oxid-reducing potential of the bottled wines after one year

The evolution of the oxid-reducing potential was different at the bottled wines from the wines kept in wood vessels. The differences that have appeared in analyzing the

bottled wines, after one year, depended on the initial composition of each wine, being influenced by the process of producing the wine.

The changes suffered by the phenol composed and the color of the red wines kept in glass bottle were less underlined as in the case of keeping it in wood vessels. The wines kept in bottles remained younger, fresh and comparing them to the basic wine (the wine before being bottled) have registered, after one year, a decrease of E_H due to the oxygen consume from the open space of the bottle and the entering oxygen while being bottled. For all the twelve samples the decrease of E_H values were more or less significant depending on the type of the wine.

The wide range of factors depending on the different composition of the twelve wines obtained through diverse technological operations have made the E_H value to oscillate between the limits until the 22mV.

Thus the sample No. 7 has registered the biggest decrease of the oxid-reducing potential, that of 22mV; the same wine has also had a lower total polyphenol content (fig.5 and 6).

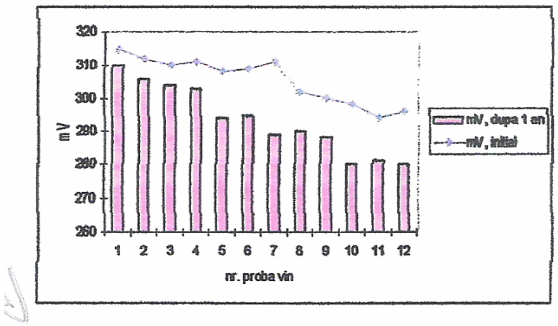

Fig. 5. The evolution of the oxid-reducing potential of the bottled wines after one year

It has been noticed that the lowest decrease of the content by only 6,4 mg/l was registered with the sample No. 11 while with the sample No. 9 it was registered the highest decrease of the content by 21,3 mg/l.

The organoleptical analysis of these wines confirmed the results of the chemical determinations, all the wines being considered as being young and fresh.

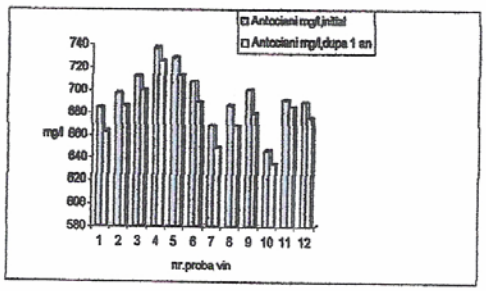

Fig. 6. The evolution of the content of the bottled wines after one year

CONCLUSIONS

- The oxid-reducing potential has a different evolution during the getting old process of the wines depending on the type of the recipient where it is kept.
- Getting older in the bottles, the wines have a lower oxid-reducing potential than by getting older in wood vessels.
- The value of the oxid-reducing potential has to constitute a reference element together with the other physic-chemical features, important for qualifying the quality of a wine.

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THE COMPARATIVE STUDY OF SOME EVALUATIVE METHODS OF THE ASTRINGENT FEATURE OF THE RED WINES

Valentina Danisor[1], Maria Avramescu[2]

KEY WORDS: astringency, red wine, tannin

ABSTRACT

One of the most important sensory attributes of a red wine is the astringent feature, that in most of the cases is estimated by tasting the product. The esteem has to be done by a group of expert tasters and it is not always objective.

There is the possibility to make some esteems,by determining the "Index of the gelatin", (Glories, 1978) or by using an other new method, elaborated by a group of scientists from Spain (Llaudy and the collaborators 2003).

In this work we present by comparison, the results obtained by using three methods of evaluation of the astringent characteristics of some red wines from the 2002 harvest, obtained by the treatment with enzymes at I.C.D.V.V., Valea Calugareasca.

INTRODUCTION

The present oenolog has well defined technologies and modern practices, which allow him to elaborate wines with organolepctical features wanted by him or by the consumer. The astringency of the young red wines is not always a well-appreciated feature, specially by the wine consumer who doesn't know very well the changes that take place during wine maturing and getting older process.

To evaluate the astringency of the red wines obtained by the experiments with enzymes preparations from the autumn 2002 at I.C.D.V.V. Valea Calagureasca, we have made a comparative study of the results obtained through sensory analysis and through two chemical methods.

In order to evaluate the level of their astringency after the methods described by Glories ("the index of gelatin", 1978) and Llaudy and the collaborators (2003), there have been chosen and analyzed twelve red wines from the twenty-eight experimental varieties obtained with different enzymes preparations at different work conditions.

At the same time with the lab analysis, a group of five expert authorized tasters have done the organoleptical evaluation of the wines. The total polyphenol concentration of the studied wines was determined as the Index of the total polyphenol (Ipf) by measuring the absorbing waves of the diluted wines 1/100 at 280 nm.

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[2] I.C.D.V.V. Valea Calugareasca

MATERIAL AND METHODS

The twelve red analyzed wines were that from Cabernet Sauvignon variety and were obtained through the classical process of fermentation of the grapes in metal rotative vessels by the help of the enzyme preparations, with different contact time depending on the temperature. The analyzed wine samples were the following:

1. Basic wine – without SO_2 and without pectolitical enzymes
2. Wine – 60mg/l SO_2 without pectolitical enzymes
3. Wine – 60 mg/l SO_2 with pectolitical enzymes (Vinozym)
4. Wine – 60 mg/l SO_2 with pectolitical enzymes (Ultrazym)
5. Wine – 90 mg/l SO_2 with pectolitical enzymes (Vinozym)
6. Wine – 90 mg/l SO_2 with pectolitical enzymes (Ultrazym)
7. Wine – 90 mg/l SO_2 without pectolitical enzymes
8. Wine - 120 mg/l SO_2 with pectolitical enzymes (Vinozym)
9. Wine – 120 mg/l SO_2 with pectolitical enzymes (Ultrazym)
10. Wine – lower ph (Ca carbonate added) with pectolitical enzymes (Vinozym)
11. Wine – 60 mg/l with pectolitical enzymes (Vinozym) with short time fermentation
12. Wine – 60 mg/l with pectolitical enzymes (Vinozym) long term fermentation

The obtained wine have been analyzed and submitted to some conditioning and stabilizing operations and surveyed during the maturation process for 1 year.

THE DETERMINATION OF THE "INDEX OF GELATIN"

As it is known the tannins have the property to react with the proteins forming stable combinations; Glories (1978) applied this property for determining the "index of gelatin ".

Work method: in 50 ml of wine there have been added 5 ml from a solution of "solved gelatin" of 70 g/l; this corresponds to a concentration of 7 g gelatin in a litre of wine. It has been kept for three days at 10^0 C for completing the tannin reaction with the gelatin and then used with the centrifuge for 10 min at 3500 rpm and it was determined the content of tannins. The tannins were determined in the diluted wine 1/50 through the acid dipolymeric reaction and by measuring the red color at wave length of 550 mm obtained due to the formation of the composed (Vivas and the collaborators, 2003). There have been determined the tannins from the basic wine (to 50 ml of wine there have been added 5 ml of distilled water and was diluted 1/50).

The aggressive tannins have been calculated by the difference from the total concentration in the tannins and the tannins remained after deposing the gelatin.

So the index of the deposed tannins with gelatin or the "index of gelatin" is given by: $I = [(C_0 – C)/ C_0]*100$ where C_0 represents the concentration (g/l) of the tannins from the basic wine and C represents the concentration (g/l) of the tannins after the reaction with gelatin.

The "index of gelatin" can be seen as a reflection of the astringent feature of the wine and when it is higher than 50, the wine can be considered rough, astringent.

THE METHOD WITH THE OVALBUMIN (Llaudy and the collaborators 2003)

In the method elaborated by Llaudy and the collaborators there have been used ovalbumin solutions (with concentration between 0,0 and 8,0 g/l) and tannic acid (with

concentration between 0,0 and 1,0 g/l) which had been prepared in a synthetic wine solution (4 g/l of tartric acid. 95 g/l ethanol, adjusted at ph 3,5 with NaOH). The solutions of tannic acid (0; 0,2; 0,4; 0,6; 0,8 and 1 g/l) have been used as standard. As protein for deposing the astringent tannins, it has been used the ovalbumin solution of different concentrations 0; 0,4; 0,8; 1,6; 2,4; 3,2; 4,0; 4,8; 5,6; 6,4; 7,2 and 8,0. There have been taken twelve test tubes where have been put the ovalbumin solution (0,0 and 8,0 g/l). In each test tube has been added the tannic acid solution or the analyzed wine. After shaking and letting it for 10 min, it has been used the centrifuge and the solution was diluted 1/50 with distilled water. Then it was measured the absorbing wave length at 280 nm in a quartz bath with the optical stratum width of 1 cm.

THE SENSORY ANALYSIS

As a result of the sensory analysis made by the five experts in testing the wines from I.C.D.V.V. Valea Calugareasca, the astringency of each wine was marked with values from 1 to 100 points.

All the data were statistically expressed by an average of three repeated tests.

THE RESULTS AND DISCUSSIONS

The obtained results by the two chemical methods were compared to those resulted after the organoleptical determination.

THE METHOD OF DETERMINING THE "INDEX OF GELATIN" "GLORIES"

From fig.1 it can be seen that the sample with the most astringent feature of the wine, regarding the value of the "index of gelatin" and the organoleptic determination, was sample No.12 and the sample with the less important astringency of the wine was sample No.1. There have been noticed significant differences in evaluating the astringency of the wines using the two methods with the samples No. 7, 10 and 11. So the sample No.7 seems less astringent than it is indicated by the "index of gelatin" and sample No. 11seems to be the most astringent sample.

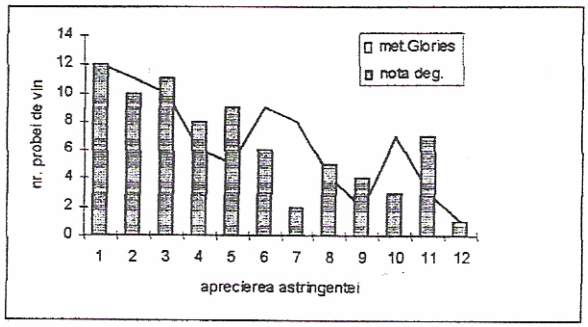

Fig. 1 The estimation of the astringency using the method of the "index of gelatin" (Glories, 1978) and the mark after tasting

THE METHOD WITH OVALBUMIN

Following the results presented in fig. 2, it has been noticed as above, that the sample No. 12 took the first place, as being the most astringent sample, and the sample No 1 took the last position, as being the less astringent sample. This time the differences

between the organoleptical determination and the determination of the tannins after ovalbumin deposing were less significant than presented above. A close difference has been noticed with the sample No. 11 between the organoleptical determination and the tannin determination after gelatin or ovalbumin deposing.

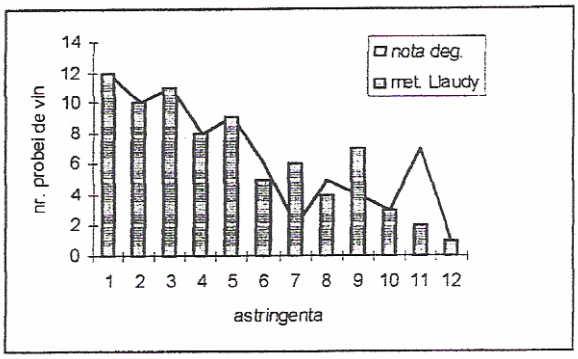

Fig. 2. The determination of the astringent feature using the method with ovalbumin and the mark after tasting

THE SENSORY ANALYSIS

The marks obtained after the organoleptical determination of the twelve samples varied from 25,2 (the wine No. 1 the less astringent wine) and 75,1 (the wine No.12 the most astringent wine) (table 1)

The marks obtained at tasting the wines for samples No. 1 and 12 depended on the total polyphenol content expressed through Ipf as well as the content in tannins remained after deposing the gelatin and ovalbumin.

Table 1

The results in evaluating the astringency of the analyzed wines

Sample No.	Ipf	Igelat	The ovalbumin method (g/l – acid tanic)	Tasting mark
1	21,87	29,4	0,129	25,2
2	36,89	42,5	0,144	44,5
3	31,26	34,8	0,156	38,3
4	35,46	45,7	0,192	39,7
5	37,56	53,5	0,294	42,1
6	41,02	57,9	0,289	51,4
7	29,08	39,2	0,172	35,3
8	37,04	49,6	0,329	58,6
9	38,29	51,2	0,318	56,8
10	63,26	61,5	0,381	68,2
11	69,07	64,3	0,338	66,5
12	66,64	65,4	0,571	75,1

The differences among the three methods of evaluating the astringency feature of the wines have been evidenced in fig. 3.

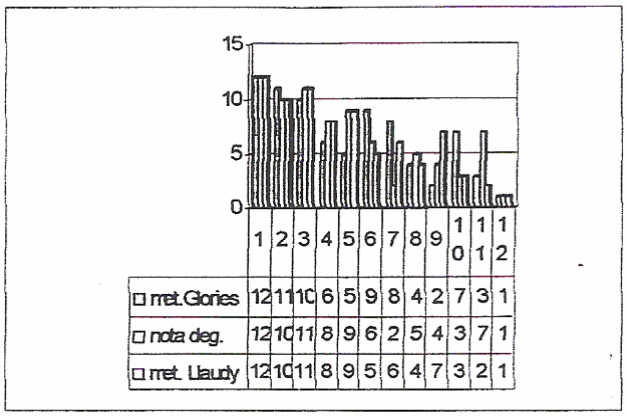

Fig. 3. The evaluation of the astringency feature of the wines by the three methods

It has been noticed (fig. 3) that the most astringent wine was sample No.12 and the less astringent one was sample No. 1. The 2nd and the 3rd places were taken by the samples No. 11 and 12, with little differences between them, significant differences between the astringentcy appeared in the samples No. 4 – 7 and No. 9 – 11. So, after the Glories method, sample No. 2 took the 9th place comparing to the 7th place according to the tasting mark and according to Llaudy method took the 11th place. As a result, this sample of wine was considered by the expert testers as being more astringent than it was been considered after using the other two methods.

The sample No. 8 took the 4th place after the organoleptical determination (tasting mark = 58,6) and after the Llaudy method, and according to the Glories method it was situated on the 7th place. The sample No. 5, after tasting mark was situated on the 8th place, as well as the sample No. 4th but after using the other methods took the 5th place (Glories) and 6th (Llaudy).

If we compare the top of the wines in their astringent decreasing order, obtained through each of the two chemical methods comparing to the tasting mark, we notice that by using the methods Llaudy, the positions 1-5, 10 and 11 were identical to those from the organoleptical determination; after using the Glories method only the positions 1 and 12 have been the same with the results after tasting the wines.

CONCLUSIONS

- For improving the technologies of processing the black grapes in order to obtain red wines of high quality it is necessary using a more precise method to evaluate the astringency of the wines.
- In general there is a well defined relation between the sensory analysis and the astringency of the red wines.
- If we compare the tasting mark to the results obtained through the other two methods, we can say that sometimes the evaluation of the astringency using the sensory analysis can be subjective.
- To firmly pronounce upon the precision and results of this new method, of deposing the astringent tannins with ovalbumin, there have to be always made

more determinations comparing to the “Index of gelatin” and sensory analysis in more labs for studying the chemistry of the wine in our country.

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MICROBIOLOGICAL QUALITY OF WATER USED IN SOFT DRINKS PRODUCTION IN DOLJ COUNTY

Calutu Mirela[1] Popa A.[2]

KEY WORDS: microbiological quality, drinking water, risk management approach

ABSTRACT

The quality of water used in the production of soft drinks has an important influence on the quality of the finished product considering the large share of the finished product but also in the process of manufacturing.

INTRODUCTION

Sources of water can be used underground (groundwater) and the surface water. Water obtained from the phreatical canvas located at a depth of less than 30 meters high has hardness and a microbiological dubious content. It is preferable to use water depth, obtained from approximately 100 meters.

Sources of water must ensure the necessary quantities and qualities of water, with fewer treatments and low investments.

In Dolj County are authorized to manufacture soft drinks a total of 4 units. Two of them use the water-city network for preparing the food, and other two units have their own water sources, a tap source. Their deepness differs, between 10 meters and 18 meters.

MATERIALS AND METHOD

Although the water quality is supervised by the manufacturer, due to the network's length to consumer, materials from which they are made and the maintenance of the equipment installation and operation of the unit production significantly may reduce microbiological quality of water used in drinks production.

By self check-up programs, the units samples water as well as finished product, to certify product compliance with legal rules in force.

All units have bulletins which attest that microbiological quality of water is in accordance with the Law on the quality of drinking water No. 458/2002 and 311/2004.

These parameters are presented in table 1.

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[2] University of Craiova

Table 1

Microbiological analysis of the drinking water

No.	Parameter /UM	Result	Maximum allowance value	Methods of analysis/ Standard ISO
1.	Bacteria coliform (no./100 ml)	0	0UFC/100ml	SR EN ISO 9308-1:2004
2.	Escherichia coli (nr./100ml)	0	0UFC/100ml	SR EN ISO 9308-1:2004
3.	Enterococcus faecalis (nr./100 ml)	0	0UFC/100ml	SR EN ISO 7899-2:2002
4.	Number of colonies at 37^0C/ml	0	No change abnormally	SR EN ISO 62222:2002
5.	Number of colonies at 22^0C/ml	0	No change abnormally	SR EN ISO 62222:2002
6.	Clostridium Perfingens/100ml	0	0UFC/100ml	Law no 458/2002 and 311/2004
7.	Other determinations	-	-	-

RESULTS AND DISCUSSION

The quality of water sources vary greatly from one locality to another, there is numerous deficiencies in keeping quality standards of urbane water networks. In accordance with the recommendations of Good Practice Guidelines for hygiene in soft drinking industry, supply of drinking water must be achieved through contracting with an authorized manufacturer to ensure an adequate quality and quantity water, and , more of that, monitoring and control in laboratories registered at the Ministry of Public Health. Furthermore, all types of water used as a source for the manufacture, processing, preservation and marketing products or substances for human consumption must be drinking.

The quality of water captured from natural sources are correct in installation of water treatment in the execution of different operations. Disinfection of water came from the city network is made by chemical plants, chlorination, because chlorine has a good efficiency at a low cost price, and the operation is relatively simple.

Microbiological quality of the food received is based on totally quality raw materials and ingredients used. Although currently register a tendency to supply a healthy and natural, synthetic aromas have a wide use in industry production of soft drinks, with all its shortcomings.

Although, sweeteners and flavorings used in production and are accompanied by health certificates accrediting their production in accordance with Good Manufacturing Practice (GMP) under sanitary conditions appropriate to food products and the products are produced according to the HACCP-regulations, microbiological quality of water can not be guaranteed, this can have very serious repercussions on the quality of the finished product.

Microbiological quality of food results should be based on precise measurements, accuracy and continue. Even if microbiological analyses of the finished products were in accordance with the standards legislation, just a single unit of production presents a sterilizer with UV lamp, built to disinfect water with ultraviolet light, used as a final step in the treatment for the purpose of resulting safe water with no microbiological risks.

This unit having opinion of the Ministry of Public Health, cancel this issue of contamination of drinking water on the route to final consumer, but also the negative impact of obsolete and battered installations and exploitation facilities inadequate. Although consumption of soft drinks or carbonated still meet a significant increase in common with other sectors of the market outlets, a food safety product must be in accordance with the concept of a risk analysis and critical control points (HACCP).

Water supply and wastewater treatment have made significant progress but also reveals increasing knowledge unrecognized problems. These can take form of both microbial and chemical contaminants and can arise from natural and anthropogenic sources or as a by-product of the water supply process. The introduction of water safety plans will inevitably bring its own problems. The publication of the third edition of WHO guidelines for drinking water Quality (2004) and the articulation of the water safety plan approach has provided renewed impetus to the consideration of quality and safety. The two approaches are closely aligned and derive from the application of Hazard Assessment and Critical Control Points, developed by Pilsbury Company for the manned space programme, and widely adopted by the food industry. The water safety plan requires that in order to achieve the primary objective of "providing good safe drinking water that has the trust of consumers" there is a need to manage water supply from catchments to tap.

The use of a risk management framework is considered a very effective means of managing water quality risks. There are many risk-based systems available to water utilities such as ISO 9001 and Hazard Analysis and Critical Control Point (HACCP).

In 2004, the World Health Organization's Guidelines for Drinking Water Quality recommended the use of preventive risk management approaches to manage water quality risks.

The contamination of drinking water by pathogens causing diarrhoeal disease is the most important aspect of drinking water quality. The problem arises as a consequence of contamination of water by faecal matter, particularly human faecal matter, containing pathogenic organisms.

The quality of drinking water and possible associated health risks vary throughout the world. Whilst some regions show high levels of arsenic, fluoride or contamination of drinking water by pathogens m elsewhere are very low and present no problem for human health. Marked variation in levels of contamination also occur more locally, often as a result of agricultural and industrial activities. The differences in health risks that represent these variations lead to different priorities for treatment or provision of drinking water. Microbial contamination of drinking water remains a significant threat and constant vigilance is essential.

CONCLUSIONS

The contamination of drinking water by pathogens causing diarrhoeal disease is the most important aspect. The quality conditions for still or carbonated drinks refer to organoleptic, physical, chemical and microbiological product’s property. In terms of production practices, must be taken into account especially microbiological quality of the product, knowing the possibility of alteration of water quality easily**.**

Delivering safe and acceptable water, therefore, is a key target in improving public health. Increased knowledge has shown the complexity of many of the issues that are related to drinking water and health, especially when drinking water is the first in terms of food production (soft drinks). Overall, however, it is evident that the supply and maintenance of safe drinking water remain key requirements for public health.

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PHENOLIC COMPOSITION OF NOVAC WINES DURING AGING THAT WERE OBTAINED FROM GRAPES AT DIFFERENT PHASES OF RIPENING

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KEY WORDS: wine, anthocyans, pigments, tannin, polyphenols, flaviliu

ABSTRACT

During the evolution phase of ripening Novac wines, obtained from grapes at different phenophases of ripening and using the same type of primary wine preparation biotechnology, have difference at phenolic composition and some of them are considerable. In the conditions of the same area, the same variety and the same primary technology, the differences have values depending on the phenophase of grapes ripening.

INTRODUCTION

In the phase of red wines aging, the main factor due to all specific changes happen is the oxygen from air. With the oxygen other factors operate: the temperature, some bio-catalytic agent and even elements with changeable valence, the iron and the copper (Gheorghita M. and col. – 2002). The oxygen importance is considerable at red wines, especially at the ones that are rich in polyphenols, compounds that are against oxidation changes (Cotea D.V., Sauciuc H.J. – 1988).

Concerning the phenolic compounds changes, during the aging phase of the red wines, changes that have strong effects on compositional and organoleptic aspect, the study realized by Glories Y. (1980), Somers .C.T. (1983) are very valuable for wine industry and science.

MATERIAL AND METHOD

The wines were obtained from Novac grapes from Dragasani vineyard, yield of 2007, harvested at: complete ripeness + 7-8 days (E1), complete ripeness + 14-15 days (E2), complete ripeness + 20-21 days (E3). For all the phases the primary wine preparation biotechnology contained: crushing, unclustering, adding of selected yeasts in the mixture resulting after pressing, SO_2 – 75 mg/l, 6 days (144 hours) of maceration. After 9 months from wine preparation, the complex analyse of the phenolic composition was realized, including the up-to-date spectrophotometer methods.

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RESULTS AND DISCUSSIONS

The elements of anthocyanic complex from Novac wines, after 9 months from wine preparation are shown in table 1. depending on the ripening phase, in which the grapes were picked, the total anthocyans were between 436 mg/l (E1) and 593 mg/l (E3). Although in the third phase, the anthocyans contents from the grapes were lower than in E1 and E2, due to the higher extraction the technological reserve grew, too, and it is in higher proportions in the wines from E3.

Table 1

The elements of the anthocyanic colouring complex from Novac wines that were obtained from grapes picked at different ripening phases (Dragasani – 2007)

The components of the anthocyanic complex	**The phenophases of the grapes ripening**		
	E1	E2	E3
Total anthocyans mg/l	436	551	593
Free anthocyans mg/l	262,1	310,8	319,6
Free anthocyans %	60,1	56,4	53,9
Combined anthocyans mg/l	173,9	240,2	273,4
Combined anthocyans %	39,9	43,6	46,1
Index of pvp %	16,4	19,4	24,1
Index of pp %	37,2	38,4	38,7
DO 420 nm	0,466	0,467	0,485
DO 520 nm	0,765	0,768	0,801
DO 620 nm	0,143	0,147	0,165
Yellow pigments %	33,9	33,8	33,4
Red pigments %	55,7	55,6	55,2
Blue pigments %	10,4	10,6	11,4
Colouring intensity	1,374	1,382	1,451
The colour tonality	0,609	0,608	0,605
Flaviliu cations	59,93	59,93	59,50

The proportions of combined anthocyans are all the more so the total anthocyans are in higher contents. This aspect is confirmed also by the values of the pvp % index. Concerning the polymerized anthocyans (the pp % index) it comes out the same evolution as sense, being in direct ratios with the total anthocyans.

Regarding the chromatic structure of the anthocyanic complex, in ratio with the colouring intensity: the yellow pigments decrease from E1 to E3; the red pigments proportions decrease the same as the yellow component, unsignificant from E1 to E3; the

blue component increase from E1 to E3. In this context: the colouring intensity increase from E1 to E3, the tonality of colour (DO 420/DO 520) decreases slowly from E1 to E3; the flaviliu cations (dA%) records an unsignificant diminution from E1 and E2 to E3.

The polyphenolic values shown in table 2, also record differences at the wines obtained from the three phenophases of the grapes ripening. The accumulation of polyphenolic compounds in grapes, until over-ripening, is shown by the values obtained from the analysed wines. It comes out that all the components of polyphenolic complex increase from E1 to E3. at total polyphenols the increases from E1 to E3 were of 9,4 %, and at tannin the increase was of 8,9 %. Increases from E1 to E3 comes out also at flavonic and unflavonic polyphenols.

Table 2

The polyphenolic composition of Novac wines that were obtained from grapes picked at different ripening phases (Dragasani – 2007)

The polyphenolic compositions	**The phenophases of the grapes ripening**		
	E1	E2	E3
Total polyphenols g/l (galic acid)	2,97	3,16	3,25
Flavonic polyphenols mg/l (catechina)	1,356	1,435	1,614
Unflavonic polyphenols mg/l (cafeic acid)	361	398	403
Tannin g/l	2,91	3,06	3,17
Indexes of tannin			
Index of HCl %	13,4	14,6	15,1
Index of gelatina %	56,4	57,3	59,2
Index of OH %	15,3	14,9	14,2

The condensed and very condensed tannins (index of HCl %) and the astringent tannins increase from E1 to E3, and the tannins associated with the salts and the polysaccharides decrease from E1 to E3 showing a reserve evolution comparatively with the one from the tannins.

CONCLUSIONS

- ✓ The different phenolic composition of Novac grapes in different phases of over-ripeness, keeps the same measure also in the wines that are aged.
- ✓ All the more the grapes had a longer period of over-ripening, so the total anthocyans contents, the total polyphenols and the tannins contents are more important, not only at the separation of the must phases and even in the ripening phase of the finite produces.

- ✓ Comparatively with the values of the colouring intensity, the yellow and the red components decrease from the earlier phase to the latest phase. For the blue component the direction is opposite.

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THE RESEARCHES REGARDING THE VARIABILITY OF TOTAL POLYPHENOLS CONTENTS FROM GRAPES ON A VINE AND FROM THE VINES ON A BLACK VARIETIES PLANTATION

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KEY WORDS: vine, grapes, variety, total polyphenols

ABSTRACT

On the grapes of a vine and on the vines yield from the plantations with Cabernet Sauvignon, Merlot, Feteasca neagra and Novac varieties situated in Dragasani vineyard, the contens in polyphenols present a variability more or less extensive, depending on variety. Also, depending on variety, the minimum and maximum absolute values of this phenolic complex are different.

INTRODUCTION

It is well known that the base particularities and the general aspect of red wines are defined, in essential way, of phenolic compounds, in a much bigger ratio than white wines. In the phenols family, less than specific pigments of the anthocyanic complex are total polyphenols, the ones who give firmness and gustative specificity to the red wines.

In the last years, scientific researches brought important data both theoretically and practically, in which concern total polyphenols (**Ionică Laura** – 2006, **Vladu Cristina** – 2007, **Nicolaescu C.** – 2007, **Popa Ionela** – 2008).

Regarding the variability of this compounds on the grapes of a vine and of all vines from a plantation, the results from this study are the first concerning the base sort of red wines from Dragasani vineyard.

MATERIAL AND METHOD

At technological ripening, in 2006 year it was proceeded, according to official methods recommanded from OIV, there were determined the contents in total polyphenols from all grapes on several vines and from individual productions of more vines to: Cabernet Sauvignon, Merlot, Feteasca neagra, Novac varieties, in Dragasani vineyard.

RESULTS AND DISCUSSIONS

❖ In the table 1 are presented the contents in total polyphenols of grapes from the same vines for the four red wines varieties, in the Dragasani vineyard.

- At Cabernet Sauvignon grapes, the total polyphenols contents are situated between two limits: inferior limit 3,10 g/kg beans and superior limit 4,06 g/kg beans, on the whole grapes achieving an average (A) of 3,706 g/kg beans. The degree of variability of total polyphenols contents at Cabernet Sauvignon is writen in the following relations: $V_M - V_m = 0,960$; $V_M - V_{med} = 0,354$; $V_{med} - V_m = 0,606$.

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- At Merlot variety, the total polyphenols contents achieved the lower values comparing with grapes of Cabernet Sauvignon variety. The scale of variability is situated between inferior limit of 2,98 g/kg beans and superior limit of 3,71 g/kg beans, the average of the 14 values representing the grapes of the vine being 3,304 g/kg beans. In this case the dimensions of variability at total polyphenols results from the values of the differences: $V_M - V_m = 0,730$; $V_M - V_{med} = 0,406$; $V_{med} - V_m = 0,324$.

It also results that, the average value of total polyphenols at Merlot is less than the value of Cabernet Sauvignon, with 0,402 g/kg beans, the two varieties being cultivated in the same vineyard and analised in the same calendar date.

- At Feteasca neagra variety, the contents in total polyphenols from grapes of the vine have 2,96 g/kg beans the inferior limit and 3,81 g/kg beans the superior limit.

It is interesting to show that, the variability degree is more emphasized at Feteasca neagra, from grapes in the same vine, comparing with the one established for Merlot variety, the averages differing with an unsignificant value of only 0,008 %. Therefore, it results that French Merlot variety is alike Feteasca neagra variety regarding the polyphenolic potential, at level of productions on vine. This significant situation between the two varieties is pointed out at anthocyans parameter too (mg/kg beans).

- At grapes from the vine, in Novac variety, the contents in total polyphenols, in this specified conditions, are between 3,27 g/kg beans and 4,09 g/kg beans, the variability being a bit lower than registered at Cabernet Sauvignon grapes. This two varieties are quite similar regarding quality parameters. The same situations between this two varieties is also because of the polyphenols averages at vines level, practically equal (3,706 g/kg beans at Cabernet Sauvignon, 3,705 g/kg beans at Novac).

The variability formulas of total polyphenols contents from grapes of the vine, at Novac variety, are presented as follows: $V_M - V_m = 0,820$; $V_M - V_{med} = 0,385$; $V_{med} - V_m = 0,435$.

❖ Regarding an experimental protocol there has proceeded also to the determination of the total polyphenols contents from the grapes of 20 vines for each variety, uniform distributed in the wine-growing parcel. The tests analyzed has been rigorously constituted in the same mode, with strict application to the recommended standards from OIV. The data are written in table 2.

- In the case of Cabernet Sauvignon variety the contents in polyphenols at level of yield from the vines are presented values situated between 2,18 g/kg beans and 4,35 g/kg beans. The difference between the two values is really important, but vines which have the first number 2 were only two, representing only 10 %. At rest of vines, in number of 18 (90 %), the contents of total polyphenols are registered with the first numbers of 3 or 4. On this base, the average for total of vines correspond to the value at 3,546 g/kg beans. Regarding the maximum, minimum and average values, the grade of variability can be expressed like this: $V_M - V_m = 2,170$; $V_M - V_{med} = 0,804$; $V_{med} - V_m = 1,366$.

- For the Merlot variety, the contents in total polyphenols from the grapes of vines are find into a scale of which inferior limit is 2,61 g/kg beans, and the superior limit is 3,93 g/kg beans. The contents under 3 g/kg beans have presented the grapes belonging to 3 vines (15 %).To another vines, in number of 17 (85 %), the total polyphenols value is not under 3,01 g/kg beans. This way is explained the average value of 3,322, much lower than the specific of Cabernet Sauvignon variety with just only 0,224 g/kg beans.

The restrict variability of total polyphenols contents from grapes of Merlot vines, results also from relations: $V_M - V_m = 1,320$; $V_M - V_{med} = 0,608$; $V_{med} - V_m = 0,712$.

- At Feteasca neagra variety, the contents of total polyphenols from grapes of the vines are situated between 2,71 g/kg beans and 3,81 g/kg beans, realizing an average of

3,202 g/kg beans, much lower with 0,120 g/kg beans than the established one for Merlot grapes, with which is compared regarding the technological characteristics. Similar to the case of Merlot variety, just for 3 vines the grapes presented the contents of total polyphenols under 3 g/kg beans, with superior limit at value of 3,81 g/kg beans. The elements of variability between different values presents the following configuration: $V_M - V_m = 1,100$; $V_M - V_{med} = 0,608$; $V_{med} - V_m = 0,492$.

- The grapes yields from the vines of Novac incorporate the contents in total polyphenols between 3,12 g/kg beans and 4,21 g/kg beans. It is shown that also the minimum and maximum contents presents the highest values comparing to all other varieties. Also the average of all 20 vines is registered with the highest level, of 3,682 much higher: with 0,136 than Cabernet Sauvignon; with 0,360 than Merlot; with 0,480 than Fetească Neagră. In comparison with all another varieties, the variability degree is lower this situation resulting from the following formulas: $V_M - V_m = 1,090$; $V_M - V_{med} = 0,528$; $V_{med} - V_m = 0,562$.

CONCLUSIONS

In the same conditions of clima, soil, wine-growing technique and ripening phenophase of grapes, the polyphenols contents from grapes of the vine and vine yields of one plantation with black varieties presents different degrees of variability depending on the variety. For the main black varieties from Dragasani vineyard: at grapes of the vine the highest variability is registred at Cabernet Sauvignon, followed in decreasing order by Merlot, Feteasca neagra, Novac varieties; at the vine yields from the plantation the differences as absolute values between maximum and minimum are a bit closer, being sub-unitary in all cases. In decreasing order these differences which represent the degree of variability are shown in another way: Cabernet Sauvignon (0,960), Feteasca neagra (0,850), Novac (0,820), Merlot (0,730).

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Table 1

The variability of contents in total polyphenols in grapes from the same vine – Dragasani, 2006

CABERNET SAUVIGNON		MERLOT		FETEASCA NEAGRA		NOVAC	
Grapes	Total polyphenols g/kg beans	Grapes	Total polyphenols g/kg beans	Grapes	Total polyphenols g/kg beans	Grapes	Total polyphenols g/kg beans
1	3,72	1	3,25	1	3,41	1	3,82
2	3,56	2	3,43	2	3,29	2	3,91
3	3,89	3	3,16	3	3,36	3	4,05

4	4,02	4	2,98	4	3,81	4	3,88
5	3,96	5	3,22	5	3,09	5	3,31
6	3,20	6	3,71	6	3,28	6	3,72
7	3,41	7	3,06	7	2,96	7	3,31
8	3,66	8	3,61	8	3,16	8	4,09
9	3,58	9	3,25	9	2,99	9	3,66
10	4,06	10	3,09	10	3,42	10	3,89
11	3,10	11	3,41	11	3,57	11	3,96
12	3,66	12	3,29	12	3,33	12	3,30
13	3,75	13	3,18	13	3,39	13	3,27
14	3,96	14	3,62	14		14	
15	3,90	15		15		15	
16	3,88	16		16		16	
Average	3,706	Average	3,304	Average	3,312	Average	3,705

CABERNET SAUVIGNON : $V_M - V_m = 0,960$; $V_M - V_{med} = 0,354$; $V_{med} - V_m = 0,606$

MERLOT : $V_M - V_m = 0,730$; $V_M - V_{med} = 0,406$; $V_{med} - V_m = 0,323$

FETEASCA NEAGRA : $V_M - V_m = 0,850$; $V_M - V_{med} = 0,498$; $V_{med} - V_m = 0,352$

NOVAC : $V_M - V_m = 0,820$; $V_M - V_{med} = 0,385$; $V_{med} - V_m = 0,435$

Table 2

The variability of total polyphenols contents from the grapes yields of vines from wine-growing – Dragasani, 2006

CABERNET SAUVIGNON		MERLOT		FETEASCA NEAGRA		NOVAC	
Vine	Total polyphenols g/kg beans	Vine	Total polyphenols g/kg beans	Vine	Total polyphenols g/kg beans	Vine	Total polyphenols g/kg beans
1	3,96	1	3,75	1	3,81	1	4,06
2	4,10	2	3,91	2	3,66	2	3,83
3	3,06	3	3,10	3	3,20	3	3,75
4	2,18	4	3,06	4	3,16	4	3,96
5	4,35	5	3,75	5	3,46	5	3,58
6	3,16	6	3,44	6	3,23	6	3,61
7	2,95	7	3,01	7	3,09	7	3,12
8	3,76	8	2,67	8	3,11	8	4,21
9	3,88	9	2,72	9	3,01	9	4,09
10	3,45	10	3,40	10	2,81	10	3,77
11	2,62	11	2,61	11	3,18	11	3,91
12	3,12	12	3,06	12	2,75	12	3,75
13	4,18	13	3,93	13	2,71	13	3,32
14	3,67	14	3,34	14	3,09	14	3,88
15	3,77	15	3,72	15	3,12	15	3,16
16	4,19	16	3,82	16	3,19	16	3,22
17	3,81	17	3,55	17	3,43	17	3,44
18	3,55	18	3,41	18	3,36	18	3,85
19	3,20	19	3,01	19	3,71	19	3,62
20	3,96	20	3,19	20	2,96	20	3,51
Average	3,546	Average	3,322	Average	3,202	Average	3,682

CABERNET SAUVIGNON : $V_M - V_m = 2,170$; $V_M - V_{med} = 0,804$; $V_{med} - V_m = 1,366$

MERLOT : $V_M - V_m = 1,320$; $V_M - V_{med} = 0,608$; $V_{med} - V_m = 0,712$

FETEASCA NEAGRA : $V_M - V_m = 1,100$; $V_M - V_{med} = 0,508$; $V_{med} - V_m = 0,492$

NOVAC : $V_M - V_m = 1,090$; $V_M - V_{med} = 0,528$; $V_{med} - V_m = 0,562$

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THE STUDY CONCERNING THE VARIABILITY OF THE MAIN QUALITY PARAMETERS OF THE GRAPES FROM THE VINES OF CABERNET SAUVIGNON AND NOVAC FROM THE DRAGASANI VINEYARD

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KEY WORDS: grapes, variability, glucides, acidity, anthocyans

ABSTRACT

In the Cabernet Sauvignon and Novac varieties of grapes from the Dragasani vineyard, the contents in glucides, acidity and anthocyans present a variability which is obvious in many cases. In the same conditions of climate, soil and winegrowing technique, the variability of main parameters of grapes of vine composition are accentuated depending on the position of vines from the slope.

INTRODUCTION

The specific literature mentions that, in general, the main constituents of composition have a particular variability, as much as part of a bean, which represent the proper fruit and also in different parts of grapes, toggled in report with the peduncle (**Cotea D.V.** - 1985, **Pomohaci N.** and co. - 2000, **Gheorghita M.** and co. - 2006, **Muntean Camelia, Ionica Laura** - 2006).

The rigorous data has never been made, reason that caused some investigations, on the variability theme for the glucides, acidity and anthocyans contents from the grapes which belong to the same vine, and some partial results, are mentioned in this present paper.

MATERIAL AND METHOD

The study was made in the winegrowing years 2006 and 2007 being mentioned the contents in glucides, acidity and anthocyans from each grape from some vines of Cabernet Sauvignon and Novac varieties cultivated in the Dragasani vineyard (Dealul Olt).

The vines aleatory choosing have occupied different positions in the plantations situated on slopes with southeastern exposition.

For the determination of contents in glucides, acidity and antocyans from each grape of the same vine are used the official methods recommended by OIV and adopted by ICVV Valea Calugareasca.

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RESULTS AND DISCUSSIONS

The data which show the variability of quality constituents from grapes of some Cabernet Sauvignon vines placed in the inferior half of the slope (1A and 2A) and in the superior half of the slope (1B and 2B) are written in the table 1.

For the first group of grapes from the inferior part of the slope (1A), the contents in glucides are on a scale of values between 185,5 g/l and 209,6 g/l, the difference being of 20,1 g/l; at acidity the variability was between 4,07 g/l and 4,89 g/l (in H_2SO_4), between these being a difference of 0,82 g/l which in relative value represent 16,7 %. Below the acidity average (of 4,43 g/l) are placed 55,6 % from cases. At antocyans, the lowest content from the vine was of 165 mg/kg beans, and the highest of 1310 mg/kg beans, resulting a difference of 45 mg/kg beans.

In another group from inferior part of the slope (2A), as much as to glucides and also to acidity, the averages are insignificantly lower regarding 1A, but also important to anthocyans, where the difference between the maximum and minimum value is situated at 126 mg/kg beans.

In the superior part of the slope (1B and 2B) increase also the minimum and the maximum values, comparing to 1A and 2A, at glucides and anthocyans and decrease the ones of acidity.

Table 1

The variability of quality parameters from grapes of the vine of Cabernet Sauvignon variety of Dragasani (at the complete ripeness)

The situation	Grapes / vine	Glucides g/l		Acidity g/l (H_2SO_4)		Anthocyans mg/kg beans	
		L.V.	A.	L.V.	A.	L.V.	A.
1A	18	189,5 –209,6	201,6	4,07 – 4,89	4,43	1265 –1310	1294
2A	20	183,1 –210,2	199,0	4,02 – 4,71	4,41	1177 –1303	1225
1B	18	192,4 –212,4	205,4	3,95 – 4,62	4,18	1275 –1313	1304
2B	16	195,1 –214,3	204,9	4,06 – 4,45	4,23	1204 -1341	1290

A – average; L.V. – limits of variation

The degree of variability at quality elements from the grapes of the vine results clearly from data of the table 2.

Table 2

The value differences between quality parameters from the Cabernet Sauvignon grapes of Dragasani (at complete ripeness)

The Situation	Glucides g/l			Acidity g/l			Anthocyans mg/kg beans		
	V_m	V_M	$V_M - V_m$	V_m	V_M	$V_M - V_m$	V_m	V_M	$V_M - V_m$
1A	189,5	209,6	20,1	4,07	4,89	0,82	1265	1310	45,0
2A	183,1	210,2	27,1	4,02	4,71	0,69	1177	1303	126,0
Average	186,3	209,9	23,6	4,05	4,80	0,755	1221	1305	83,5
1B	192,4	212,4	20,0	3,95	4,62	0,67	1275	1313	38,0
2B	195,1	214,3	19,2	4,06	4,45	0,39	1204	1341	137,0
Average	195,9	213,4	19,6	4,00	4,54	0,530	1240	1327	88,0

The differences between the maximum and minimum values, as averages were: of 23,6 g/l and respectively 19,6 g/l to glucides; of 0,755 g/l and respectively 0,530 g/l to acidity; of 83,5 mg/kg beans and respectively 88 mg/kg beans to anthocyans. It results that, in superior part of the slope the differences at glucides and acidity are reduced, but increase at anthocyans.

For the Novac variety from Dragasani vineyard, the variability elements from grapes of one vine are mentioned in tables 3 and 4.

Table 3

The variability of quality parameters from the grapes of the vine at Novac variety of Dragasani (at complet ripeness)

The Situation	Grapes / vine	Glucides g/l		Acidity g/l		Anthocyans mg/kg beans	
		L.V.	A.	L.V.	A.	L.V.	A.
1A	15	187,5 –216,3	205,2	4,06 – 4,66	4,38	1204 – 1385	1308
2A	14	175,4 –214,1	199,5	4,22 – 4,75	4,48	1187 – 1403	1294
1B	17	173,7 –210,2	199,4	4,32 – 5,02	4,55	1193 – 1352	1273
2B	13	188,8 –213,3	204,1	4,16 – 4,61	4,31	1201 – 1405	1306

A – average; L.V. – limits of variation

In the table 3, the data represent the minimum and maximum level of glucides, acidity and anthocyans for all 4 groups of grapes / vine and the averages obtained through the summarization all values representing the grapes of the vine. Although the contents from 3 chemical compounds differ, sometimes significant, for a grape to other from the vine, the well-balanced averages are approaching enough from one vine to another.

Table 4

The value diferences between quality parameters from Novac grapes of Dragasani (at complet ripeness)

Characteristic	**Sit.**	**V_m**	**V_M**	**Average**	**Dif. V_M-V_m**	**Dif. V_M - V_{med}**	**Dif. V_{med} - V_m**	**Contents**	
								Over average	**Under average**
								%	**%**
Glucides g/l	**1A**	187,5	216,3	205,2	27,70	10,00	17,70	46,7	53,3
	2A	175,4	214,1	199,5	38,70	14,60	24,10	64,3	35,7
	1B	173,7	210,2	199,4	36,50	10,80	25,70	58,8	41,2
	2B	188,8	213,2	204,1	24,50	9,20	15,30	53,8	46,2
	Average	**181,4**	**213,1**	**202,1**	**31,85**	**11,15**	**20,70**	**55,9**	**44,1**
Acidity g/l (H_2SO_4)	**1A**	4,06	4,66	4,38	0,60	0,28	0,32	66,7	26,7
	2A	4,22	4,75	4,48	0,53	0,27	0,26	64,3	35,7
	1B	4,32	5,02	4,55	0,70	0,47	0,23	35,3	41,2
	2B	4,16	4,61	4,31	0,45	0,30	0,15	46,2	69,2
	Average	**4,19**	**4,76**	**4,43**	**0,57**	**0,33**	**0,24**	**53,1**	**42,3**
Anthocyans mg/kg beans	**1A**	1204	1385	1307,5	181,0	77,5	103,5	46,7	53,3
	2A	1187	1402	1294,3	215,0	215,0	107,3	64,3	35,7
	1B	1193	1352	1272,7	159,0	79,3	79,7	58,8	41,2
	2B	1201	1405	1306,2	204,0	98,8	105,2	30,8	69,2
	Average	**1196**	**1386**	**1295**	**189,8**	**117,7**	**98,9**	**50,2**	**49,8**

The degrees of variability for glucides, acidity and anthocyans contents from the grapes / vine at Novac variety are showed from data of the table 4. The Differences are

clearly pronounced through the application of the formulas $V_M - V_m$; $V_M - V_{med}$; $V_{med} - V_m$, but also the proportions of grapes with contents across and below the averages of the three chemical compounds.

The differences of averages resulted from formula $V_M - V_m$ were: of 31,85 g/l to glucides; of 0,57 g/l to acidity; of 189,8 mg/kg beans to anthocyans.

The differences of averages resulted through application of formula $V_M - V_{med}$ were of: 11,15 g/l to glucides; of 0,33 g/l to acidity; of 117,7 mg/kg beans to anthocyans.

Using relation $V_{med} - V_m$ the differences were of: 20,7 g/l to glucides; of 0,24 g/l to acidity; of 98,9 mg/kg beans to anthocyans.

The proportions of grapes with contents across average, to the three characteristics were of: 55,9 % to glucides; of 53,1 % to acidity; of 50,2 % to anthocyans.

CONCLUSIONS

At Cabernet Sauvignon variety cultivated in the Dragasani vineyard, the contents in glucides, acidity and anthocyans from the grapes of the same vine are presented on the large scale.

For all grapes from the vine, the contents of glucides and anthocyans are bigger, and the ones in acidity are smaller at those situated in superior part from the slope.

At Novac variety the three chemical compounds present a considerable variability as part as the group grapes from the vine, but also between different vines yields from plantation. It is possible that these large variability aspects can be also effects from heterozis character, knowing that it is obtained by sexuate crosses.

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OBTAINING CLAIRET TYPE WINES THROUGH SHORT TERM MACERATION OUT OF OLTENIAN VINEYARD VARIETIES

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KEY WORDS: variety, grapes, maceration, anthocyans, pigments

SUMMARY

Clairet type wines or cafe, much appreciated products in countries with tradition, like France, can be obtained from vineyard varieties from Oltenia, by aplying carefully conceived and applied technologies. Through the use of short-term maceration process, Clairet type wines have been obtained, whose characteristics are comparable with those obtained in the great european wine-growing countries.

INTRODUCTION

Clairet wines, from the point of view of cromatic features and other phenolic compounds, occupy an intermediary position between typical rose and red. The wine with controled nomenclature Bordeaux Clairet is situated, under the report of phenol compounds, above veritable rose. The Clairet type is a red wine, a little coloured, with moderate quantities of tannin, subtle, frutty, that can be consumed young and cooled (**Ribereau-Gayon J.** and col. – 1976**)**.

Wines containing greater than 100 mg/l anthocyans have been settled as superior to rose wines obtained through limited maceration. Above 100 mg/l anthocyans, wines will be named „Clairet" or „Cafe wines". Reputed specialists in France have produced notable results on the procedures and production techniques for rose wines and Clairet type (**Sudraud P.** – 1958, **Roson J. P**. – 1976, **Andre P.** and col. –1970).

In the present paper we will present the forst results regarding production possibilities for Clairet type wines, in several vineyards of Oltenia.

MATERIAL AND METHOD

In the 2004-2006 period for obtaining Clairet wines, Cabernet Sauvignon, Merlot and Burgund mare variety grapes from theDragasani and Samburesti vineyards have been used. In the elaboration technology, the variable factor has been teh maceration period (0, 12, 24, 36, 48, 72 hours), and for fixed factors : SO_2-75 mg/l; maceration temperature 23-24 C; adding selected leeses + pectolitic enzymes (6 million cel/ml, respectively 3g/hl); homogenising regime of musting phases (5-6 times/day).

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RESULTS AND DISCUSSIONS

The chromatic components of Cabernet Sauvignon Wines, out of South Dragasani vineyard are submitted in the tables 1 and 2.

Table 1

Contents in anthocyans chromatic structure of wines type Clairet.
Cabernet Sauvignon South Dragasani-2004

Maceration duration - Hours	Anthocyans mg/l	Yellow pigments %	Red pigments %	Blue pigments %
Mt. 0	0	-	-	-
12	47	43,1	55,6	1,3
24	102	37,07	58,12	4,77
36	136	36,54	54,87	8,58
48	168	35,45	56,52	8,03
72	289	35,1	55,92	8,96

Table 2

Chromatic features of wines type Clairet.
Cabernet Sauvignon South Dragasani – 2004

Maceration duration - Hours	D.O.420 nm	D.O.520 nm	D.O.620 nm	Ic	Tc	dA %
Mt. 0	0,00188	0,00371	-	0,00559	0,506738	-
12	0,12954	0,167	0,00396	0,3005	0,775688	60,02
24	0,18983	0,29763	0,02447	0,51193	0,637805	63,99
36	0,24609	0,39234	0,05576	0,69419	0,627236	61,53
48	0,23187	0,34818	0,05446	0,63451	0,665948	58,88
72	0,26536	0,4227	0,06772	0,75578	0,627773	60,60

According to the analysis, the chromatic parameters, characteristic to Clairet wines, are achieved at contact durations between the two musting phases of 24 to 48 hours. At these durations wines with quantities of anthocyans between 102 and 168 mg/l are obtained, with proportions of yellow pigments varying from 35 % and 37 % and blue pigments ranging from 4,77 % and 8,03 %. The flavium cathion quantities confirm a chromatic structure extremely favorable.

Using a Merlot grapes, from the same area – South Dragasani – Clairet type wines have been obtained for maceration periods between 24-48 hours (tables 3 and 4). It's interesting to mentioned that although grapes have had smaller quantities of antocyanes, in wines, these components, at comparable maceration time frames present higher proportions, probably due to more intense extractability during maceration-fermentation.

Table 3

Contents in anthocyans chromatic structure of wines type Clairet.
Merlot, South Dragasani-2004

Maceration duration - Hours	Anthocyans mg/l	Yellow pigments %	Red pigments %	Blue pigments %
12	66	47,73	52,27	-
24	116	40,85	54,84	4,3
36	168	35,93	57,27	6,79
48	196	33,67	59,66	6,65

Table 4

Chromatic features of wines type Clairet.
Merlot, South Dragasani – 2004

Maceration duration - Hours	D.O.420 nm	D.O.520 nm	D.O.620 nm	Ic	Tc	dA %
12	0,07093	0,07767	-	0,1486	0,91322	-
24	0,13688	0,18375	0,01442	0,33505	0,744925	58,82
36	0,22481	0,35826	0,0425	0,62557	0,627505	62,69
48	0,2384	0,42237	0,04718	0,70795	0,564434	66,19

The elements of antocyanic complex for the Clairet wines obtained in 2005 out of Burgund mare variety grapes, grown in the Samburesti vineyard, are produced in aprox 48 hours of maceration-fermentation (tables 5 and 6).

High proportions of yellow pigments (42,23 %) and small quantities of blue pigments make for a lively, attractive aspect, without upsetting tones for visual considerations. These aspects are demonstrated by the analysis of color tone value (0,8269), on one side and of proportions in flavium cathions (52,11), on the other.

Table 5

Contents in anthocyans chromatic structure of wines type Clairet.
Burgund mare Samburesti-2005

Maceration duration - Hours	Anthocyans mg/l	Yellow pigments %	Red pigments %	Blue pigments %
12	32	66,72	33,28	-
24	69	50,38	49,62	-
36	91	49,99	46,36	3,64
48	130	42,23	51,06	6,67

Table 6

Chromatic features of wines type Clairet.
Burgund mare Samburesti – 2005

Maceration duration - Hours	D.O. 420 nm	D.O. 520 nm	D.O. 620 nm	Ic	Tc	dA %
12	0,03904	0,01947	-	0,05851	2,005136	-
24	0,05573	0,05487	-	0,1106	1,0115673	-
36	0,1048	0,0972	0,00764	0,20964	1,078189	42,16
48	0,14874	0,17986	0,02351	0,35211	0,826976	52,11

For fermentation-maceration perios between 36-58 hours Clairet wines may result but this may be the product of Cabernet Sauvignon variety grapes cultivated in teh central area of the Dragasani vineyard (tables 7 and 8). Remarcable proportions of flavium cathions (58,95 - 64,76) and small quantities of blue pigments offer the guarantee that the final product wil corespond entirely to the established goal.

Table 7

Contents in anthocyans chromatic structure of wines type Clairet.
Cabernet Sauvignon Dragasani – Olt Hill 2006

Maceration duration - Hours	Anthocyans mg/l	Yellow pigments %	Red pigments %	Blue pigments %
0	6,7	79,75	20,25	-
12	46	50,76	48,96	-
24	88	43,66	56,34	-
36	109	41,1	54,92	3,97
48	167	36,33	58,66	5,01
72	219	35,9	58,15	5,92

Table 8

Chromatic features of wines type Clairet.
Cabernet Sauvignon Dragasani - Olt Hill 2006

Maceration duration - Hours	D.O. 420 nm	D.O. 520 nm	D.O. 620 nm	Ic	Tc	dA %
0	0,03916	0,00997	-	0,0491	3,927783	-
12	0,06647	0,06415	-	0,1310	1,036165	-
24	0,09374	0,12089	-	0,2146	0,775415	-
36	0,16654	0,22247	0,0161	0,40511	0,748595	58,95
48	0,19334	0,31217	0,2664	0,53215	0,619342	64,76
72	0,23441	0,37975	0,3872	0,65288	0,617274	64,03

In the technological conditions of equal primary wine-making, the Merlot variety cultivated in the Dragasani vineyard is able to „offer" comparable Clairet wines, under the report of chromatic parameters with those produced out of Cabernet Sauvignon variety grapes grown in the same area. These aspects are noticeable in Tables 9 and 10. For time duration of macerations-fermentation between 36 and 48 hours, the wines contain anthocyans of 126 mg/l and respectively 181 mg/l, of notable proportions for red and yellow pigments and little quantities of blue pigments. The colour tone (0,6809 - 0,6950) and the cathions of flavium (60,09 - 58,79) are in agreement with the required norms of a quality coloring.

Table 9

Contents in anthocyans chromatic structure of wines type Clairet.
Merlot, Dragasani – Olt Hill 2006

Maceration duration - Hours	**Anthocyans mg/l**	**Yellow pigments %**	**Red pigments %**	**Blue pigments %**
12	65	46,78	52,84	0,40
24	92	40,36	55,25	4,38
36	126	37,87	55,6	6,51
48	181	38,09	54,81	7,07

Table 10

Chromatic features of wines type Clairet.
Merlot, Dragasani - Olt Hill 2006

Maceration duration - Hours	**D.O.420 nm**	**D.O.520 nm**	**D.O.620 nm**	**Ic**	**Tc**	**dA %**
12	0,10399	0,1175	0,00087	0,22236	0,885021	55,37
24	0,14837	0,20313	0,01609	0,36759	0,730418	59,51
36	0,19971	0,29326	0,03434	0,52731	0,680999	60,09
48	0,22483	0,32348	0,04173	0,59004	0,695035	58,79

CONCLUSIONS

- Oenoclimatic and edafic conditions in the Dragasani- Valcea and Samburesti-Olt vineyards are completely profitable for the production of wines with quantities of anthocyans, amidts which are the Clairet types.
- Cabernet Sauvignon, Merlot and Burgund varieties, cultivated in the above mentioned vineyards benefit form a real technologic potential in comparison with the conditions that are required for Clairet wines.
- Among the techological factors of primary wine-making, adding selected leeses, pectolitic enzymes and SO_2, presents a notable importance in obtaining products with well-balanced physic-chemical and chromatic composition.

- Depending on the qualitative potential of the primary material and the way the mentioned factors are applied, the timing periods between the musting phases, within 24 - 48 hours can trigger exceptional results.

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BIOTECHNOLOGICAL MEANS OF OBTAINING CLAIRET TYPE WINES BY MODIFYING THE RATION BETWEEN THE MUSTING PHASES

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KEY WORDS: grapes, oozing phases, wine, anthocyans, pigments

SUMMARY

Clairet type wines, generally, are not found in our country, can be obtained at required quality level by versed consumers also by modifying ration between oozing phases and leading to drying of fermentation in conditions of low proportion of peels, core and seed.

INTRODUCTION

Specialized literature from France mentions the fact that among the procedures of Clairet type wines elaboration it is included that of modifying ration between oozing phases leading to drying of fermentation.

This change consists in the diminishing of the peel ratio against the liquid fraction. As a fact, it is realized a wine making in demi-red, the separation of the two fractions in changed proportion takes place at the ending of the alcoholic fermentation (**Andre P.** – 1976, **Ribereau-Gayon J.** and col. – 1976).

On this theme the facts in this paper are presented.

MATERIAL AND METHOD

During the grape processing of the Cabernet Sauvignon and Merlot from the Dragasani vineyard, the solid phase from the oozing component represented 10, 15, 20, 25, 30, 35 %.

In the oozing with the phases with modified proportions, sulfite with 60 mg/l SO_2 there were added selectioned leeses (7 million cel/ml) and pectin enzymes 2,5 g/hl. The fermentation- maceration was lead to be dry, in the temperature conditions of 23 and 25 C degrees.

RESULTS AND DISCUSSIONS

The chromatic characteristics of Clairet wines obtained through the modifying of the proportions between the oozing phases (Merlot – Dragasani).

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Table 1

Chromatic features of Clairet type wine obtained by ratio modifying between oozing phases (Merlot-Dragasani)

Variant % Solid phase	Anthocyans mg/l	I.c.	T.c.	dA %	Pigments Categories		
					Yellow Do 420 nm	Red Do 520 nm	Blue Do 620 nm
100 (Mt)	687	1,635	0,569	49,62	32,6	57,2	10,2
10	56,4	0,269	1,560	50,36	55,1	35,3	9,6
15	101,6	0,438	1,459	50,12	53,4	36,6	10,0
20	132,9	0,516	1,233	49,68	49,1	39,8	11,1
25	169,7	0,699	1,132	51,12	47,9	42,3	9,8
30	199,3	0,486	1,045	50,46	45,8	43,8	10,4
35	233,2	0,564	1,000	50,25	44,9	44,9	10,2

The data from the Table 1 show the possibility of obtaining Clairet wines, from Merlot grapes from Dragasani, when the solid phase represents proportions from 15 and 25 % with dry fermentation. In such situations, the anthocyans contents are between 101,6 and 169,7 mg/l, and the proportions of yellow and red pigments realize tonalities with values between 1.132 and 1.459, very attractive under visual bearing.

Table 2

The main features of composition of Clairet obtained by modifying the proportions between the oozing phases (Merlot - Dragasani).

Variant % Solid phase	Alcohol % vol	Total Acidity g/l H_2SO_4	Volatile Acidity g/l H_2SO_4	Unred. extract g/l	Ash g/l	Glycerol g/l	Glycerol X 100 /alcohol	Ash X100 /extr
100 (Mt)	12,72	4,41	0,43	36,9	3,59	10,2	10,16	9,72
10	12,65	4,40	0,39	21,6	2,06	10,1	10,12	9,53
15	12,67	4,42	0,39	22,3	2,09	10,4	10,41	9,37
20	12,51	4,38	0,38	22,8	2,12	10,2	10,33	9,29
25	12,68	4,40	0,37	23,1	2,20	10,0	10,00	9,52
30	12,65	4,36	0,37	23,4	2,29	10,3	10,32	9,78
35	12,66	4,39	0,36	23,7	2,31	10,0	10,01	9,74

In Table 2 there are registered values of the main physical-chemical parameters of Clairet wines, obtained from Merlot at proportions of solid parts, between 15 and 25 %. On the whole of the variants, the alcohol contents, total acidity, volatile acidity and glycerol does not differ only a little.

While the control – when the solid phase has occupied 100 % – the contents of non-reducing extract and ash differ quite a lot. These oenological measures, at wines which meet the chromatic conditions, are between 22,3 and 23,1 g/l and 2,09 and 2,20 g/l.

Table 3

Chromatic features of Clairet wines obtained by modifying the proportion between the oozing phases (Cabernet Sauvignon - Dragasani)

Variant % Solid phase	Anthocyans Mg/l	I.c.	T.c.	dA %	Pigments Categories		
					Yellow Do 420 nm	Red Do 520 nm	Blue Do 620 nm
100 (Mt)	766	1,841	0,581	50,34	33,0	56,8	10,2
10	71	0,270	1,487	48,95	54,6	36,7	8,7
15	112	0,473	1,351	49,51	52,3	38,7	9,0
20	146	0,566	1,318	48,80	51,8	39,30	8,9
25	175	0,663	1,145	49,60	48,7	42,5	8,8
30	210	0,554	1,031	49,40	46,2	44,8	9,0
35	248	0,651	1,008	48,75	45,9	45,5	8,6

Using the grapes of Cabernet Sauvignon from Dragasani vineyard, by modifying the proportion between the oozing phases, the proportions of solid phase between 15 and 25 % have proved to be efficient, as it shows in table 3, concerning the chromatic complex of the products. The contents of anthocyans situated between 112 and 175 mg/l and the tonality values between 1,145 and 1,351 consists doubtless arguments in this way.

The chromatic features of the wines mentioned in table 3 are accompanied by physical-chemical parameters registered in table 4.

Regarding wines from Merlot, those from Cabernet Sauvignon are more extractive (23,0-23,6 g/l) and richer in mineral substances (2,16-2,26 g/l). The differences are minor.

Table 4

The main composition features of Clairet wines obtained by modifying the proportion between the oozing phases (Cabernet Sauvignon - Dragasani)

Variant % Solid phase	Alcohol % vol	Total Acidity g/l H_2SO_4	Volatile Acidity g/l H_2SO_4	Unred. extract g/l	Ash g/l	Glycerol g/l	Glycerol x 100/alc.	Ash x 100/extr.
100 (Mt)	12,70	4,62	0,42	37,80	3,72	10,4	10,37	9,87
10	12,60	4,54	0,36	22,35	2,01	10,2	10,26	8,99
15	12,65	4,52	0,37	23,00	2,16	10,3	10,32	9,39
20	12,60	4,53	0,35	23,25	2,20	10,1	10,16	9,42
25	12,55	4,56	0,37	23,60	2,26	10,4	10,50	9,57
30	12,65	4,60	0,38	23,90	2,30	10,1	10,12	9,62
35	12,70	4,58	0,38	24,10	2,34	10,3	10,27	9,70

CONCLUSIONS

- Involving the procedure by modifying the proportion between the oozing phases in order to Clairet wines, the bankable results are acquired when the solid phase represents 15 – 25%, with the fermentation-maceration dry of the mixture, in the certain structure.

- By acting this way, from the Merlot and Cabernet Sauvignon grapes from Dragasani, Clairet wines can be obtained, with anthocyans contents between 102 mg/l and 175 mg/l, with chromatic characteristics and physical-chemical content highly valuable.

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THE ASPECT REGARDING THE EVOLUTION IN TIME OF THE PHENOLIC COMPOUNDS FROM PINOT NOIR WINES OBTAINED IN OREVIȚA – VÂNJU MARE VINEYARD

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KEY WORDS: wine, anthocyans, pigments, polyphenols, tannin

ABSTRACT

The hight degree of favorability for winegrowing from Orevița – Vânju Mare vineyard and remarcable technological potential of Pinot noir variety are found in the exceptional quality of the wine – finished product.

During the Pinot noir wines ripening, the phenolic compounds, those which confer the particulatites and the general aspect, present a positive evolution, with significant improvments, specialy of chromatic, olfactive and gustativ order.

INTRODUCTION

During red wines ripening, the most „spectacular" transformations are actioning on the phenolic compounds. The reference material mentions that in the period of red wines ripening, the phenolic compounds has the following modifications: the condensation and decomposition of some part of anthocianic complex and tannins; the reactions of condensation between phenolic compounds with acetic aldehyde contribution; the reactions of the C – C liaison scissions; the forming of xantiliu derivates and chinonic compounds; the colour, from the point of visual, performs, in raport with monomer and polimer pigments proportions from anthocyanic complex, the nuances passing from intense red, to brick red, in a much higher ripening period (Glories Y. – 1980; Cotea D.V. and co. – 1988; Somers T.C. and co. – 1990).

Concerning the evolution of phenolic compounds from Pinot noir wines of Orevița vineyard, during the ripening, there are registered even these results which make the object for this study.

MATERIAL AND METHOD

The Pinot noir wines, the 2007 yield, obtained through the implication of three periods of contact between must phases: 5 days (V1), 6 days (V2), 7 days (V3) were analized at 9 months period, the results being compared with the ones registered at must fractions of separation. Were to aim, in both case, the componentes of anthocianic complex and from tannins and total polyphenols of constitution.

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The nominalisation of the 13 chromatic proportions and of the 7 polyphenolic proportions are find with results. For these determinations we used the methods recommended by OIV, in especially one the high degree spectrophotometric methods.

RESULTS AND DISCUSSIONS

The chromatic elements of the wines at 2 moments are quantified in the table 1. In one 9 months the contents in total anthocyans are diminished: with 8.4 % to V1; with 17.5 % to V2; with approximate 13 % to V3. It comes out, in generally, the proportions of losses of colour have tendency of increasing, as the initial contents are much importants. In this case the rule is partial valid.

For this aging of the wines, the proportions of combined anthocyans are much bigger as the total anthocyans are bigger. This aspect is marked by index of pvp % evolutions with values between 18.4 (V1) and 23.1 % (V3). The same meaning is in the case of polymerized anthocyans evolution (index of pp %).

The three category of pigments present different evolutions. For the same wine, in the 9 months: the yellow component increase, the red component decrease, the blue component increase. These evolutions in chromatic structure tend at: decreasing of colouring intensity, increasing of tonality (DO 420/DO 520 nm) and decreasing of flaviliu cations proportions (dA%).

The values of polyphenolic compounds at separation of phases and at 9 months are written in table 2.

Between the moment of separation of phases and the aging of 9 months of wines, the total polyphenols have decreased with 0.107 g at V1; with 0.345 g at V2; with 0.393 g at V3. Therefore, the decreasings in relativ values are much bigger as the initial contents were much higher. The decreasings for all wines have registered also flavonic, unflavonic polyphenol and tannin components too. At tannins the losses were: of 0.138 g at V1; of 0.182 g at V2; of 0.262 g at V3, the similar situations like meaning with from total polyphenols.

During the aging of wines, the condensed and very condensed tannins being degreased in generally with 3 – 5 % (index of HCl %), the astringent tannins decreased with 7 – 8 % (index of gelatine), the colloidal tannins, that meens associated with salts and polysaccharides increased with nearly 3 – 3.5 %.

Table 1

The levels of anthocyanic complex elements at wines
(at separation of phases and 9 months from elaboration)

The characteristics	**V1 Macerated 5 days**		**V2 Macerated 6 days**		**V3 Macerated 7 days**	
	At the phases separation	**At 9 months**	**At the phases separation**	**At 9 months**	**At the phases separation**	**At 9 months**
Total anthocyans mg/l	322	295	389	351	402	349
Free anthocyans mg/l	235	180.2	276	213.1	277	210.8
Combined anthocyans mg/l	87	114.8	113	137.9	125	138.2

Free anthocyans %	73	61.1	71	60.7	69	60.4
Combined anthocyans %	27	38.9	29	39.3	31	39.6
Index of pvp %	12	18.4	14	19.2	16	23.1
Index of pp %	31	40	33	44	36	45
Yellow pigments %	28.4	33.1	28.0	32.4	29.3	32.0
Red pigments %	62.4	56.8	63.0	56.8	62.9	57.8
Blue pigments %	9.2	10.1	9.0	10.8	7.8	10.2
Ic	6.310	5.836	6.416	5.994	6.389	5.968
Tc	0.455	0.582	0.444	0.570	0.465	0.553
dA%	69.7	61.86	70.4	61.85	70.3	63.31

Table 2

The polyphenolic composition of the wines
(at separation of phases and 9 months from elaboration)

The characteristics	**V1 Macerated 5 days**		**V2 Macerated 6 days**		**V3 Macerated 7 days**	
	At the phases separation	**At 9 months**	**At the phases separation**	**At 9 months**	**At the phases separation**	**At 9 months**
Total polyphenols g/l	1.795	1.688	2.479	2.134	2.886	2.493
Flavonic polyphenols g/l	1.275	1.213	1.401	1.360	1.536	1.451
Unflavonic polyphenols g/l	206	196	238	223	296	263
Tannin g/l	1.701	1.563	2.189	2.007	2.755	2.493
Index of HCl %	14.6	11.1	15.4	11.7	17.1	12.1
Index of gelatina %	56.6	49.6	60.4	52.4	64.7	58.1
Index of EtOH %	12.6	15.4	13.1	16.4	13.4	16.1

CONCLUSIONS

During the wines aging of Pinot noir, on the background diminutions of anthocyans, total polyphenols and tannins are produced: increasing from anthocyans proportions in report with total anthocyans to the respective moment; increasing of yellow pigments proportions, decreasing of red pigments, increasing of blue pigments.

The modifications from chromatic structure have presented: the decreasing of couloring intensity and of flaviliu cations and increasing of tonality.

The astringency of wines continuously decreased, instead, the softness character and delicate taste increased, because of the associated tannins with salts and polysaccharides.

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THE INFLUENCE OF SOME BIOTECHNOLOGICAL FACTORS OF PRIMARY VINIFICATION ON COMPOSITION AND QUALITY OF FETEASCA REGALA WHITE WINES FROM DRAGASANI VINEYARD

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KEY WORDS: must, yeasts, activators, fermentation, wine

ABSTRACT

Indifferently of the yeasts type that were involved in the must fermentation, the period of glucides metabolization decrease with the increase of the fermentation temperature. It also came out that the adding of selected yeasts and fermentation activators in must that were rich in glucides, has as results: the decrease of the glucides transformation periods; the increase of the alcohol and glycerol contents; the diminution of the volatile acidity, acetic aldehyde and residual sugar contents, and also of the fermentation output.

INTRODUCTION

In the last decades, in the modern wine industry, the provocated and controlled fermentations, took the place of the spontaneous fermentation which most of the time have uncertain results.

The provocated and controlled fermentations suppose a professional and total control of the biotechnological factors that determine the transformations of the must in wine, which is known as "the birth" of wine. Among the factors, the microbiological one had the most attention of the researches. Concerning this, the studies regarding the using of selected yeasts in the fermentation process of the grapes must and the role of some factors that can intensify the activity during the glucides metabolization had priority (Ciolfi G., Cecchini T. – 1989; Giudici P., Zambonelli C. – 1992; Ribereau-Gayon P. and co. - 1998).

Regarding the optimalization of the biotechnologies, the transformation of the white must that are rich in glucides is studied in this work and the results are shown below.

MATERIAL AND METHOD

The research took place in 2007 viticultural year, using must of Feteasca regala that was obtained from grapes in the phenophase of late over ripening.

At vinification the must had a glucides content of 235 g/l and an acidity of 4,03 g/l (in H_2SO_4). After static clearing under the action of SO_2 (75 mg/l) at low temperature, the decanted must was used in two biotechnological experiments of primary vinification. In the first experiment there were involved indigene yeasts (IY – from spontaneous microflora)

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and selected yeasts (SY – R 73/P breed). For the two types of yeasts there were established 5 levels of fermentation temperatures. They will be mentioned with the results. In the second experiment we used the two types of yeasts (IY and SY) together with the activators of fermentation: Actibiol, yeasts cells walls, thiamine, oxygen, Actibiol+oxygen.

RESULTS AND DISCUSSIONS

The conjugated influence of the yeasts types and the fermentation temperatures is shown in table 1.

For all levels of temperature, the selected yeasts involve superior results regarding the wines composition. The advantages of induced fermentations are: better alcohol production, due to lower fermentation outputs; lower contents of volatile acidity and unreduced acetic aldehyde in the last phase of fermentation; higher glycerol contents and better marks at tasting.

For the same level of temperature the periods of glucides metabolization are smaller with 2 – 4 days.

Due to the selected yeasts, the alcoholic fermentation can progress in good conditions and at temperatures of lower than 20^0C, that is very advantageous for white wines.

It also comes out that in the wines obtained with the action of the selected yeasts, for the same level of temperature, the contents of residual sugar are evidently lower than in the wines fermentated with IY, but without entirely ensuring the biological stability.

Using the same must we realized another experiment and the results are quantified in table 2. For both types of yeasts, in the fermentation medium we also added activators and growth promoters for yeasts. Both in the case of indigene yeasts and also of selected yeasts, the activators had a positive influence on the process of glucides metabolization. The periods of fermentation decrease evidently both at indigene yeasts action and also at induced fermentation.

The output of fermentation improves in all situations. Using only the activators, the walls of the yeasts cells exert the most important influence on the fermentation output. Regarding the ratio of glucides / alcohol, the most favourable result was obtained using the combination: Actibiol + oxygen. Due to the diminution of the fermentation ratio, the alcohol productions increased, but in bigger amount when we used both activator and selected yeasts.

Increases were also recorded at glycerol contents and also more important diminutions at volatile acidity and acetic aldehyde, comparatively with the results from the first experiment.

With all the advantages of the activators, at spontaneous fermentations, residual sugar contents between 5,03 g/l and 6,26 g/l remained unmetabolised, making unsure the biological stability without important doses of SO_2. The using of selected yeasts (SY) + activators had as result in all cases the glucides metabolization to “dry”, the residual sugar contents being situated between 1,43 g/l (SY + Actibiol + oxygen) and 2,47 (SY + thiamine).

The results are such as the ones obtained in similar situations (Popa Ionela– 2008).

Table 1

The conjugated influence of the yeasts types and fermentation temperature on the composition and the organoleptic features of Feteasca regala wine – Dragasani, 2007

Type of yeast	Temp. of ferm. - ^{0}C	Period of ferm. - days	Compositional elements							Mark at tasting
			Alcohol %vol.	Total acidity g/l H_2SO_4	Volatile acidity g/l H_2SO_4	Acetic aldehide mg/l	Glycerol g/l	Residual sugar g/l	Ratio of ferm.	
Indigene yeasts (IY)	9-10	20	12,90	4,33	0,435	48	8,36	13,10	17,20	16,96
	14-15	18	13,08	4,28	0,416	45	8,78	11,21	17,11	17,78
	19-20	17	13,25	4,16	0,402	43	8,90	9,22	17,04	18,60
	22-24	16	13,37	4,12	0,397	42	8,93	7,08	17,05	18,52
	26-28	15	13,17	4,18	0,413	52	8,69	8,02	17,23	17,06
Selected yeasts (SY)	9-10	17	13,33	4,27	0,411	42	9,26	8,06	17,03	17,11
	14-15	16	13,43	4,25	0,402	36	9,55	7,12	16,97	18,40
	19-20	14	13,48	4,09	0,386	31	9,60	6,88	16,92	18,75
	22-24	13	13,42	4,11	0,375	30	9,83	6,72	17,01	18,02
	26-28	11	13,32	4,14	0,398	46	9,72	7,15	17,11	17,13

Table 2

The conjugated influence of the yeasts type and the fermentation activators on the composition of the wines obtained from a must that was rich in glucides (Feteasca regala – Dragasani, 2007)

Type of yeast	Activators of ferm. - ^{0}C	Period of ferm. - days	Compositional elements							
			Alcohol %vol.	Total acidity g/l H_2SO_4	Volatile acidity g/l H_2SO_4	Acetic aldehide mg/l	Glycerol g/l	Residual sugar g/l	Output of ferm.	Free SO_2
Indigene yeasts (IY)	Actibiol	14	13,41	4,21	0.381	36	10,27	6,26	17,05	21,7
	Cell walls	14	13,46	4,20	0.379	35	10,30	6,00	17,01	21,3
	Thiamine	13	13,43	4,23	0,382	36	10,28	6,07	17,04	21,6
	Oxygen	14	13,42	4,24	0,377	35	10,20	6,12	17,05	21,4
	Act.+oxy	13	13,53	4,19	0,351	32	10,36	5,03	17,00	24,1
Selected yeasts (SY)	Actibiol	12	13,70	4,16	0,325	30	10,40	2,44	16,97	23,6
	Cell walls	12	13,75	4,15	0,316	31	10,44	2,33	16,92	23,9
	Thiamine	12	13,71	4,18	0,320	29	10,39	2,47	16,96	24,2
	Oxygen	12	13,69	4,10	0,319	29	10,36	2,50	16,98	25,1
	Act.+oxy	11	13,84	4,12	0,306	26	10,51	1,43	16,87	27,4

Conditions of experiment: glucides in must – 235 g/l; acidity in must – 4,03 g/l (H_2SO_4); SO_2 – 75 mg/l; LSA – 6-6,5 mil cells/ml; temperature of fermentation – 20-22^{0}C

CONCLUSIONS

- In the case of white wines riched in glucides, the fermentations with selected yeasts involve superior results comparatively with the ones obtained from fermentations with yeasts from spontaneous microflora.

- The advantages of fermentation with selected yeasts action are: the shortening of the fermentation period; the diminution of the fermentation ratio; higher contents of alcohol and glycerol; lower contents of volatile acidity and acetic aldehyde.

- Adding the activators of fermentation even in musts that are rich in glucides, the parameters of fermentation and wines composition improve considerable. Concerning this aspect, on the first place is situated the variant: selected yeasts (SY) + Actibiol + oxygen.

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