A Review of Forests, Wood Products and Wood biotechnology of Iran and Germany

Edited by: Prof. Dr. Ali Reza Kharazipour, Dr. Cora Müller and Dr. Christian Schöpper

Foreword

Since the year 2001 the University of Göttingen has signed a "Memorandum of Understanding" (MOE) with the north Iranian University of Mazandaran and since the year 2005 with the Research Institute of Forests and Rangelands. This memorandum implies cooperation in science and research between the Faculty of Natural Resources of the University of Mazandaran, the Research Institute of Forests and Rangelands and the Faculty of Forest Sciences and Forest Ecology of the University of Göttingen.

Hereafter many different activities took place like study internships, summer school and supervision of doctoral students. The requirement for this collaboration was an implementation of international accepted degrees like Bachelor and Master of Science by the Faculty of Forest Sciences and Forest Ecology of the University of Göttingen. The Faculty posses a long tradition and a far-reaching experience in Forestry and wood industry and can rely on a wide-ranging and competent local and international operating network.

The cooperation between the University of Göttingen, the University of Mazandaran and the Research Institute of Forests and Rangelands should be enhanced in the special program „German-Arabian/Iranian Education Dialogue" of the German Academic Exchange Service (DAAD). This program is for encouraging partnerships of Universities in Arabian countries and the Iran and emerged from the special program "European/Islamic Culture Dialogue", which was found in the year 2001.

Furthermore it is desirable to establish a research and development network in close collaboration with the "Kompetenznetz Holz e.V, which should contribute to solutions of interdisciplinary questions

Target of this special program is to advance a professional cooperation and an intercultural dialogue. Thereby it should be able for junior scientists, especially women, from both countries to get a multifarious and modern education. Lasting science and research objectives should be started for developing and enlarging master and postgraduate programs of study in the Iran. An additional and essential issue of this project is a purposeful transfer of technology and an establishment of environment-friendly and preserving resources processes in forestry and wood industry.

Hence, an assignment is to get together different delegates of forestry and wood industry, of research, industry and politics from both countries and to discuss problems and dares of a sustainable forestry and wood industry and to develop sustainable oriented strategies. Thereby processings and technologies, which are tested in Germany, could to be used in Iran.

The present book shows a partial balance of our activities within the scope of this project in 2006. The book contains the sessions and talks of junior scientists from Iran and Germany. Also these activities should emerge further collaborations between scientists and industrial partners of both countries.

The realisation of this project was possible by the work and help of numerous persons and institutions both in Germany and Iran. My particular thanks apply the director of the Research Institute of Forests and Rangelands, Teheran, Dr. Asareh and the responsible person for partnership in Iran, Mr. (M.Sc.)

Göttingen, January 2007.

Prof. Dr. Alireza Kharazipour, Project Manager

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"Investigation on potential of long fibre Pinus eldarica Kraft Pulp"

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Abstract

Kraft pulping of Pinus eldarica wood was carried out at 18% active alkali, 25% sulphidity and pulping temperature & Time of 165 C and 90 minutes pulp was compared with imported unbleached long fibre pulp with the kappa number of 34. Pinus eldarica pulp (500 ml csf) and imported long fibre pulp 120 g/m2 hand sheets were prepared for testing. Blending ratios were kept at 5, 10, 15 and 20 % for both long fibre pulps.

Upon mixing 5, 10, 15, 20% Pinus eldarica pulp with hardwood pulp, breaking length increased to 6.9, 7.77, 8.01 and 9.14 Km. Respectively, and corresponding figures for imported pulp addition were 6.71, 8.2, 8.25 and 9.27 Km respectively. Tear index is also measured. Adding 5, 10, 15, 20% Pinus eldarica pulp to local hardwood pulp produced tear indexes of 5.93, 6.95, 6.65, 7.08 mNm2/g respectively and corresponding figures for imported pulp 5.38, 5.61 and 5.60 kPa.m2/g with blending above percentages of P. eldarica pulp and 4.78, 5.27, 5.38 and 6.23 kPa. m²/g upon addition of imported pulp.

The results of this investigation indicated that blending 5% of either P. eldarica or imported pulp did not improve hand sheet strengths, but other mixing ratio improved the strengths. However since no statistically significant difference was observed between 15 and 20% addition, it can be concluded that blending 15% P. eldarica Kraft pulp is sufficient.

Introduction

The strategic importance of paper and paper products is to be searched in social – economic development of this community. This fact necessitates the development of paper industries based on local and regional resources of cellulose fibres. In such efforts national research and development activities helped efficient utilization of unknown fibre supplies. Pinus eldarica has been one of the

unknown species with good potential in supply of long fibre wood for pulp production which requires applied research to discover its industrial potential.

Previously Tabatabaei and Teror (1970) had investigated the tracheids geometry of Pinus eldarica wood and expressed the tracheids diameter of early wood and latewood at 22 and 32.5 /m respectively and cell wall thickness of these woods were measured at 2.5 and 2.4 /m. The average tracheids length was calculated at 1.339 mm. Habibi (1991) studied the tracheids length and cell wall thickness variation of this wood. The results of this study indicated that the tracheids length increases with the height of the sample in tree stem. The average length and cell wall thickness of early wood tracheids were calculated at 1.27 mm and 5.13 mm and respective values for latewood were 2.24 mm and 5.44 mm. Ksir & Haly (1988 – 1989) studied the anatomical features of P. pinea. P. brutia P. eldarica and P. halopensis and expressed that P. eldarica with the highest growth rate and latewood is the most suitable species for pulping. Pulping characteristics of Pinus teada was studied by Lobosky & Ifju (1981). This study showed that early wood fibres increased the tensile and burst strength of the pulp and latewood fibres improved

The thinner cell wall

The thinner cell wall, plasticity and flexibility of early wood fibre were anticipated to be the cause of improvement in tensile and burst strength of hand sheets. McFarlane & Sera fin (1966) studied the influence of tree age on pulping characteristics of Pinus *patula* wood grown in Kenya. Trees in age class of 7, 12, 15, and 22 years were selected for pulping and pulps were refined to 500 ml CSF freeness prior to hand sheet making. The results of this study revealed the superiority of wood from 7 years old tress. The highest yield of 52.4% at kappa no. of 42 was obtained and the breaking length. Burst factor and tear factor of 12800 m. 92 Kpa.m²/g and 98 mN.m²/g respectively were the highest among different ages. Young (1964) also investigated the influence of tree age on pulping properties of Pinus *radiata* grown in Australia. Pulps prepared from woods of 8, 13, 18, and 24 years old trees were refined to 450 ml CSF and the strength of hand sheets were measured. Young (1964) expressed that age did not influence the tear factor. But burst factor was increased with age. Chittenden and Palmer (1960) studied the relationship between wood specific gravity and the pulp properties of Pinus teada. Trees with the age of 14 and 25 years with respective specific gravity of 0.4 and 0.45 were pulped. Breaking length increased from 5600 to 8200 m. burst factors increased from 36 to 55 Kpa.m²/g and tear factor increased from 120 to 130 mN.m²/g as the specific gravity increased from 0,4 to 0.45.

Materials & Methods

Sampling

Three 23 years old *Pinus eldarica* trees were felled from exotic tree adaptation project site implemented at Peakaleh-Zaghmarz ground and one meter long bolt was cat from the bottom of stem. These anatomical and chemical characteristics of sample trees are summarized in table 1.

Table 1

Annual Growth	Specific Gravity Dry	Tracheids Geometry				Chemical Composition			
		Length	Diameter	Lumen	Cell wall thickness	Cellulose	Lignin	Extractive	Ash
mm.		mm.	m.	m.	m.	%	%	%	%
5.4	0.4	3.64	49.9	36.19	7.52	49.95	21.40	6.53	3.9

Bolts were chipped in a Pallmann drum chipper and chops were screened and then stored in polyethylene plastic bags prior to pulping. Kraft pulping was carried out with 4- cylinder rotating digester. Pulping variables were 18% A.A. (Na_2O basis), 25% sulphidity, 160 °C cooking temperature, 90 minutes cooking time and liquor to wood ratio of 5:1.

At the end of each pulping period pulp was refined in 8 inch sieve. *Pinus eldarica* pulp yield and kappa no. before and after screening is summarized in table 2.

Table 2: Yield and kappa No. of *Pinus eldarica* Kraft pulp before and after screening

	Kappa No.	Yield
Before	35.50	39.13
After	32.00	33.62

Local supply hardwood Kraft pulp with the kappa no. of 85.2 and 663.38 ml CSF was refund to 350 ml. CSF and long fibre imported pulp prepared *Pinus eldarica* pulp with given kappa no. were refined to 500 ml. CSF. *Pinus eldarica* and imported pulps were mixed with locally produced hardwood Kraft pulp and then hand sheets were prepared.

In order to evaluate the potential of *P. eldarica* Kraft pulp as long fibre pulp additive to reinforce hardwood Kraft pulp 5, 10, 15 and 20% addition of long fibre pulps was investigated.

All experiments were in accordance with TAPPI testing procedures as follow:

-Laboratory Beating	T-248-cm-85
-Freeness	T-227-om-92
-hand sheet preparation	T-205-om-88
-Test sample preparation	T-220-om-88
-Breaking Length	T-294-om-88
-Tear Strength	T-414-om-88
- Burst Strength	T-403-om-91
- Folding endurance	T-423-om-89

Table 3: Average Strength Properties of hand sheets from various blends of Pulps

Kraft Pulp (%)		Breaking Length (Km)	Tear Strength (mNm2/g)	Burst Strength (Kpa.m2/g)	Folding Endurance
Pinus Eldarica	5	6.90	5.93	4.82	48.5
	10	7.77	6.95	5.38	69.25
	15	8.01	6.65	5.61	68.50
	20	9.14	7.06	5.60	74.25
Imported Pulp	5	6.71	7.56	4.78	45.00
	10	8.02	8.10	5.27	64.57
	15	8.52	8.38	5.38	73.25
	20	9.27	9.36	6.23	90.50

Results and Discussion

In order to investigate the possible utilization of *Pinus eldarica* Kraft pulp to replace imported long-fibre pulp, the strength properties of locally produced mixed hardwood pulp (c), imported long-fibre pulp (A),laboratory produced *Pinus eldarica* Kraft pulp (B) were measured.

Then hand sheets from various proportions of pulp A or B with C were prepared and the strength of these hand sheets was measured. The results are summarized in table 3 and plotted in figures 1 thru 4. The results of this study indicated that the addition of either pulp A or B to pulp C will improve breaking length. Statistical analysis of data for breaking length reveals the significant difference at 1%

level between breaking length of long fibre pulps and various mixtures of long fibre pulps with locally produced mixed hardwood Kraft pulp as compared with pure mixed hardwood Kraft pulp. Average breaking lengths of hand sheet from various mixtures were tabulated using DMRT.

Accordingly average breaking length of hand sheets from imported long fibre pulp (A), *Pinus eldarica* Kraft pulp (B) and mixed hardwood Kraft pulp (C) are in three different groups and the highest value of 10.1 km belongs to pulp A followed by 9.1 km for pulp B and the lowest value of 6.4 km was obtained from hand sheets produced from mixed hardwood Kraft pulp.

Upon addition of pulp A or B to pulp C at various ratio between 5 to 20%, breaking length increased. However no statistically significant difference was observed between 15 and 20% addition of either pulp A or B. of course the rate of improvement with addition of either pulp A or B was the same up to 20% addition which indicate the potential of P*inus eldarica* pulp as substitute for imported long fibre pulp.

Tear strength of hand sheet was also measured. Tear strength was improved with the addition of either pulp A or B. statistical analysis of tear strength of hand sheets prepared from pulp A, pulp B, pulp C and also various mixtures of either pulp A or B with C indicated the significant difference at 1% level, with the highest value belonging to pulp A followed by pulp B and C.

Increasing the rate of addition of pulp A to pulp C from 5 to 10% significantly increased the tear strength, but from 10 to 15 or 20% addition no significant increase was observed. However the highest tear strength was observed with 20% addition of pulp A. on the other hand the addition of 5% pulp B pulp C did not affect the tear strength.

Furthermore there was no difference between 10, 15 and 20% addition of pulp B, but the relevant values are lower than the addition of pulp A, but higher than pulp C.

Average burst strength values of pure pulp A, pulp B and pulp C and mixture of pulp A with C are different at 1% level. The burst strength of pulp B is higher than pulp A or pulp C, and addition of 5 to 15% pulp A to pulp C increased burst strength, but there was no difference between 15 and 20% addition. Similar changes were seemed with the addition of pulp B to pulp C. However no significant difference was observed between 10, 15 and 20% addition of pulp B. The highest burst strength was observed with 20% addition of either pulp A or B. Folding endurance of hand sheet was measured and the results indicated the statistical difference at 1% level between pure pulp A, B and C. The highest values belong to pulp B followed by pulp A and C.

Addition of 5 to 20% of either pulp A or B to pulp C increased folding endurance and the highest values were observed with the addition of 20% either pulp A or pulp B.

Fig. 1 Differences of Breaking Length
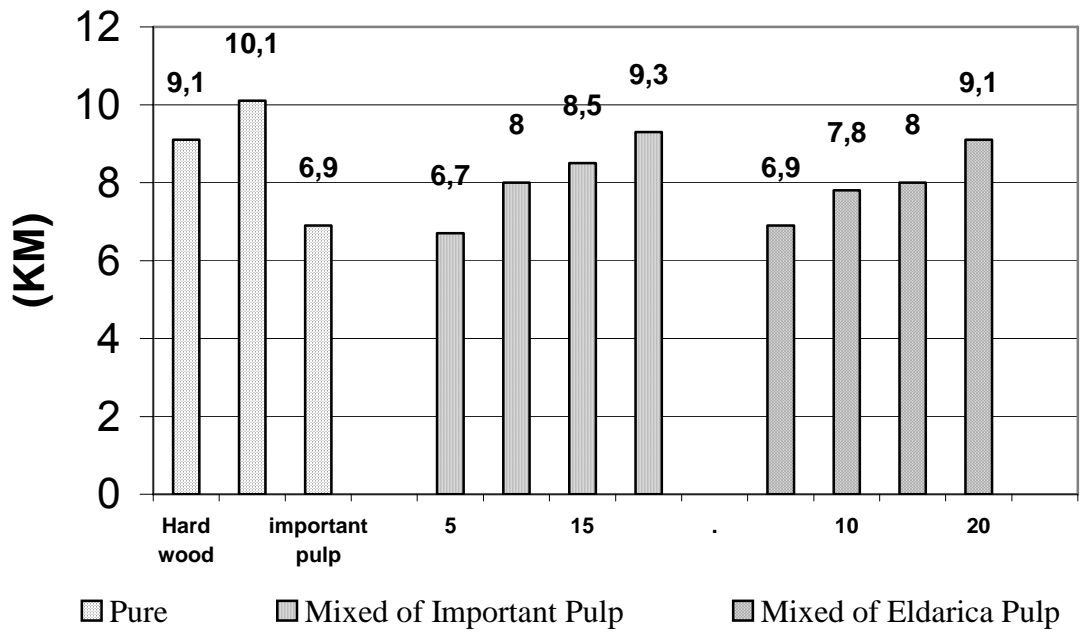
Fig. 2 Differences of Anatomical Properties
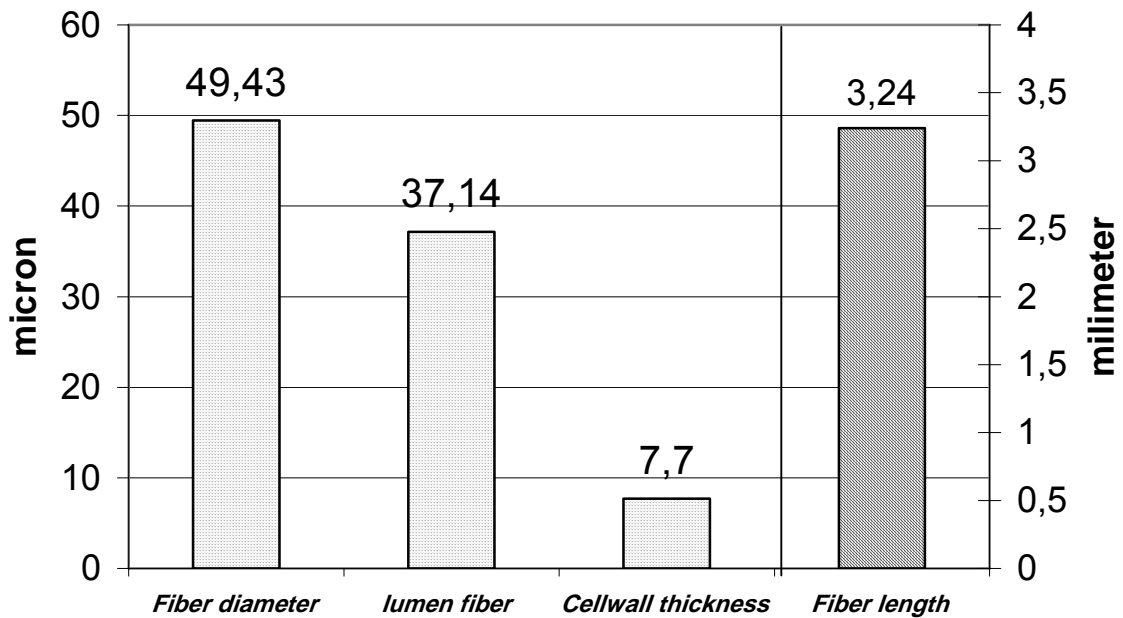
Fig. 3 Differences of Burst Factor

Bar chart showing Burst Factor values:
- Hard wood (Pure): 5,3
- important pulp (Pure): 6,7, 4,6
- 5: 4,9, 5,3
- 15: 5,4, 6,3
- . (10): 4,8, 5,4
- 20: 5,6, 5,6

Legend: Pure | Mixed of Important Pulp | Mixed of Eldarica Pulp
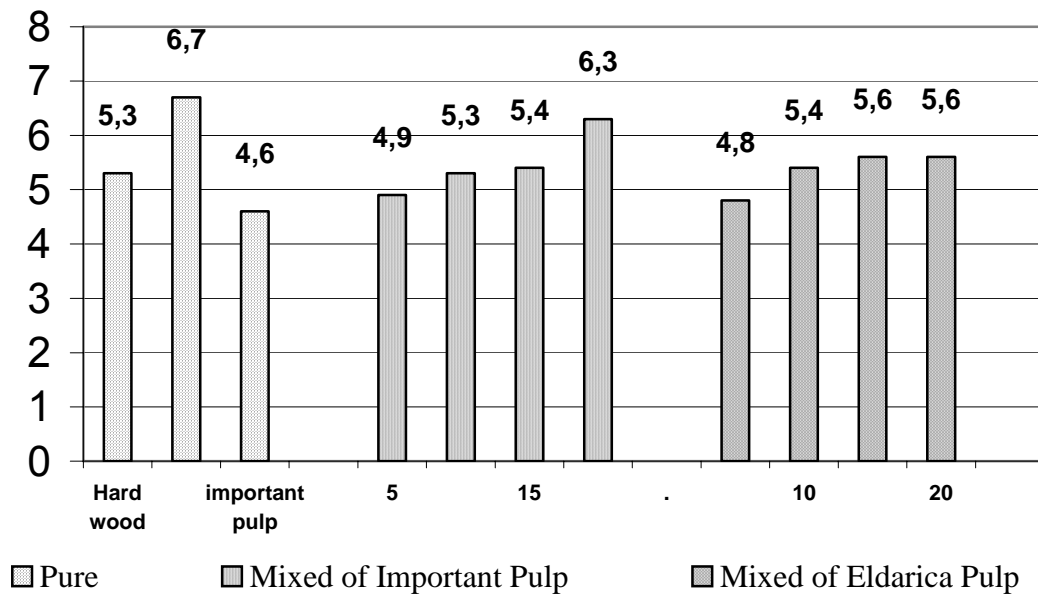
Fig. 4 Differences of Tear Factor

Bar chart showing Tear Factor values:
- Hard wood (Pure): 10
- important pulp: 8,5, 5,7
- 5: 7,6, 8,1
- 15: 8,4, 9,4
- . (10): 5,9, 7
- 20: 6,7, 7,1

Legend: Pure | Mixed of Important Pulp | Mixed of Eldarica Pulp
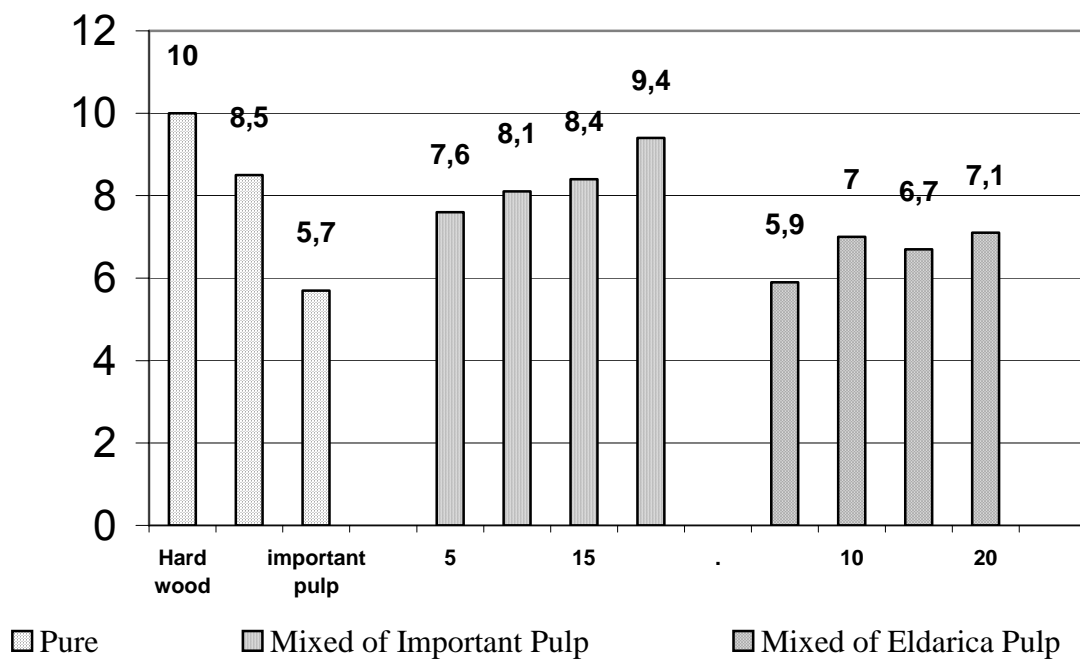
Fig. 5. Differences of Folding
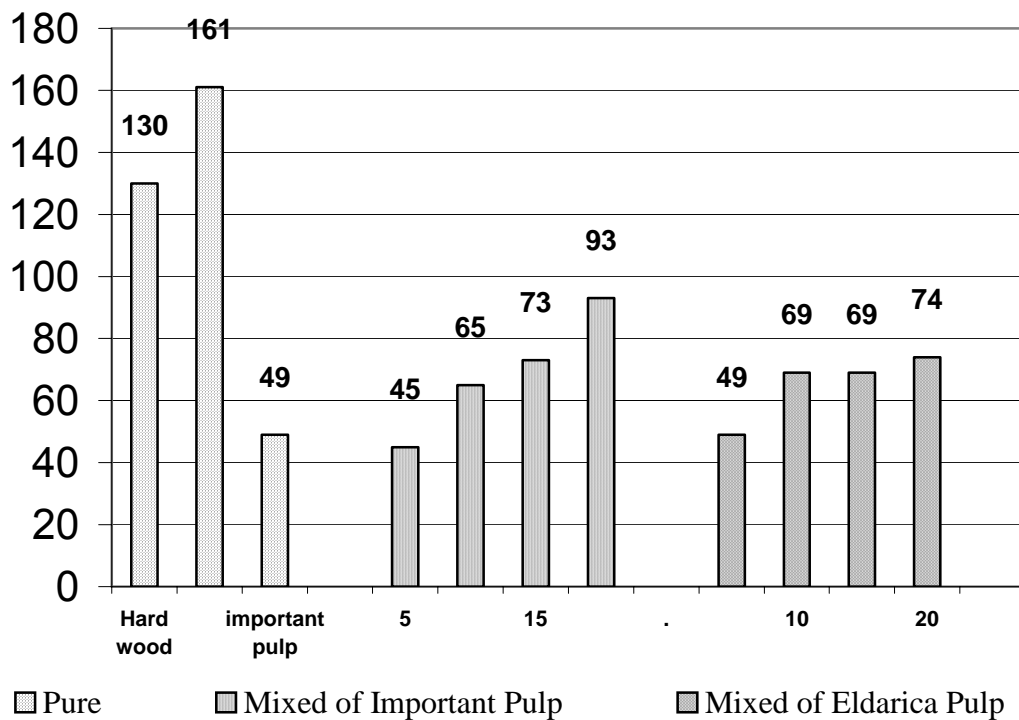
Conclusions

The results of this study revealed the potential of *Pinus eldarica* Kraft pulp to substitute imported long fibre pulp. Except 5% addition of *Pinus eldarica* Kraft pulp to locally produced mixed hardwood pulp. Addition of 10, 15 and 20% improved strength properties of long fibre pulp or *Pinus eldarica* Kraft pulp was the same.

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"Development of a wheat protein based adhesive for the production of medium density fibreboards"

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Introduction

The medium density fibreboard was developed in the United States. In the mid sixties the first industrial MDF plant station was build in Deposit, New York by the company Allied Chemical. Mainly two reasons were responsible for the development of this kind of wood based panel. One reason was the worse mechanical properties of the hard density fibreboards produced in the Unites States and the few application possibilities involved. Another reason was the bad quality of the three layered particleboards produced in the United States (LAMPERT, 1966). Because of the used low quality raw materials and the non optimised production parameters for particleboard production the mechanical-technological properties of the particleboards were not satisfying. Based on this situation there was a market gap opened for medium density fibreboards (DEPPE & ERNST, 1996).

Medium density fibreboards were produced in the beginning with thicknesses from 3 mm to 100 mm and densities from 600 kg/m³ to 900 kg/m³. By further technical development it was possible to produce thinner MDF boards on the basis of calendar- or conti-roll press procedures. Over the years the technical process and the raw materials for the production of medium density fibreboards were continuously processed. Up-to-date the medium density fibreboard itself and the production process are highly developed. Nevertheless processing on MDF boards and production parameters is still in progress and necessary for the wood based panel industry to be able to compete in future (DEPPE & ERNST, 1996).

The production amount of medium density fibreboards in Europe steadily increased in the last years. In 1998 the European MDF industry produced about 6 million m³. 2004 the production amount was already 14 million m³. The latest prognoses quote that the production amount of medium density fibreboards will further increase in the next years to satisfy the needs of MDF (EUROPEAN PANELBOARD FEDERATION, 2004).

The increase of the required demand on medium density fibreboards is founded in the advantages that MDF boards have compared to three layered particle boards. Advantages are the possibilities of direct printing, direct painting, edge veneers are not required and there are more application possibilities for medium density fibreboards than for three layered particleboards. Main market areas are the furniture industry and the building industry. Almost 54% of all produced medium density fibreboards are used in the furniture industry, 29% are required in the building industry (EUROPEAN PANELBOARD FEDERATION, 2004).

The nowadays used resins e.g. urea formaldehyde, phenol formaldehyde or melamine urea formaldehyde are all based on the non renewable resource crude oil. These resins are all highly developed for the production of wood based panels and by using these resins derived timber products with special mechanical-technical properties can be achieved. Due to the steadily increasing oil price the price for these resins increases steadily, too. Actually up to 20 % of the total production costs are caused by the resin price depending on the kind of resin that is required. The wood based panel industry is forced to develop new and cheaper bonding agents that are not based on crude oil to compete in future. Last but not least the utilisation of environmentally friendly resources is an ecologically benefit that could bring advantages for wood based panel industry in the coming years.

The production of low-formaldehyde medium density fibreboards is of current interest for the wood based panel industry (DUNKY, 2005). Formaldehyde emissions are quite a problem as well for producers as for end consumers (ROFFAEL, 1982; SUNDIN & ROFFAEL, 1989; ROFFAEL et al., 1993). Formaldehyde was classified as carcinogen suspicious for a very long time but latest studies done by the INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC) in the United States showed that formaldehyde is carcinogenic (IARC, 2004). Based on these cognitions the wood based panel industry is forced to produce low-formaldehyde or non-formaldehyde wood based panels. There are two possibilities to realise this kind of derived timber products. One opportunity is to decrease the amount of formaldehyde in the common used petrochemical based resins. Formaldehyde is always required as reaction partner in these adhesives. By decreasing the amount of formaldehyde in the resin the properties of the produced wood based panels will deteriorate. Another opportunity for the production of formaldehyde free wood based panels is the use of natural bonding agents that contain no formaldehyde.

In recent years natural bonding agents gain more in importance because of their wide application areas. The earlier economical advantage of the petrochemical based bonding agents, the low crude oil price, becomes nowadays less important by the ever increasing price of crude oil. In contrast to this aspect the ecological advantage, the sustainable utilisation of renewable resources, becomes more and more important for the producers as well as for the consumers.

To develop a natural formaldehyde free bonding agent for a later application in medium density fibreboard production this project was initiated and applied by the PFLEIDERER HOLZWERKSTOFFE GmbH & Co. KG, Arnsberg in cooperation with the INSTITUTE FOR FOREST BOTANY, University of Göttingen and the CERESTAR company, Krefeld. The main objective of this project was to optimise a by-product of the starch industry and to develop an alternative adhesive, the so called wheat protein suspension, for a later utilisation as bonding agent in the wood based panel industry as substitute for the nowadays used petrochemical based resins.

Research Focus

The main research focus of this project is to create a natural bonding agent based on wheat protein for a later production of medium density fibreboards that fulfil the requirements regarding to their mechanical-technological properties and formaldehyde emissions given by the DEUTSCHES INSTITUT

FÜR NORMUNG and European norms. Actually there is no economical utilisation for this by-product of the starch production that occurs in huge amount throughout the whole year. In minor quantities it is used as feeding material in livestock breeding.

Due to the fact that the starch production is a very well developed process a constant level according to the product quality of this by-product that is based on renewable resources is given. The price for the wheat protein suspension is lower than the price of urea formaldehyde so that an industrial utilisation of this product would increase the economic efficiency of this product and the wood based industry would be able to reduce their production costs by using wheat protein as bonding agent. The bonding properties of proteins in generally do not depend on a certain pH value so that an utilisation of the wheat protein suspension with urea formaldehyde or phenol formaldehyde should be possible. The monomers in the wheat protein suspension can react to stable polymers by using a certain temperature and pressure. All these reasons are advantages that theoretically allow an utilisation of the wheat protein suspension as natural bonding agent for the production of MDF boards.

To develop a natural bonding agent for a later production of wood based panels' e.g. medium density fibreboards many research focuses have to be recognised during this development. The solid content of the bonding agent should be comparable to nowadays used petrochemical resins. The main amount of water that is added by the required bonding agents, fibres and additives during the production process of wood based panels has to be dried for the later hot pressing process. Never the less it is very important to hold the viscosity of the bonding agent on a more or less constant level during decreasing the water amount. The viscosity of a natural bonding agent has to be comparable to the viscosity of resins to make sure that it is possible to use it in industrial panelboard production processes. Otherwise it would not be possible to spray this natural bonding agent on the fibre material during the gluing process, the distribution of the bonding agent would not be homogeneous and in the end well mechanical-technological properties could not be achieved. Therefore the water amount in the natural bonding agent should be decreased to an optimum to avoid expensive drying costs during the production process. The optimised solid content in a bonding agent is reached when a further reduction of water is not possible without downgrading the viscosity.

Another important aspect during the development of natural bonding agents is that the natural bonding agent is compatible to additives like hydrophobic substances, fungicides, curing agents or fire protecting substances that are added during the production process to achieve certain properties of the wood based panels. Even an utilisation of the natural bonding agent with petrochemical resins (mixed condensates) should be possible to analyse the maximum possible substitution amount of these resins in combination with the new natural bonding agent.

By the use of different so called mixed condensates consisting of the natural bonding agent and nowadays used petrochemical resins the use of the natural bonding agent as formaldehyde catcher can be analysed. Therefore several mixed condensates consisting of different ratios of wheat protein suspension and urea formaldehyde respectively different ratios of wheat protein suspension and phenol formaldehyde are analysed in line with this project.

Last but not least later recycling possibilities for wheat protein bonded wood based panels are very important. Since June 2006, given by the TA-Siedlungsabfall, it is no longer allowed to store materials

that contain more than 5 % organic components on dumps. Due to this fact new recycling possibilities or disposal possibilities for wood based panels are required.

Materials & Methods

The aims of this project should be achieved by dividing the research and development into two main focuses. One main focus is on the development and analysis of the wheat protein suspension. The other main focus is on the production of medium density fibreboards in pilot scale by using the wheat protein suspension and mixed condensates as bonding agents. For checking purposes medium density fibreboards bonded with pure urea formaldehyde respectively with pure phenol formaldehyde are produced in pilot scale under the same industrial production parameters than the wheat protein bonded boards. The produced boards are later tested regarding to their mechanical-technological properties and their formaldehyde contents.

The main focus development and analysis contains increasing the solid content, measuring the viscosity and analysing the main ingredients of the natural wheat protein suspension. This work is done in cooperation with CERESTAR, Krefeld and the INSTITUTE FOR FOREST BOTANY, Göttingen. The solid content of the wheat protein suspension should be increased by evaporating the water under low temperatures so that the proteins do not denature during this process. Furthermore a preservative should be added to this bonding agent to optimise the storage stability of the wheat protein suspension.

For the production of medium density fibreboards the full automatically MDF pilot plant station of the Competence Network for Sustainable Utilisation of Wood (www.kompetenznetz-holz.de) is used. By using this pilot plant station it is possible to produce medium density fibreboards under controlled industrial production parameters in pilot scale. It is possible to use different kind of bonding agents, additives and fibre materials on this pilot plat station. Medium density fibreboards with end thicknesses from 3 mm up to 20 mm can be produced analogue to the wet, dry or semi dry method. The fibres and the bonding agent are mixed in a new blender system. After mixing the fibres, bonding agent and additives the fibres can be dried in a 60 meter long drying line using different temperatures. After drying the glued fibres are collected in a bunker and than strewed to a non woven fibre mat in different thicknesses according to the later end thickness and density of the panelboard. This non woven fibre mat is pressed in a first step without temperature and in a second step pressed in a hot press to medium density fibreboards.

In line with this project medium density fibreboards with thicknesses from 4 mm up to 16 mm are produced. The average density of these medium density fibreboards is about 750 kg/m³. The board dimensions are 800 mm x 450 mm. The quantity of bonding agent is about 10 % to 12 % related to absolute dry fibre material. Paraffin is used in quantities from 0.5 % up to 2 % related to absolute dry fibre material. The average moisture content of glued and dried fibres is 9 %. The medium density fibreboards are cold pressed with 50 bar for 2 minutes and than pressed at 195 °C with a pressure of 5,8 N/mm². Press time is minimum 18 seconds per mm board thickness and maximum 30 seconds per mm board thickness. As hot press a SIEMPELKAMP electrical heated press with spacers is used. Each

production run consists of six boards under the same conditions so that the results are statistically firm.

Results and Perceptions

Research and development of the wheat protein suspension

The chemical analyses of the wheat protein suspension regarding to the main ingredients showed that this by-product of the starch industry mainly consists of saccharides, hemicelluloses and proteins. Related to the solid content the wheat protein suspension contains 45 % saccharides, 30 % hemicelluloses and 25 % proteins in average (see Figure 1). These relations did not change during the later increase of the solid content. Even the latest developed wheat protein suspension with a solid content of 43.5 % (November 2004) shows the same ratios for proteins, saccharides and hemicelluloses. That leads to the result that there is no shift in the composition of the wheat protein suspension related to the main ingredients by increasing the solid content.

Main ingredients of the wheat protein suspension
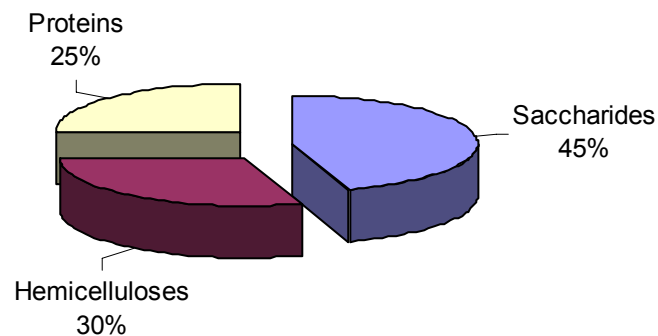
Figure 1: Main ingredients of the wheat protein suspension

In the beginning of the project term the solid content of the wheat protein suspension was increased from 20.3 % in January 2004 up to 43.5 % in November 2004 by the CERESTAR Company, Krefeld (see figure 2). The water amount was much reduced by using low temperatures and pressure. This is a very important development to guarantee a later efficient and cost optimised industrial production using wheat protein suspension as bonding agent for wood based panels.

At this stage (December 2006) the solid content of the wheat protein suspension is further increased up to 55 %. Our aim is it to increase the wheat protein suspension until the end of this project up to a solid content of 66 % that would be comparable to the solid content of nowadays industrial used urea formaldehyde. By using a natural bonding agent with an equivalent solid content compared to urea formaldehyde the costs for drying and the parameters for the production of medium density fibreboards would be nearly the same than in the present production of wood based panels.
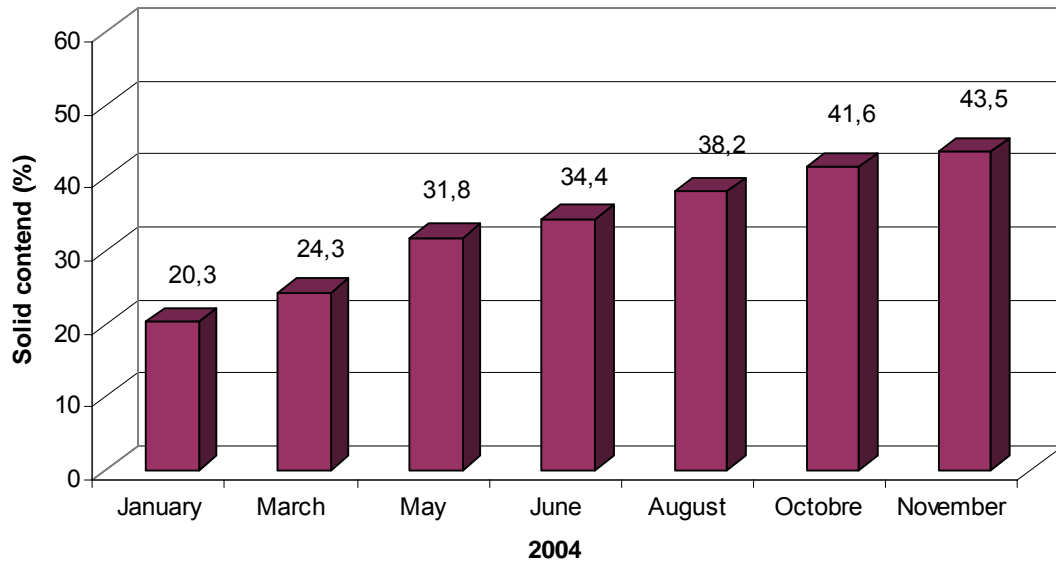
Figure 2: The increase of the solid content of the wheat protein suspension from January 2004 until November 2004

The viscosity of the wheat protein suspension was first analysed in the beginning of the project (see Figure 2) and later during the increase of the solid content. The results of the viscosity analysis show that the wheat protein suspension has an average viscosity of 200 mPa·s to 250 mPa·s in the beginning of the project. This viscosity is lower than the average viscosity of urea formaldehyde (400 mPa·s) but still very well for an application as bonding agent for the production of wood based panels. By increasing the solid content the viscosity raised up to 350 mPa·s.

Even adding additional components like hydrophobic substances, fungicides, flame retardants or hardeners leads to very well viscosities. That is a proof that the wheat protein suspension can be used in combination with additional components during a MDF production process but it is not a proof that these combined components work in the way they are expected to do.
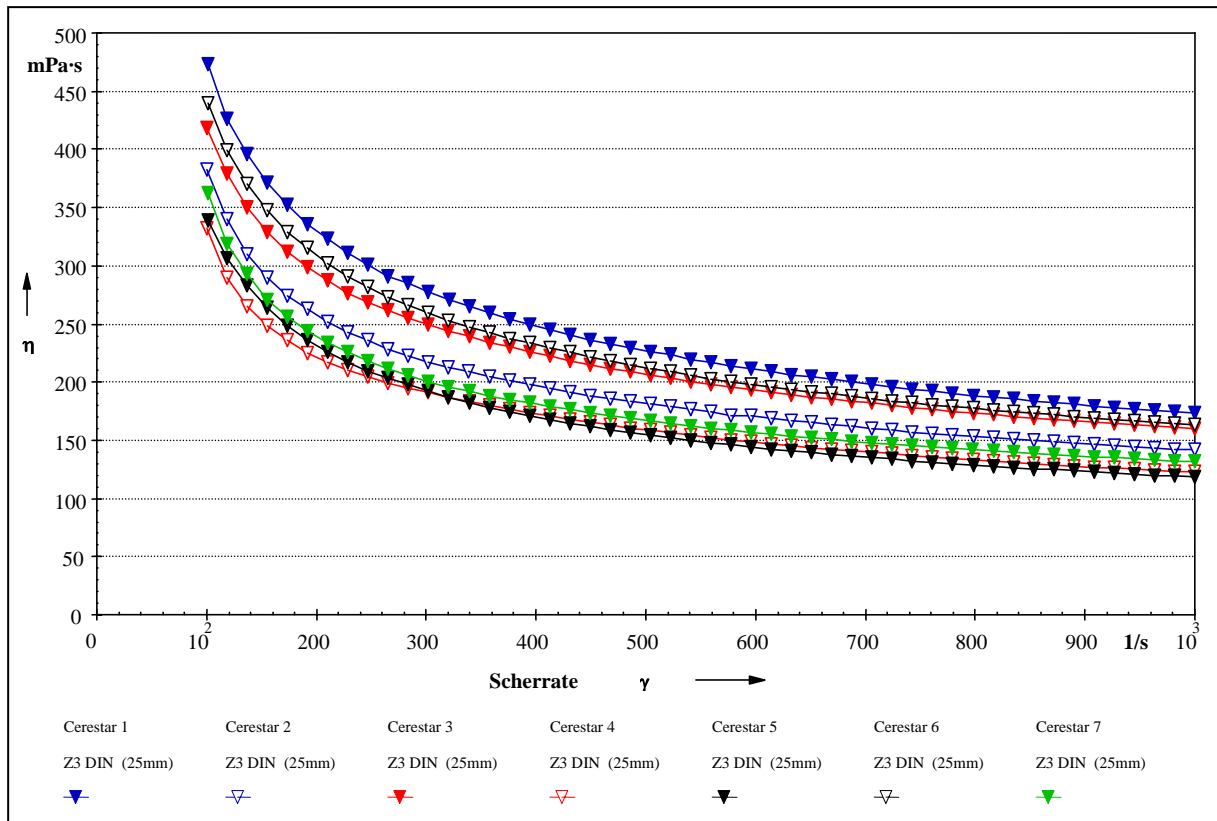
Figure 2: Viscosities of the wheat protein suspension

Development of wheat protein bonded medium density fibreboards

In Figure 3 the mechanical-technological properties of wheat protein bonded medium density fibreboards are represented. The internal bond strengths of nearly all these boards fulfil the requirements given by the DEUTSCHES INSTITUT FÜR NORMUNG (DIN) and the European norms (EN). Only the 16 mm thick medium density fibreboards do not reach the supposed internal bond strength. The press temperature (195 °C) was obviously to low so that the bonding agent was not able to react in the middle of these boards. To achieve well bonding strength in wheat protein bonded medium density fibreboards with thicknesses more than 16 mm a press temperature of 210 °C or more must be chosen.

The swelling properties of the wheat protein bonded show a significant developing. The thicker the medium density boards are the higher are the swelling results after 24 hours. In most cases the swelling results do not fulfil the required properties given by DIN / EN. Up to a board thickness of 6 mm it is possible to use pure wheat protein suspension as bonding agent. The swelling properties after 24 hours and the internal bond strengths are very well. Due to the fact that the used press temperature of 195 °C was to low for the production of thicker boards in pilot scale the internal bond strengths and the swelling properties after 24 hours are negatively influenced. These results lead to the production of medium density fibreboards in pilot scale under the utilisation of mixed condensates in which only a certain ratio of the total required urea formaldehyde is substituted by the wheat protein suspension.
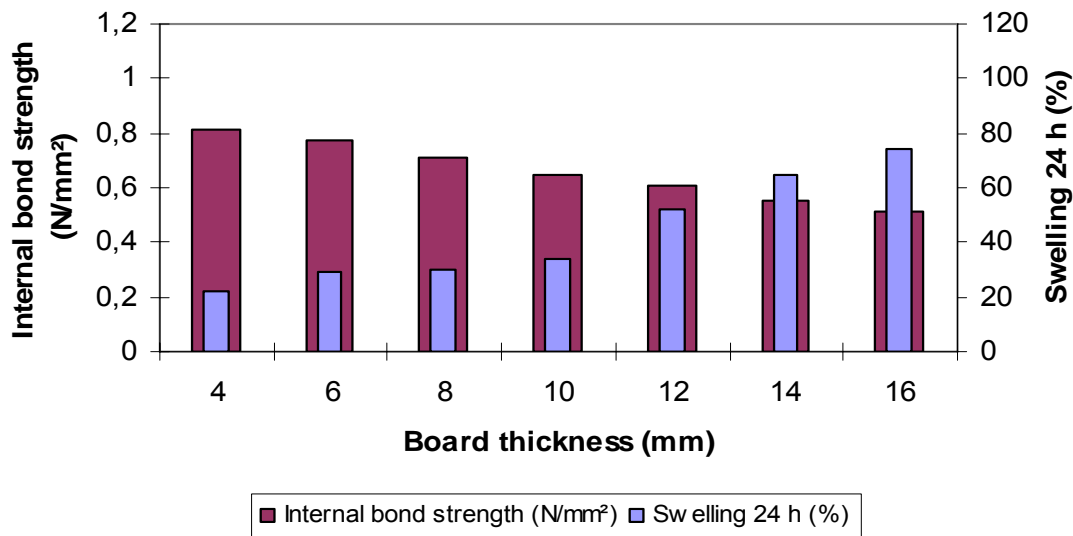
Figure 3: Mechanical-technological properties of wheat protein bonded medium density fibreboards

The mixed adhesives consist of different ratios of wheat protein suspension (WP) and urea formaldehyde (UF) respectively wheat protein suspension (WP) and phenol formaldehyde (PF). The ratios are varied in 25 % steps and as controls medium density fibreboards bonded with 100 % wheat protein respectively 100 % urea formaldehyde or 100 % phenol formaldehyde were produced. The best mechanical-technological results were achieved with mixed condensates consisting of 75 % urea formaldehyde and 25 % wheat protein suspension respectively 75 % phenol formaldehyde and 25 % wheat protein suspension. The results of these medium density fibreboards are shown in Figure 4.
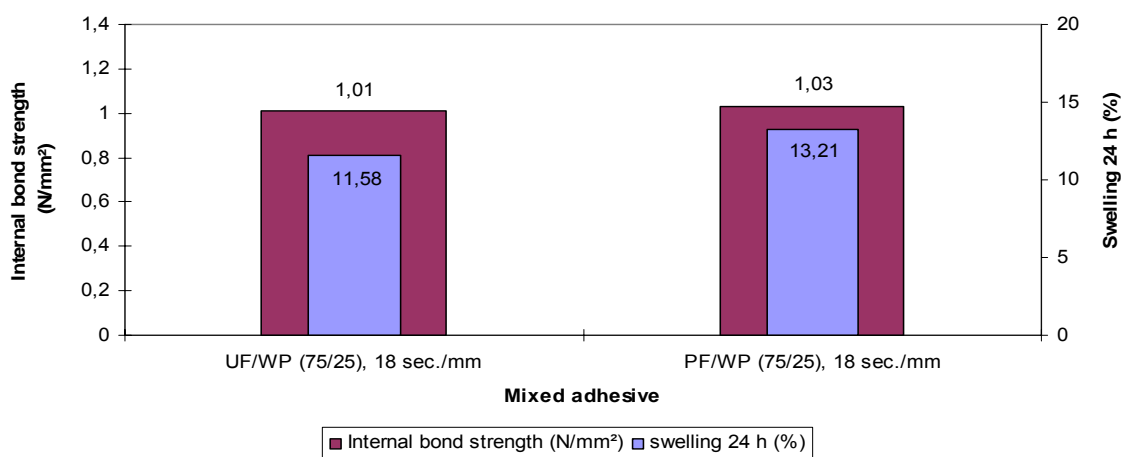
Figure 4: Mechanical-technological properties of 10 mm thick wheat protein (WP) and urea formaldehyde (UF) bonded respectively wheat protein (WP) and phenol formaldehyde (PF) bonded MDF with 750 kg/m³ density

The utilisation of mixed condensates leads to very high internal bond strengths and very low swelling properties after 24 hours without using any kind of hydrophobic substances. All properties fulfil the required values after DIN / EN. The press time of 18 seconds per mm board thickness used on an

electrical heated press is comparable to industrial used press times of 9 seconds per mm board thickness on a Conti-roll press. By increasing the amount of the wheat protein suspension up to more than 25 % in the mixed condensates the swelling properties deteriorated and did not fulfil the requirements given by DIN / EN.

By substituting the total required amount of conventional bonding agents by the natural wheat protein suspension for the production of medium density fibreboards it is possible to reduce the formaldehyde emissions of these wood based panels. The formaldehyde emissions of the medium density fibreboards produced with mixed adhesives consisting of wheat protein suspension (WP) and urea formaldehyde (UF) and wheat protein suspension (WP) and phenol formaldehyde (PF) are shown in Figure 5. By substituting 25 % of the total mount of conventional resins by the wheat protein suspension it is possible to reduce the formaldehyde emission. The fact that the formaldehyde emissions in these medium density fibreboards are reduced at more than 25 % is a proof that the wheat protein suspension can react as a formaldehyde catching substance.

The utilisation of the developed natural wheat protein suspension in combination with urea formaldehyde or phenol formaldehyde for the production of medium density fibreboards results in lower production costs, lower formaldehyde emissions and very well mechanical-technological properties.
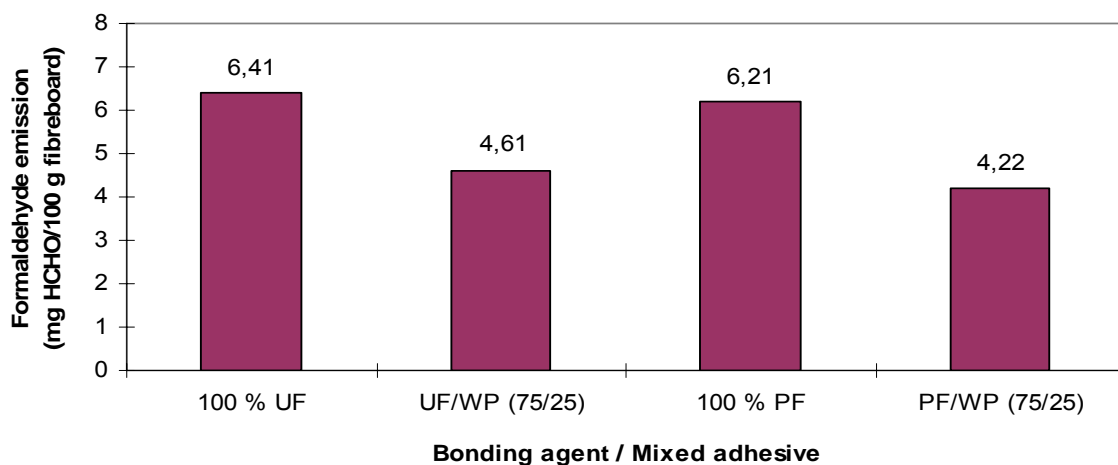
Figure 5: Formaldehyde emissions from wheat protein (WP) and urea formaldehyde (UF) respectively wheat protein (WP) and phenol foramldehyde (PF) bonded MDF

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"Enzymatic modification of wood fibres to activate their ability of self bonding"

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Introduction

Lignin is a renewable raw material which is the determining factor for interior wood strength. Cell wall gets comprehensive strength and lignin inhibits a penetration of water, furthermore it protects against microbial degradation. Natural lignin is hardly accessible but it can be degraded by enzymes of some special fungi. This non-chemical reaction is the key for using natural lignin as adhesive.

Tests had shown that an enzymatic activation of lignin is basically suitable for bonding of three layered particle boards and fibre boards. The more natural lignin is the better activation is. This depends on the changes of chemical structure of lignin by decomposition processes.

The production of fibre material usually works on refiner-method. Therefore mainly chips of softwood are used. The chips are compacted using a screw feeder into small plugs which are heated for 30 to 120 seconds to soften the wood, then fed into the defibrator. The defibrator consists of two counter wise rotating plates each with radial grooves that get smaller as they get closer to the circumference. The plug is fed into the centre and gets broken down as the centrifugal forces push it toward the outside of the plates where the groves are finer. The feeding devices at the entrance and exit to the defibrator maintain suitably high pressure and temperatures about 150 °C. By using high temperatures the required energy for defibrating the wood is decreased as there is a softening of lignin that facilitates fibre separation along the middle lamellae. The steam is then separated from the pulp the total time in the defibrator is about one minute. The pulp may pass through a secondary refiner to ensure the fibres meet pre-determined levels of freeness. This method is named Thermomechanical Pulp Process (TMP). Because of the relatively gentle TMP pulping process the use of this fibre material for a later enzymatic activation of lignin is suited very well for the production of fibreboards.

Laccase-Enzyme

Laccase (polyphenoloxydases; E.C. 1.10.3.2.) is the most widely distributed of large blue copper-containing oxidases, being found in higher plants and many fungi (THURSTON, 1994). Laccase reduces dioxygen to water, coupled to the one-electron oxidation of a wide range of phenolic and related

compounds. A numerousness of plants (*Rhus vernicifera, Acer pseudoplatanus, Pinus taeda,* etc.) and bacteria (*Azospirillum lipoferum*, *Sinorhizobium meliloti,* etc.) and also some insects are able to produce Laccase. In addition to ligninperoxidases (E.C. 1.11.1.7.) and manganperoxidases (E.C. 1.11.1.13.) Laccases belong to the most important lignin decaying and lignin polymerising enzymes which are mainly common in fungi. White-rot fungi are suited very well for production of Laccase in a technical scale.

Methods for low cost cultivating of white-rot fungi in a bio reactor were developed in different works (KHARAZIPOUR, 1983; KRUSENBAUM, 1991; SHEKHOLESLAMI, 1991; KHARAZIPOUR, 1996). It was possible to apply the elaborated methods as basis for a production of Laccase enzymes as a result of close co-operation between the Institute of Forest Botany of the University of Göttingen and the Schering Company (Germany), Novozymes (Denmark) and Genencor (USA). In the meantime most of these enzymes are produced by genetically modified organisms like yeasts and ascomycete hyphal fungi.

Laccase has the advantage, concerning to biological uses, that it requires oxygen as oxidiser, whereas peroxidases need hydrogen peroxide which can cause some problems in biotechnological practices, because of its harmful properties (MAI et al., 2004). A disadvantage by using Laccases is their low redox potential. Due to their low redox potential Laccases normally require a free phenol group at the aromatic ring for oxidation. This is unfortunately for Laccase using in lignin biotechnology, because in natural lignin most of the phenol groups are substituted by reason of synthesis of the lignin macromolecule (LEONOWICZ et al., 2001; ROCHEFORT et al., 2004). BOURBONNAIS und PAICE (1990) were the first who could show, that after an addition of low molecular redox compounds (so-called mediators) Laccase is able to attack non-phenolic compounds.

Laccases are used in a lot of technical processes, e.g. "biological bleaching" in pulp and paper industry (CALL und MÜCKE, 1997; MESSNER et al., 1993, MESSNER, 1993 and MESSNER and SREBOTNIK, 1994) and in textile industry (GALANTE und FORMANTICI, 2003). Also wood based composites were produced without any additives (KHARAZIPOUR & HÜTTERMANN, 1993, KHARAZIPOUR et al. 1997 and 1998; FELBY et al., 1997, 2002; WIDSTEN, 2002) by using lignin and Laccase as bonding agent. Laccase has a function to cause radicals for polymerising lignin. Until now Laccases from white rot fungi were primarily used for this process (KHARAZIPOUR, 1983 und 1996). Laccases from white rot fungi act extra cellular that means the most important steps of lignin depolymerisation occur beyond the cells of the fungus. Therefore these extra cellular enzymes can be isolated very simple and cheap from culture media. Furthermore phenoloxidase is widely stable against heat and does not loose its activity very fast, so it can be used over a longer period. Production of extra cellular phenoloxidases can be induced by phenolic compounds.

Enzymatic activation of lignin

Since the end of 2nd millennium B.C., Japanese already used the sap of lacquer tree (*Rhus vernicifera,* syn.: *Toxicodendron verniciflua*) for protection and decoration of hand-crafts and buildings and for

bonding paint. The using of lacquer sap was by-and-by improved and is known as urushi technique. For urushi technique the sap which colour range from colourless to amber, is coated in numerous thin layers on the surfaces. A red lacquer is achieved by admixing iron oxide and vermilion and a black lacquer by soot. Japanese created a special gold lacquer by admixing gold and silver dust.

The sap of lacquer tree contains 60 – 65 % urushiol, 0.1 - 1% Laccase, 6.5 – 10 % resins and 20 – 30 % water. The hardening of lacquer results from a polymerisation of urushiol with the collaboration of Laccase. The cured lacquer is resistant against water, alcohol, solvent and acids, permanently elastic and food safe and has a fungicide effect against moulds. But strong direct insolation causes a destruction of the lacquer.

The utilisation of sap is problematical because of urushiol. It is a strong contact allergen which effects large area of skin irritation. The chemical structure of urushiol is a compound of different related organic constituents. The basic module is 1,2-dihydroxybenzene (pyrocatechol und guaiacol) with a residual of different alkyl groups. 1,2-dihydroxybenzene is a bivalent phenol and harmful to health. Therefore the idea was to swap the harmful phenol for a non harmful phenol that is cheap and available in large quantities. In this case lignin is a practical substitute, because it is not harmful for health and lignin accumulates in great quantities during the production of pulp.

For one thing on this idea bases the application of a two-component-adhesive for making derived timber products. This adhesive consists of technical lignin which is enzymatic activated by Laccase. For another thing native, wood own lignin ought to transfer in a reactive form by phenoloxidases. Substrate of phenoloxidases is lignin that lies on surface of fibres. This lignin was former a component of the middle lamellae. By refining the chips during fibre production, lignin gets a vitreous structure caused by high temperature. Laccase effects an exposure of functional groups, especially phenolic hydroxyl groups. Contemporary with a radicalisation these modifications of the chemical structure of lignin molecule facilitate a formation of strong physical and chemical compounds in future fibre linkages. An optimal enzymatic treatment of fibres is achieved when the reaction obtains a maximum of chemical modification and radicalisation and at once a merest removal of lignin molecules from cellulose fibrils.

Enzymatic modification of wood fibres

Lignin is a natural occurring polyphenol and it was already used as an adhesive together with a chemical redox system (e.g. H_2O_2) for derived timber products since the beginning of the seventies (NIMZ et al., 1972). The disadvantage of this process was that huge amounts of H_2O_2 were required which foreclosed the utilisation of this method in an industrial scale. In the beginning of the eighties chemical redox systems were replaced by biological radical maker. These were Laccases from white rot fungi (HÜTTERMANN & HAARS, 1980; KHARAZIPOUR, 1983; KHARAZIPOUR 1996, KHARAZIPOUR & HÜTTERMANN, 1998). Derived timber products which were produced with the two-component-adhesive showed sufficient mechanical-technological properties but the values of thickness swelling were

unacceptable. The utilisation of water-insoluble lignin, e.g. Kraftlignin and small amounts of conventional resins made it possible to produce derived timber products which showed acceptable DIN-properties (KHARAZIPOUR u. HÜTTERMANN, 1998). However this process was not usable for industrial scale at that moment caused by missing custom-made enzymes which show a higher activity and a higher range of pH-tolerance including a better tolerance of temperature. An enzymatic activation of the ability of fibres for self-bonding is necessary because lignin of middle lamellae got a glassy crust by thermal treatment during decomposition. On the surface of the treated fibres a caramelisation caused by high temperature occurred. This causes an inactive surface on cellulose fibres which are bound in lignocellulosic complex and can not react in eventual bonding.

Basically it is possible to modify the inactive crust of middle lamellae lignin by an incubation of wood fibres with phenoloxidases. Lignin will be activated so that it is feasible to get acceptable technical properties by conventional pressing (KHARAZIPOUR & HÜTTERMANN, 1993; KHARAZIPOUR et al., 1994, 1997).

This process was already tested in pilot scale by the competence network for sustainable utilisation of wood [NHN] e.V. in Göttingen and was optimised in view of process engineering and different production parameters. Fibres from industrial production were incubated with the enzyme Laccase and afterwards they were dried with low temperature for avoiding unpreferred caramelisation processes on fibre surfaces. Also these fibres could process in conventional proceeding (KHARAZIPOUR, 2004). The fibreboards showed acceptable mechanical and technological properties but they did not reach always the required DIN-norms. This circumstance and the long duration for incubation inhibit an implementation on industrial scale.

The chemical process is an elimination of one electron of substrate by the enzyme Laccase. The Laccase charges negative and the substrate is transferred in a radical cation. This radical cation mostly reacts continuing unspecific non-enzymatic when Laccase oxidises reverse by transferring one electron to oxygen which is reduced to water later (LEONOWICZ et al., 2001). The advantage of this process is that Laccase needs oxygen as oxidant in opposition to peroxidases which need hydrogen peroxide as oxidant. The disadvantage by using Laccase is the low redox potential of this enzyme (not higher than $E° = 800$ mV) (SOLOMON et al., 1992).

Due to the low redox potential Laccases need free phenolic group at the aromatic ring for oxidation. But using Laccase in lignin biotechnology is a problem based in the fact that most of lignin groups are substituted by radical reactions during synthesis of lignin macro molecules (LEONOWICZ et al., 2001; ROCHEFORT et al., 2004). However Laccase is able to attack non-phenolic compounds by addition of low molecular redox-compounds (so-called mediators). BOURBONNAIS und PAICE (1990) were the first who showed that the utilization of ABTS (2,2'-azinobis(3-ethyltbenzthazolin-6-sulfonat) as mediator is possible. By Laccase-mediator-system (LMS) the mediator assumes an intermediary effect by the oxidative degradation of lignin while mediator is oxidized by enzyme. This implicates that a radical and heavy oxidising bonding occurs because this oxidation is a one-electron process on the mediator caused by Laccase. The mediator radical is now able to oxidise lignin and thereby the mediator will be

reduced. MAJCHERCZYK et al. (1999) showed that during the utilisation of ABTS as mediator this ABTS-dication decayed and therefore an irreversible step exists in this process. For this reason it is needful to know potential decomposition products of mediators during the use of Laccase-mediator-systems to avoid sewage problems.

Within the framework of an actual project it is a main target to produce medium density fibreboards without any conventional adhesives. For this purpose the improvement and optimisation of a system of Laccase enzymes and mediators should be developed for reaching a higher reactivity during activating the ability of self bonding of wood fibres. On the base of renewable raw materials respectively fibre lignin mediators and technical enzymes are supposed to enable a cost-effective production of sustainable wood derived timber products. All in all in this project the following targets are important:

Climate protection by CO_2-neutrality of the products and protection of fossil resources

Reducing the production costs for adhesives

Prevention of problems that can occur during the process of composting or recycling by residues of synthetic components of the used adhesives

Development of novel products consisting of domestic and renewable primary products

Improvement of the mechanical and technological properties of the produced fibreboards

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"The utilisation of Heterobasidion annosum in vivo degraded spruce wood as a plant substrate and peat substitute"

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Background

In Germany about 10 Mio m³ of peat are excavated and utilised each year (VOGTMANN, 2005). This equates an area of about 400 to 1.000 ha peat land. Provided that these excavation rates will not alter in the future, it can be stated that white peat will completely be dissipated in about 25 years. The resources of black peat, the peat where the herbal structures are no longer visible, are exhausted in about 40 years.
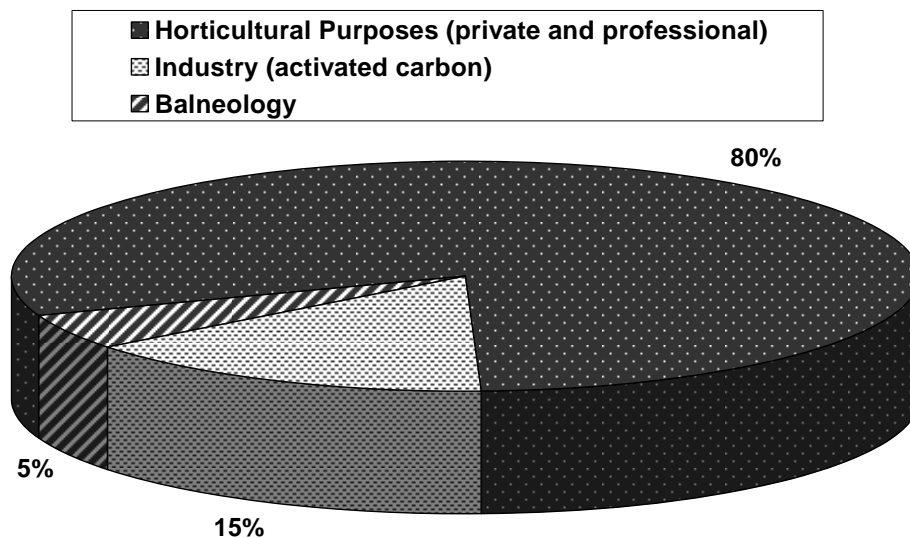
Figure 1: Utilisation of peat (FALKENBERG, 1990).

As shown in Figure 1, the majority of peat is used for horticultural purposes, either private or professional use. 15 % of the peat are used by the industry e.g. for the production of activated carbon. The remaining 5 % are used for Balneological purposes.

Keeping the excavation rates and the fact that peat only regrows at an amount of 1 mm/ year in mind, one can say that it is necessary to look for peat substitutes. In other words the peat management as it is done nowadays is not sustainable, because due to KRAMER´s (1985) definition sustainability is:

„The capability of the forest enterprise to fulfil the manifold benefits of the forest constantly and optimal for present and future generations."

This definition first occurred in a forestry context, because managing forest means planning for decades. The sustainable forestry is based on the idea that only the annual growth is harvested each year. This also means that the old trees are replaced by natural regeneration.

EICHHORN (1969) observed in sub alpine spruce stands, that the growth of young spruce trees on root-rot wood entails the following advantages:

- ✓ good accretion due to spontaneous mycorrhization

- ✓ high percentage of germination

- ✓ low death rate of seedlings

- ✓ good physical properties (no strong soil compaction; slowed evaporation and water storage as well as good concurrence conditions between the seedlings)

Replacing or at least substituting peat by wood should generally be possible. But for this attempt normal wood is too expensive. The saw mill industry increases the added value more then the wood fibre substrate industry could do. Thus it is necessary to use timber of lower quality.

Wood which is infested by the fungus *Heterobasidion annosum* is of lower value and normally, depending on the degree of disintegration, remains in the forest. This wood is suitable for the peat substitute production.

Heterobasidion annosum

Three distinct groups of *Heterobasidion annosum* occur in Europe. The groups are originally named according to their principal host tree as the P (pine), S (spruce) and F (fir) group (KORHONEN, 1978; CAPRETTI et al., 1990). NIEMELA and KORHONEN (1998) recently raised the groups to the species level: the P group as *Heterobasidon annosum*, the S group as *Heterobasidon parviporum* and the F group, as *Heterobasidon abietinum*. In this context the fungus is simply called *Heterobasidion annosum*.

The most important tree species that are attacked by the fungus in Europe are pine and spruce. The blatant difference is that pine trees die pretty fast due to the attack of the fine root system. Whereas spruce can life many years with the fungus.

There are several ways of infection. The fungus can get into the tree via damages at the bark, e.g. caused by yarding or deer striping, or simply colonise tree stumps via its spores. From these infested trees or stumps *Heterobasidion* can infect other trees via root-to-root contact. The fungus grows in a conical way from the roots to the top of the trees.

Generally this fungus is categorised to be a white rot fungi, this means it disintegrates lignin and hemicelluloses. In an advanced stage of disintegration the typical appearance of the fungi can be seen. The wood structure is broken into little cubes due to lack of lignin, which serves as a natural glue between the fibres.

WOODWARD et al. (1998) estimate that about 20 % of the spruce timber in Germany is infested. This leads to a financial loss of about 80 to 100 Mio. Euro annually. Worldwide the fungus causes a damage of about 1 billon Euro (WOODWARD et al., 1998).

Till nowadays all attempts were based on fighting the fungus with different methods. Now this project is the first try to work with and not against the fungus. In this case *Heterobasidion annosum* serves as some kind of in vivo bio reactor.

Wood fibre substrate

For the production of wood fibre substrates in the context of this project not the whole logs were used but merely the lowest segment. The lowest 2.5 m to 3 m of the trunk show two advantages for the production. First of all the degree of disintegration in rather high compared to the rest of the log because the fungi grows from the roots to the crown of the tree. This wood shows a loose structure and can easily be disintegrated thermo-mechanically. And second of all the amount of infested wood at the ratio of the whole segment is very high. Furthermore those segments, depending on the degree of disintegration, normally remain in the forest because the costs for yarding are higher then the selling price.

In order to increase the added value of the log segments it was investigated if it is possible and recommendable to separate the non infested wood areas from the infested areas. Therefore the logs were brought to a saw mill and sawed in a vertical saw. The idea was to get boards that are strong enough for construction purposes and chips of the infested areas for the wood fibre substrate production.

This industrial scale test revealed that increasing the added value with the separation of the non infested and infested areas is generally possible, but there also occurred some problems. Depending on the degree of disintegration in the centre of the log it is capable or not to saw boards out of the non infested areas. If the disintegration is in an advanced stage and the wood structure is very loose, it might happen that the log brakes while entering the saw. A separation in this stage is neither capable

nor recommendable. All in all the conclusion can be drawn that it might be easier just to chip the logs completely without separating the non infested areas and infested areas.

The chips from this industrial scale test were brought to the industrial partner Toresa® Deutschland GmbH. The wood fibre substrates were manufactured in an Extruder, which is a thermo-mechanical process. Prior to the thermo-mechanical disintegration different additives could be added, e.g. fertiliser. The disintegration itself runs at a temperature of about 90 ° C to 95 ° C, which means that the substrate afterwards is not totally sterile. Nevertheless the fungus *Heterobasidion annosum* is killed during the disintegration. For this reason a new infection caused by the wood fibre substrate can be excluded.

Investigations with the wood fibre substrate

The wood fibre substrates used in this project were fertilised with a special NPK fertiliser made by the Intertoresa in Switzerland. The fibres were coated with the fertiliser and were a bit darker in colour then the none fertilises fibres. Those non fertilised fibres were used for investigations with different compost/ peat substrates. One investigation in this context was done with tomato seedlings.
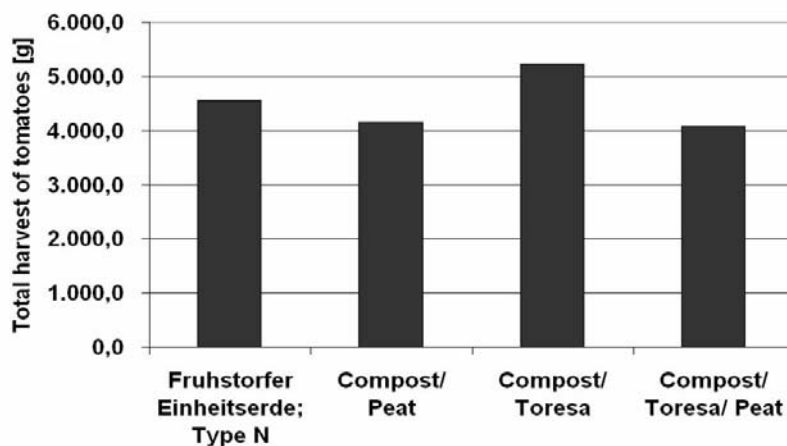
Figure 2: Total harvest of tomatoes grown on different compost, peat and wood fibre substrates (Ludwig, not published).

Figure 2 shows that the growth of tomatoes can be increased by using a mixture of 50 vol. % compost and 50 vol. % wood fibre substrate, called Toresa. The total amount of tomatoes harvested on this substrate is about 5228 g whereas the reference substrate (Fruhstorfer Einheitserde; Type N) only provided 4562 g of tomatoes. Also investigations with other vegetable plants showed that the combination of wood fibres with compost is very good.

But not only vegetable plants had an improved growth in wood fibre substrates. Investigations with conifers and other tree species showed that wood fibres are also suitable for forest plant nurseries.

The growth of *Thuja occidentals* `Smaragd` for instance was quite different after a cultivation time of about 12 month. Right at the beginning the rooted cuttings have had the same height (X = 77 mm). Five substrates have been investigated.

Table 1: Composition of the substrates used in an investigation with Thuja occidentalis `Smaragd`.

Substrate	Composition
1	Fruhstorfer Einheitserde (FE); Type N + 4 g/ l Osmocote
2	FE – Toresa with ITAG Mix 2.22 (50 vol. %- 50 vol. %) + 4 g/ l Osmocote
3	FE – Toresa with ITAG Mix 2.22 (50 vol. % - 50vol. %) + 4 g/ l Perl Humus + 4 g/ l Osmocote
4	Toresa with ITAG Mix 2.22 + 4 g/ l Osmocote
5	Toresa with ITAG Mix 2.22 + 4 g/ l Perl Humus + 4 g/ l Osmocote

The substrates were fertilised with the depot fertiliser Osmocote[®] Exact[®] Standard (16+11+11+3 MgO+ Te) by Scotts[®] International B.V. from Heerlen, Holland. The fertiliser lasts for 3 month and after this period the substrates were fertilised again.

The humic acid product „ PerlHumus[®]" was manufactured by HUMINTECH GmbH from Düsseldorf. According to the description of the manufacturer Perlhumus[®] is an organic soil conditioner based on Leonardite. Leonardite is a natural raw material containing a high content of humic matter (humic acids and fulvic acid). These are formed in specific sedimentation layers of soft brown coal.

Already after three month of cultivation the plants in substrates with wood fibres (2 and 3) or 100 % wood fibre substrates (4 and 5) showed a bigger height then in the reference (Fruhstorfer Einheitserde; Type N + 4 g/ l Osmocote). This trend lasted for the following months. Generally it can be stated that the wood fibre substrates are suitable for this kind of cultivation.
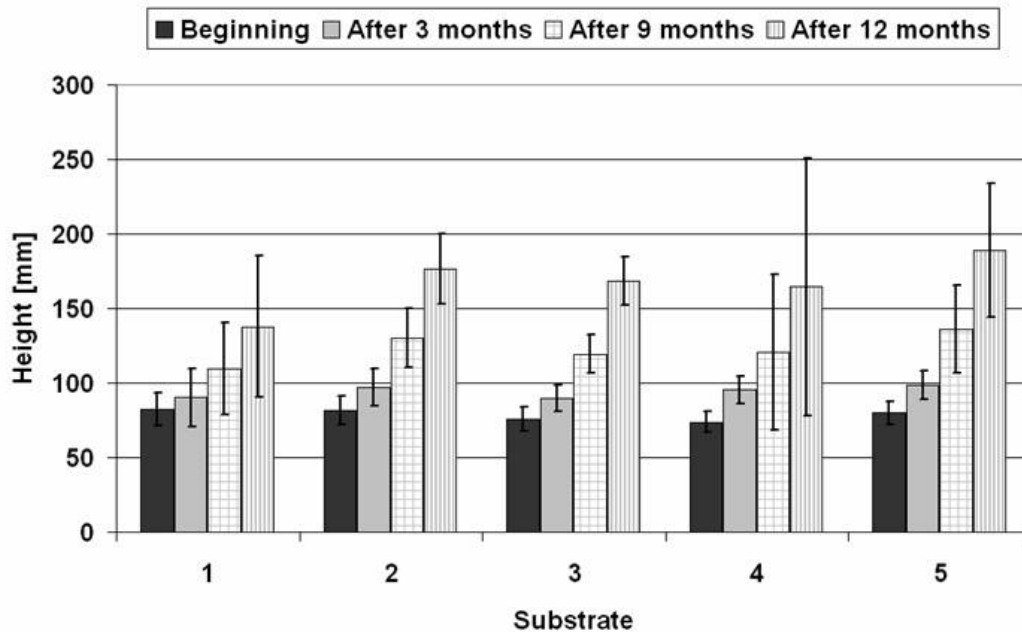
Figure 3: Height of Thuja occidentalis `Smaragd` after cultivation in different substrates. Substrate compositions see in Table 1.

Conclusion

As stated in the previous chapters the peat management as it is done nowadays is not sustainable. The majority of the peat is used for horticultural purposes and therefore it is necessary to look for alternatives for this utilisation. One way might be the substitution by wood fibre substrates. It could be shown that the wood fibre improves the properties of the culture substrate. In all investigations the growth of the plants could be increased.

Generally it can be stated that the implementation of wood fibres in the substrate entails some advantages. The substrates have an increased porosity and better substrate stability. Those two facts lead to an improved rooting. Furthermore the wood fibre substrates have a reduced specific weight. And last but not least the substrates with wood fibres have an improved water balance.

Due to those advantages at least a substitution of peat in parts is feasible. A complete substitution of peat is still aspired but probably not realisable.

Acknowledgment

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"Panel board production by the use of silanes"

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Organic functional silanes are particularly suitable as adhesives to produce panel boards with huge mechanical technological properties. The manufaction and use of these panel boards is free of toxic emissions. The combination of organic functional silanes with natural based adhesives like proteins opens the opportunity to establish panel boards especially medium density fibreboards which are able to reach the respective European standards (EN).

Silanes exist by a silicon framework and hydrogen. Their body can be branched or none branched. To create inorganic compounds with organic properties the hydrogen is replaced by an organic moiety.

Investigations on gluing fibres with an organic functional silane showed internal bond strengths (IB) of the manufacted fibreboards at about 1.48 N/mm². By gluing the same fibers with an usual urea formaldehyde resin (UFR) fibreboards were produced with IB properties of only 0.92 N/mm². Thickness swelling (TS) of the investigated fibreboards was 7 % for the silane bonded fibreboards and 24 % for the UFR bonded ones. Fewer values (0.3 mg/100 g) of formaldehyde (HCHO) concentration were investigated for the aminosilane bonded fibreboard samples. The UFR samples had values of about 6,5 mg/100 g HCHO. Silanes could not only act as adhesives exclusively. Less percentage additions of an aminosilane to a glue mix could influence the properties of the manufactured fibreboards favourable. Fibreboards bonded with wheat protein without silane addition showed IB of 0.48 N/mm² and TS of 86 %. By addition of 1 % of an aqueous aminosilane to the protein glue mix IB was 0.70 N/mm² and TS was narrowed to 14 % which means EN achieved.

Introduction

For the manufacturing of wood panels predominantly synthetic organic adhesives are used (DEPPE/ERNST, 2000). About 90 % of these bonding agents are urea-formaldehyde resins despite of the negative character of methanol (formaldehyde). In detail 85 % urea formaldehyde resin, 7 % phenolic formaldehyde resin and about 5 % of melamine (urea) formaldehyde resin is used for the world production of panel boards. The remaining fraction is occupied by the isocyanates and some natural adhesives like tannins, particularly in some South American countries like Brazil. Lately the formaldehyde problem has been very present. The well-known World Health Organisation (WHO) recommended in a press release in 2005 that formaldehyde should be newly classified. Today

methanol belongs to class 3, "probably carcinogenic", but according to a study of HAUPTMANN (2004) the European Chemical Bureau holds an offer of France to upgrade formaldehyde to class 1, "carcinogenic". Because of this background it makes sense to develop and apply adhesives with a very low or without any formaldehyde potential.

Urea formaldehyde resin (UFR)

UFRs are the most important glue types of the Aminoplast resins. Since the thirties they are set up in large lots and meanwhile occupy a very important role in the field of material bonding. The raw materials for the production of these resins are relatively readily available and can be produced in great amounts. Since urea is used worldwide in large amounts as artificial fertilizer, it is very economical. This is the reason why UFRs could maintain their leading position in the past compared with other synthetic resins. Quantitatively, UFRs currently make up the larges share of the adhesives which are used for panel board production in the world. The worldwide share of particleboards produced with acid-curing UFR is approx. 90 %.

Urea and formaldehyde, which react with each other in a diluted solution in the pH range of alkalescent to neutral, serve as the raw material for the production. Basically the reaction would lead to high-molecular methylol-ureas, but it is interrupted at the stage of the mono- and di-methylol-ureas. These compounds represent the actual adhesive and were offered as solution with a solid content of approx. 66 % or as powder.

In the acidic pH field these dimethylolgroups continue to react, forming condensation polymerization products. In practice this is achieved through the addition of chemical catalysts, so-called hardeners. Mostly ammonium sulphate is used as the catalyst, more seldom ammonium peroxide sulphate or magnesium chloride.

As aforementioned the manufacture of particleboards is a very important application for UFRs. The glue mix is generally composed of a liquid resin to which water was added in order to decrease viscosity and facilitate spraying, plus small amounts of hardener. Furthermore, quantities of insecticides, wax emulsions and fire-retarding agents (such as ammonium phosphates) are added before spraying the adhesive onto the wood particles. Pressing temperatures and maximum pressures for producing particleboards are in the range of 150 to 200 °C and 0,2 to 3,5 N/mm². The moisture content of glued furnish chips is 7 to 8 % for the middle layer and 10 to 12 % for the surface layer. The resin contents used are 6 to 8 % for the middle layer and 10 to 11 % for the surface layer (PIZZI/MITTAL, 1994).

UF resins are non-toxic in their cured state. Urea is also harmless. However, free formaldehyde and formaldehyde generated by slow hydrolysis of the amino plastic bond are highly reactive and combine easily with proteins in the human body. This may cause a painful inflammation of the mucous membranes of the eyes, nose, and mouth (MARUTZKY, 1989). Even a low concentration of

formaldehyde vapour in the air can cause disagreeable irritations of the nose and eyes. High temperatures and high relative humidity can result in odour problems in a room containing panel board manufactured with UF resins (MARUTZKY, 1989). The release of formaldehyde, for example from UFR particleboards, is caused by two factors. It can either be due to free formaldehyde in the board that has not yet reacted or due to formaldehyde formed by hydrolysis of the amino plastic bond as a result of temperature and humidity (PIZZI, 1983).

Table 1: Sources of formaldehyde in panel boards

Types of formaldehyde that can exist in amino plastic bonded panel boards

- gaseous formaldehyde in cavities of the boards
- formaldehyde solved in the moisture of the boards
- low bonded formaldehyde:
    o methylol groups (end or within the polymeric chain)
    o hemiacetal groups
    o Disposal of formaldehyde takes place during slow hydrolysis (because of the moisture content of the boards) even at common environmental conditions
- methylene and ether bridges in the UF molecule: a separation of formaldehyde only takes place under strong hydrolysis conditions (impact of high moisture content particularly at higher temperatures)

UFRs for panel boards currently have urea/formaldehyde (U/F) molar ratios of 1:1,05 to 1:1,1. In former times UF resins with U/F molar ratios of 1:1,4-1,5 were used for producing particle- and medium density fibreboards. These boards had a very high formaldehyde level of 50-70 mg HCHO/100g board (PIZZI/MITTAL, 1994) and it was not possible to achieve today's European standard (6,5-7 mg HCHO/100 g board) for particleboards and/or medium density fibreboards. However, currently used UF resins have, compared to the "old" ones, the disadvantages of a longer curing time and a lower level of dilution with water. Furthermore, the produced panel boards have a lower water durability because they are more vulnerable to hydrolysis.

Table 2:   **Properties of a typical UF resin**

Appearance	Milky cloudy
Solid content	68±1 %
pH-value (20 °C)	7,5-9,5 (ISO 1148)
Density (20 °C)	1,29-1,31 g/cm³ (ISO/FDIS 2811-3)
Viscosity at filling in factory (20 °C)	400-600 mPa s
Hardening temperature	104 °C
Required hardener addition	Yes
Storage time	4-6 $^{1/2}$ weeks
Price (kg)	0,35 Euro

Melamine Formaldehyde resins

Melamine formaldehyde (MF) and melamine urea formaldehyde (MUF) resins are among the most commonly used adhesives for exterior and semi exterior wood panels and for the preparation and bonding of both low- and high-pressure paper laminates and overlays. Their much higher resistance to water attack is their main distinguishing characteristic from urea formaldehyde resins. MF adhesives are expensive. For this reason MUF resins, which have been cheapened by addition of a greater or lesser amount of urea are also often used (PIZZI/MITTAL, 1994). The applied mix condensates mostly consist of 45 % MF resin and 55 % UF resin. These mix condensates have advantages when used in particleboard which must be reckoned against pure UF resins and phenol formaldehyde resins through a short, temporary moisture exposition (DEPPE/ERNST, 2000). However, at continuing moisture influence the danger of hydrolysis effects exists, which cannot be prevented by an extra addition of phenol formaldehyde resin (PERESTIFILIPPO/PIZZI, 1996).

MF resins are used as adhesives for exterior and semi-exterior-grade plywood and particleboard. In this application their handling is very similar to that of UF resins for the same uses, with the additional advantage of their excellent water and weather resistance. MF resins are also used for the impregnation of paper sheets in the production of self-adhesive overlays for the surface of wood-based panel products and of self-adhesive laminates.

The condensation reaction of melamine with formaldehyde is similar to the reaction of formaldehyde with urea. As for urea, formaldehyde first attacks the amino groups of melamine and methylol. Compounds are formed. But the formaldehyde addition to melamine occurs more easily and completely than does the addition to urea. Melamine with its amino group accepts easily up to two formaldehyde molecules. Thus, complete methylolation of melamine is possible in contrast to urea (PIZZI, 1983).The methylolation step leads to a series of methylol compounds with two to six methylol

groups. In the reaction the hydrophilic stage proceeds more rapidly than urea because melamine is less soluble in water. Therefore hydrophobic oligomers appear early in the reaction. MF condensation to resins and the final curing can occur under acidic, neutral and even slightly alkaline conditions. In the main curing, intermediates transform into insoluble and infusible MF resins through the reaction of amino and methylol groups, which are still available for reaction. Curing temperature of MF resins starts at about 100 °C and ends completely at approx. 150 °C. UF resins which cure under the same conditions evaporate a larger quantity of formaldehyde than MF resins (PIZZI/MITTAL, 1994).

Phenolic Formaldehyde resins

Apart from UF resins the phenolic formaldehyde resins have a significant importance for the panel board industry. The phenolic resins have the broadest application range of all synthetic resins due to their high adaptability. They possess high dry- and wet bonding strengths and an enormous adhesion to wood. Their main application field is the manufacturing of wet proof particleboards. PF resins are also used for the production of weatherproof fibreboards, plywood, lagging boards and for the generation of wafer and strand boards.

The reaction of phenol with formaldehyde thus leads to the polycondensation product phenolic resin. Phenolic resins were the first true synthetic polymers, which were developed commercially. Today their structure is not completely clear, because the polymers derived from the reaction of phenol with formaldehyde differ in one important aspect from other polycondensation products. The polyfunctional phenols can react with formaldehyde in the ortho and para positions to the hydroxyl group. Because of this condensation products exist as numerous positional isomers for any chain length (PIZZI/MITTAL, 1994).

The production of PF resins takes place in a discontinuous process. The condensation of phenol with formaldehyde is carried out in presence of sodium hydroxide (NaOH). The molar ratio of phenol to formaldehyde is about 1:2,5. Because of the high hygroscopic character of the NaOH alkaline salt, the balance moisture content of wood raises.

The final curing of PF resins occurs under the elimination of water between the reactive hydroxymethyl groups and the separable hydrogen at the phenolic core. New methylene bridges are formed. Linear structures and, with growing condensation level, cross linking bridges are created.

PF resin starts the final curing at approx. 130 °C. The speed of curing is linked to temperature. With increasing temperature the curing speed becomes progressively shorter. In contrast to UF resins the panel boards bonded by PF resin can harden further after hot pressing in pack storage, because hardened PF resins cannot be damaged hydrolytically by moisture or heat. Because of this, the necessary star coolers can be economised in the industrial manufacturing.

Equilibrium moisture content of PF resin bonded particleboards is up to 10 % higher than that of UF resin bonded (ROFFAEL/SCHNEIDER, 1978).

The final cured PF resins are hygienically harmless and do not need any conditioning procedure. Not completely cured bonding layers and those, which may release free phenols, are not allowed for the use of furniture or rooms in which foods are stored, because phenols affect flavour (ZEPPENFELD, 1991). Phenol causes chemical combustion on the skin, and it is a neurotoxin and a cell toxin. A subsequent elimination of formaldehyde from PF resin bonded panel boards is approx. 10 % smaller, than that of UF resin bonded panel boards (CHERUBIN, 1978).

Isocyanates

Organic isocyanates have been used since the beginning of the seventies as adhesives mainly in the particleboard fabrication. Basically the polymeric methylene diphenyl diisocyanate (PMDI) is applied (LARIMER, 2006). The isocyanates are not adhesives like the previously mentioned thermosetting cured bonding agents. They do not contain any cold adhesiveness and cure with water only to a foamily compound with lower strength. Therefore special emulsifiers have to be used as solvents to create a usable paste for panel boards. PMDI can react with wood and other lignocellulose containing materials to build powerful adhesive joints while hot curing. In doing so PMDI reacts with the water enclosed in these materials to polymeric ureas or with available hydroxyl groups to polyurethanes. The forming of a strong particle or fibre compound respectively inside a panel board occurs like a chemical bond between PMDI and wood. Investigations have shown that isocyanates can react with lignin as well as cellulose (BOEGLIN et al., 1995).

PMDI is produced by phosphor gelification of aniline formaldehyde condensates. It reacts at conditions above 25 °C with water in gelification kind. However, if the curing is conducted at higher temperatures and pressures, a hard coating is built up. In these processes 20-50 % of PMDI are changed into polyurethane, the remainder is polyurea. The level of cross linking between PMDI and the material rises with increasing temperature. PMDI bonding joints are stronger and more (moisture) durable compared to UF resin, because PMDI does not only crosslink with itself, it also reacts with molecules of assembly components and establishes main valence bindings (DEPPE/ERNST, 2000; ZEPPENFELD, 1991).
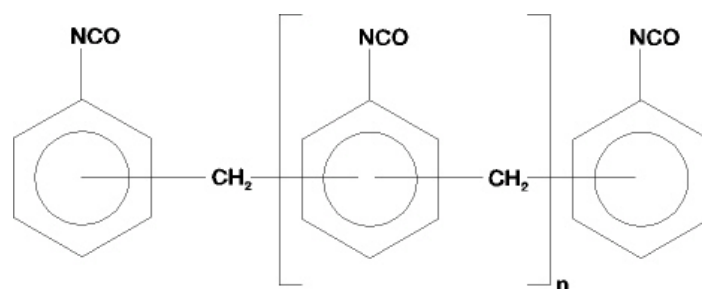
Figure 1: Structure of PMDI

Some problems arise with the application of isocyanates. Due to their high reactivity the risk of sticking to metallic parts of the hot press exists while using PMDI for bonding panel boards. Thus, releasing agents or special coated conveyor belts must be used.

Another negative aspect is the possible health risk, which occurs while applying isocyanates. Significant employment protection is necessary. When inhaling isocyanates they can react with amino- and hydroxide groups in the human body. The cell membranes can be mutated and destroyed. Strong respiratory diseases can occur. Furthermore, isocyanates can convert into carcinogenic amines in case of high humidity.

When isocyanates come in contact with the skin eczemas can arise (ZEPPENFELD, 1991). Economically negative is the high price (approx. 1,60 Euro/Kg) compared to UF resin.

Silanes

Organofunctional silanes are used as adhesion promoters between inorganic materials like glass, minerals and metals and organic polymers (thermo sets, thermoplastics, elastomers). They are further used as surface modifiers of inorganic and organic materials. In this application field silanes due to a hydrophobation of the treated material by an improved wetting or a protective coating. At least organofunctional silanes can act as cross linking agents for moisture cross linking of polymers (HüLS, 1990).

Silanes are silicon organic compounds based on silicon. While their synthesis silica sand (SiO_2), the minerally raw material for silicon recovery, is transformed via an electrothermic reaction with carbonate as catalyst to pure silicon. While these process the oxygen is separated from the carbon under high temperatures (1800 °C). The needed silica sand is obtained from the Scandinavian countries because of its pureness. The required energy for the purification reaction is mostly supplied by water power stations. Base material for synthesis of organofunctional silanes is trichlorosilane (H-$SiCl_3$). By giving of pure hydrogen chloride, to pure silicon under defined conditions these compound will be received. Tricholorsilane is transformed into the organofunctional silane by hydrosylation, esterification and if necessary substitution.

The properties of organofunctional silanes are due to their special molecular structure and reactions. In the next figure (2) the chemical structure of an organofunctional silane is shown.

$$Y - (CH_2)_n - Si\ X_3 \qquad n = 0\text{-}3$$
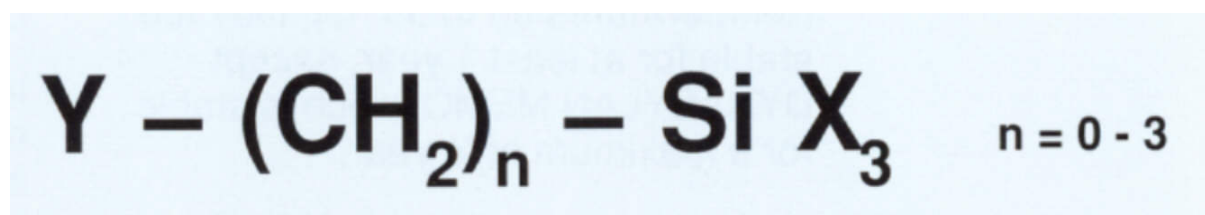
Figure 2: Structure of organofunctional silanes

The silicon as central unit of a silane is combined with two different functional groups. The organofunctional group **Y** is strongly bound to the silicon via a stable unreactive carbon chain. The adhesion to a polymer occurs via this group. Examples for organofunctional groups are: Amino, epoxy, vinyl, methacrylic and mercapto groups. The silicon functional group **X**, which is mostly an alkoxy group and which is attached to the silicon directly, can react after it's hydrolysis with active centres of an inorganic substrate or condensation with other silicon compounds. So stable bonds can be formed. Mostly organofunctional silanes with two silicon functional groups are used.

For the investigations about the applicability of silanes for the production of wood based panels four types of organofunctional silanes were tested. All of them had two silicon functional groups with special effects. Because of their properties, like complete solubility in water, the equal functionalities within a wide pH-range and their increased shelf life of 6 to 12 month (UFR only 5 weeks) these silanes could be used like common adhesives.

Viscosity changes

By addition of 1 % silane to an urea formaldehyde resin and a phenolic formaldehyde resin which are usually used in the fibreboard production, the viscosity of the adhesives were reduced at about 31 %. The viscosity measurements were done with a rotational rheometer and the values were read at 500 s^{-1}. A viscosity reduction may have positive effects of the sputtering process in the panel board production.

Production of fibreboards

At the investigations 10 mm fibreboards were produced on a pilot plant station. After grinding the surface of boards on each side of 0.5 mm the thickness was about 9 mm. The fibreboards were bonded with common adhesive level but exclusively with an organofunctional silane. Four types of silanes were investigated of their application for panel board production. The pressing time was adjusted at 24 s/mm and the pressing heat was about 195 °C. An electric heated press was used. The results of the IB test is shown in figure 3.
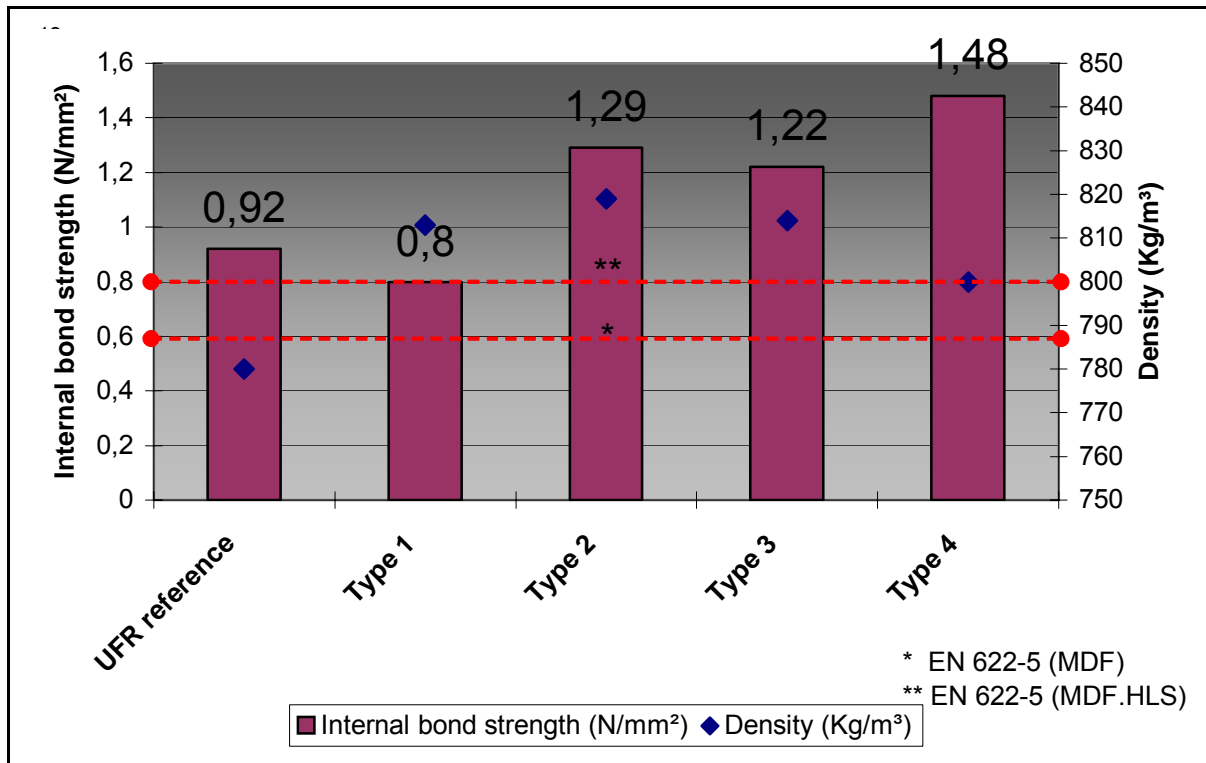
Figure 3: IB of silane bonded fibreboards

In figure 3 it is shown that all produced fibreboards were able to achieve the guidelines of the European standards, concerning fibreboard use for furniture (IB 0.6 N/mm²) and for high stress able uses (0.8 N/mm²). The best results could be achieved with type 4 silane as adhesive. But all in all, three of the investigated silane showed better results as the reference fibreboards which were UFR bonded.

At investigations about the thickness swelling after 24 h water storage it was shown that type 4 bonded fibreboards had the best results, too. With a thickness swelling of about 6.99 % these samples underbid the European standard value for furniture (15 %) obviously. The other samples of type 2 and type 3 silane bonded fibreboards had swelling values of about 7.48 % and 7.54 % respectively. Only type 1 silane showed inferior results (33,15 %). Overall it must be said that all produced fibreboards were made without giving of a hydrophobic substances like paraffin wax to the glue mix. The samples of the UFR reference showed with 24.05 % worse results compared to the silane type 2 – 4 bonded fibreboards.

Because of the very well properties of the exclusively organofunctional silane bonded fibreboards, further investigations with type 4 silane were done. The glue mix of type 4 silane was modified. This modification had positive effects on the adhesive application. The bonding agent level could be reduced down to 4 % without missing the guidelines of the European standard EN 622-5 [MDF] (DIN, 1999). The pressing time could also be reduced to 15 s/mm and 12 s/mm respectively. The pressing temperature had to adjust at 215 °C. With these properties it was possible to manufacture fibreboards

with an IB of about 0.67 N/mm² (12 s/mm) and a thickness swelling after 24 h of about 15.88 % (15 s/mm).

Wheat protein as adhesive

Because of the very positive results with silanes as stand alone adhesives for fibreboards the opportunity should be investigated to upgrade the properties of natural adhesive. Because natural adhesives have some advantages compared to adhesive on petrochemical origin like UFR or PFR.

Bonding agents from renewable resources are better integrated in natural material cycles and their use and waste disposal won't lead to an accumulation of toxic substances like formaldehyde. Furthermore the price for adhesives of renewable resources is not depending on the oil price (world political situation). But the positive qualities of the UF-resins should be hold up as far as possible. So close to nature bonding agents are searched which can substitute conventional adhesives particular or completely.

Investigations with a wheat protein adhesive were done. This wheat protein is a waste product of the starch industry. The solid content of the adhesive is made up of 40-50 % specific high quality saccharides, 20-30 % proteins and 30-40 % hemicelluloses. Fibreboards in a thickness of 9 mm after grinding were produced without and with giving of a small amount of type 1 silane to the glue mix, which contained in both cases 1 % of a hydrophobic substance on base of paraffin. The pressing time was adjusted with 24 s/mm and pressing temperature was 195 °C. The silane addition changed the board properties immensely. The IB of the boards without a silane giving was about 0.48 N/mm² and the belonged thickness swelling was about 86 %. With a addition of an organofunctional silane the IB raised up to 0.70 N/mm² and the swelling were reduced under the European standard with a value of about 14 %. The results showed that it was possible to produce panel boards with an adhesive from renewable resources with board properties which were able to fulfil the needs of the relevant European standard 622-5 (MDF). Furthermore the wheat protein bonded fibreboards and the exclusively silane bonded fibreboards did not have crude oil as raw material for the synthesis of their adhesives. Because of that, very less amounts of harmful formaldehyde is outgased of the boards. In the next figure this relationship is shown.

Formaldehyde
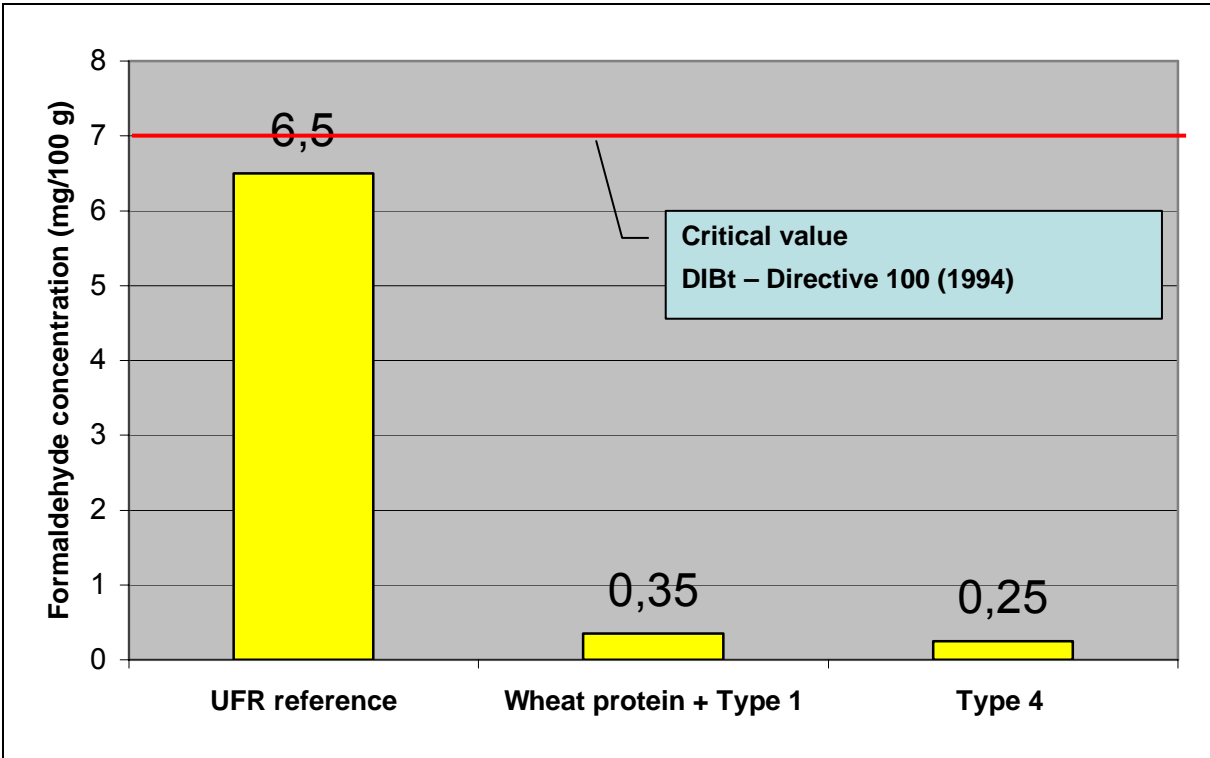
Figure 4: Formaldehyde concentration of fibreboards

The formaldehyde values shown in figure 4 were determined with the perforator method EN 120. It is clear that UFR reference has a much higher formaldehyde potential as the other measured samples. Obviously UFR reference was able to reach the critical value of the DIBt-Directive 100 barely. The samples of the other fibreboards had a very low formaldehyde potential. From this background it makes sense to substitute much as possible of panel boards which are produced with conventional adhesives and harmful formaldehyde. Silane modified natural adhesives could be a real alternative to aminoplast adhesives.

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"FTIR-spectroscopy and -microscopy as tools for analysis and control of wood properties and production processes"

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Introduction

Wood products and derived timber products are used for interior constructions, furniture production, as packing or building materials and are mainly made of coniferous wood (Marutzky & Thole 2003). The properties of the intermediate and final wood products depend as well on the raw materials as on the methods of production. For technical wood use profound knowledge on the structure and chemical composition of wood and its products is of great importance. This holds especially, if novel tree species, for which little experience exists, were introduced into the production processes.

Wood is primarily composed of cellulose, lignin, hemicelluloses and minor amounts of extractives (Böttcher 1993). Cellulose, the major component, is a high-molecular-weight linear polymer consisting of (1-4) ß-linked glucose monomers. The cellulose molecules are arranged into fibrils, which are organized into elements that make up the cell wall of wood fibres. Most of the cell wall cellulose is crystalline (Forest Products Laboratory 1999). Lignin is the substance that binds the cells together and generates high stability. It is a three-dimensional phenylpropanoid polymer. The hemi-celluloses are low-molecular-weight polymers composed of several different kinds of pentose and hexose sugar monomers, whose amounts vary with the tree species. Extractives are organic and inorganic components. Organic components contribute to wood properties as colour, odour, taste, decay resistance, density and influence hygroscopicity and flammability.

Extractives include tannins and other polyphenolic, colouring compounds, essential oils, fats, resins, waxes, gum starch, and simple metabolic intermediates. The inorganic components are calcium, potassium and magnesium. Trace amounts of phosphorus, sodium, iron, silicon, manganese, copper, zinc, and perhaps a few other elements are usually present (Forest Products Laboratory 1999).

Methods for wood analysis- state of the art of science and technology

Standard methods

It is of great importance for technical wood use that wood properties of a species match to product requirements. Therefore, efficient utilization of wood products is possible through an understanding of product properties and this requires laboratory analyses of the anatomy and of wood composition by

wet chemical methods. Established standard methods are e.g. the quantification of cellulose (TAPPI T 203 om-93), lignin (TAPPI T 222 om-98), hemicelluloses (TAPPI T 203 om-93), extractives (TAPPI T 264 cm-97) and ash content (TAPPI T 264 cm-97). In addition to these methods there are many other techniques in use. However, since they are labour and time intensive procedures, they are not appropriate for screening of the material involved. In practise resolving numerous problems is possible by microanalysis of wood, e.g. glue or lacquer incompatibilities during processing of wood (Böttcher 1993).

FTIR-spectroscopy as optical method

Fourier Transform infrared spectroscopy is a well established method for analyses of different organic substances (Salzer, 2000). The technique is widely used in both research and industry for quality control and dynamic measurement. FTIR-spectra of solid, liquid or gaseous samples are generated in a few minutes. In the wave number area between 1800 and 600 cm^{-1} (fingerprint area), the bonds can be directly interpreted and ascribed to a particular chemical composition in the sample.
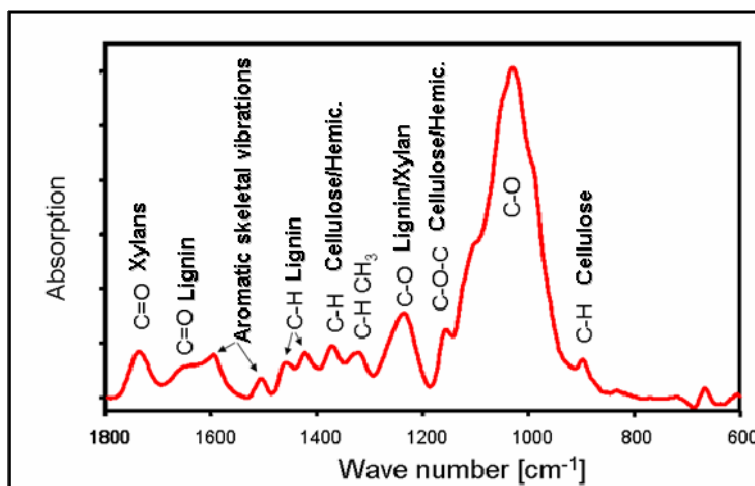
Fig.1: Illustration of a typical FTIR- spectrum of wood in the fingerprint-area. The spectrum shows different absorbance units. Each bond can be seen as typical for a particular chemical component in the sample e.g. xylans. The bonds have already been specified (Faix 1991).

FTIR-spectroscopy is a non-destructive method of the molecular composition of a sample. This means that it is possible to analyse the sample several times. Furthermore, little or no sample preparation is required.

FTIR-spectroscopy offers a large potential for wood analysis because it is a fast and quite simple method for determination of the chemical composition of complex samples. The FTIR-spectra of wood and wood products are determined by biochemical and physical properties of the material. For spectroscopic measurements the middle infrared range is mostly used. In this range the basic

vibrations of the molecules are activated. The chemical composition of the wood sample can be derived from the detected vibrations caused by IR-light (Bruker 1995).

FTIR-spectroscopy

Operating mode of a FTIR- spectrometer

A FTIR-spectrometer is working with Infrared (IR)-radiation, which is an electromagnetic radiation characterized by wave length or frequency. The wave length is defined as distance of two maxima in a sinusoidal wave. Frequency is defined as the number of vibrations of a wave per time unit (Setz 2005).

The FTIR- spectrometer is composed of the following components (Fig.2):

- ✓ A radiation source mostly of silicon carbide (an electrographic ceramic). An alternative is a nickel-chrome resistance which is used predominantly in simple spectrometers because of the lower radiation intensity.

- ✓ An interferometer which consists of a beam splitter and a HeNe laser for detecting the place of the moving mirror.

- ✓ A fixed and a moving mirror which modulate the delay and the propagation of the beam

- ✓ A detector which turns the energy of the incoming photons in electric power.

- ✓ A personal computer to accomplish the Fourier-transformation of the measured electric signal and to get a spectrum (Bruker, 1995).

The core of a spectrometer is the so called Michelson-interferometer. IR-light in the middle infrared range (4000-500 cm^{-1}) is emitted by the globar. The beam is divided in two single beams by the beam splitter. One beam is sent to a fixed mirror, the other one to a moving mirror. Afterwards, both beams are reflected and recombined. Then the beams pass the sample and are focussed into the detector (Fig.2). If the split paths of the light differ by a whole number of wavelengths, there is a constructive interference and a strong signal at the detector. If they differ by a whole number and half wavelengths, there is a destructive interference and a week signal. Result of the data acquisition is the digitized interferogram (Fig.3), which is transformed in a spectrum by means of an arithmetic operation named Fourier transformation (FT).
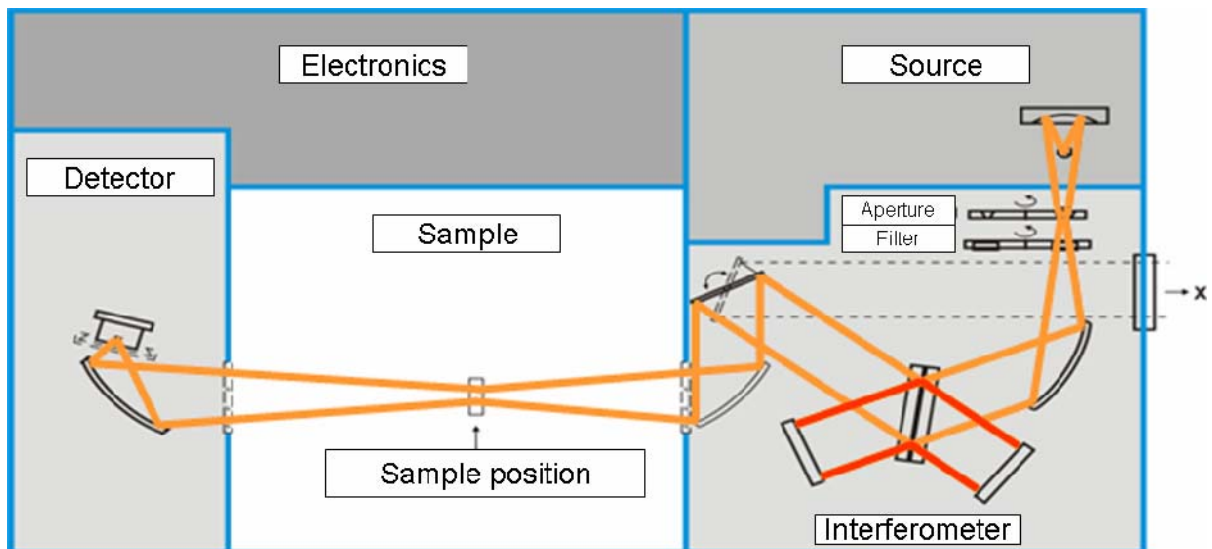
Fig.2: Illustration of the operating mode of a FTIR- spectrometer. The components of the spectrometer are displayed. The course of beam is illustrated in yellow and red colour (Bruker 1995).
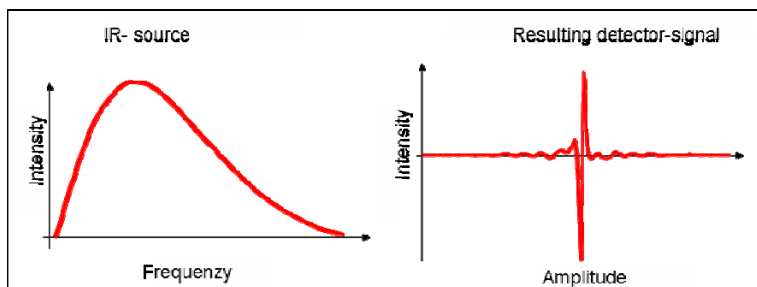
Fig.3: Illustration of the untreated source signal (left) and the interferogram as resulting detector signal (right). The source emits a specific frequency. After passing the Michelson-interferometer and the sample, the interferogram is detected. This electric signal is transformed in a spectrum by FT (Bruker 1995).

Analysis of spectra

There are three possibilities to interpret and evaluate spectra.

a) **Quantitative identification of a substance**

Analysis of quantities of wood components in the sample

b) **Qualitative identification of a substance**

Educt-product comparison to follow reactions

Direct interpretation of spectra (the absorption bonds are assigned to an index of significant chemical bonding)

Comparison with a reference spectrum

c) **Semi- quantitative identification of absorption ratios at two wave numbers**

Calculation for e.g. of lignin-cellulose-ratio

FTIR- spectroscopy with the Attenuated Total Reflectance - unit (ATR-unit)

Fig.4: Bruker Equinox 55 FTIR- spectrometer with unit

Fig.5: ATR- unit with an attached ATR- Plunger on the bottom side which is pressed on the sample with constant pressure.

Until recently it was necessary to produce KBr (potassium-bromide) pellets for sample analysis with FTIR spectroscopy. This required time-consuming sample pre-treatment. The sample had to be milled for fine wood powder. Afterwards, it had to be pressed into small pellets mixed with a calculated amount of KBr powder. This chemical substance served as background for the spectroscopic measurements.

Innovative units, such as ATR- units (**A**ttenuated **T**otal **R**eflectance) have been developed lately. Spectroscopic measurements are possible without any pre- treatment of a sample. It is possible to measure e.g. wood blocks with plane surface and wood powder to determine different wood components like lignin and cellulose. The technique allows determining the structure of substances with the interpretation of the frequencies of functional groups and is convenient to detect the chemical modifications of raw materials and mixtures. A DTGS- detector, frequently used in modern FTIR spectroscopes, allows analyzing measurements of spectra within a frequency range from 600-4200 cm^{-1}.

Mode of measurement of the Attenuated Total Reflectance-unit

If IR- light is passing the interface of a matter with high index of refraction (e.g. ATR crystal) to a matter with lower index of refraction (sample), the radiation is reflected into the more dense matter. Part of the radiation is hitting the surface of a matter with less density (sample). If the beam is totally reflected the process is called total reflectance.

However, the ATR- technique is based upon the principal of attenuated total reflectance (Fig. 6). This means, that a part of the radiation is absorbed by the sample and is missed in the reflected beam afterwards. The difference in intensity is recorded and compared to the standard beam (ATR crystal without sample) (Bruker 1995).

The solid sample is pressed into direct contact with the ATR- crystal by a plunger. A beam of infrared light (middle infrared range) passing the ATR- crystal (composed of zinc selenite) in the ATR- unit is absorbed by the molecules in the sample and excites molecular vibrations (Fig. 6). The reflected IR-light is detected by the DTGS- detector. The detected signal depends on the molecular structure of the chemical components in the sample and results in a spectrum of different absorbance units. The spectra show the chemical components of the sample (e.g. wood) in the so-called fingerprint area, the wave number area between 1800 and 600cm^{-1}. Each bond can be seen as typically for a particular component in a sample.
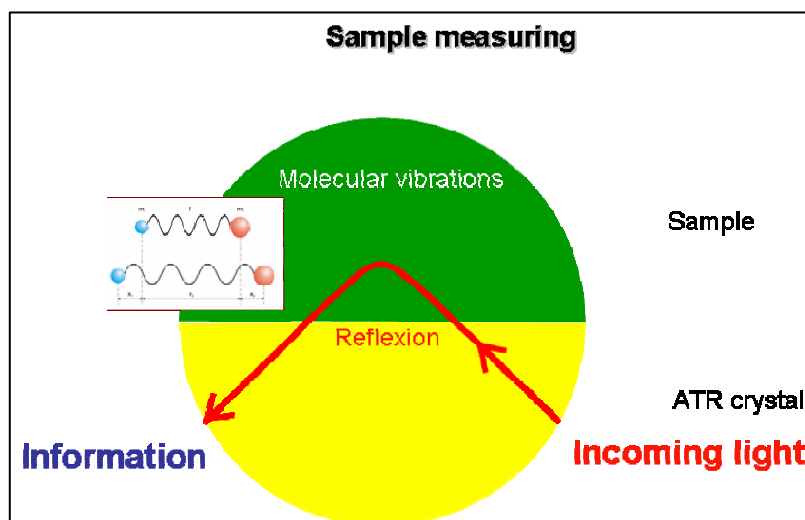
Fig.6: Illustration of the mode of measurement of FTIR -spectroscopy with ATR- unit. The IR-light is passing the crystal (high index of refraction) and entering the sample (lower index of refraction). The beam causes molecular vibrations and is reflected afterwards. The incoming signal is detected at the DTGS-detector.

Application of FTIR-spectroscopy for timber

FTIR-spectroscopy allows a quick analysis of wood components. Due to qualitative differences in the spectra it is possible to assign the chemical structures of timber to a single wave number as e.g. that

for lignin, extractives or polysaccharides. It is also possible to distinguish spectra of timber of coniferous wood or hardwood due to different compositions of lignin and hemicelluloses (Faix 1991).

FTIR- spectroscopy was used to distinguish wood of different habitats and proveniences of *Eucalyptus* spp. and for screening of cell wall mutants (Chen et al. 1998). Mycosis of wood is detectable as well as the identity of fungi decomposing wood (Naumann et al. 2005). The changes in crystalline structures of cellulose in cell walls and the composition of the chemical structures of various tree species can be defined. The chemical composition of wood, its primary, composed and degradation products can be analyzed (Windeisen et al. 2003). FTIR- spectroscopy allows to detect the influence of binders on wood quality and the accumulation of additives.

Another example for the use of FTIR- spectroscopy is the quality control of pulp and paper (Böttcher 1993). Absorption bonds of substances are generated during each analysis and can directly be assigned to a certain molecular bond (e.g. C=O) or to frequencies of functional groups (e.g. G/S lignin) and identify the substance (Gottwald & Wachter 1997; McCann et al. 1997; Windeisen et al. 2003).

FTIR -spectroscopy in combination with FTIR- microscopy

A relatively new technique used for sample analyses is FTIR- microscopy. This allows rapid analysis of organic samples. The result of a measurement is a pseudo colour image which reflects the allocation of the intensity of the bonds of the scanned area. High and low intensities are thereby encoded by particular colours. The resolution of the image is dependent on the size of a single detector element out of the 4096 elements, which has an area of 4x4 µm. A maximum resolution of 4µm is possible depending on the wavelength.

FTIR- microscopy with FPA-Detector (focal plane array)

The FPA-Detector (**f**ocal **p**lane **a**rray) is used to obtain high resolution chemical images from a large sample area. To characterize wood and wood products, woodcuts of about 10µm of thickness or less are used. Imaging is the illustration of the spatial distribution of the chemical composition in the measured wood area. The FPA- detector contains 64x64 (4096) detector elements and generates 4096 independent spectra per scan (Fig.7).
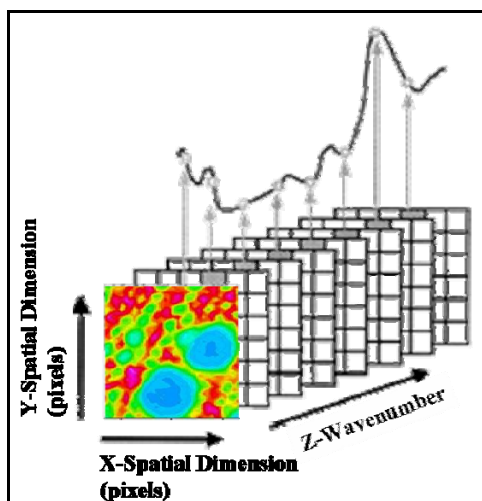
Fig. 7: Spectroscopic imaging data cube. Each slice of the data cube in the xy- directions represents a spectral image taken by the detector array at a particular wavelength or at a particular mirror position in the case of a Fourier transform instrument. A spectrum is obtained from the series of consecutive pixels in the z- direction. (Salzer 2000)

The measured sample area is 256x256 µm, because there are 64 detector-elements on each detector side with a longitude size of 4 µm (64x4µm=256µm).

The data are evaluated with "OPUS" software developed by BRUKER. An easy selection of the area of interest and a convenient control of the data acquisition is possible. FPA is a true imaging technique, acquiring both spatial and spectral information simultaneously. The huge gain in measurement speed in imaging is based on the simultaneous detection of several thousand spectra by individual pixels across the detector array in 1 to 15 data sets.

One image consists of 4096 single spectra and can be evaluated with two methods.

a) Integration Method

This method integrates the 4096 spectra for a particular wave number range which is considered to be typical for a chemical component in the sample. The result of the evaluation is an area below each single spectrum for a selected wave number area (Fig. 8). Thus, for an image 4096 single areas are received.
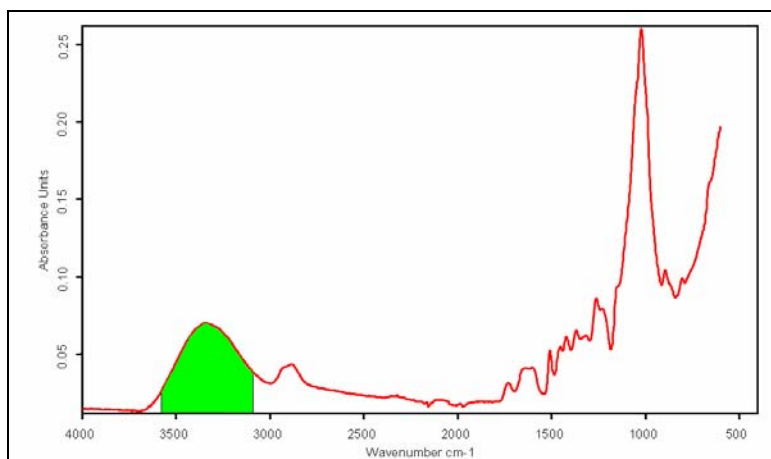
Fig.8: Result of the integration of the wave number area between 3100 and 3600 cm^{-1}. The received area (green) below the FTIR- spectrum (red) of wood was evaluated with the integration method. Depending on the height of the absorbance unit, the size of the area below the spectrum is differing, and because of that also the colour in the image.

The calculated area below each FTIR- spectrum is illustrated in the image with different colours. Depending on the size of the 4096 single areas the colour in the images differs. Red colour indicates the highest values of a substance, green colour middle values of a substance and blue colour the lowest values. Therefore the coloration of an image reflects the abundance of the component of interest (Fig.9).

b) Correlation method

The other evaluation method is the so called correlation method. With this method, a "trace" is loaded into the FTIR-image and is correlated with the 4096 spectra.

A trace is defined as an average spectrum of several (at least three) FTIR-ATR spectra of an individual substance for example lignin, cellulose or UF resin. This average spectrum is loaded into the image and is correlated with the 4096 spectra. The resulting colour in the image indicates the correlation coefficient. A high correlation coefficient of spectra and trace is illustrated in red colour, a low correlation coefficient in blue (Fig.9). Compared to the first method, not a calculated value but an organic substance is used as basis for the imaging.
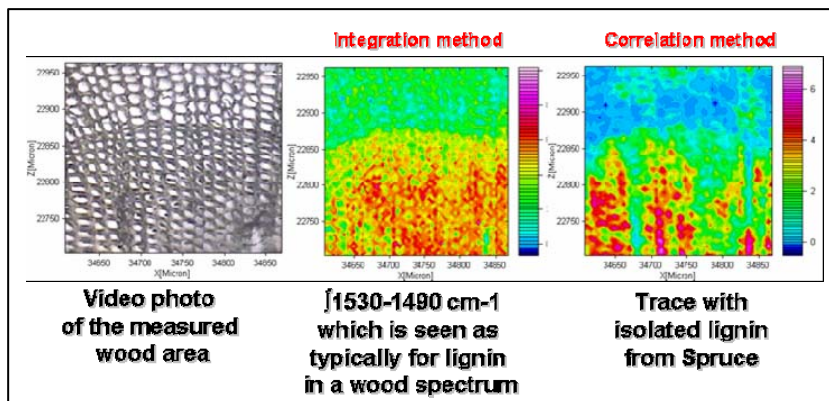
Fig.9: Video photo of a measured wood area (10μm woodcut of solid wood of spruce, spring-wood late-wood are intersection) and FTIR- images (middle, right) imaging the different illustration of lignin depending on the used evaluation method.

At present analyses are made in research whether the illustration of the correlation method represents the spatial distribution of the chemical composition in the measured wood area of a sample in a better way than the integration method or not.

FTIR- microscopy with MCT-single-element-detector (Mercury cadmium telluride)

Another modern technique for microscopic measurements of sample composition is achieved by use of a MCT-single-element-detector. This technique of measuring is called mapping. In comparison to imaging, the mapping technique relies on one single detector.

The measured area is not predetermined as it is in the FPA detector. While moving the sample one spectrum is recorded. A fast measurement of an area in a wood cut is possible, because just one spectrum is recorded for the whole area. Recording one spectrum for a larger sample area causes a better signal to noise ratio, even better than the ratio of a DTGS-detector. An example is shown in Fig. 10.

The main disadvantage is that the spectra are not measured simultaneously but one after another. This is much more time-consuming than FPA- imaging. The measurements are adversely affected by a limited area of spectra and permanent cooling of the instrument with liquid nitrogen.
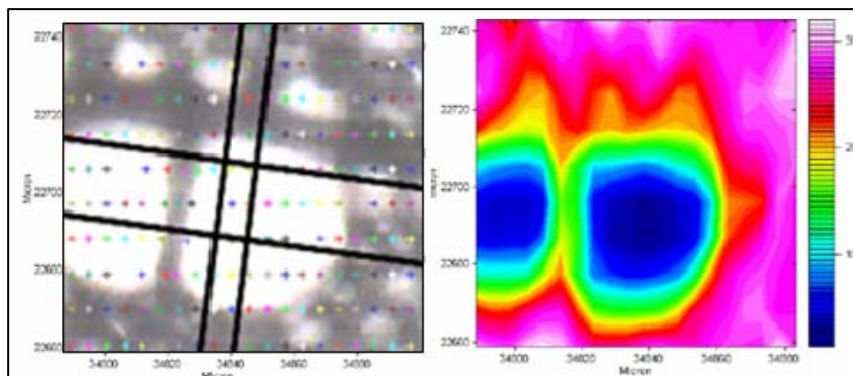
Fig.10: Video photo of a measured wood area (left, woodcut of solid wood of poplar) and FTIR- image measured with MCT- detector with a resolution of 8cm and 150 scans. In the right picture, the spatial distribution of lignin is illustrated evaluated with the integration method.

Future prospects

The number of publications concerning FTIR- spectroscopy of wood and wood components continually is increasing. FTIR- spectroscopy of wood and wood components is qualified for various analyses, because no pre- treatment of samples is necessary and little material is required. In combination with mathematical and statistical evaluation procedures FTIR- spectroscopy is established as a routine method for quantitative analyses in wood chemistry. FTIR- microscopy represents a basic technique in wood science, in particular for the analyses of surface properties of composed wood products.

Acknowledgements

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„Biotechnological research on basidiomycete fungi"

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Introduction

Fungi play an important role in our daily live. They have medicinal values as source for pharmaceuticals (Wasser 2002) and are used in the food industry (e.g. in the production of bread, cheese, yoghurt, beer, mushrooms, Quorn[TM], vitamins, flavours, acetic acid, etc.) (Janssens et al. 1992, Trinci 1992). They also have their place in composting organic waste materials and in bioremediation of industrial contaminated soils and of water effluxes (Pointing 2001). The highest developed class in the fungal kingdom is that of the basidiomycetes that by their ecological role can be divided into three groups: saprophytic, symbiotic (mycorrhizal) and parasitic fungi. In industry, the saprophytic species find most interest.

Saprophytic fungi use dead organic material as a nutrient source, like wood, litter or dung. The mycorrhizal (Greek: fungal roots) basidiomycetes, such as *Laccaria bicolor*, are able to exchange nutrients with their tree symbionts and protect the roots of their hosts against attack by other microorganisms. Parasitic fungi attack other organisms as nutrient resource and they may kill their host. Regardless of their ecological role, many of the higher basidiomycetes (hymenomycetes) are capable of forming fruiting bodies (mushrooms) for sexual reproduction. Many of these fruiting bodies are edible. Mushrooms of saprophytic basidiomycetes may be produced in industrial cultivation. *Agaricus bisporus*, *Pleurotus ostreatus* (Fig. 1), and *Lentinula edodes* are species with worldwide production rates of 1,956, 1,564 and 876

Fig. 1 Fruiting body of *Pleurotus ostreatus* grown on wheat straw substrate

Fig. 2 Young *Coprinopsis cinerea* mushrooms as preserved food in Thailand

tons, respectively (Chang 1999). Mainly in Asia, many more species are used for industrial mushroom production (Rühl & Kües 2006), amongst *Coprinopsis cinerea* (Fig. 2). Saprophytic hymenomycetes have enzymatic systems which act in degrading lignocellulolytic waste: Species which can attack only cellulose and leave the lignin undigested are called brown rot fungi, due to the brown colour of the remaining lignin. Species that preferentially degrade lignin leaving the cellulose intact or degrade

both substances simultaneously are known as white rots. Species used in industrial mushroom production are in most instances white rots.

Research in our group concentrates on enzymes involved in the degradation of lignin, in particular in redox-enzymes such as Laccases and peroxidases. The fungus *C. cinerea* acts as a model organism to study different aspects in enzyme production for biotechnological applications as well as in fruiting body development. Genome analysis of several different basidiomycetes (*C. cinerea*, *L. bicolor*, *P. ostreatus* and *Heterobasidion annosum*) and techniques of fungal proteomics are used to address questions in fungal growth and development, substrate degradation and ecology.

Fruiting body development in Coprinopsis cinerea

C. cinerea is a fast growing hymenomycete that completes its whole life cycle within two weeks. This fact makes the fungus a very useful tool to study basic biological functions. As shown in Fig. 3, the basidiospores (sexual spores) germinate into a monokaryotic (one nucleus per fungal cell) mycelium that is not able to form a fruiting body. By fusion of two compatible monokaryotic hyphae (filaments of fungal cells) a dikaryotic (two different nuclei per fungal cell) mycelium arises that is able to form a fruiting body. In the cap (pileus) of the fruiting body, basidia form as specific cells on gills which give rise to the basidiospores.
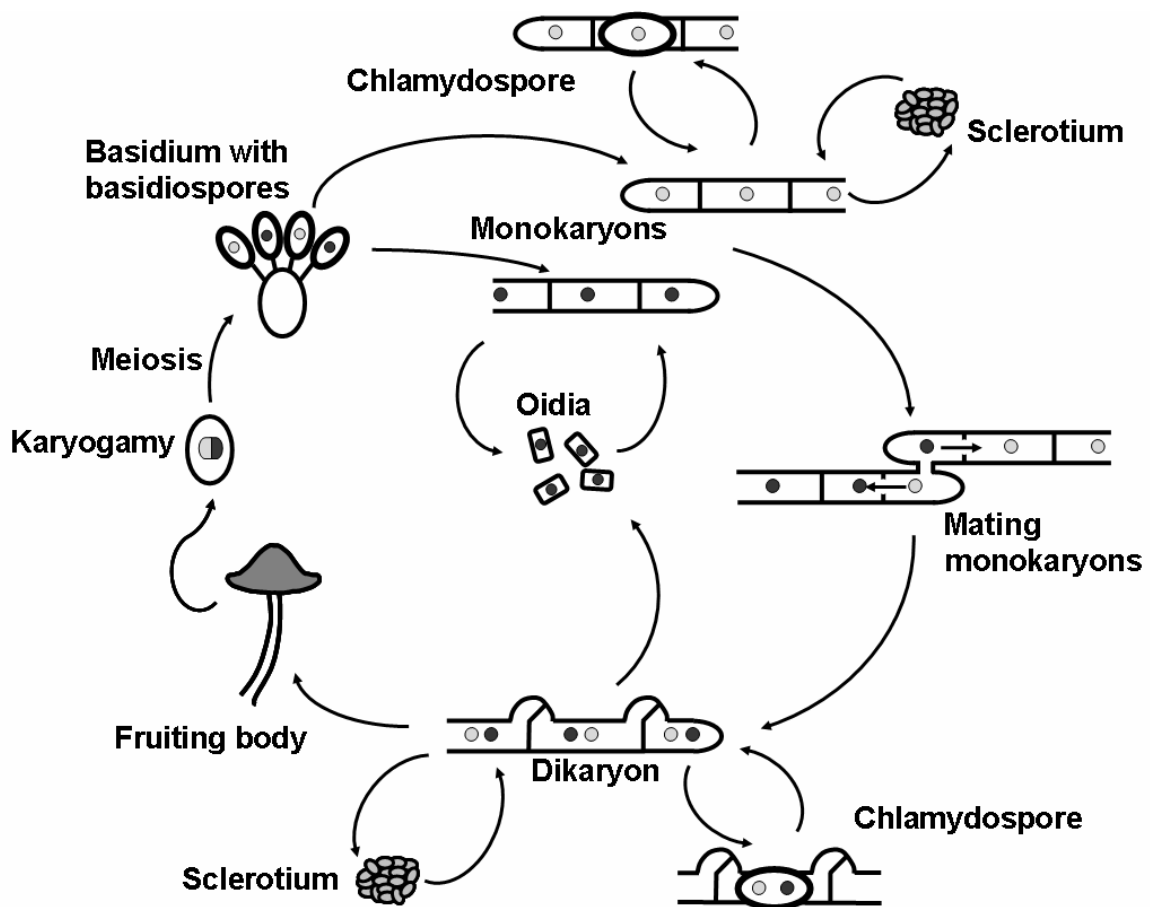
Fig. 3 Life cycle of *Coprinopsis cinerea*
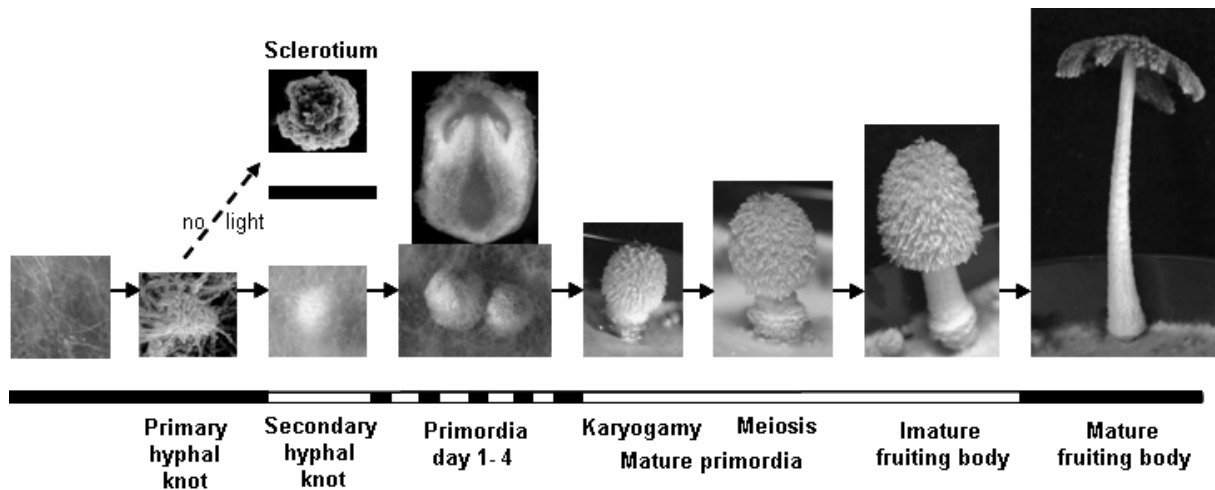
Fig. 4 Fruiting body development in *Coprinopsis cinerea* occurs within 6 days: dark/white boxes = day and night periods; a day is defined from the start to the end of a 12 h light period

Fruiting body development is regulated by environmental factors (nutrients, temperature, light, humidity). Light is needed to synchronize development within the day/night rhythm (Fig. 4). On the dikaryotic mycelium, primary hyphal knots (agglomeration of hyphae) form in the dark. If kept in the dark light, they transform into sclerotia (dense hyphal bodies, Fig. 4) for survival under adverse environmental conditions. When light is provided, primary hyphal knots convert into the more compact secondary hyphal knots in which tissue differentiation occurs. In the resulting primordia, the different parts (cap, stipe) of a fruiting body become visible. In mature primordia, karyogamy and meiosis occur in the basidia, leading to formation of the basidiospores. In parallel, the stipes elongate and the caps expand to a mature fruiting body (Kües 2000). Little is yet known on the cellular and molecular events that determine this complex course of development – mutants in fruiting body development help to clone genes acting in the processes (Liu et al. 2006).

Identification of fungi in wood

Worldwide, fungal damage on wood is enormous, since these organisms can destroy the structure of the wood in living trees or in dead wood including timber in use. In order to apply appropriate treatments and precautions, techniques for fast detection of wood infecting fungi and early recognition of an infection are required. In our laboratory, two different techniques are developed for identification of fungal attack on wood.

The molecular method: ITS sequences for detection of fungal infections

Within the genomes of any organism, identical sets of genes coding for the ribosomal RNAs (in eukaryotes: 18S, 5.8S, 28S, respectively) occur in tandem repeats in up to a few thousands copies.

These so-called rDNA repeats (Fig. 4) are separated by non-transcribed spacers (NTS). Furthermore, the three ribosomal RNA genes are separated from each other by internal transcribed spacers (ITS). Due to a high genetic variation between species, these ITS sequences are used for molecular systematics (Fig. 5). Every species has their own defined ITS sequences.
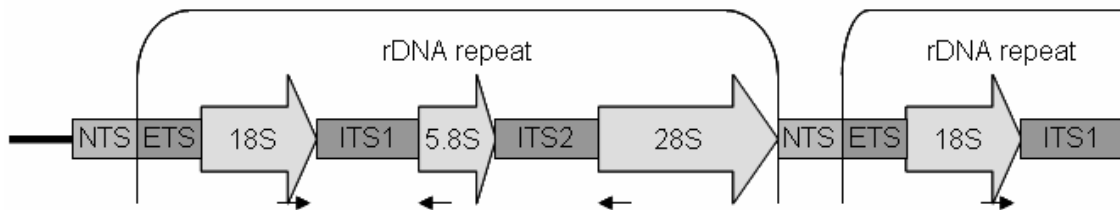
Fig. 5 rDNA repeats with the genes for the three ribosomal RNAs in the genome of an eukaryotic organism. NTS (non-transcribed spacers); ETS (external transcribed spacers); ITS (internal transcribed spacers); arrows indicate primer positions for the amplification of ITS sequences by PCR

To identify the ITS sequence, we first have to extract the DNA from infected wooden samples or from fungal cultures, for example from *H. annosum* infected spruce wood (Fig. 6). Afterwards, the ITS regions are amplified by using specific primers (short DNA fragments which bind to a sequence motif in the ribosomal RNA genes) in PCR (Polymerase Chain Reaction which provides by an enzymatic reaction a multiplication of DNA sequences). Next, the PCR-product is sequenced and compared with a sequence database with suitable computer programs. If the search of the database gives a hit, the amplified ITS sequence can be assigned to a species. With no hit in the database, the kind of the fungus has to be determined for an exact identification, for example by microscopy. About 1.5 million fungi are estimated to exist in nature (Hawksworth 2004). Many of these will be wood rotting and wood inhabiting fungi. For a best functional database, the number of entries of ITS sequences has to be expanded with new ITS-sequences of as many of these fungi as possible.

Fig. 6 *Picea abies* samples infected by *Heterobasidion annosum*; from left to right: early infection with no visible damage, first discoloration and late stage of decay

The optical method: FTIR spectroscopy and microscopy

Another possibility for identification of fungal infection of wood is the detection of specific spectroscopic patterns being characteristic for wood decay or for presence of a fungus. To this end, we are using Fourier Transform Infrared Spectroscopy (FTIR) within a microscope (for further explanation see chapter "FTIR spectroscopy and microscopy as tools for analysis and control of wood properties and production processes"). Presence of fungal hyphae within wood vessels can be detected by FTIR due to the different chemical composition of the fungal hyphae compared to the cell walls of the wood (Naumann et al. 2005).

Fungal Genomics and Proteomics

The complete genomic sequences from three homobasidiomycetes are currently available: that of the saprophytic fungus *C. cinerea* (http://www.broad.mit.edu), that of the white rot *Phanerochaete chrysosporium* (http://genome.jgi-psf.org) and of the ectomycorrhizal species *L. bicolor* (http://genome.jgi-psf.org). With the brown rot *Poria placenta* and the white rots *Schizophyllum commune*, *P. ostreatus* and *H. annosum*, within the next year four more complete genomes will be available (http://genome.jgi-psf.org). Currently, we help in annotation of the genes in *L. bicolor* with the target to compare the different sets of genes in the different species to define components specific to the different ecological roles of the fungi. Searching the genome of *C. cinerea* allowed for example the identification and cloning of seventeen different Laccase genes (Kilaru et al. 2006, Fig. 7).

The main aim of our group in the field of fungal proteomics is the analysis of secreted proteins in *C. cinerea*, *P. ostreatus*, and other white rot fungi. Secreted proteins either occur freely in culture supernatants or they are bound to the fungal cell wall or to the surrounding gelatinous sheet. Proteins harvested from the supernatant or extracted from cell wall fractions or the hyphal sheet are separated in 2D gel-electrophoresis by their IEP (Iso-electrical Point – 1st Dimension) and MW (Molecular Weight – 2nd Dimension) (Fig. 7). Subsequently for ESI-LC-MS (Electro-Spray-Ionisation/Liquid-Chromatography/Mass-Sepectrometry) analysis, protein spots are cut from the gel and digested with trypsin (a certain peptidase) to obtain specific peptides. The sample is analysed by ESI-LC-MS and the obtained peptide sequences are used for possible identification by Mascot database searching, thereby making use of a protein sequence database deduced from the *C. cinerea* genome (see Fig. 8 as an example for *C. cinerea* Lcc1 peptide sequences).

Match to: **Lcc1** Score: **401**
Cc Genome (Lcc1)

Nominal mass (M$_r$): **58634**; Calculated pI value: **5.17**
NCBI BLAST search of Lcc1 against nr
Unformatted sequence string for pasting into other applications

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: **17%**

Peptides sequences obtained by ESI-LC-MS

Matched peptides shown in **Bold Red**

```
  1 MFKNLLSFAL LAISVANAQI VNSVDTMTLT NANVSPDGFT RAGILVNGVH
 51 GPLIRGGKND NFELNVVNDL DNPTMLRPTS IHWHGLFQRG TNWADGADGV
101 NQCPISPGHA FLYKFTPAGH AGTFWYHSHF GTQYCDGLRG PMVIYDDNDP
151 HAALYDEDDE NTIITLADWY HIPAPSIQGA AQPDATLING KGRYVGGPAA
201 ELSIVNVEQG KKYRMRLISL SCDPNWQFSI DGHELTIIEV DGQLTEPHTV
251 DRLQIFTGQR YSFVLDANQP VDNYWIRAQP NKGRNGLAGT FANGVNSAIL
301 RYAGAANADP TTSANPNPAQ LNEADLHALI DPAAPGIPTP GAADVNLRFQ
351 LGFSGGRFTI NGTAYESPSV PTLLQIMSGA QSANDLLPAG SVYELPRNQV
401 VELVVPAGVL GGPHPFHLHG HAFSVVRSAG SSTYNFVNPV KRDVVSLGVT
451 GDEVTIRFVT DNPGPWFFHC HIEFHLMNGL AIVFAEDMAN TVDANNPPVE
501 WAQLCEIYDD LPPEATSIQT VVRRAEPTGF SAKFRREGL
```

[Show predicted peptides also]

[Sort Peptides By]   ⦿ Residue Number ◯ Increasing Mass ◯ Decreasing Mass

Start – End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence	
42 – 55	708.58	1415.14	1414.84	0.29	0	AGILVNGVHGPLIR	(Ions score 35)
194 – 211	916.14	1830.26	1829.95	0.31	0	YVGGPAAELSIVNVEQGK	(Ions score 47)
194 – 212	980.22	1958.43	1958.05	0.39	1	YVGGPAAELSIVNVEQGKK	(Ions score 52)
261 – 277	1050.72	2099.42	2099.01	0.41	0	YSFVLDANQPVDNYWIR	(Ions score 32)
285 – 301	838.11	1674.20	1673.88	0.31	0	NGLAGTFANGVNSAILR	(Ions score 53)
285 – 301	838.11	1674.21	1673.88	0.32	0	NGLAGTFANGVNSAILR	(Ions score 53)
285 – 301	838.56	1675.10	1673.88	1.21	0	NGLAGTFANGVNSAILR	(Ions score 22)
285 – 301	838.56	1675.10	1673.88	1.22	0	NGLAGTFANGVNSAILR	(Ions score 32)
428 – 441	735.94	1469.87	1469.71	0.15	0	SAGSSTYNFVNPVK	(Ions score 25)
428 – 441	735.96	1469.91	1469.71	0.19	0	SAGSSTYNFVNPVK	(Ions score 38)
428 – 442	814.03	1626.04	1625.82	0.22	1	SAGSSTYNFVNPVKR	(Ions score 50)
428 – 442	814.03	1626.05	1625.82	0.23	1	SAGSSTYNFVNPVKR	(Ions score 12)
428 – 442	814.57	1627.12	1625.82	1.30	1	SAGSSTYNFVNPVKR	(Ions score 14)
443 – 457	780.57	1559.12	1558.82	0.30	0	DVVSLGVTGDEVTIR	(Ions score 92)

Lcc1

Fig. 7 Protein band of Coprinopsis cinerea Laccase Lcc1 (left) separated by 1D-gelelectrophoresis. The band was cut from the gel, digested by trypsin, analysed in ESI-LC-MS and the protein identified by Mascot search (right) with the obtained peptide sequences (bold red)

The incident that the genome of *C. cinerea* is already released to the public and that the *P. ostreatus* genome will be available soon, helps us to easily identify also unknown secreted proteins. In *C. cinerea*, we could identify several proteolytic and hydrolytic enzymes, which could be of interest for biotechnological studies (Fig. 8).
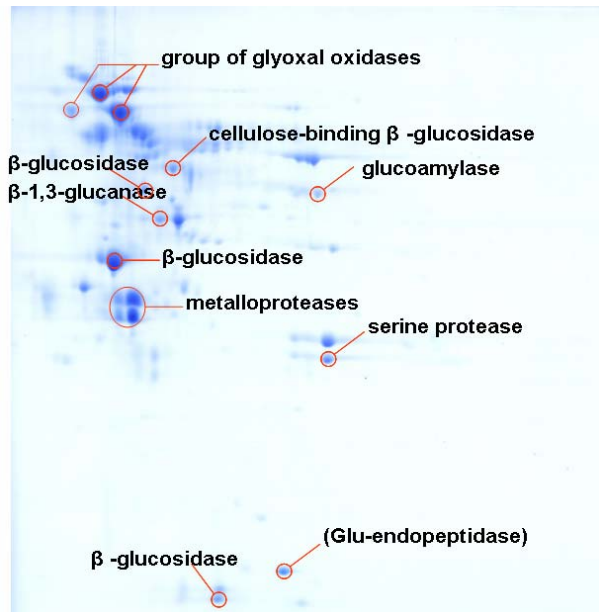
Fig. 8 2D-Gel of *Coprinopsis cinerea* proteins from culture supernatants. Identified proteins are indicated.

Laccase production by transformants of Coprinopsis cinerea

In nature, Laccases occur in fungi, plants and insects (Mayer & Staples 2002), but also in bacteria (Claus et al. 2003). Laccases are multi-copper oxidases that oxidize phenolic and a number of other organic compounds (Fig. 9). Because of their wide substrate range, Laccases are interesting for various biotechnology applications in the food, the textile and the pulp and paper industries, in soil bioremediation and in wood composite production (Hüttermann, Mai & Kharazipour 2001, Fig. 10).
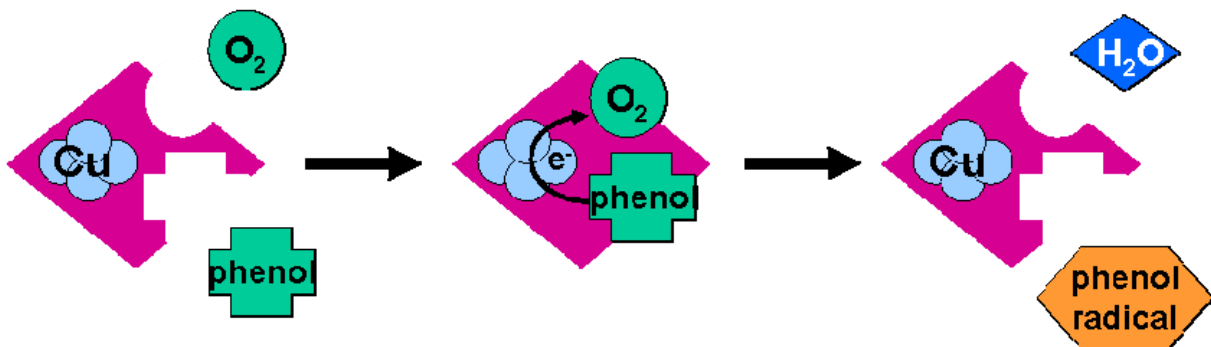
Fig. 9 Schematic diagram of the oxidation of phenol by Laccase (for explanations of the enzymatic reaction, see Chapter "Enzymatic modification of wood fibres to activate their ability of self bonding")
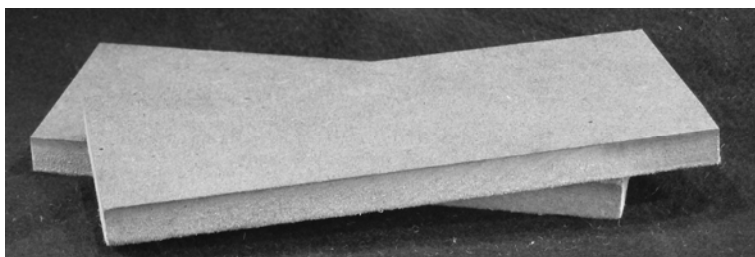
Fig. 10 Medium density fibreboards (MDF) produced with laccase as binding agent

For natural enzyme production, fungal strains are selected that are most efficient in secretion of enzymes of the required characteristics. Most of the "natural" Laccases are won from basidiomycetes in fermentation, usually after treating the fungal cultures with suitable inducers of enzyme production (e.g. copper, phenolic compounds). *P. ostreatus* and *Trametes versicolor* are used as native producers for Laccase and other lignin degrading enzymes in submerged fermentation. Spent substrate (wheat straw) of *P. ostreatus* mushroom production (Fig. 1) can also be a source of Laccase - we obtained Laccase activities of approximately 1.5 U/ml in the substrate liquor.

Recombinant Laccases in *Coprinopsis cinerea*
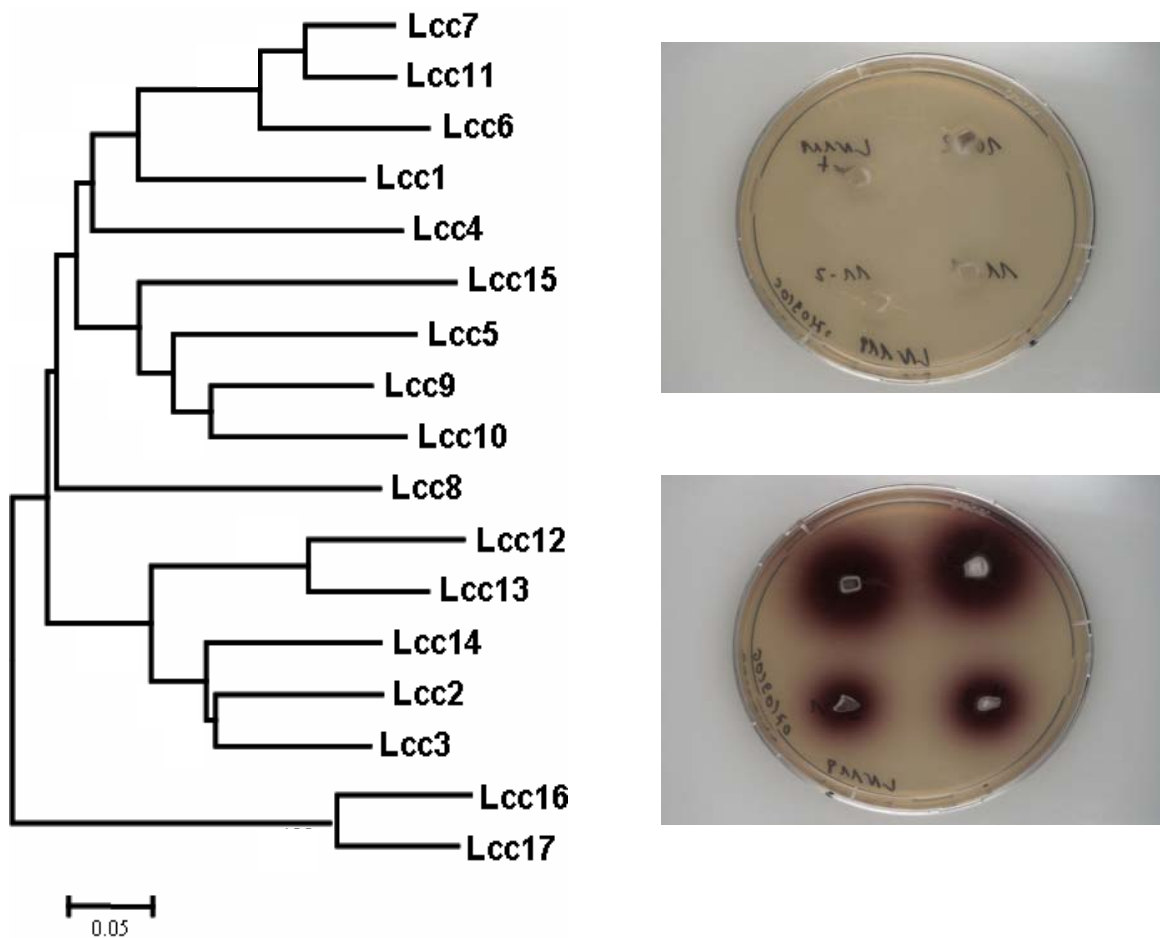
Fig. 11 (left) Phylogenetic tree of *Coprinopsis cinerea* Laccases calculated with the program "megasoftware" from aligned amino acid sequences that reflects the distance of relatedness between the proteins. The scale bars shows the probability of an interchange in one amino acid position. (right) Transformants of *Coprinospis cinerea* on agar plates supplemented with the colourless Laccase substrate ABTS (2,2'-Azinobis(3-ethylbenzthiazoline-6-sulfonate)): the wild type strain (control shown at the top) shows no reaction whereas Laccase transformants stain the agar by enzymatic oxidation of ABTS.

Laccase yields from natural production are often not high enough. Overexpression of these enzymes in fungi upon gene transformation is an alternative way for high-level enzyme production (Kilaru 2006).

Heterologous expression of basidiomycete Laccase genes in ascomycetes has been tried with limited success: yields were low and enzyme properties were altered (Kilaru 2006). Therefore, a basidiomycete expression system seems to be more adequate. We use *C. cinerea* as an expression host for various homologous and heterologous Laccase genes.

Biotechnological applications demand for different Laccases which are distinct in their biochemical and physical properties. The genome of *C. cinerea* has 17 different genes coding for Laccases (Kilaru, Hoegger & Kües 2005, see Fig. 11). These enzymes differ to some extend from each other in amino acid sequence which is reflected in the phylogenetic tree shown in Fig. 11. Properties of the enzymes are determined by their amino acid sequences and differences in sequence can result in other characteristics. Having 17 different Laccase genes offers therefore special opportunities for their utilization. All of these Laccase genes were transformed into *C. cinerea* under control of a very active constitutive (i.e. always operating) promoter. Transformants of nine of the genes showed Laccase activity on agar plates and six also in liquid cultures.

The best clones were chosen for further characterisation studies and, up to now, two Laccases were biochemically characterised. The optimal pH for enzymatic reactions varies, depending on the used substrate, between pH 4.0 and 8.0. pH stabilities are between 5.0 and 12.0. Further studies have the goal to characterise the other Laccases and to test their potential in biotechnological applications. For these purposes, highest Laccase yields in large amounts are needed. Therefore, liquid cultivation of *C. cinerea* for optimal production of recombinant Laccases is studied.

Production of recombinant Laccases in liquid culture

Our aim is to obtain higher yields of Laccase activity in liquid cultures. For this purpose, different experiments are carried out concerning fungal growth and Laccase secretion. The media composition is probably one of the most important factors influencing the cultivation of *C. cinerea* and, thus, the amount of produced and secreted enzyme. As it is shown in Fig. 12, a soybean based media for example gives much higher Laccase yields as the standard growth medium for *C. cinerea* (YMG – Yeast extract, Malt, Glucose).

Further to the provided nutrients, also the temperature can have an influence on the production of Laccases. In a yeast-extract based medium, production yields at 25 °C where higher than at 37 °C which is the optimal growth temperature. Further studies have to reveal why there is a higher activity at the lower temperature, whether there is a higher stability of the Laccase enzyme at 25 °C, a lack of proteolytic enzymes or any other reason. The temperature influences the growth morphology of the mycelia (Fig. 13) which also might contribute to the phenomenon.
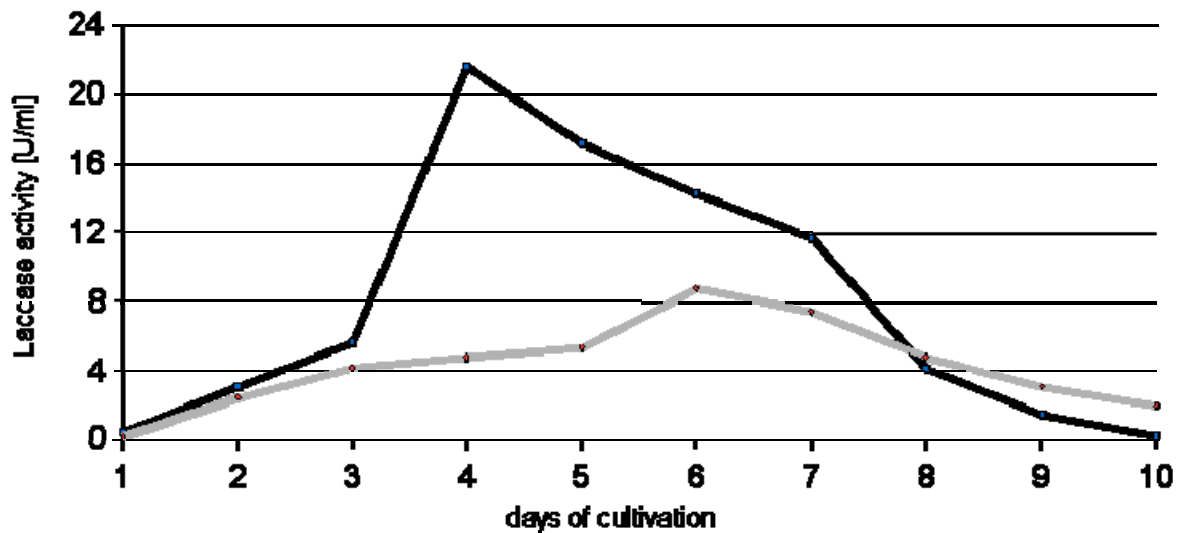
Fig. 12 Laccase activity of a *Coprinopsis cinerea* transformant in YMG (grey) and FG4 (black) media

Other of our studies focus on the production of the basidiomycete in a stirring vessel. To define best cultivation conditions for Laccase production by *C. cinerea*, factors influencing growth, like the oxygen concentration, pH, temperature, media composition, stirring velocity and shear forces, will be considered.
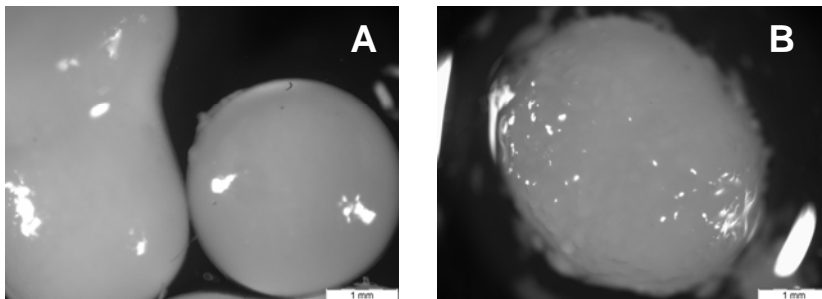
Fig. 13 Mycelial pellets of a *Coprinopsis cinerea* transformants grown at 25°C (A) and 37°C (B). Highest Laccase activity at 25°C ~ 11 U/ml and 37°C ~ 3 U/ml

In cooperation with Prof. Kharazipour and his co-workers, recombinant Laccases in sufficient amounts will be tested as a natural bonding agent for the production of Medium Density Fibreboards (MDF) in order to replace formaldehyde as a binder. Laccases act on the lignin of the fibers, thereby producing phenolic radicals which in turn may interact with other phenolic compounds on adjacent fibres resulting in polymerisation and cohesion of the fibres (see Chapter "Enzymatic modification of wood fibres to activate their ability of self bonding").

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"A short review of Forests of Iran"

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Introduction

Iran is an ancient country in the Middle East (map 1); with an area of 1.6 Million Km². Forests of Iran with an area about 12.4 million hectares comprise 7.4% of the whole country area which is classified as a low forest cover countries (LFCC). While the forest cover of Iran is considered poor as compared with other countries, it is a unique country regarding plant diversity and genetic reserves. Approximately 8000 plant species have been identified in Iran. Climatic diversity especially from the land structure viewpoint is such that geographers have called Iran the global climates bridge. The phyto geographical regions that concern the flora of Iran (map 2) are as follows:

1. The Euxino-Hyrcanian Province of the Euro-Siberian Region
2. The Irano-Turanian Regions
3. The Saharo-Sindian Regions

Map 1: Map of Iran
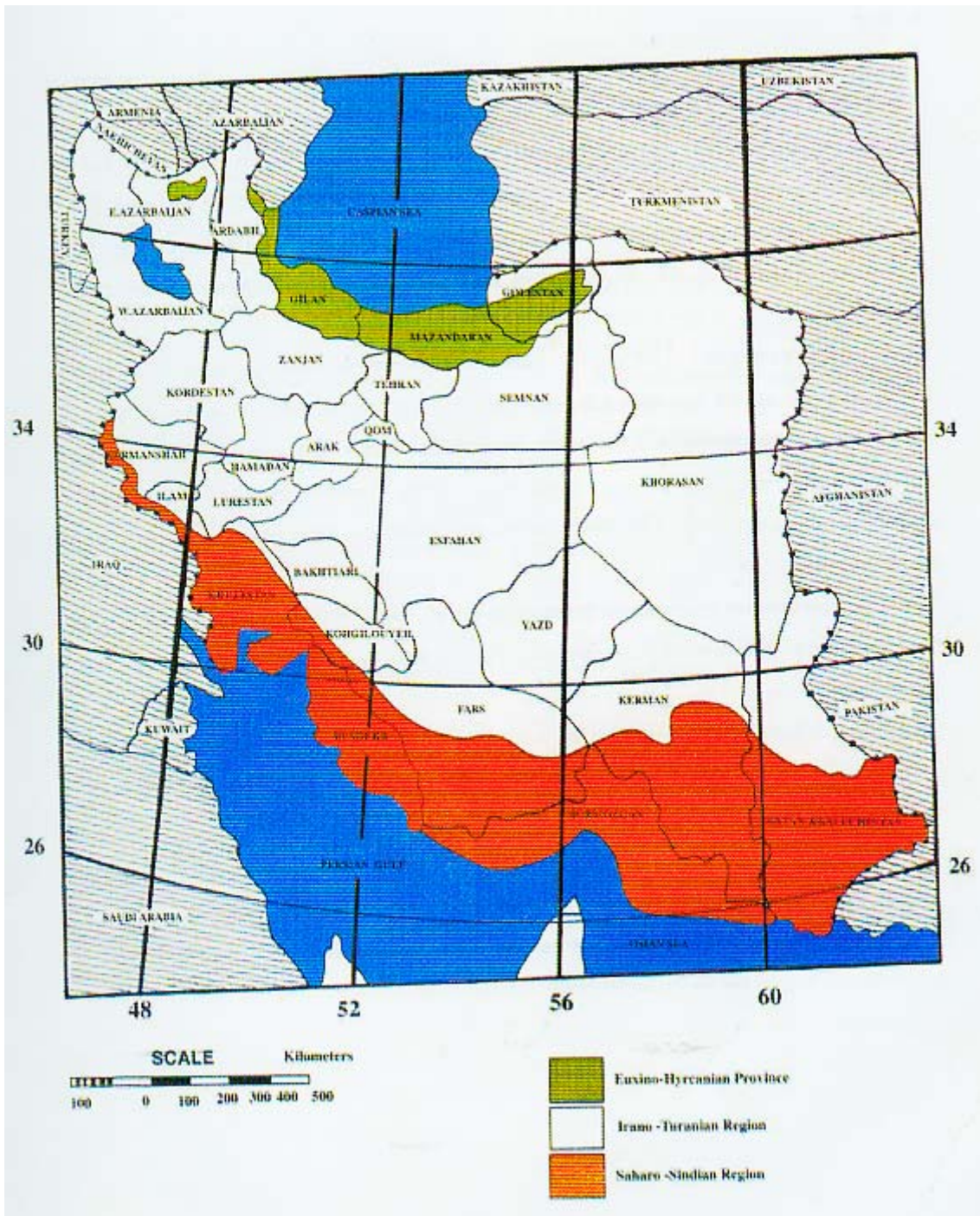
Map 2: Three phytographical regions of Iran.

Euxino-Hyrcanian Province

In Iran, the Euro-Siberian region is represented by the Euxino-Hyrcanian Province. It is confined here to the coastal surroundings of the Caspian Sea and occupies three main habitats: alluvial flats of the coastal plain, the northern slopes of the Alborz Mts. and the sub alpine meadows of these mountains. The most outstanding feature of this area is the broad-leaved deciduous forest, which ranges in altitude from sea-level to 2800m above sea-level.

The province is well distinguished from other areas by high annual precipitation (600-2000mm), a considerable part of which falls in summer. The high air humidity and the higher winter temperatures at the lower altitudes make the greater part of this area most favourable for mesic forest, not unlike those of western or southern Europe. The rather high number of species, endemic to the Euxino-Hyrcanian Province gives this forest type a distinctive character. This phyto geographical region consists of two separate forest zone: Hyrcanian and Arasbaran.

Hyrcanian zone

Picture 1 shows a view of Hyrcanian forest and Hyrcanian vegetation zone is a green belt stretching over the northern slopes of Alborz mountain ranges and covers the southern coasts of the Caspian Sea. This area stretches from Astara in the northwest to Gorgan vicinity in the northeast of Iran. Based on the latest data from the Iranian Forests and Rangelands Organization, this area is approximately 800km long and 110km wide and has a total area of 1.85 million ha comprising 15% of the total Iranian forests and 1.1% of the country area. Alborz mountains interception between the Caspian Sea and Iran plateau has resulted in a climate with distinct vegetation cover. Hyrcanian forests stretch out from sea level up to an altitude of 2800m and encompass different forest types thanks to their 80 woody species (trees and shrubs). The area is rich in hardwood species; however there are only four genii of endemic softwood trees including *Taxus* sp., *Juniperus* sp., *and Thuja* sp. And *Cupressus* sp. The primary function of these forests other than wood production is supportive and environmental and their vital role in soil and water sources conservation as well as natural balance distribution in this extremely considerable. The Hyrcanian zone is a humid zone in the north of Iran. The average annual rainfall ranges between 530mm in the west reaching up to an occasional record of 2000mm in the west. Based on the climatic data from meteorological stations, the maximum annual rainfall is experienced during spring and late fall and winter. Relative humidity is also constantly high with an average value fluctuating from 74.6% in the east to 84.6% in the west, rarely dropping below 60% at the hottest hours. Also it should be mentioned that according to the climatic data from meteorological stations, the average annual temperature in the Hyrcanian region has varied from 15 °C in the west to 17.5 °C in the east over the past decade. The warmest month temperature ranges between 28 °C to 35 °C while that of the coldest month is between 1.5 °C to 4 °C. Summer temperature ranges between 20 °C to 30 °C. General speaking, the Hyrcanian climate is warm Mediterranean in the east and temperate and semi- temperate Mediterranean and occasionally temperate xeric in the central and western parts.

Picture 1: A View of Hyrcanian forests

The importance of the Hyrcanian Forests

This area along with similar North American and East Asian forest communities remained intact nowadays seen as a Tertiary Period deciduous belt containing communities formerly associated with each other. Among these, the Hyrcanian region is quite intermediate thanks to its species. There are 65 different tree species in the region among which, some relic species, indicator of the Tertiary Period such as *Zelkova carpinifolia, Parrotia persica* and *Pterocarya fraxinifolia* are found. Of 80 woody plants reported in the region, 45 species (ca 60%) belong to the late Pleistocene. The extent of the Hyrcanian forests minimally changed during the entire Quaternary Period at least until the end of the Ice Age. During this time, the entire area was dominated by a single indicator forest system including various Tertiary Period polar elements. Different ice ages have undoubtedly ruined temperature susceptible species such as *Eucommia, Ginkgo* and *Taxodium.*

Vegetation

In contrast to the Euxino-Caucasian forest lands, no high altitude softwood forest stands are observed in Alborz region and the timber line is characterized by a hardwood species (*Quercus macranthera*). No softwood seeds were found in the archeological digs in Lar region whereas *Fagus, Carpinus, Alnus, Corylus, Quercus,* and *Betula seeds* were abundant. *Acer cappadocium, Alnus subcordata, Buxus hyrcana, Fagus orientalis, Fraxinus excelsior, Parrotia persica, Populus caspica, Quercus castaneifolia, Taxus baccata, Ulmus glabra,* and *Zelkova carpinifolia* are abounded in the Hyrcanian forests.

High species diversity in the region has given rise to various plant communities. The most important tree and shrub communities in the Hyrcanian zone are *Querco-Buxetum, Querco-Carpinetum, Parrotio-Carpinetum, Fagetum hyrcanum, Carpinetum orientale, Cupressus sempervirens* and *Thuya orientalis* communities.

Arasbaran zone

Arasbaran forest site previously covering a vast area, currently constitutes a limited territory of Kalibar, Ahar and Jolfa with an area of 140000ha. Despite the limited area, 1080 plant species and 97 woody species have been identified in the region. This, along with the presence of rare fauna and Arasbaran protected area with an area of 78560ha covering 56% of the region has placed the region among the nine Iranian biosphere reserves under the UNESCO Man and Biosphere (M & B) program. The climatic diversity of Arasbaran region is due to the main mountain directions and the wind speed and direction resulting in the entrance of humidity from the Caspian Sea from the east, the Mediterranean from the west and Siberian low pressure fronts from the north. While the average annual rainfall is estimated to be around 300-500mm. and generally speaking, according to Emberge method, the climate of the region is humid and cold.

Vegetation

Arasbaran region is an intermediate region due to the presence vegetation elements associated with various climates. The most important woody plants in this site are as follows:

Quercus macranthera, Q. petraea, Carpinus betulus, Acer campestre, Acer hyrcanum, Juniperus foetidissima, Taxus baccata, Pistacia mutica, Paliurus spina-Christi, Sorbus torminalis, Ulmus glabra, Cotinus coggygria, Viburnum lantana and *Cornus mass.(Picture 2).*

Some of the above mentioned species such as *Cotinus coggygria, Viburnum lantana, Juniperus foetidissima, Q. petraea* and *Cornus mass* are indigenous to Arasbaran region. 33 communities are identified in Arasbaran with the followings as the most important tree communities:

Secondary woodlands: Includes *Paliurus spina-Christi* along with *Q. petraea, Juniperus foetidissima* and *Bothriochloe ischaemum* immediately growing above valleys exploited for farming and grazing. These communities develop due to the clear cutting or slash and burn methods used for primary woodlands. Due to the abolishment of these methods since 15 years ago, a very thick tree cover has developed lying at 350-1000m altitudes.

Primary woodlands: Consisting of *Carpinus betulus, Q. petraea, Acer campestre, Q. macranthera* and *Acer hyrcanum* mainly coppiced prior to the application of protective measures 15 years ago. This community lies at an altitude of 1000-1800m.

Acer velutinum

Parrotia persica

Quercus castaneifolia

Alnus subcordata

Tilia platyphyllus

Zelkova carpinifolia

Picture 2: A View of the most important woody plants in Euxino-Hyrcanian Province

Irano-Turanian Region

The Irano-Turanian Region has always been distinguished from the adjacent Euro-Siberian and Mediterranean Regions by a series of floristic and vegetational characteristics. Most of the Irano-Turanian Region is dominated by a continental climate, widely ranging in temperature. Rainfall is confined to the winter season which is less extreme in its temperature. Its central and eastern parts have very extreme winter temperatures, and their rainy season is spring and early summer, to which the growing season is thus limited, while winter and late summer are generally resting periods. The local climatic differences are partly responsible for the differences in the flora and vegetation of which should be looked upon as relics of a former climatic period. Floristically, the Region is characterized by a very large number of genera, sections and species. This vast region consists of two major forest zones: Zagros and Irano-Turanian.

Zagros zone

Zagros vegetation covers a vast area of Zagros mountain ranges. Zagros forest zone are classified as semi-arid forests, Zagros forests with an area of 5 million ha account for almost 40% of the country is forests. The main influence of these forests is on water supply, soil conservation, climate alteration and socio-economical balance of the entire country. Seven first grade rivers having 34.5 billion cubic meters water accounting for 40% of the total ground water of the country initiate in Zagros Mountains and flow into the fertile plains. At about 10 million people reside in this region with 1.5 million living inside the forested areas extensively affecting the ecosystem. The forests are currently considered as

degraded forests with firewood production and livestock feeding recognized as the main causes. According to the statistics presented by the State Bureaus of Natural Resources there are 14.6 million stationary and nomadic livestock units feeding on Zagros forests every year. This has led too the gradual death of the wildlife due to the food supply restrictions, soil bed and its organic surface materials degradation and the subsequent shortage of food elements and soil erosion. Non-irrigated farming is also another major ecosystem degrading factor. The rain and snowfall in Zagros region stem from fronts passing from the Pacific Ocean and the Mediterranean Sea and occasionally from northern Europe towards the region. This results in a long dry summer. Based on De Martin dryness index there are four climates in the region namely humid, semi-humid, Mediterranean and semi-arid climates. It should be mentioned that the average annual temperature ranges between 9 °C to 25 °C depending on the latitude and altitude.

Vegetation

Formation of Zagros forests dates back to 5500 years ago resulting from increased precipitation and decreased temperature in the existing savannah. The most important tree and shrub species in Zagros region are: Persian oak (*Quercus brantii*), Aleppo oak (*Q.infectoria*), Lebanon oak (*Q.libani*), Montpellier maple (*Acer monspessulanum*), pistachio (*Pistacia mutica* and *P.khinjuk*), almond (*Amygdalus scoparia*), Caucasian nettle (*Celtis caucasica*), Daphne (*Daphne sp.*), juniper (*Juniperus polycarpos*) and pear (*Pyrus sp.*). It should be also noted that Quercetum persicum, and *Acer monspessulanum, Amygdalus scoparia* are the main vegetation communities in this region. Picture 3 shows a view of Oak forest in this zone.

Picture 3: A view of Oak Forests in Zagros zone

Zagros region accounts for the most area for the utilization of forest by-products. In fact, many areas could be found in which forest by-products have higher value than wood. Walnut and olive have gained considerable attention thanks to their value and previous high population. More investments are being made on pistachio, almond, wild cherry and wild pear. It should also be mentioned that 45% of the country is poplar plantations lay in Zagros area.

Irano-Touranian zone

More than one third of the entire area of the country is dominated by desert and Kavir, playing an important role in balancing the ecosystems of the country and even the region. For this reason, the forest site in this region with an area about 3300000ha is the vastest vegetation region of the country. This region includes the whole central Iranian plateau stretching. Due to the vast extent of this zone and climatic variation, it is divided into mountainous and plain forest types. In mountainous parts Juniper, Pistachio and Almond grow, while in plain parts saxsaul, tamarisk, bean caper and quail bush are dominant. The main forest utilization processes are firewood production, fruit picking and livestock grazing. The Touranian biosphere reserve, one of the nine Iranian biosphere reserves with an area of 1.8 million ha, is located in this region. So far 604 plant species are identified in this reserve among which 46 species are endemic.

Based on Emberge method, Irano-Touranian forest climates include four climates namely, cold dry, temperate dry, temperate desert and warm desert. Furthermore, according to the statistics obtained from the closest meteorological stations, the average annual rainfall is 130mm (between 84 to 260mm) with the maximum in the northern parts reaching up to 800mm in highlands.

Vegetation

These forests are often highly degraded and they are hardly ever considered as industrial forests. These forests are distributed from the altitude of 400m to 3500m above the sea level. In southern low lands, tropical temperature-susceptible species such as *Prosopis spicigera, Ziziphus spina-christi, Dalbergia sissoo, Calotropis procera* and *Calligonum* spp. are found. At higher altitudes the vegetation greatly changes and species like *Olea* spp., *Pistacia khinjuk* and *Amygdalus* spp. replace them. Between the altitudes of 1800m and 2500m trees and shrubs adapted to cold semi-arid regions such as almond (*Amygdalus lycioides*) Montpellier maple (*Acer monspessulanum*), pistachio (*Pistacia mutica*) and milkvetch (*Astragalus* spp.) are dominant. Above 2000 m, juniper dominates.

The *Haloxylon* sp. Vegetation, The *Tamarix articulate* association, The *Zygophyllum atriplicoides* association, Zone of the *Amygdalis reuteri, Berberis integerrima, Crataegus* spp., The association of the *Pistacia vera,* The zone of *Pistacia mutica, and Juniperus excelsa* zone are the main communities of this region.

Saharo-Sindian Region

In this Region there are representatives of Saharo-Arabian, Sudanian and also Irano-Touranian species. Rainfall is limited to the winter season and does not exceed 100mm per year in most of this Region. The summer is long and extremely hot and dry. The flora is very poor in species, and it has never been an important centre of speciation.

As well as Saharo-Arabian and Sudanian elements, some Irano-Turanian elements are seen at higher altitudes and in the northern part of this Region. The ecological reasons for a concentration of some sub-tropical elements in this part of Iran are the winter temperature, altitude and the rainfall of this area as compared with the central plateau. This region is generally known as the Khalijo-Omanian zone in Iran. Having an area of 2130000 ha, this region is stretched as a narrow band. The altitude ranges from sea level to 200 m. and the main vegetation elements in the region belong to sub-tropical elements and have Sahara-Sindian origin. This vegetation region is divided into two territories namely Khaliji and Omani due to the distinct ecological differences. Climatic differences also exist with higher temperatures in Omani coasts. In fact, the average temperature increases from the west to the east in this region. Mangrove forests are one of the most important vegetation communities in this region, gaining higher extension from the east to the west.

Three geological zones exist in this region are Khuzestan plain zone, Zagros folded area zone,. and The Eastern Iranian Zone-Makran Mountain ranges.

Annual precipitation increases from the east to the west of Khalijo-Omanian forest zone. The minimum temperature average increases from the east to the west, while the average minimum temperature decreases. The region has warm summers and humid winters. Based on the Gaussian method, the eastern parts have severe semi-desert, the middle areas in the vicinity of Booshehr have mild semi-desert and the western parts have severe semi-desert climates. The climatic characteristics of the coast band indicate that Chabahar climate is similar to sub-tropical climate with dominating desert characteristics toward the west.

Vegetation

Climatic changes along the southern coasts of the country have influenced the vegetation, so that the main species of the Khaliji territory are:

Arabian jujube (*Ziziphus spina-Christi*), Indian mesquite (*Prosopis spicigera*) and Euphrates poplar (*Popolus euphratica*) with Arabian jujube as the dominant species. In Omani territory the most important vegetation types are Indian mesquite and various kinds of acacia (*Acacia* spp.).

At higher altitudes, the population of these species decreases and other element such as almonds (*Amygdalus scoparia*) and (*Amygdalus lycioides*) and pistachios (*Pistacia mutica*) and (*Pistacia khinjuk*) replace them. The most important community of this region is Mangrove (picture 4).

Iranian mangrove forests are spread through the southern coasts, covering an area of 20000ha. The biggest continuous communities with the higher diversity lie in Qeshm Island and Bandar-e-Khamir covering an area of 10000ha including dense and semi-dense forests. Due to the destruction of other tree and shrub species as a result of harsh site conditions, *Avicenia marina* and *Rhizophora macronata* are the exclusive species in the Iranian mangrove forests. The mangrove ecosystem of Iran consists of unique fauna communities such as different crabs, shrimps, fishes, reptiles, birds and amphibians with Mudskipper (*Periophtalmus* sp.) as the most important one.

Due to the multi-purpose role of this ecosystem, the Iranian mangrove forests are under protection and attention of global environmental conventions namely wetlands, biodiversity and world heritage conventions so that their protection has been given priority over any other ecosystems. Furthermore, the Iranian mangrove forests are one of the nine biosphere reserves of the Man and Biosphere (M & B) program and are supervised by the Environment Department.

The optimal temperature for mangroves growth is 22 °C to 26 °C, 240 to 260 day a year.

Picture 4: A view of Mangrove Forests.

In the end it should be also added that UNESCO defines biosphere reserves as coastal or terrestrial regions internationally recognized to serve as a factor stabilizing and developing a balanced relationship between people and nature under the Man and Biosphere program. Nine region in Iran are recognized as biosphere reserves including; Ararsbaran, Arjan Plain, Mount Geno, Golestan Forest, Mangrove Forest, Kavir Plain, Lake Urmia, Lake Miankaleh and Touran Plain.

Plant species are classified into several categories based on the UN conservation priority. Endangered (EN) and Vulnerable (VU) are the most important categories among them.

Based on this list, the two species of yew (*Taxus baccata L.*) and boxwood (*Buxus hyrcana* Pojark) are the endangered species of Iran and the following species are on the vulnerable list:

Cornus sanguinea L., Pyrus kandevanica Ghahreman & Khatamsaz, *Pyrus mazenderanica* Schonbeck, *Pyrus turcomanica* Maleev, *Quercus robur* L., *Rhizophora mucronata* Poir., *Thuja orientalis* L.

Many other Iranian species are endangered or on the verge of extinction due to the site limitations, degradation intensity and other problems. Some of these species are as follows:

Acer platanoides, Betula pendula, Castanea sativa, Dalbergia sisso, Prunus avium, Prunus mahaleb, Quercus iberica, Quercus magnusquamata, Sorbus aucoparia, Sorbus torminalis, Ulmus glabra and Zelkova carpinifolia

For further information you are referred to the below mentioned books:

- Red data book of Iran : a preliminary survey of endemic, rare & endangered plant species in   Iran / by Adel Jalili & Ziba Jamzad ; with contributions from S.C. Shaw ... [et al.].

-Trees & Shrubs of Iran. By Habiboallah Sabeti.

- Forests of Iran. Khosrow Sagheb-Talebi.

"Investigation on possibility of MDF production from Reeds"

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Abstract

In this study, MDF was produced from Reed (*Phragmites australis*). Reeds are collected from Hor-Alazym. The treatments conditions for fibre preparing were as following:

- Steaming temperature 170 ºC, 180 ºC
- Steaming time 5, 10, 15 minutes
- The other production variables were constant

The anatomical properties of Reed such as fibre length, fibre diameter, lumen cell diameter , and cell wall thickness were measured. L/D ratio of Reed fibre was measured 73.69. The physical and mechanical properties of MDF were determined according to DIN standard. The measurements of bending properties and IB of MDF revealed that increasing steaming time and steaming temperature caused to decrease MOR, MOE and IB.

Also the results of MDF dimensional stabilities indicated that Thickness Swelling after 2 and 24 hours decreased upon increasing steaming time and steaming temperature. Degradation of hemicelluloses chains may be the reason of decreasing Thickness Swelling.

Key words: Medium Density Fibreboard, Fibre, MOR, IB, Thickness Swelling

Introduction

Medium density fibreboard belongs to the composite board families. MDF production is feasible almost using all of woody and lignocellulosic material. Mixing a several preliminary substances, a low price fibre and a homogeneous material is prepared, and at last a high quality production with excellent specifications is produced. MDF has numerous applications in machinery due to its good physical characteristics and favourable ability.

According to the unique features of this production, its production has been rapidly increased through the last years, so that only the MDF share of the composite board productions' market has been 6%

and about 7.5 million m^3, that its production has been increased to 19 million m^3 in 2001 [2]. Investigations also has shown that the MDF consumption of the country (Iran) has been increasingly grown from 1995 to 2001 and had about 400 times increase and the ascending manner would be continued so that the MDF consumption of the country is predicted to be 200,000 m^3 in 2006 [3].

It should be noted that since the wood of existing forests is not able to satisfy the needs of industry portion and if the new units are initiated this problem will be doubled. In the following years the main and the most significant of lignocellulosic sources of the country, in addition to the north forests of the country, would include utilizing populous planting, eucalyptus planting, and residues of agricultural plants also non-wood raw material.

Reed stem is one of lignocellulosic source. The primary studies were promising on the field of economic investigating of Hor-Alazym's Reeds in order to use in lignocellulosic industries [1]. The above Hor-Alazym's Reeds area is estimated to be 60 to 70 thousand hectares and the dry material per hectare is estimated to be 55 to 65 tons [1].The studies conducted by Familian et .al (1994) associated to Reeds anatomical properties of Hor-Alazym and Talab-e-Anzali areas are the witness of the utility in order to be used in fibreboard and paper industries. In this study fibbers dimension of Reeds were measured. The average fibre length, fibre diameter, lumen cell diameter, and cell wall thickness of fibre for Hor-Alazym and Talab-e-Anzali areas were 1.32 mm, 19.17, 3.91, 7.63 microns, 1.45 mm, 18.77, 3.37, 7.7 microns respectively. Atchison (1987) also reported in his work the average fibre length of some of non wood species including liquorices, corn stock, sun flower stock, wheat straw, barley chaff, and rice straw, as 1.1, 0.79, 1.14, 0.94, 1.1, and 0.95 mm respectively.

According to the preliminary investigations of reed stem, it seems necessary to research about using this material to produce medium density fibreboard. On the other hand since quality of fibre preparation has a significant influence on the medium density fibreboard properties, so this study aimed at the influence of fibre treatment conditions on the properties of the production. Some of the conducted researches have been addressed below.

Short et. al (1978) determined the physical and mechanical properties of fibreboards made out of (a) wet chips and (b) chips of up to 50% moisture. MDF was made out of the mixture of *Pinus teda* and hard woods. In the case (b) the boards of hard woods indicated better internal bond and the values of MOR, internal bond, water absorption, and thickness swelling in the boards of case (b) showed an increase with compared to boards of case (a), but the linear expansion and MOE decreased. Refining of under pressure chips of case (b) showed better fibre for hard wood and soft wood than case (a) and consequently the refining dried *Pinus teda* chips indicated an improved MDF board.

Roffael and Dix (1992) studied the properties of MDF made out of young poplar woods. These researchers noted hat fibreboards made out of 16-year poplar fibres had more mechanical strength and less thickness swelling than fibreboards made out of 5-years poplar woods with the same colon. Results show that it is possible to produce the medium density fibreboard with acceptable strength properties, out of poplar juvenile wood in the proper construction process condition.

Laboskey et. al (1993) studied the influence of various levels of steam pressure inside the double disk refiner (50, 60, 70, 80, 90, 100 psi) and the resin content of formaldehyde urea (6%, 8%, 10%, 12%) on the MOR, MOE, internal bond ,water absorption, and thickness swelling in medium density fibreboard resulted from *Acer rubrum* and concluded that increasing the steam pressure of refining didn't have a considerable influence on the MDF strength properties and dimensional stabilities of the board, while the resin content had a considerable influence on the all of the board properties, so that increasing resin content from 6 to 12% lead to 174 % increase in internal bond, 68 % increase in MOR and 40 % increase in MOE. They also concluded that there is another important factor, other than resin, that affects MDF properties and that is the moisture content of chips during the refining of fibres.

Okamoto et. al (1994) investigated the influence of high steam pressure on the mechanical and physical properties of MDF boards as well as steaming pressure treatment effects on the chemical composition of MDF. They concluded that the dimensional stabilities of MDF improve by increasing the steaming time as well as increasing steaming pressure and mechanical properties of MDF decrease. It has also been observed that the longer the steaming time and the greater the steaming pressure, the less amounts of hemi-cellulose and alpha cellulose will be, while lignin composition doesn't so vary. The best condition for under pressure steam injection in range of 60 – 90 sec is identified to be in steam pressure of 11 kg / cm^2 or 90 -180 sec in steam pressure of 6 kg / cm^2.

Cao et. al (1999) performed a research about fibres refining process and identifying the refiner segments pattern to make MDF. Toward this end three soft wood species, three refiner segments pattern were used in the forms of small-medium, medium, and coarse. The chips were steamed under fixed pressure of 0.4 – 0.65 MPa for 3, 6, 9, 12 minutes. The distance of refiner plates was adjusted about 0.1 mm. The results of the experiment showed that the value of medium-long fibre (remainder on the screen with mesh 32 - 115), was 50 – 70 %. The refiner plates of the medium type produced better fibres. Steaming time had not a significant effect on the fibres quality.

Habibi et. al (2002) in a research titled as the effect of Bagasse fibre properties on the medium density fibreboards (MDF) quality noted that the fibres strengths properties decrease and their water absorption improve by increasing steaming time and steaming temperature. They ascribed this problem to the destruction of cellulose and hemi-cellulose chains caused by hydrolyze reactions.

Kargarfard et. al (2003) made medium density fibreboard (MDF), using poplar wood in three steaming time of 15, 20 and 25 minutes and three press time of 4, 5 and 6 minutes and in two resin content consumption (9% and 11 %) .The results showed that the maximum bending properties and internal bond have been obtained in short steaming time (15 minutes).

Faraji (1998) made medium density fibreboard using Bagasse and in the conditions of steaming temperature of 170 and 180 °C and steaming time of 5, 10, and 15 minutes. The research results showed that the maximum bending properties and internal bond have been obtained in steaming temperature of 170 °C and steaming time of 5 minutes. He noted that the values of fibreboards' thickness swilling were decreased by increasing steaming temperature and steaming time.

Zahedi (2000) investigated the medium density fibreboard properties made out of the residue of roots of liquorices. He used three steaming time of 15, 20, and 25 minutes, three press time of 5, 6, and 7 minutes and two resin content of 10 and 12 % to produce medium density fibreboard. The boards prepared in the steaming time condition of 20 minutes, press time of 7 minutes, and resin content of 10 % had the maximum strength properties.

Rassam (2004) investigated the possibility of using wood and recycle fibre from paperboard in producing fibreboard by the wet method. In this survey the effect of four production process factors were analyzed, including the raw material, resin, press temperature, press time on the physical and mechanical properties. The test boards with raw material including the mixture of wood fibre and recycle fibre were produced in 5 levels, and two kind of resin phenol formaldehyde in the levels of 1 and 2 %, and lignin craft in the levels of 5 and 10 %, press temperature in the levels of 190 and 200 $^{\circ}$C, and press time of 8 and 10 minutes. Based on the obtained results, using recycle fibre had an undesirable influence on water absorption and thickness swelling after 2 and 24 hours of immersion, while resin content increasing, press temperature increasing and press time increasing had a desirable effect on the above properties. The strength properties of boards improved, once they were just made of recycle fibre, but using these fibre mixing with wood fibre leads to the reduction of the properties. Resin content increasing, temperature, and pressing time caused improvement of mechanical properties in all of the boards.

Xiaobo (2004) studied the physical, mechanical, and chemical properties of bamboo (*Phyllostachys pubescent*) and investigated its ability of use for fibreboard production. The Physical and mechanical properties of the boards improved by resin increase consumption. The aging effect of bamboo on properties of boards was also significant. The boards produced from 1-year bamboo had the greatest MOR and MOE compared to the boards produced in higher compaction ratio, and longer fibers. The boards produced from 5-years bamboo with resin content of 8% had the greatest internal bond.

Material and Methods

The required reeds were supplied from Hor-Alazym area and then transported to laboratory of Wood and Paper Science Researches. Since the dominant species of area was *Phragmites australis*, so the researches were performed on this species. Although the reed fibre dimensions have been previously measured by other researchers, this experiment was done to assure more certainty. Reed fibre preparation to measure them was performed by Franklin method (1938). Toward this end 300 fibre lengths, fibre diameters and lumen cell diameters and cell wall thicknesses were measured. The samples were supplied from different areas of Hor-Alazym and different heights of Reed stem.

Variable parameters: In this survey the two steaming temperature (170 and 180°C) and three steaming time of 5, 10, 15 minutes were used.

Constant parameters: In this survey the press temperature of 165 °C, board density of 0.7g/cm^3, resin content of 10% (based on dried fibre) were used constantly for all treatments.

Stages of Experimental Boards Construction

In order to prepare fibre, reeds were chipped into proper chips by a drum chipper of the type *Pallman* and treated with variable steaming temperatures and steaming times. Then reed chips were refined by an experimental refiner and their fibres were separated. After that drying process was done by a rotary dryer with an angular velocity of 3rpm. The final fibres moisture content before spraying resin was about 1%.

Spraying resin was performed horizontally and with angular velocity of 20rpm. To form the fibres mat, the wooden cast of the dimensions 30cm*32cm*25cm were applied. Fibre were weighted using the scale with the accuracy of 1g and were sprinkled within the cast uniformly. The height of fibres mat was smoothed and balanced in all directions. After spraying resin stage, two samples from fibres mat were prepared, to control the mat moisture content. After forming the fibres mat, the experimental press of the type Burkle-L100 was used for compressing and constructing boards.

The boards were put in the experimental condition for 2 weeks and then were cut based on DIN-68754 from test samples. Then the physical and mechanical properties of boards including MOE, MOR, internal bond and thickness swelling after 2 and 24 water immersions were determined. The results of this survey were analyzed using Factorial experiment in the form of a completely random design and comparing the average values using Duncan test.

Results

Anatomical properties: The average of reed fibre dimensions of Hor-Alazym area including fibre length, fibre diameter, lumen cell diameter, and cell wall thickness are presented in table 1. L/D ratio of reed fibre was calculated 73.69.

Table 1- Fibre dimensions of Reed in Hor-Alazime area

Fiber length (mm)	Fiber diameter (μ)	Lumen cell diameter (μ)	Cell wall thickness (μ)
1.28	17.37	6.61	5.38

Physical and Mechanical Properties of fibreboards: All of strength properties and stabilities dimension of boards in different treatments are summarized in table 2.

Table 2 - Mechanical and physical properties of MDF produced in various treatments

Steaming Temperature (°C)	Steaming Time (min)	MOR (MPa)	MOE (MPa)	IB (MPa)	Thickness Swelling after 2 hours (%)	Thickness Swelling after 24 hours (%)
170	5 10 15	13.45 11.01 10.89	1480 1220 1200	0.301 0.3 0.268	26.11 22.68 20.79	28.59 25.35 23.75
180	5 10 15	10.98 8.85 7.22	1201 1086 975	0.301 0.228 0.186	18.86 17.85 17.72	21.75 21.06 20.34

MOR

The results derived from variance analyses showed that the independent effect of steaming temperature and steaming time on the MOR is statistically significant at the level of 1%, and the interaction effect of these two variables on MOR is not statistically significant (CV=8.78%).Maximum MOR was observed at the temperature steaming of 170º C (fig 1).The results derived from the comparison of averages by Duncan test are presented in table 3 for different steaming times.
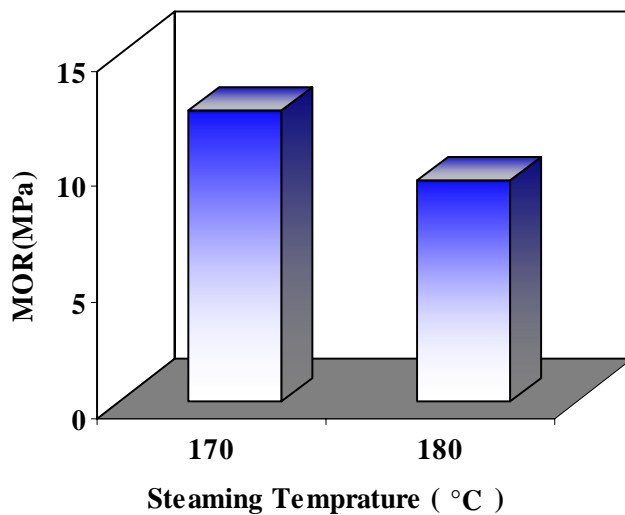
Fig 1: The effect of steaming temperature on MOR
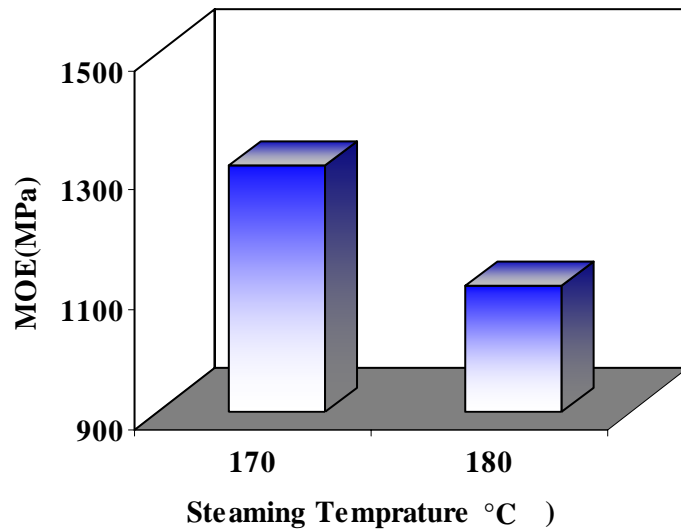
Fig 2: The effect of steaming temperature on MOE

Table 3 - Duncans grouping test of MOR based on different steaming time

Steaming time (min)	MOR (MPa)	Duncans grouping
5	12.21	A
10	9.93	B
15	9.05	B

MOE

The results derived from variance analyses showed that the independent effect of steaming temperature and steaming time on the MOE is statistically significant at the level of 1%, and the interaction effect of these two variables on MOE is not statistically significant (CV=5.13%).Maximum MOE was observed at the temperature steaming of 170º C (fig 2).The results derived from the comparison of averages by Duncan test are presented in table 4 for different steaming times.

Table 4 - Duncan's grouping test of MOE based on different steaming time

Steaming time (min)	MOE (MPa)	Duncans grouping

Internal bond

The results derived from variance analyses of the variable's effect on the internal bond showed that the independent effect of steaming temperature and steaming time on the internal bond is statistically significant at the level of 5%. The interaction effect of these two variables on internal bond is not statistically significant (CV = 18.49%). Fig 3 shows the effect of steaming temperature on the internal bond. The results derived from the comparison of averages by Duncan test are presented for different steaming times.
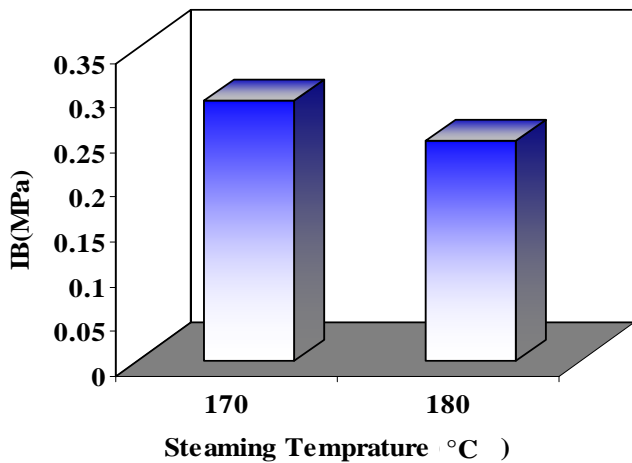
Fig 3: The effect of steaming temperature on IB

Table 5 - Duncans grouping test of IB based on different steaming time

Steaming time (min)	IB (MPa)	Duncans grouping
5	0.307	A
10	0.264	AB
15	0.227	B

Thickness swelling after 2 and 24 hours

The results derived from variance analyses of the variables' effect on the Thickness swelling after 2 and 24 hours showed that the independent effect of steaming temperature and steaming time on the above properties is statistically significant at the level of 5%. The coefficient of variance for thickness swelling after 2 and 24 hours are 7.65% and 7.10% respectively.

The interaction effect of the said variables on the above properties is not statistically significant. Figures 4 and 5 show the effect of steaming temperature on the thickness swelling after 2 and 24

hours, respectively. The results derived from the comparison of averages by the Duncan test on the said properties are presented in tables 6 and 7 for different steaming times.
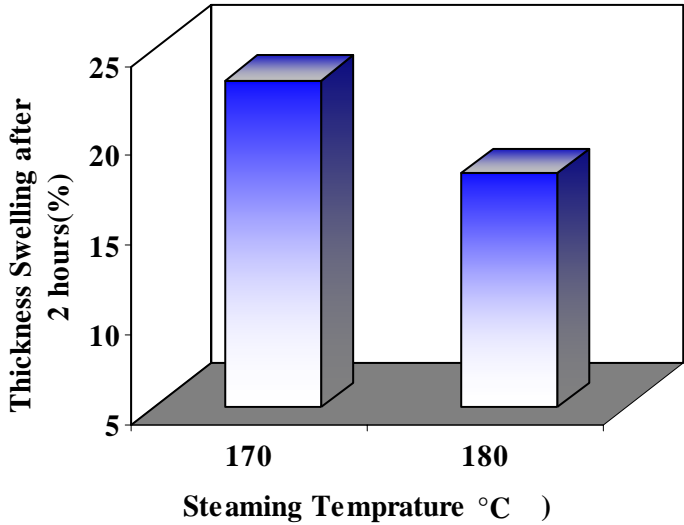
Fig 4: The effect of steaming temperature on thickness swelling after 2 hours
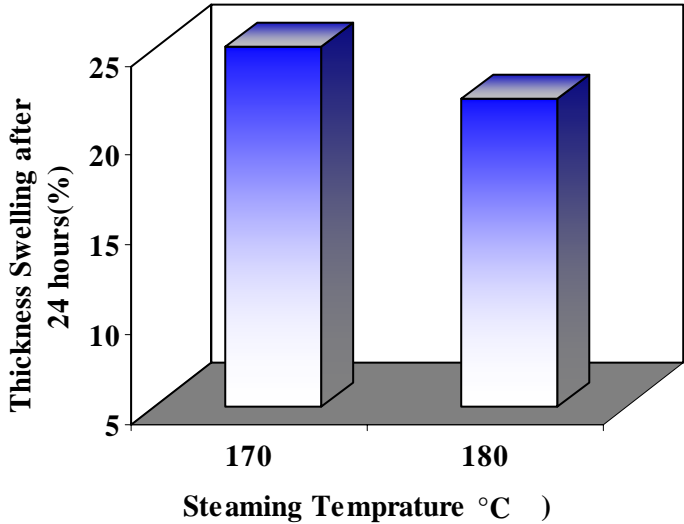
Fig 5: The effect of steaming temperature on thickness swelling after 24 hours

Table 6 - Duncan's grouping test of thickness swelling after 2 hours based on different steaming time

Steaming time (min)	Thickness swelling (%)	Duncans grouping
5	22.48	A
10	20.20	B
15	19.32	B

Table 7 - Duncan's grouping test of thickness swelling after 24 hours based on different steaming time

Steaming time (min)	Thickness swelling (%)	Duncans grouping
5	25.15	A
10	22.83	B
15	22.39	B

Discussion

Reed fibre length and diameter (*Phragmites australis*) of Hor-Alazym area were measured 1.28 mm and 17.37 microns respectively, that is similar to the Familian's researches [8]. Table 8 show the priority of reed fibre length than a number of nonwood species.

Table 8 - Fibers length some of nonwood species

Species	Licorice root	Cornstalk	Sunflower stalk	Wheat straw	Barley straw	Rice straw
Fiber length (mm)	1.1	0.79	1.14	0.94	1.1	0.95

The longer the fibre length, the greater the contact area within them will be, and the physical and mechanical properties of board will be improved. One of the main factors that contribute a considerable share in fibreboard quality is the length to diameter ratio. This L/D ratio varies from 20 to 150. The greater the ratio, the higher mechanical properties of the obtained board. The L/D ratio of the reed fibres was calculated as 73.69.

The results derived from investigating the effect of steaming temperature on the bending properties of the boards showed that increasing the steaming temperature decreases MOR and MOE, so that the maximum of these properties observed at the temperature of 170º C. The value of decrease of MOR and MOE caused by increasing the steaming temperature was 30.74%, and 19.59% respectively. It can be noted in this case that by increasing the steaming temperature, the properties of fibre strength decreases and at last the bending properties of the boards also decreases. The above results are similar to their results derived by the researches of Habibi, Kargarfard, Faraji, and Okamoto [10, 11, 9, and 13]. Increasing the steaming time also decreases the MOR and MOE of the boards. The maximum of these properties was observed in steaming time of 5 minutes.

Furthermore, the MOR and MOE of the boards resulted from the fibers derived in steaming time of 5 minutes were classified and separated from the steaming time of 10 and 15 minutes according to Duncan test (Tables 3 and 4). Increasing the steaming time from 5 to 15 minutes causes the decrease of MOR and MOE to 34.92%, and 23.28% respectively. Increasing the steaming time, the reed chips were under pressure and temperature for longer time and this caused the strength properties and the quality of the fibre to be decreased. The said results are similar to the works of Habibi, Kargarfard, Faraji, and Okamoto (4, 8, 9, and 16).

The internal bond is and indicator of connection within fibres. The results derived from the investigating of the influence of steaming time and temperature on the internal bond, showed that increasing both factors, decrease the internal bond by 23.53% and 35.24% respectively. Increasing the steaming temperature and time, decrease the strength properties of fibre by destructive reactions, and this reduction causes dropping of internal bond. Habibi, Kargarfard, and Faraji obtained the same results [10, 11, and 9]. Because the mechanical properties of composite materials are affected by the mechanical properties of the forming elements.

 Changing the dimensions of lingo-cellulosic productions caused by the absorption and desorption of water by the cell wall, particularly the productions with high density is considered as unfavourable properties. Productions such as fibreboard are swelled in the direction of thickness. The results derived from investigating the steaming temperature and steaming time on the thickness swelling after 2 and 24 hours showed that increasing both factors, have improved these two properties, so that the minimum value of each noted properties was observed in steaming temperature of 180º C and steaming time of 15 minutes. Increasing the steaming temperature and time, apparently causes the destruction of hemicellulose chains, and therefore by destructing OH agents, the water absorption of fibre and consequently the boards, decreases. The results of researches of Habibi, Faraji, and Okamoto also confirms this. [10, 9, 13]. Since the maximum of strength properties of medium density fibreboard, made out of Reed fibre obtained in the steaming temperature and time of 170º C and 5 minutes, so using the said condition is advised to produce the medium density fibreboard. It is necessary to note that using paraffin is advised to improve thickness swelling.

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"Nutrient relation in some damaged landscape trees on National Botanical Garden of Iran (NBGI)"

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Abstract

The NBGI was founded in 1968 in an area of about 150 hectares which was allocated to the garden at an altitude of 1320m. In this study the soil physical and chemical characteristic and the leaves analysis of *Acer negundo* and the *Uulmus glabra* was done to understand if the nutritive condition of soil is the cause of declining the number of trees in NBGI. Soil chemical and physical properties are displayed in tables 1 and 2. The soil texture is Sandy loam or Sandy, which is very poor in organic mater in both profiles near the damaged and healthy trees. The soils near the healthy trees tended to have highly levels of total soil N and P available especially in top soil (0-12 Cm). The concentration of N and P in damaged trees is lower compared with group 1, which have better appearance. Soil and foliar analysis showed that the low fertility of soil is a major problem for damaged trees in NBGI and the organic and inorganic fertilizer can increase the soil fertility and therefore trees vitality.

Introduction

The NBGI was founded in 1968 in an area of about 150 hectares which was allocated to the garden at an altitude of 1320m.

Climate is dry with an average annual precipitation of about 240mm falling between November and May. Temperature reaches as much as 42-43°C during July and August. During winter the temperature may fall to -10°C or lower. The NBGI is planned to be the main centre for horticulture and plant taxonomy in Iran.

In botanical garden around the cities the most conditions are similar to urban forests and it mitigated many problems of urban developments such as, air pollution, water deficiencies, nutrients deficiencies, soil compaction, and temperature extremes.

The amount of nutrients that soil can hold and the availability of these nutrients is greatly influenced by physical characteristic of the soil (Harris 1992), that is its texture, structure, CEC, and pH.

The relation between physical and chemical characteristics of soils and growth of trees were the subjects of numerous studies (Augosto et al 2002, Nys et al 2000, Frank et al 1998, Binkley and Giardina 1998, Binkley 1995). In this study the soil physical and chemical characteristic and the leaves analysis of some trees species was done to understand if the nutritive condition of soil is the cause of declining the number of trees in NBGI.

Material and Methods

The study was performed in the NBGI in an area of about 150 hectares with different trees and shrubs species. We have selected 10 different tree species and the result of two species, the *Acer negundo* and the *Uulmus glabra,* will be presented in this poster. The soil classification of the site is entisoil and poor in organic mater, with pH between 7.6-8.2 and EC < 5.1 ds/m and sandy texture.

Soil sampling and analysis

The following depths were sampled: 0-12, 12-30 and 30-90 Cm. Soil texture. SP, pH, EC. CEC, OM, %T.N.V, Total N and available P, K, Ca and Mg were determined. Soil samples were air-dried and ground to pass a 2-mm sieve. The available Ca and Mg were determined with complexometry method, K with ammonium acetate extraction and flame photometry and the total nitrogen was determined using the micro-kjeldahl method. Soil phosphor with Olson method. Soil Ph was determined using a glass electrode in a 2:1 soil: water mixture. % TNV using volumetric method (KCl 1%). Organic mater using Wallcey & Black method. For determination of cation exchange capacity (CEC), the soil was percolated with 1N acetate ammonium.

Foliar sampling and analysis

Leaves were harvested by tree climbers at the August 2004. They were taken from three approximately healthy trees (group 1) and seven damaged trees (group 2). The healthy trees showed fewer than 20% yellow leaves, not completely green and the damaged trees showed moderate damage of between 20 and 60% leaf loss. For each tree one composite sample was made, consisting of three sub samples from different branches of upper crown.

Foliage was oven dried to a constant weight at $65°C$, and then ground to pass s 1.mm sieve. The ground tissue was dry-ashed at $500°C$, dissolved in 6N HCl and analyzed for P, K, Ca, Mg, Fe and Mn. Total nitrogen was determined by using the micro-kjeldahl method.

Results and Discussion

Soil properties

Soil chemical and physical properties are displayed in tables 1 and 2. The soil texture is Sandy loam or Sandy, which is very poor in organic mater in both profiles near the damaged and healthy trees. The soil organic mater near the healthy trees is slightly higher than the soil around the damaged trees. The soils near the healthy trees tended to have highly levels of total soil N and P available especially in top soil (0-12 Cm). The pH is height in both stands (between 7.76-7.96).

Table 1- Soil properties in different depths of healthy trees

Dept (Cm)	Clay (%)	Silt (%)	Sand (%)	Tex	O.C (%)	CaCO$_3$ (%)	SP (%)
0-12	1.5	34	64.5	S.L	2.24	1.88	44.02
12-30	3.5	26	70.5	S.L	0.37	1.96	13.05
30-90	3.5	4	92.5	S	0.23	1.96	27.74

Dept Cm)(	EC (ds/m)	pH	N %	Extractable (mg/kg)				CEC (Meq/100gr)
				K	P	Ca	Mg	
0-12	0.65	7.85	0.23	499.2	44.2	980	42	29.74
12-30	0.66	7.76	0.04	327.2	22.2	868	42	19.36
30-90	1.25	7.93	0.02	135.5	1.6	798	67.2	17.29

Table 2- Soil properties in different depths of damaged trees

Dept cm	Clay %	Silt %	Sand %	Tex	O.C %	CaCO$_3$ %	SP %
0-12	7.5	18	74.5	S.L	0.88	1.52	37.59
12-30	9.5	20	70.5	S.L	0.24	2.05	25.43
30-90	7.5	20	73.5	S.L	0.25	1.88	21.09

Dept (Cm)	EC (ds/m)	pH	N %	Extractable (mg/kg)				CEC (Meq/100gr)
				K	P	Ca	Mg	
0-12	0.79	7.96	0.09	347.1	12.6	798	109.2	4.83
12-30	0.99	7.84	0.02	314	3.2	980	25.2	21.44
30-90	0.80	7.85	0.03	406.6	1.6	10.22	42	21.44

Foliar nutrient levels

Foliar nutrient levels of *Acer negundo* and *Ulmus glabra* are presented in tables 3 and 4. In *Acer negundo* trees, foliar N, P, K and Ca ranged from 1.61 to 1.97, 0.16 to 0.29, 2.13 to 1.64 and 3.32 to 3.96 percent in group 2 and 1 respectively. N, P and Ca foliar levels were higher in group 1 whereas K foliar level is higher in group 2.

In *Ulmus glabra* trees foliar N, P, K and Ca ranged from 1.84 to 2.02, 0.14 to 0.16, 1.73 to 2.43 and 2.16 to 1.83 percent in group 2 and 1 respectively. N, P and K foliar levels were higher in group 1 whereas Ca foliar level is higher in group 2.

The result of soil analyses showed that the soil texture in the NBGI is Sandy soil in which the sand percentage ranged from 65% to 92.5%, therefore the soil capacity for absorption of nutrient elements is very low. The low levels of CEC that ranged between 4.83 and 29.74 referred to the insufficient soil fertility. Comparisons with literature data show that the level of N and P at different layers of soil is very low especially in soil near the damaged trees (Marx et al, 1999).

Table 3- Foliar nutrient levels of two sampling trees groups in *Acer negundo* trees

		N (%)	P (%)	K (%)	Ca (%)	Fe (mg/g)	Zn (mg/g)	Mn (mg/g)
group 1	Mean	1.97	0.29	1.64	3.96	0.06	0.17	0.11
	(std)	0.23	0.21	0.44	1.02	0.05	0.018	0.017
group 2	Mean	1.61	0.16	2.13	3.32	0.13	0.14	0.09
	(std)	0.33	0.03	0.65	0.83	0.13	0.03	0.03
total	Mean	1.72	0.21	1.99	3.52	0.11	0.15	0.10
	(std)	0.34	0.12	0.62	0.89	0.11	0.031	0.03

Table 4 - Foliar nutrient levels of two sampling trees groups in *Ulmus glabra* trees

		N (%)	P (%)	K (%)	Ca (%)	Fe (mg/g)	Zn (mg/g)	Mn (mg/g)
group 1	Mean	2.02	0.16	2.43	1.85	0.02	0.10	0.07
	(std)	0.13	0.021	0.48	0.26	0.015	0.028	0.007
group 2	Mean	1.84	0.14	1.73	2.16	0.023	0.11	0.092
	(std)	0.30	0.03	0.69	0.58	0.01	0.017	0.009
total	Mean	1.90	0.14	1.94	2.06	0.021	0.11	0.086
	(std)	0.27	0.03	0.69	0.49	0.011	0.02	0.012

The foliar levels of nutrient elements showed that:

- The concentration of N and P in damaged trees is lower compared with group 1, which have better appearance.

- The species of those trees haven't got the same trend of concentration in nutritive elements. The concentration of Ca in *Acer negundo* is approximately twice compared with *Ulmus glabra*.

Soil and foliar analysis showed that the low fertility of soil is a major problem for damaged trees in NBGI and the organic and inorganic fertilizer can increase the soil fertility and therefore trees vitality.

Fig. 1. Image of damaged *Acer negundo tree*

Fig. 2. Image of damaged *Uulmus glabra tree*

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"Application of geostatistics for estimation of forest growing stock in the Caspian region of Iran"

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Abstract

This study investigates application of geostatistics for estimating forest growing stock in the broad-leaved Caspian forests of Iran.

Field sampling was performed based on a 150m by 200m systematic rectangular grid of 5 clustered plots (50m away). Each sample plot consisted of two concentric circles. Overall, 720 sample plots were measured in 500 hectares. Experimental variogram for forest growing stock were calculated and plotted using the geo- referenced inventory plots. The variogram showed a large nugget effect, implying weak spatial auto- correlations between samples, even in short distances. Estimations were made using ordinary block kriging and spherical variogram models. Cross- validation results showed that all the estimations are with low precision, because of the large nugget effects and weak spatial structures in the experimental variogram. Therefore, kriging could not make accurate estimation due to high spatial variability of forest growing stock in this heterogeneous and uneven-aged forest.

Keywords: Forest growing stock, Geostatistics, Kriging, Spatial variability, Variogram.

Introduction

Estimation and mapping of forest resources is an inescapable premise of management, planning and research. Time and cost constraints do not usually allow exhaustive measurements; hence, sampling schemes need to be designed and implemented to estimate population values (Husch *et al*. 1982).

Accurate knowledge of spatial structures is needed to inform silvicultural guidelines and management decisions for long term sustainability of forests. Furthermore, geostatistics is a useful tool to describe and map of spatial variability as well as estimation of forest variables.

Geostatistics was developed to study variables that are distributed continuously in space, called "regionalized variables"(Isaak & Srivastava, 1989; Goovaerts, 1997). It provides a natural framework for estimation techniques in forest inventory sampling (Mandallaz, 1991).

The first contributions to forest inventory were due to Guibal (1973) who applied geostatistics for estimation of forest stock in a tropical uneven aged forest in Gabon. Jost (1993) compared, under systematic sampling, the classical error estimate with their geostatistical counterparts in the forests of Germany. Biondi *et al.* (1994) found that basal area could be measured as a regionalized variable in U.S. old- growth forest. Gunnarson *et al.* (1998) showed that hardwood volume in old stands is an example of a variable that has no or little useful spatial auto-correlation in Swedish forest. Tuominen *et al.* (2003) found that geostatistical interpolation in the boreal forests of Finland did not result in any further improvement in the accuracy of the estimates. Montes *et al.* (2005) used ordinary kriging for estimation of cork oak production in Spain.

The aim of this study is to investigate the spatial variability of the forest growing stock, and using of kriging (geostatistical approach) for forest growing stock estimation.

Material and Methods

Study area

Data used in this study were collected from a part of the 8000 hectare educational and research forest station of Tehran University, located in the Caspian forests in northern Iran. Geographical coordinates for the approximate centre of the area are 51˚ 35' E longitude and 36˚ 34' N latitude (Figure 1). Elevation varies from 700m to 1200m above sea level and slope from 5% to 65%. The forest is typical of mixed hardwood stands of Caspian forests, currently under active management by selective cutting regime since 30 years ago.

The inventoried forest area (500 ha) is composed by a mix of broad- leaved deciduous tree species. The species composition and structure of the forest have been influencing by human intervention and management activities such as animal husbandry and harvesting. Dominant species are beech (*Fagus orientalis* Lipsky) and Hornbeam (*Carpinus betulus* L.) alongside Maple (*Acer velutinum* Boiss.), Alder (*Alnus subcordata* C.A.M.) and Oak (*Qercus castaneifolia* C.A.M.).

The average annual increment of the forest is about 8m^3/ha with mean growing stock of 460m^3/ha. Mean annual temperature, precipitation and relative humidity are 12.23˚C, 1450 mm and 83%, respectively. Climate is cold and wet in winter and temperate in summer without dry season. The site is naturally seeded, old-growth and uneven-aged. It is so heterogeneous in nature with large topographic variations.
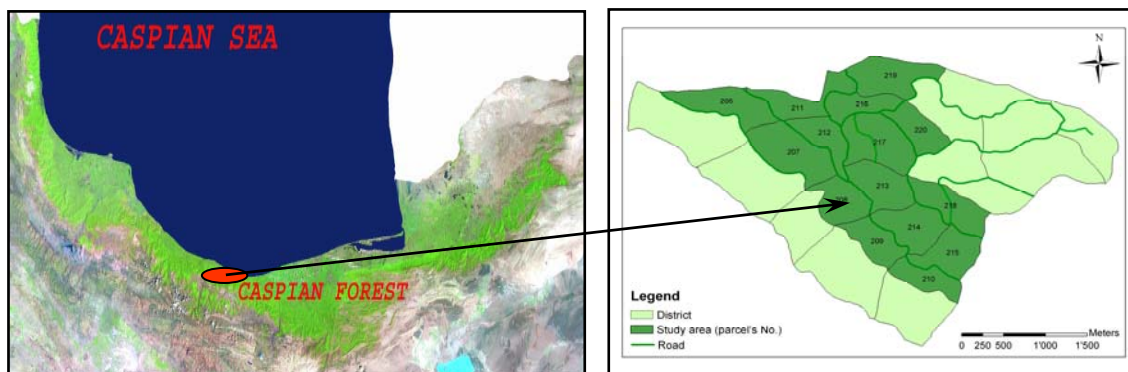
Fig. 1. Study area

Methods

Design-based approach

Systematic sampling was employed using a design of 5 plot clusters for the whole area: from the central plot, 4 plots were taken 50m away in the W-E and N-S directions. The central cluster plots were on a 150m (W-S) by 200m (N-S) systematic rectangular grid (Figure 2).

Each plot in a cluster consisted of two concentric circular samples with surface areas of 300m^2 (9.77m radius) and 700m^2 (14.93m radius). Diameter at breast height (DBH in 1.3m), species, distance and azimuth from centre, as well as other qualitative variables were recorded on each tree in the plot whose DBH exceeded of 7.5cm till 37.5cm at the small plot and greater than 37.5cm at the bigger one. In total, 720 sample plots were measured. The coordinates of each sample plot were determined using global positioning system (GPS) equipment with differential correction. Using concentric circles approximates a Probability Proportional to Size (PPS) inclusion rule and is therefore more efficient for forest growing stock estimation.
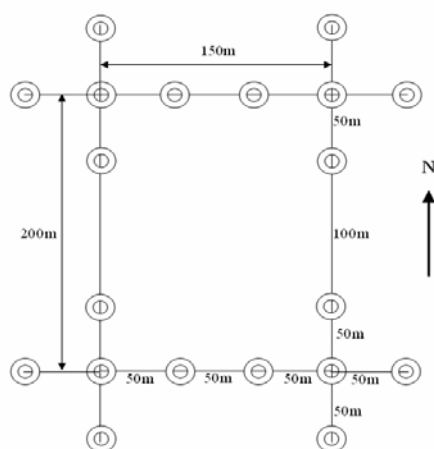
Fig. 2. Arrangement of sample plots in the study area

Geostatistical approach

The basic principal of geostatistics is that correlation between values of a regionalized variable will decrease as distance between the sample points increases. The semi-variogram or simply variogram indicates the degree of similarity among the values of a regionalized variable when they are located in given separation distance (lag) as well as direction away from each other. The spatial structure is analyzed by means of experimental variogram and it is calculated by the formula:

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} \left[z(x_i) - z(x_i + h) \right]^2 \qquad (1)$$

Where $\hat{\gamma}(h)$ is the semi-variance estimator for N data pairs, separated by a particular lag vector of h. $z(x_i)$ and $z(x_i + h)$ are the values of regionalized variable z at locations of i and $i + h$.

The parameters of the theoretical variogram can be estimated by fitting a model to the experimental variogram. When spatial dependence is present, the modelled variogram will generally increase with distance until a constant value called the *sill*. The distance at which the sill reached is referred to as the *range*. Theoretically, the variogram should pass through the zero variance. However, in practice, there is often a nonzero variance known as the *nugget effect*, which represents the random component of the spatial structure. The nugget effect can also caused by spatial variability at distances shorter than the smallest sampling interval as well as by measurement errors. In the current study, the models considered in fitting the variogram were spherical, exponential and Gaussian (Cressie, 1993), and fitted to the experimental variogram using the weighted least squares (WLS) method. The spherical model, that is the most commonly used to describe environmental data variability, showed a good fit with experimental variogram. It is defined as:

$$\gamma(h) = c_0 + c \left\{ \frac{3h}{2a} - \frac{1}{2} \left(\frac{h}{a} \right)^3 \right\} \qquad \text{for } 0 < h \leq a$$

$$\gamma(h) = c_0 + c \qquad \text{for } h > a \qquad (2)$$

Where c_0, c and a represent nugget variance, structural variance and range, respectively.

Prediction or estimation is the task for which, geostatistics was initially developed and it is generally called Kriging after D.G. Krige (1951). Kriging is a procedure for estimating regionalized variables at un-sampled locations, based on initial data value. However, ordinary kriging, the workhorse of geostatistics, is the most common type of kriging in practice, particularly in environmental sciences (Webster & Oliver, 2000). It is given by:

$$\hat{z}(x) = \sum_{i=1}^{n} \lambda_i z(x_i) \qquad\qquad (3)$$

Where, λ_i is the weight associated with each sample location value.

The estimator may be used for estimation at a single point (point kriging) or over an area (block kriging).

In this study for estimation, ordinary block kriging without trend were used, where as the mean is assumed stationary and unknown as well as no large-scale trend was observed. A 25m× 25m grid was used to discretize the area to estimate average values over the blocks. The size of the grid was chosen to be approximately the same as that of the sample plots to emphasize the local variation around the sampling plots.

The estimations were done on the nearest 16 data plots, within the maximum search radius (400m), which corresponded to the scale of auto-correlation.

To evaluate the results of kriging usually, a Jack-knife cross-validation approach is used. All the samples are excluded one by one from the data set and estimated again by kriging, using the remaining samples. Then measured and estimated values are compared to evaluate the kriging results (Webster & Oliver, 2000).

In this study, the accuracy of kriging is measured using Root Mean Squared Error (RMSE):

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left[z(x_i) - \hat{z}(x_i) \right]^2} \qquad\qquad (5)$$

and cross-validation is evaluated by calculation of Mean Error (ME) which should ideally be equal to zero, because kriging is unbiased (Webster and Oliver, 2000):

$$ME = \frac{1}{N} \sum_{i=1}^{N} \left[z(x_i) - \hat{z}(x_i) \right] \qquad\qquad (6)$$

The software package used for geostatistical analysis were Gs$^+$ version 5.3b (Gamma Design software) and ISATIS, 2002.

Results

Descriptive statistics of the forest growing stock (Table 1) shows that data's coefficient of variation and therefore, variability is rather high with low intra-cluster correlation coefficient, ρ, among sample plots.

Table 1. Summary statistics of sampled plots.

Variable	Samples No.	Mean	Min	Max	SD	CV (%)	ρ
Growing Stock (m^3/ha)	720	460.7	6.5	1153	199.7	43.4	0.08

SD, Standard deviation CV, Coefficient of variation

ρ, Intra-Cluster correlation coefficient (optimal value: ±1)

In this study, variogram was used as a measure of spatial dependence between two points. After several lag distances have been tested, the experimental variogram was calculated for lag distance of 100m.Variogram anisotropy, as investigated through the experimental variogram surface, was not found; consequently, only omni- directional variogram was modelled using the spherical model, to which a nugget effect was added. Model parameters of the variogram is indicated in Table 2. Experimental variogram plot and fitted model are shown in Figure 3.

Table 2. Parameters of the model fitted to experimental isotropic variogram

Model	Lag distance (m)	Nugget Effect (m^2/ha)^2	Sill (m^2/ha)^2	Range (m)	SP (%)
Spherical	100	33900	38750	430m	12.5

SP, Structured part, given by the ratio: (Sill- Nugget effect / Sill) ×100
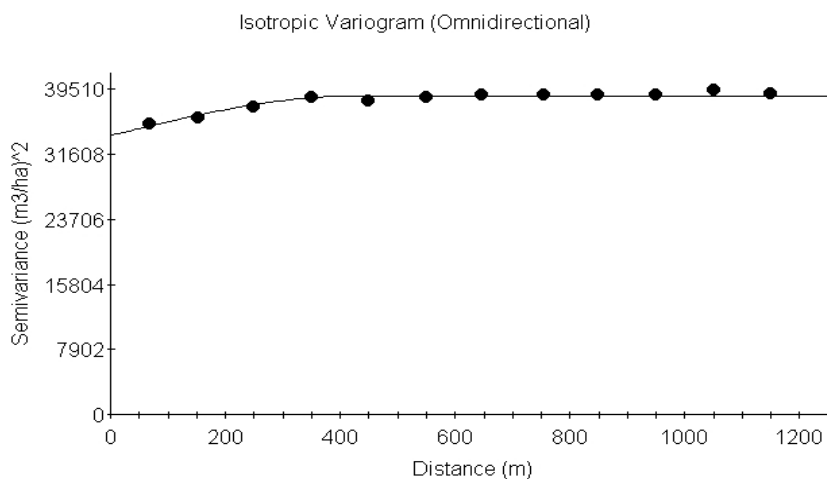
Fig. 3. Isotropic variogram (omni-directional) with the fitted spherical model using the WLS method (Filled circles represent experimental variogram and solid line represents fitted model).

Ordinary block kriging was applied to produce continuous map for the forest growing stock over the study area. The results are shown as kriged map and error map in Figure 4. Kriging results show that the estimated values have a much smaller variance than the measured data because of the smoothing effect of kriging. However, the estimated mean is close to the measured data mean (Tables 1 and 3).

Fig. 4. Kriged map (a) and error map (SD) of estimation (b)
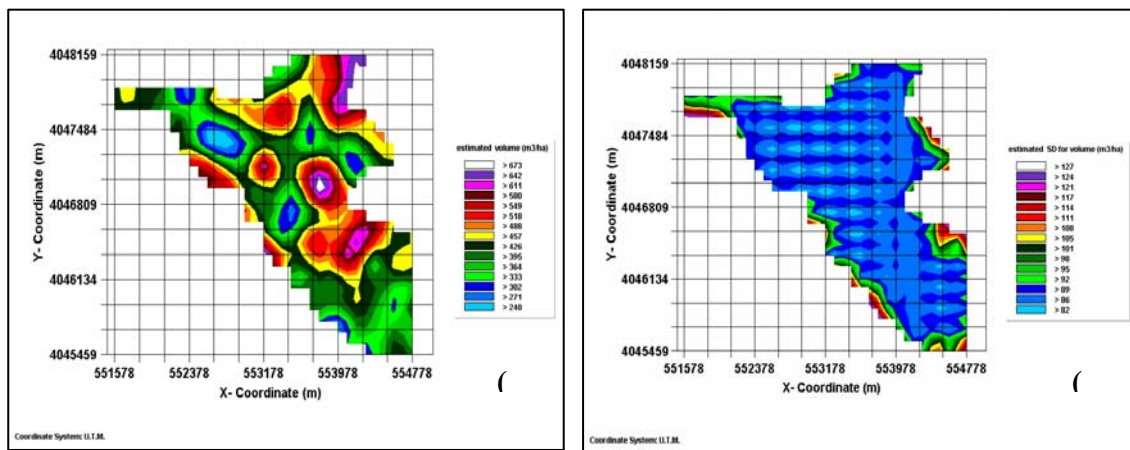
Table 3. Results of kriging and cross-validation

	Mean	Min	Max	SD	CV (%)	RMSE	ME
Estimated growing stock (m³/ha)	457.2	294.1	706.4	73.1	16	194.24	3.1

RMSE, Root Mean Squared Error   ME, Mean Error

The measured data are plotted versus the estimated values in Figure 5. A bias can be observed, which agrees with the high nugget effect in the spherical models (Table 2) and low intra-cluster correlation coefficient, ρ as well (Table 1). The RMSE (194.24) appears to be large and ME (3.1) is far from zero, indicating that kriging did not produce an accurate estimation for each point.
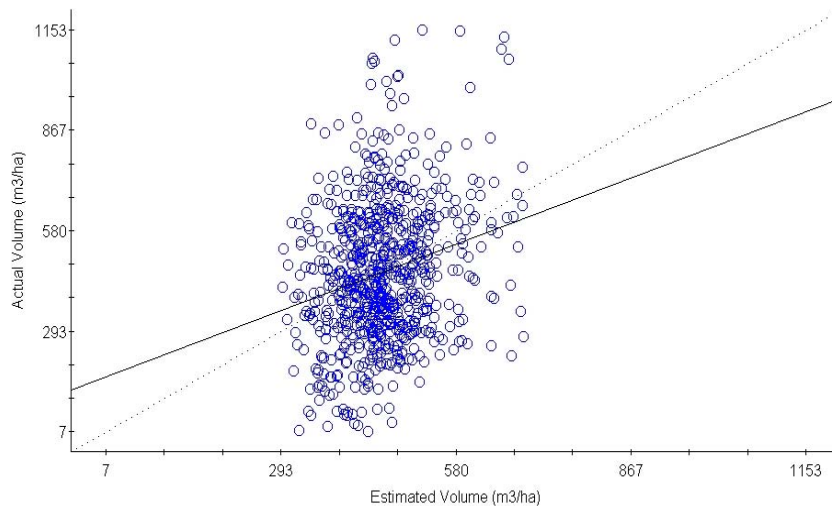
Fig. 5. Comparison between measured and estimated standing volume, using cross-validation

Discussion

In this research, forest growing stock showed weak spatial structure with large nugget component, which indicates large variability in short distances. It means that, the hardwood trees' stock has scattered randomly over the area without any significant continuity, because of two main reasons:

Firstly, human intervention, such as selective cutting operation, road making and animal husbandry, and secondly, natural causes such as wind throw and outbreaks of insects and diseases. Furthermore, physiographic and topographic variations are so big. In fact, the area is quite heterogeneous in nature. These factors produce abrupt spatial variations. Kriging result showed that estimated variance is much lower than data variance. However, the estimated mean was close to the data mean. Cross-validation results showed that all the estimations are scattered vertically around the data mean and it produce low precision in estimation. Therefore, kriging is not a suitable alternative for estimating forest growing stock in the study area, because forest growing stock does not behave like a regionalized variable. This result confirms the results of Gunnarson *et al*. (1998) and Tuominen *et al*. (2003) as they said:

Weighting procedures based on spatial auto-correlation do not generally perform very well when (growing stock related) variables are estimated in managed forests. The main reason is that human interventions produce abrupt changes in the forest, whereas geostatistical methods are best suited for data, in which the value of the measured attribute changes slowly in stages.

Therefore, it is proposed to apply geostatistical approach in un-managed natural forests, such as forest reserves (lack of abrupt changes), to obtain insight of natural processes which is necessary for naturalistic forestry.

However, there is an ongoing similar research in a forest plantation in Iran by *Maple*, and the initial result is optimistic.

Acknowledgements

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"Properties of Populus deltoides. Delignification and paper making"

Col 77.51 in three rotation period

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Abstract

Three, four, six and 12 years old _Populus deltoides_ trees were randomly selected and felled from the Safrabasteh Research Station located in Gilan province.

Samples were transferred to the Wood chemistry and paper making of research Institute of forest and rangelands.

By using of Kraft processing, percentage of sulphidity rate of 25%, and the temperature of 165 °C and addition to modification of effective alkali up to 14%, 17%, 20% and cooking time at maximum temperature of 60 min. , 120 min , 180 min , with a rate of liquid to lignocelluloses material equivalent at 5/1 (L/W).

Averages of yield and kappa number of pulps were measured between 43.83%-52.20% and 14.85%-30.48% respectively.

Pulps were refined up to the freeness degree of 350 c.s.f. the mechanical properties of 60 grams hand-made papers include breaking length, burst index, tear index and folding were also measured.

Key word: _Populus deltoides_, Kraft process, yield and kappa number, burst index, tear index.

Introduction

Paper and paper products have had a very important role in human Society, Economic, Progress and job opportunity. Population growth and technology progress demand a great deal of paper increasing, while the destructive factors and irregular wood harvesting have decreased the forest level. In order to compensate the shortage of lignocelluloses raw material needed for wood and paper Industrial, planting and training of fast growing trees which can produce high yield of wood in the shortest time, have been considered by specialists.

Fast growing poplar species have suitable technical quality and some of them have agricultural flexibility such as selection, fecundation and increasing wood production.

Today, progress in genetic science, producing these trees with possibility of using in industry have increased and it is necessary to study the properties of these of this source as a raw material for paper making industry.

In following table, the average consumption of paper in the world, Iran, America and Japan has been showed.

Table 1 Average consumption paper in the world, Iran, America and Japan

Average Consumption (kg)	Name of Country
16.4	Iran
52.6	World
239.4	Japan
347.2	America

The advantages of poplar wood in paper making

 - Poplar is planted in many parts of our country in traditional and industrial case.

 - Poplar generally have soft, light weight, light colour wood and to change them to pulp. They need less energy (chemical and mechanical) in comparison with hard wood.

- Poplar grows very fast and they take short time to achieve the ability in pulping industry. In general, the main reason for global using of Poplar in paper industry is fast growing and its application in agro-forest

Survey

In general, the main reason for global using of Poplar is fast growing and its application in agro-forest. The first application of Poplar soda pulp began about 100 years ago. An American company produced poplar soda pulp with about 18-24 t/d Capacity in Manayunk Producing pulp from Poplar has been continuing yet and due to its fast growing and technological characteristics and having more than 50% cellulose , 30% hemicelluloses and less than 20% lignin , the use of Poplar have been developing.(Macleod 1988). In 1988 in Canada 500.000t Kraft pulp from Poplar produced.

Rahmany and Hemmaty (1998) , On investigating the most suitable harvesting period project for _Populus deltoids_ col. 77.51 stated , the 4 years old _Populus deltoids_ col. 77.51 had the highest yield of wood production compare to 6 and 12 years trees.

Materials and Methods

Sample was prepared from the most suitable period operation of Populus deltoids col. 77.51 from research Safrabasteh station in Gilan province. The condition of Kraft cooking of 4, 6 and 12 years old Populus deltoides are as follow

Effective alkali	14%, 17% and 20% (base on Na2o)
Cooking time	1, 2 and 3 hours
Cooking temperature	160°C
Age of trees	4, 6 and 12 years old
Sulphidity	25% (Na2o base)
L/W	5/1

- In order to produce 60 grammage papers, pulps were refined at 350 M.L. (CSF)

- The pulp condition to make hand sheet is as follow

Effective alkali	14 % and 20 % (Na_2O base)
Cooking time	1 hour
Cooking temperature	160 °C
Age of tree	4, 6 and 12 years old
Sulphidity	25 % (Na_2O base)
L/W	

- The applied standard methods were as bellow

Kappa number	according to T 236 cm-85 TAPPI test method
Freeness	according to T 227 om-92 TAPPI test method
Refining	according to T 205 om-85 TAPPI test method
Tear strength	according to T 414 om-88 TAPPI test method
Burst strength	according to T 403 om-91 TAPPI test method
Folding	according to T 403 om-81 TAPPI test method
Breaking length	according to T 320 om-88 TAPPI test method
Preparing wood for analyzing	according to T 257 cm-85 TAPPI test method
Chemical analysis	according to T 264 om-88 TAPPI test method
Ash	according to T 211om-85 TAPPI test method
Lignin	according to T 222om-88 TAPPI test method
Extractives	according to T 204om-88 TAPPI test method

- The result was analyzed using factorial randomized block complete design method

Results and discussion

Chemical composition

chemical composition of Populus deltoides wood
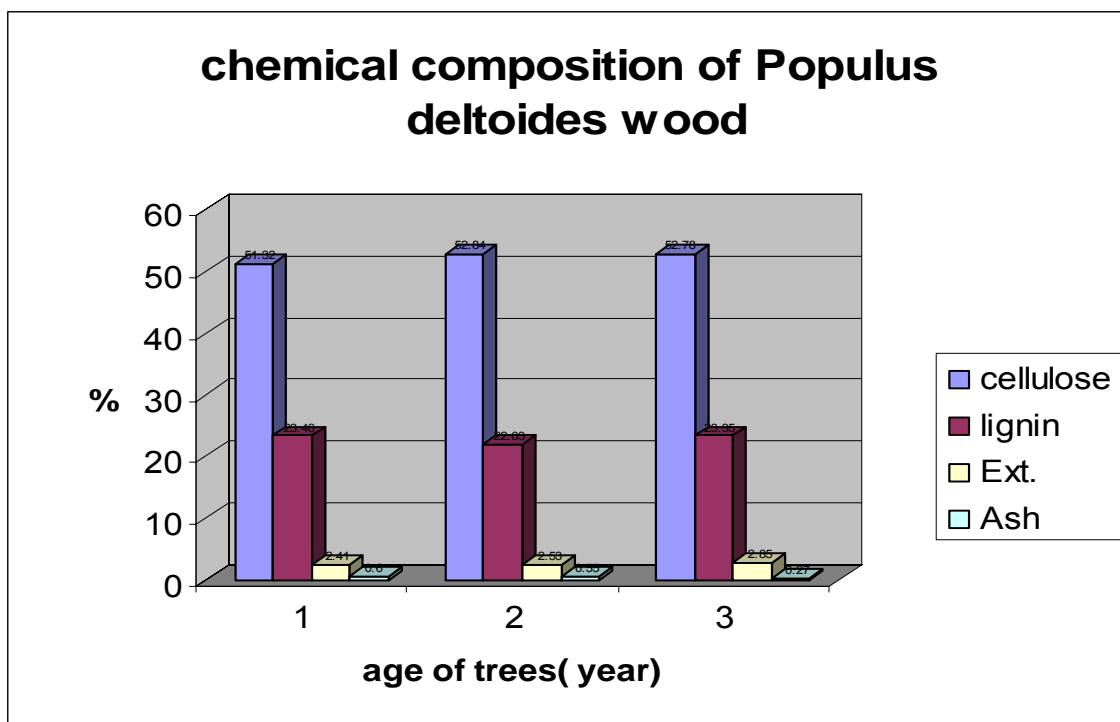
Table 2 Average of chemical composition of *Populus deltoids*

Ash (%)	Extractives (%)	Lignin (%)	Cellulose (%)	Age of trees (a)
0.6	2.41	23.46	51.32	4
0.55	2.53	22.03	52.84	6
0.27	2.85	23.35	52.78	12

Specific gravity of Populus deltoides
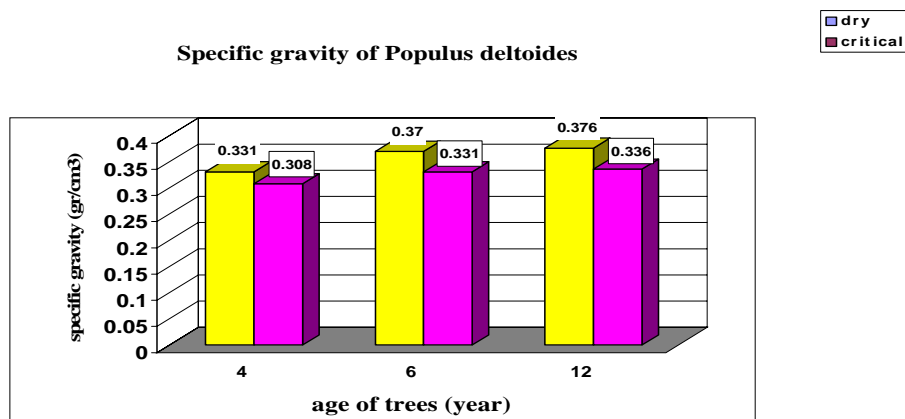
Table 3 Average of Critical specific gravity (g/cm)of *Populus deltoides*

Critical Specific Gravity (g/cm^3)	Dry Specific Gravity (g/cm^3)	Age of Trees (a)
0.308	0.331	4
0.331	0.370	6
0.336	0.376	12

Table 4 Average of fibre dimension of *Populus deltoides*

Cell Wall Thickness (µm)	Fibre Diameter (µm)	Fibre Lumen (µm)	Fibre Length (mm)	Age of Trees (a)
3.993	28.657	20.593	1.025	4
4.922	29.409	19.738	1.177	6
6.245	34.461	22.028	1.299	12

Table 5 Yield and kappa number of 4 years *Populus deltoides* wood

Kappa Number			Yield (%)			EA %	Cooking time (h)	*Age of Trees (a)*
Average	Replication		Average	Replication				
	2	1		2	1			
29.81	28.30	31.33	50.36	50.93	49.80	14	1	4
21.49	21.05	22.83	48.41	47.71	49.11	17		
18.37	18.12	18.63	47.76	47.42	48.11	20		
23.26	22.34	24.18	48.80	48.67	48.94	14	2	
18.22	18.03	18.42	46.51	46.59	46. 43	17		
16.71	16.98	16.45	45.92	46.57	45.27	20		
20.54	20.57	20.23	47.33	47.20	47.47	14	3	
17.89	18.75	17.03	46.31	46.43	46.20	17		
14.85	15.27	14.43	43.83	44.35	43.31	20		

Table 6 Yield and kappa number of 6 years *Populus deltoides* wood

Kappa Number			Yield (%)			EA%	Cooking time (h)	*Age of Trees (a)*
Average	Replication		Average	Replication				
	2	1		2	1			
28.33	27.56	29.10	49.98	49.21	50.76	14	1	6
20.47	20.93	20.02	47.96	48.07	47.85	17		
18.24	18.82	17.66	46.34	46.45	46.23	20		
18.75	18.83	18.68	49.16	48.85	49.48	14	2	
16.68	17.23	16.13	46.99	47.52	46.46	17		
15.34	14.92	15.76	45.76	45.51	46.02	20		
21.86	22.30	21.43	49	49.48	48.52	14	3	
18.16	18.04	18.28	45.73	46.02	45.44	17		
15.76	15.95	15.57	43.84	43.82	43.87	20		

Table 7 Yield and kappa number of 12 years *Populus deltoides* wood

Kappa Number			Yield %			EA%	Cooking Time (h)	*Age of Trees (a)*
Average	Replication		Average	Replication				
	2	1		2	1			
30.48	31.54	29.42	52.20	52.88	51.53	14		
19.58	20.53	18.64	50.43	51.01	49.86	17	1	
16.65	16.50	16.80	49.38	49.58	49.18	20		
19.13	21.03	17.24	50.16	51.14	49.19	14		
16.26	16.55	15.97	48.57	49.07	48.08	17	2	12
15.85	14.50	17.20	47.56	47.53	47.59	20		
18.68	18.84	18.52	49.01	49.49	48.53	14		
15.84	16.43	15.26	48.27	48.87	47.67	17	3	
14.52	15.23	13.81	46.30	47.67	45.14	20		

Table 8 Comparison of average pulp yield

EA (%)	Grouping (average)
14	A = 48.70
17	B = 47.08
20	*C = 45.84*

Table 9 Comparison of average pulp yield

Cooking Time(h)	Grouping (average)
1	A=48.71
2	B=47.08
3	*C=45.83*

Table 10 Comparison of average pulp yield

Age of harvesting(a)	Grouping (average)
1 = 4	49.10 = A
2 = 6	47.21 = B
3 = 12	47.12 = B

Table 11 Papers Strength properties

Fold			Burst Strength Kpa m/g			Tear Strength mNm²/g			Breaking Length (Km)			EA (%)	Age of Trees (a)
27	28	29	6.18	6.09	6.12	7.83	7.97	7.63	8.14	7.89	8.02	14	4
30	30	31	5.89	6.37	6.19	8.52	7.95	8.31	8.32	8.14	8.28	20	
32	33	32	5.13	5.97	5.56	7.98	7.41	7.72	8.73	8.51	8.69	14	6
35	36	35	6.82	5.96	6.27	8.93	8.36	8.75	8.97	8.65	8.81	20	
35	36	37	5.22	5.42	5.23	7.83	7.52	7.76	8.26	8.32	8.53	14	12
36	36	37	6.11	6.42	6.23	8.79	8.92	8.83	8.52	8.49	8.37	20	

Results of cooking showed that in constant cooking temperature and chemical charge, with increasing age of tree, yield of pulps increased and there were significant difference at level 1%.Average pulp yield of 12 years old was 49.10 percent and it was the highest yield and pulp yields of 6 and 4 years old trees were 47.21 and 47.12 percent respectively. The reason of decreasing in yield was due to more lignin extraction from wood and also due to sensitiveness of 4, 6 years old trees elements to alkali destruction. With increasing in age of trees and constant cooking temperature and chemical charges, the kappa number of pulps decreased and there were significant difference at level 1%.With increasing in cooking temperature and in chemical charges, yield and kappa number of pulps due to more lignin extraction from fibres cell wall decreased. With respect to yields and kappa number of pulps, it is observed that in 17 and 20 percent active alkali, 25% sulphidity and 2 hours cooking time, it is possible to obtain suitable pulp for bleaching and make printing papers from Populus deltoids in 4, 6 and 12 years old trees. In these conditions average pulp yields were obtained between 48.57 to 45.76 percent

and kappa number was among (18.22 to 15.39). Rahmani, R, Hemati, A. in investigation on determination of the best harvesting period for *Populus deltoides* col. 77.51 showed that the highest production average was related to 4 years old Populus whereas the production rate of it in acre is 30 tons higher than 12 years old Populus. In the view of return property harvesting period of it is reduced to one third of 12 years old Populus. So it is economical for producer to investigate on harvesting 4 years old.

Results show that Populus deltoids col. 77.51 has short fibre (1-1/3 mm) so it is necessary to add some long fibre pulp to it in order to produce pulp with good strength. With the aim of investigated strength properties of hand sheets of this species, pulps which produced in 14 and 20 percent active alkali were refined to 350 ml (csf). Marlon , R. et al. (1968) were reported that burst , tear and breaking length factors of the Populus Kraft pulp are Kpa m2/g 55 , mNm2/g. 63 and Km 7.3 respectively. The results of this research show that it is possible to use of *Populus deltoids* col. 77.51 woods, because of it is fast growing, having light colour and suitability for making pulps with proper yield and kappa number. In order to increase strengths, it is necessary to add some long fibre pulp to it.

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"Application of ovary and ovule culture in Populus. euphratica Oliv. x Populus alba L. hybridization"

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Introduction

Hybridization is currently used to combine desirable traits and to achieve hybrid vigour in many crops and trees. There has been a long and sustained interest in the hybridization of poplars largely because of the benefits derived from capturing heterosis and combining desirable traits to improve the quality and amount of wood production (Stettler 1980).Because of its resistance to drought and salinity, *Populus euphratica* Oliv. has been chosen as a parental species in *Populus* breeding before, however, incompatibility has been observed between this species and some other poplar species (Willing and Pryor 1976). During the last decade, a number of observations have been made on pollen-stigma interactions in interspecific crosses of *Populus*, with emphasis on the sections *Aigeiros, Leuce* and *Tacamahaca* (Knox *et al.* 1972; Heslop-Harrison 1975; Guries and Stettler 1979; Gaget *et al.* 1984).

Incompatibility in tree species is usually due to premature abscission of flowers, early dehiscence of the capsules, pollen mortality failure of grafting and to unknown physiological disorders occurring within the flowering branches (reviewed in Ramming 1990 for fruit trees). In addition, with interspecific and intergeneric crosses, and crosses between diploids and tetraploids, the endosperm often develops poorly or not at all. This problem can be overcome by aseptically culturing the embryo in a nutrient medium, a technique used in many different crosses. It seems to be amenable to large-scale application in poplar hybridization (Li *et al.* 1983; Kouider et al. 1984; Li and Li 1985; Noh *et al.* 1986, and Savka *et al.* 1987). The *in vitro* method developed for *P. alba* x *P. euphratica* hybrid plant induction (Jafari Mofidabadi *et al.* 1998) was used to produce *P. euphratica* x *P. alba* hybrid poplar (reciprocal crosses).

Materials and methods

Artificial pollination was carried out between *P. euphratica* Oliv. and *P. alba* L. Pollen grains of *P. alba* were collected in March from the Research Centre of Alborz - Karaj, Iran. The female inflorescent buds of a *P. euphratica* tree were covered with transparent paper before anthesis to avoid contamination. Then pollination was carried out on covered female inflorescence by intensively dusting *P. euphratica* stigma with collected *P. alba* pollen grains. Artificial pollination was carried out using the bottle grafting, and twig and pot techniques (described by Kouider *et al*. 1984; see also Rajora and Zsuffa 1984) on a mature tree. A control pollination was made on the same tree in different branches. Catkins were

collected from branches 10, 30 and 45 days after pollination. Closed capsules, still attached to the axis of the catkins, were disinfected for 12 min in a 2.6% solution of calcium hypochlorite followed by three 5-min rinses in sterile distilled water. To rescue fertilized embryos, isolated ovaries and ovules were transferred to a 50% MS agar medium in 100 x 15 mm petri dishes. Cultures were incubated at 24°C under a 16 h photoperiod with light provided by cool white 40-watt fluorescent lamps (4000-5000 lux). Plantlets 1-2 cm in height were transferred to jars containing the same medium and kept for two months under the same conditions before being acclimatized (Fig.1).

The tested medium for the embryo germination and plantlet development contained a 50% concentration of MS inorganic salts (Murashige and Skoog 1962) with FeEDTA 10-4M and pH adjusted to 5.8; two concentrations of sucrose (0.06M and 0.17M) were used. No growth regulators were added to the medium. It was autoclaved for 20 min at 120°C and then dispensed in sterile petri dishes and jars with 10 and 20 ml respectively.

Results and discussion

Germination of embryos was observed when half-capsules of ovaries and isolated ovules were placed on the surface of an agar medium. The method used in our experiment differed from the culture techniques described by Li *et al.* (1983), Kouider *et al.* (1984), Li and Li (1985) and Savka *et al.* (1987), as these required the excision of individual embryos. This step is time consuming, requires technical ability and can also damage the integument of the embryos. We simply cultured the intact ovary and ovule and obtained a high number of plantlets. The highest embryo germination rate (more then 90%) was observed on the 50% MS medium containing 0.17M sucrose in both ovule and ovary culture. The same result was reported in the embryo germination of *P. alba* x *P. euphratica* hybridization product (Jafari *et al.* 1998).

Kouider *et al.* (1984) and Li and Li (1985) used a culture media which although based on MS components was supplemented with growth hormones, namely 3-indole-acetic acid (IAA) and 6-benzylaminopurine (BAP), as well as vitamins. This resulted in the production of calluses and multiple shoots, and made it necessary to transfer embryos to a rooting medium in order to obtain plantlets. To simplify the composition of the media, Raquin and Troussard (1993) only used media that contained basic components, i.e., mineral salts, water and sucrose water, and no growth regulators. In order to simplify and avoid the variation caused by callusing, we used 50% concentration MS agar medium without plant growth regulators.

Embryo age (number of days post-pollination) affected the ability of the embryos to respond to culture media. Due to the long period of *P. euphratica* embryo development, embryos younger than 45 days (10 and 30 days old) did not respond to the culture media (Table 1). The mean effect of explants (ovary and ovule) and sucrose concentration on embryo germination and plantlet production was compared (Table 2) and significant differences between explants and sucrose concentration (P<0.01) were found. Pollination of *P. euphratica* x *P. alba* was successful only on a mature tree in contrast to the *P. alba* x

P. euphratica cross where pollination was successfully carried out by using the twig and pot system (Jafari *et al.* 1998). The length of time required for embryo development when *P. euphratica* is used as the maternal plant, is the reason that artificial pollination using bottle grafting and twig and pot did not produce plantlets.

In ovary culture, an average of 67% of cultured capsules produced plantlets, while this value was 90% for ovule culture. Compared to ovary culture, ovules produced only one tiny shoot each (Table 1), while due to the induction of two to three plantlets in each cultured ovary, higher numbers and yields of induced plantlets were observed in ovary culture (Table 1). Germination of ovule embryos started three to four days after culture initiation, i.e. transfer to the new media. The highest frequency of embryo germination (90%) was obtained in ovule culture harvested 45 days after fertilization.

Of the cultured ovaries, 32.5% changed to white-yellow within 2 weeks and did not produce plantlets that turned necrotic. Abnormal structures were observed in ovary culture while there were no malformed ovules on ovule culture. Malformed ovules have been described for other tree species such as sour cherry (Furokawa and Bukovac 1989) and fertilized and unfertilized ovules of apricot (Eaton and Jamont 1992). The fastest germination, 10 days in the case of 45-day-old ovaries (45 days after pollination), was observed with the combination of *P. euphratica* x *P. euphratica* (control pollination). Thirteen *P. euphratica* Oliv. x *P. alba* L. hybrid plants were successfully acclimatized in a greenhouse and transferred to the field (Fig. 2).

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Table 1. - Influence of the age of embryos on plantlet production of *P. euphratica* Oliv. x *P.alba* L. in ovary and ovule cultures on nutrient media with 0.17M sucrose

Crosses	No. of days after pollination	No. of explants isolated		No. of explants producing plantlets		Plantlets					
						% of explants producing plantlets		Total no. of plantlets		Yield[†]	
		Ovary	Ovule	Ovary	Ovule	Ovary	Ovule	Ovary	Ovule	Ovary	Ovule
P. euphratica x *P. alba*	10	120	20	0	0	0	0	0	0	0	0
P. euphratica x *P. alba*	21	120	20	0	0	0	0	0	0	0	0
P. euphratica x *P. alba*	45	80	20	54	18	67.5	90	73	13	1.35	0.61
P. euphratica x *P. euphratica* (contr.)	45	80	20	61	17	76.3	85	81	11	1.33	0.61

Table 2. Mean effect of independent variables on *P. euphratica* Oliv. x *P. alba* L. embryo germination and plantlet production, and significant differences

Variables	Embryo germination and plantlet production
Explants Ovary Ovule	% of embryo germination† 67.50 90.00
Explants Ovary Ovule	Yield (plantlets per isolated explant)† 1.35 0.61
Sucrose 0.06 M 0.17 M	% of embryo germination† 41.25 78.50

Fig.1 Plantlets in jars

Fig. 2. *Populus euphratica* OLIV. X *P. alab* L. hybrid plant in filed

"Chemical composition of the essential oils from the aerial parts, roots and seed of Lepidium latifolium L. from Iran"

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Abstract

Hydro-distilled volatile oils from the aerial parts, roots and seed of *Lepidium latifolium* L. which gathered in Touchal on mountain Alborz (Tehran province) in Iran, were investigated by a combination of GC and GC/MS. Twenty-five components were identified in the oils. The main constituents of the essential oils in aerial parts, roots and seed were allyl isothiocyanate (57.4%-81.7%and 30.5%). Although the main components of all the oils are common , their percentages are different.

Keywords*: Lepidium latifolium*; Cruciferae; Essential oil composition; allyl isothiocyanate; Iran

Introduction

Lepidium L. belongs to family Cruciferae and comprises about 150 species, which occur largely in all temperate and subtropical regions of world. Eight species of this genus are found in Iran.[1]We have studied the essential oils content and composition of aerial parts, roots and seeds of *Lepidium latifolium* in Iran. This plant is a perennial herb, the flowers are hermaphrodite and are pollinated by insects. Aerial parts, roots and seeds have edible uses.

As a medicinal plant, *Lepidium latifolium* has been traditionally used as antiscorbutic, depurative and stomachic. An infusion of the plant is used in the treatment of liver and kidney diseases, it increases cardiac amplitude, decreases frequency and regulates the rhythm. It is also used in the treatment of skin diseases.[2] The volatile oil composition of the other species of this genus were previously reported. The main components from aerial parts of *L.meyenii*[3] oil from Peru were reported to be phenylacetonitrile (85.9%). Earlier chemical work on the roots of this plant yielded macaenes, macamides, fatty acids sterols and benzyl isothiocyanate.[4] The tubers of *Lepidium meyenii* contain the benzylated derivative of 1,2-dihydro-*N*-hydroxypyridine, named macaridine, together with the benzylated alkamides [5]. Other species of the genus *lepidium* exhibit the presence of flavonoids, flavonoid glycosides.[6,7] The main alkaloids of *L. sativum* is bis-benzyl imidazole [8] and a steryl ester isolated from the aerial parts, has been identified as stigmast-5-en-3β,27-diol 27benzoate.[9] Diuretic action of an aqueous extract of *L. latifolium* were examined.[10] The effect of an integral suspension of this species on prostate hyperplasia in rats has also studied.[11] The roots of *Lepidium virginicum*

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exhibited antiprotozoal activity against *Entamoeba histolytica* trophozoites one known glucosinolate responsible for such activity. It was identified as benzyl glucosinolate.[12] The major constituents in oilseed of *lepidium campester* was fatty acid.[13] Literature search did not reveal any reference to previous work on the essential oil of different parts of *Lepidium latifolium* L.

Materials and methods

Plant material

Aerial parts, roots and seeds of *Lepidium latifolium* L. were collected from Touchal on mountain Alborz (Tehran province), at an altitue of 1900 m in may 2005. The voucher specimens have been deposited in the national herbarium of Iran (TARI).

Isolation procedure

Dried plant material (80-100g) were subjected to hydro-distillation for 3h using a Clevenger-type apparatus . The oils were dried over anhydrous sodium sulphate and stored in sealed vials at low temperature before analysis.

Gas chromatography

GC analysis was performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30m x 0.25 mm i. d., film thickness 0.25 µm). Oven temperature was held at 50 ˚C for 5 min and then programmed to 250 ˚C at a rate 3˚C/min. Injector and detector (FID) temperature were 290˚C; helium was used as a carrier gas with a linear velocity of 32 cm/s.

Gas chromatography-mass spectrometry

GC-MS analyses were carried out a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d, film thickness 0.25 µm.). Oven temperature was 40-240 ˚C at a rate of 3 ˚C , injector temperature 250 ˚C and transfer line temperature 260 ˚C , carrier gas helium with a linear velocity of 31.5 cm/s , split ratio 1/60, ionization energy 70 eV, scan time 1 s, mass range 40-300 amu.

Identification of components

The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds, or with data published in the literature . [14,15]

Results and discussion

Table 1 summarized the identified compounds, their retention indices, their percentage composition and the method of identification. The constituents are arranged in order of their elution on the DB-5 column. The yields based on dry weight of samples were as follows: aerial parts, 0.8% : roots, 0.6% and seed, 0.3%. Twenty-three compounds were identified, constituting 94.6 % *Lepidium latifolium* aerial parts oil. The major components of this oil were found to be allyl isothiocyanate (57.4%), benzylthiocyanate (7.8%), phenylacetonitril (5.9%) and butyl isocyanate (4.9%). The deep yellow root oil was characterized by large amounts of allyl isothiocyanate (81.7%) and butyl isothiocyanate (3.8%).

Twenty-five compounds were identified constituting 95.3% seed oil. The main constituents of pale yellow seed oil were shown to be allyl isothiocyanate (30.5%), phenylacetonitril (17.3%), benzylthiocyanate (13.2%), geranylacetone (7.3%) and cedrene (1.7-epi-α) (6.2%). Comparison between the percentages of some main components in the oils is shown in Fig. 1. The first major compound of the oils were allyl isothiocyanate. It is used principally as a flavouring agent in a variety of foods, an alcohol denturant and in external analgesic products. The other main component in the seed oil was phenylacetonitril (17.3%) that was found in aerial parts and root oil at percentages of 5.9% and 0.6%. Phenylacetonitril is known to be a degradation product of benzyl glucosinolate.[16]

Some of the other phenolics including benzylthiocyanate which was found in aerial parts (7.8%), roots (0.4%) and seed (13.2%) have also been reported as degradation products of benzyl glucosinolate. Comparing the oil composition of these parts showed that geranyl acetone, cedrene (1.7-epi-α) was found in seed oil at percentages of 7.3%, 6.2% was observed in the aerial parts and root oils a few amount and Pinocamphone (tr.-) (4.2%) was found only in seeds.

Table 1- Percentage composition of the oils of different parts of *Lepidium latifolium* L

No	Compound	RI	Aerial parts	Roots	Seed	Method of identification
1	E-2 Hexenal	858	1.1	0.1	0.2	b,c
2	4-Methylthiazol	869	3.8	2.7	0.3	b,c
3	Allyl isothiocyanate	883	57.4	81.7	30.5	b,c
4	Butyl isothiocyanate	935	4.9	3.8	1.2	b,c
5	Benzaldehyde	965	0.2	0.1	0.3	b,c
6	Sabinene	981	0.4	0.3	0.1	a,b,c
7	1-Butene,4-isothiocyanato	985	0.5	0.1	0.2	b,c
8	p-Cymene	1028	0.7	0.2	0.4	a,b,c
9	Limonene	1033	0.6	0.4	0.2	a,b,c
10	1,8 Cineole	1035	1.2	0.5	0.3	a,b,c
11	γ-Terpinene	1064	0.6	0.3	0.4	a,b,c
12	Nonanal	1108	2.4	0.4	3.6	a,b,c
13	Phenylacetonitril	1142	5.9	0.6	17.3	b,c
14	Pinocamphone(tr.-)	1160	-	-	4.2	b,c
15	Estragol	1200	1.6	0.6	0.8	a,b,c
16	Decanal	1206	t	t	0.4	a,b,c
17	Cumin aldehyde	1246	0.7	0.2	0.1	a,b,c
18	Pregijerene	1290	1.1	0.7	1.2	b,c
19	Thymol	1294	0.6	0.3	0.4	a,b,c
20	Benzylthiocyanate	1368	7.8	0.4	13.2	b,c
21	β-Cubebene	1393	0.3	0.7	2.2	b,c
22	Cedrene(1,7-epi-α)	1397	0.4	0.9	6.2	a,b,c
23	β-Caryophyllene	1425	0.6	0.4	2.1	a,b,c
24	Geranyl acetone	1461	1.2	0.3	7.3	b,c
25	n-Pentadecane	1472	0.6	0.5	2.2	a,b,c
total			94.6%	96.2%	95.3%	

RI: Retention indices relative to C_9-C_{24} n-alkenes on DB-5 column

a: compared with retention time of authentic samples or components of reference oils,

b: retention indices relative to n-alkane on DB-5 column

c: compared with mass spectra, t: trace, less than 0.05

Acknowledgements

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"The Effect of Poplar wood fibres quality in thermoplastic composites"

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Abstract

In order to utilize wood fibers effectively as fillers or reinforcements in thermoplastic composites, a fundamental understanding of the structural and mechanical properties of poplar wood fibers/polypropylene composites is required. Characterization of aspect ratio and FTIR spectroscopy as well as surface tension properties as indicators of wet ability become important . The results indicate that the interfacial bond between reinforcing fibers and polymer matrix modified MAPP play significant role in improving the mechanical properties. The FTIR spectroscopy showed that the copolymer was bonded to the fibers by ester linkages and hydrogen bonds at 1705-1735 cm-1.

Keywords: Wood, Fibre ,poplar ,Composites ,Thermoplastics , FTIR

Introduction

Attractive properties of wood and paper residues such as biodegradability, recyclability and low cost and density favour the utilization of this material as reinforcement. It has been shown in earlier study (1,5,7) that wood and paper have good potential as a reinforcing fillers and fibers for polymers. Unfortunately these composite materials due to low impact and other properties and difficulty in mixing to obtain a uniform dispersion of the cellulose fibers in the polymer matrix are not yet been widely used. These shortcomings depend on poor interfacial adhesion between wood and synthetic polymer surfaces (2). If this interaction is improved, composites with acceptable mechanical properties and better fibers dispersion in the matrix can be produced (6,8). One way to improve adhesion between incompatible polymers with cellulosic fibre is by addition compatibilizer. Of course melt temperatures should be kept below 200oC because prolonged exposure to high temperatures can result in release of volatiles, discoloration, odour, and degradation of the wood component (3.4). The objective of this study was to determine the effect of different levels of poplar fibers addition to composite mixtures at three of mixing temperatures to improve the adhesion between poplar fibers and polypropylene, and produce composites with acceptable properties.

Material and Methods

Fibers: poplar fibers (*populus deltoides*) are produced by refiner mechanical pulp (RMP). Fibre length, diameter and aspect ratio were measured at 0.706 mm , and 34.62 respectively.

Thermoplastics: Polypropylene (poliran P10800) was received from Emam port, Petrochemical Company. The melt flow rate of polypropylene was 230o c, 2.16kg gr/7-10min. and it is used for house ware, stationary, toys, hygienic, packaging.

Coupling agents: Maleated polypropylene (MAPP) was supplied by Eastmann as Epolene PMG-3003 polymer with 6% acid anhydride and was added at 2% of polymer by weight.

Initiator: Dicumyl peroxide (DCP) from Aldrich was added at 0.1% of polymer by weight.

Composite Preparation

The mixtures of polypropylene and poplar fibres were compounded at 180oC, 190oC and 200oC for 10 min. and 50 rpm in a Haake Rheomix SIS 90 equipped with a roller blade rotor. After mixing polypropylene and MAPP and, as soon as the registered torque indicated that the polymer melt had reached a steady state, fibre was added. Then dicumyl peroxide was than added to the mixing head. Tensile, flexural and impact specimens were produced by compression moulding using in a carver press at 190o C under a pressure of 4 MPa followed by cooling in another press equipped with refrigeration facilities. Rectangular specimens were cut from pressed sheets.

Testing Procedure

Strength measurements of samples were carried out in a MTS, M/10 tester model, and Zwick impact tester model. Following testing procedures were used: Tensile strength, Young's modulus; ASTM D-638, Flexural and modulus of elasticity ASTM D-747, Izod impact strength ASTM D-256 .The tensile load was measured at 2mm/min strain rate and flexural strength at 1mm/min strain rate. The Izod impact strength was tested with an impact tester (Zwick). Mechanical properties were analyzed using factorial experimental design at completely random and DMRT test, and regression equations. FTIR spectroscopy is used to investigate the bond formation mechanism and its influence on mechanical properties.

Results and Discussion

Tables 1 show the tensile, flexural and notched Izod impact strength of PP/poplar fibers composites containing up 40% (by weight) fibre, and mixed at 180°C, 190°C, and 200°C. The results indicate the

effect of cellulose fibers and mixing temperature on these strength properties. Results of this study revealed that of poplar fibre up to 40% by weight can be incorporated into PP/fibre composites, and the utilization of 10 to 40% poplar fibers improved the properties of composites. Various parameters influence the properties of poplar fibre reinforced composites including the fibre aspect ratio, fibre matrix adhesion, stress transfer at the interface and mixing temperatures.

Table 1 – Mechanical Properties of Composites of PP/poplar fibres

Strength Properties	Mixing Temp. °C	Fiber Content % (By Weight)			
		10	20	30	40
Tensile Strength (MPa)	180	22.94	28.29	31.00	31.03
	190	26.05	29.43	32.89	34.00
	200	23.98	27.44	32.06	32.06
Tensile Elongation at Yield (%)	180	3.16	3.82	3.75	3.04
	190	4.00	3.64	3.69	2.84
	200	5.25	3.43	3.60	2.99
Tensile Modulus (MPa)	180	1135	1167	1412	1718
	190	1227	1399	1521	1746
	200	1139	1367	1521	1576
Flexural Strength (MPa)	180	44.50	47.08	44.15	45.30
	190	44.74	47.59	52.84	48.11
	200	42.98	42.96	46.42	45.69
Flexural Modulus (MPa)	180	2074	2049	1935	1994
	190	2120	2140	2190	2113
	200	2040	2028	1974	2020
Impact (J/m)	180	21.09	18.11	15.88	10.60
	190	24.61	20.00	16.36	13.29
	200	22.50	19.74	15.91	11.27

Mixture Condition: 10 Min., 50 rpm X (Fibre contents)

Original PP properties: Tensile Strength (MPa): 28.5, Tensile Elongation at Yield (%): 4.75,

Tensile Modulus (MPa): 1250,Flexural Strength (MPa): 38.5,Flezural Modulus (MPa): 1150

Figure 1 shows relationship between fibre content and tensile strength of composites. Tensile strength of composites with 40% poplar fibre is higher than that of PP filled with 10% poplar fibres, which indicates the reinforcing effect of these fibres. The tensile strength is sensitive to the matrix properties, whereas the modulus is dependent on the fibre properties. To improve the tensile strength, a strong interface, low stress concentration, higher fibre aspect ratio is required. It is shown that with the incremental of fibre content from 10 to 40 % poplar fibers, tensile stress moderately increased.
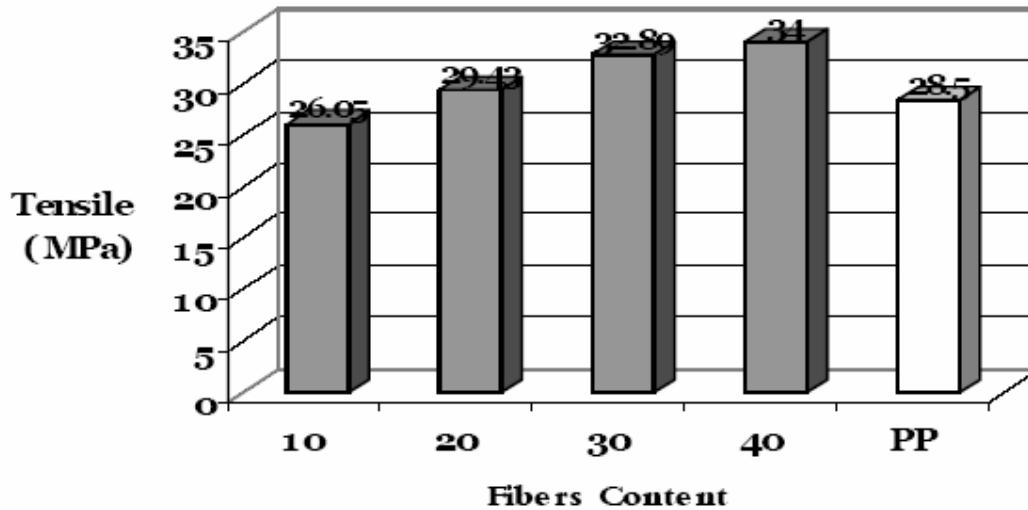
Figure 1- Relationship between Poplar fibres content (wt %) and tensile strength of composites
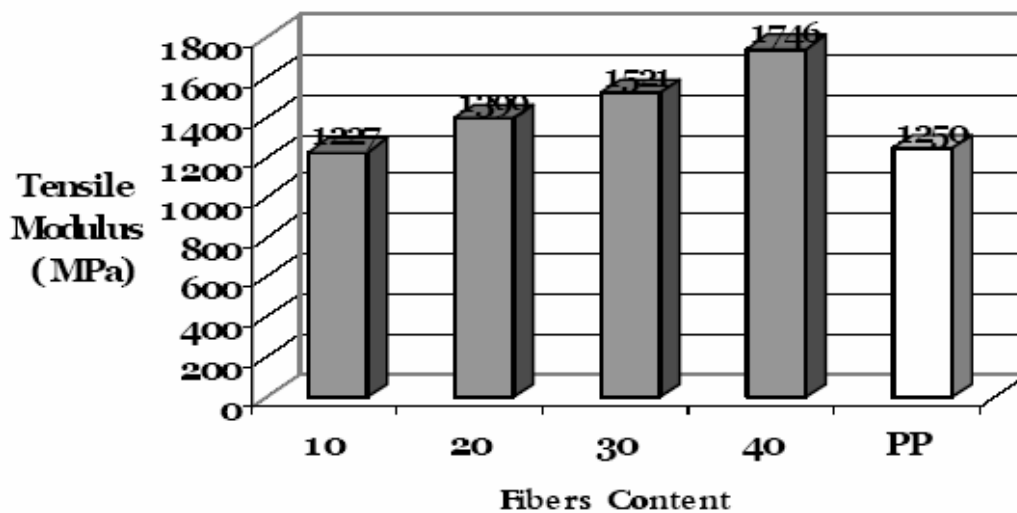

Figure 2- Relationship between Poplar fibres content (wt%) and tensile modulus strength of composites

The result of tensile modulus measurement as a function of fibre contents is presented in Fig.2. The tensile modulus of PP/poplar fibre composites shows that the modulus of PP filled with 40% fibre was higher than 10% poplar fibers in composites. The aspect ratio and fibre length are very important parameters in development of tensile modulus. In short fibre reinforced composites a critical fibre length is required under the full stressed condition in the polymer matrix. Fibre lengths shorter than this critical length lead to failure due to de-bonding at the interface at a lower load. On the other hand for fibre lengths greater than the critical length, the fibre is stressed under applied load and thus results in a higher strength of the composites. Composites made from poplar fibers exhibited improved strength

properties because the fibres were more conformable, thus creating more fibre-to-fibre and fibre-MAPP – plastics contact and therefore increasing the potential for bonding. Fig. 3 and 4 shows the result of flexural modulus and flexural strength measurement as a function of fibre contents. Flexural strength and modulus strength reached the maximum value at 30% of poplar fibre content. However, the addition of 30 and 40 % poplar fibers to PP significantly improved the flexural strength and modulus of PP/poplar composites as compared to 10 and 20% of poplar fibers. It is shown that incremental addition of 10 to 40% fibers, flexural strength and modulus sharply increased up to 30% poplar fibers. Fig. 5. Shows the result of notched Izod impact strength measurement function of fibre contents .For a good impact strength, an optimum bonding level is necessary. The degree of adhesion, fibre pullout, and a mechanism to absorb energy are some of the parameters that can influence the impact strength of cellulosic fibers/plastics composites. Notched Izod impact strength generally decreases with addition of fibers from 10 to 40%. The energy required for crack propagation was also measured. Crack propagation occurs at the PP/poplar fibre composites interface as a result of the poor interfacial adhesion between the hydrophilic poplar fibre and hydrophobic PP. FTIR spectroscopy shows that the carbonyl stretch peaks in 30% poplar fibers in composites were maximized. The results show that of carbonyl stretch peak at 1688-1755Cm-1 and anhydride exhibits a characteristics doublet due to carbonyl stretch of coupling agents.
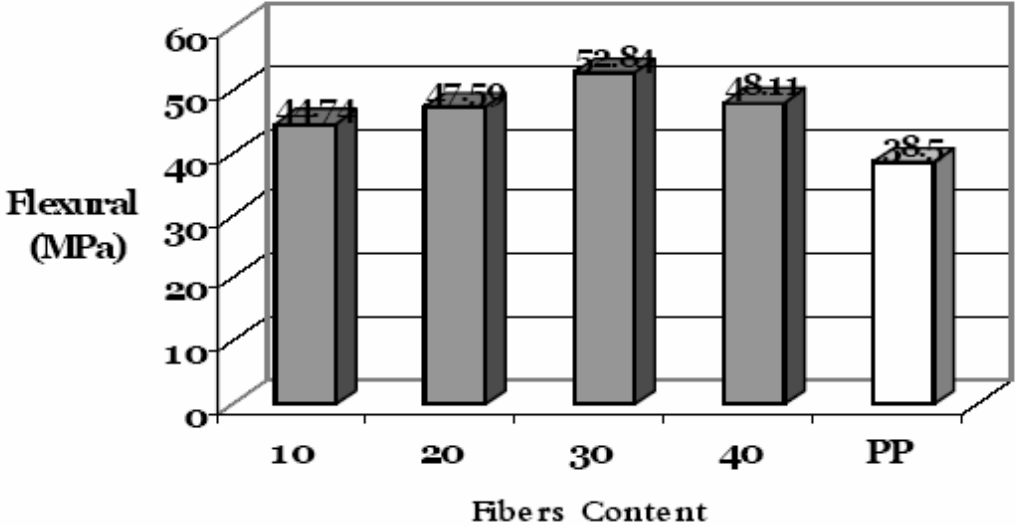

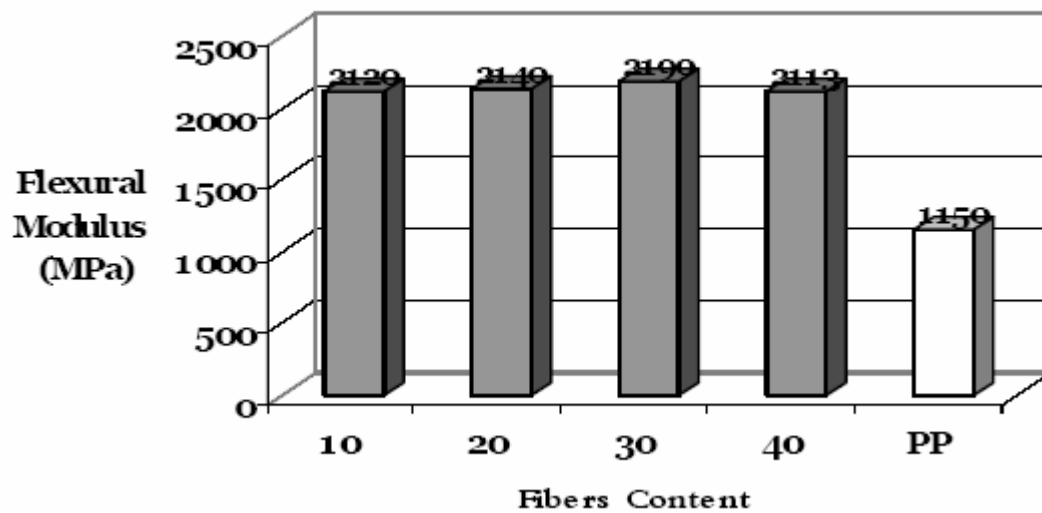**Figure 3- Relationship between Poplar fibers content (wt%) and flexural strength of composites**

Figure 4- Relationship between Poplar fibers content (wt%) and flexural strength of composites
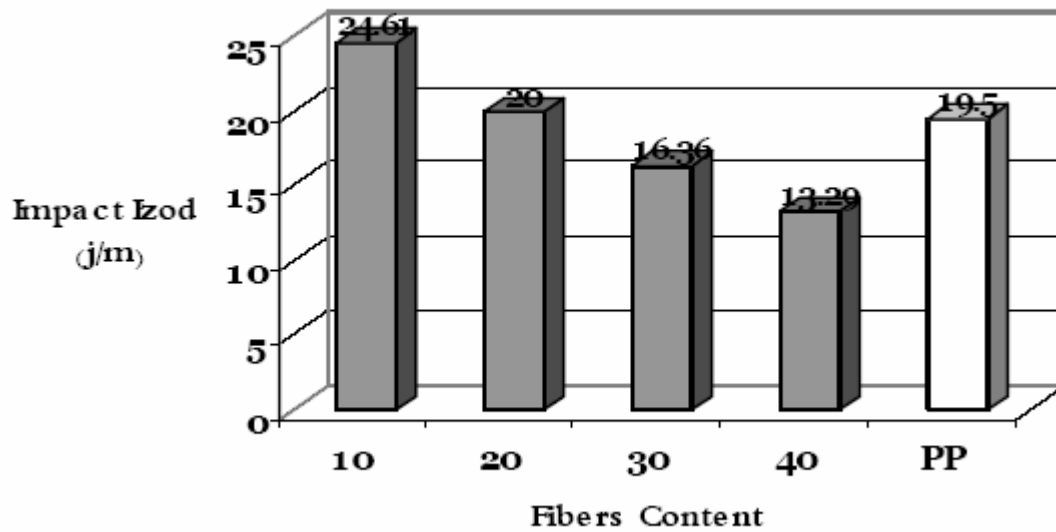

Figure 5- Relationship between Poplar fibers content (wt%) and notched Izod impact strength of composites

The effect of mixing temperatures on mechanical properties of the PP/OCC fibre composites was also investigated by changing the temperatures in the range of 180-200oC. However the rate of rotation and the mixing time during the kneading were fixed to 50 rpm and 10 min, respectively. The results have been shown in Fig.1 to 5, which indicates that maximum values of mechanical properties can be

reached at 190oC.At lower temperatures, the melt viscosity of polymers is rather high so that poor dispersion of OCC fibers in its composite results. Concerning the lower strength value of the PP/OCC composites at temperatures higher than 190oC it must be noted that, thermal degradation of the matrix polymer and OCC fibers is possible. At 200oC, the OCC fibers components such as lignin and hemicelluloses begin to depredate 3. The thermal degradation of the fibers also results in production of volatiles compounds at processing temperatures above 200oC. This phenomenon will produce porous polymer products with lower densities and inferior mechanical properties. On the other hand, the degradation of OCC fibers leads to poor organoleptic properties and consequently the deterioration of their mechanical properties. The absorption bands at 1704-1725Cm-1 (carbonyl stretch) were influenced after heat treatment of copolymer at 190oC (Fig.6).
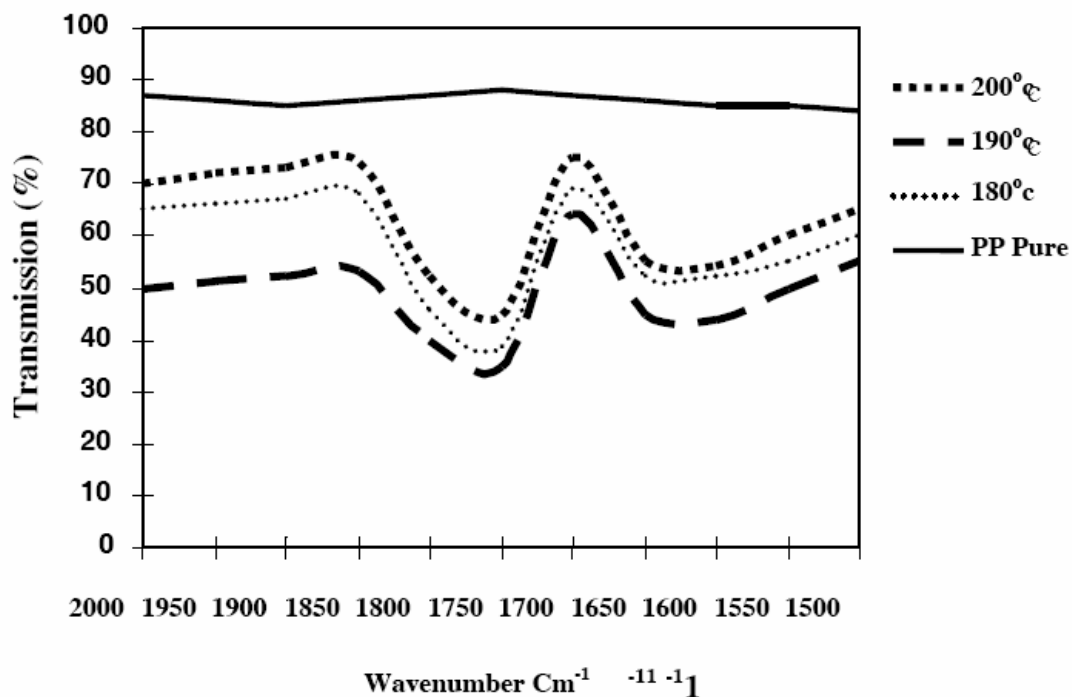

Fig.6. – Infrared spectra for PP and composites of Poplar-filled PP

Conclusion

OCC fibers should be considered as a potential source of low cost, natural fibers for composites. It has been demonstrated that the fibre content, and mixing temperatures had the greatest effect on mechanical strength of PP/OCC composites. Based on the results of this investigation, the highest mechanical properties of OCC fibre reinforced composites can be reached as follow; Both tensile, flexural strength of composites with 30 and 40 % fibre contents at 190oC were improved, While the modulus also increased with increasing the fibre content, because OCC fibre are believed to be more rigid than polymer, elongation and energy decreased So the Izod notched impact strength composites

with 10% fibre and 190oC mixing temperature increased. Consequently, optimum stress transfer between a high modulus cellulose fibre and a low modulus polymer requires an interphase region of intermediated modulus. In the present study, poor adhesion between fibre and polymer is responsible for decreasing trends in certain mechanical properties, particularly elongation and energy. The results clearly indicate the use of suitable mixing temperatures and 30% OCC fibres lead to mechanical properties increase. Regression equation shows the existence of direct relationship between variables and the properties of PP/OCC composites.

FTIR spectroscopy shows that the carbonyl stretch peak in 30% OCC and at 190oC is the highest.

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