Érica Daiuto[a]
Marney Cereda[a]
Silene Sarmento[b]
Olivier Vilpoux[c]

[a] FCA/UNESP, Botucatu – SP, Brazil
[b] ESALQ/USP – Piracicaba – SP, Brazil
[c] NGO – Raízes – Botucatu – SP, Brazil

Effects of Extraction Methods on Yam (*Dioscorea alata*) Starch Characteristics

Starch extraction from roots and tubers uses grating with water and sieves to separate the starch slurry from residual mass. The starch is recovered by decantation or centrifugation. The yam starch extraction is difficult due to high viscosity of the slurry caused by non-starch polysaccharides (NSP). The establishment of an efficient extraction process may turn yam into a competitive raw material. In this paper *Dioscorea alata* starch extracted by four methods was characterized in order to establish the impact of treatments. When the tubers were digested with an aqueous oxalic acid/ammonium oxalate (OA/AO) 1/1 solution, it was easier to separate the starch slurry from residual mass, because viscosity was reduced. For all the others methods tested, the viscosity remained almost the same. The nitrogen present in yam tubers was removed during the different extractions to a different extent. The largest nitrogen reduction was observed with OA/AO followed by the control (water). The spectrum of starch granules sizes obtained also varied according to the treatment. Results proved that NSP carries small starch granules over to the waste water. The smaller starch granules diameter varied from 1.9 μm (OA/AO extraction) to 13.5 μm (water and pectinase extractions). The larger diameter varied from 41.0 μm (NaOH treatment) to 67.7 μm (OA/AO). All starches extracted showed a RVA behavior in agreement with literature for yam starch, but with small differences due to the influence of methods. OA/AO extraction showed the best recovery (18 g of starch/100 g tuber yam) and granular variation but it interfered with the rheological behavior of starch.

Keywords: Yam; Starch extraction; Properties; Granulometry

1 Introduction

Starch is an important product with many industrial applications. The world production of starch reaches approx. 48×10^6 t. In Europe and the USA, main raw materials for the extraction of commercial starches are corn, wheat and potato. Other raw materials are used in Asia, as cassava, rice, canna [1], sweet potato, arrowroot and yam.

Almost 70% of starch is used in the food industry worldwide [2]. Canna starch, which is not considered commercial, is extracted in large quantities in China. All canna starch is used directly for preparation of noodles and is not mentioned in official statistics [1].

The starch extraction process from roots and tubers consists in grating the raw material, in order to break vegetal cells and release the starch. This step is followed by passing the fiber through sieves of different mesh sizes and subsequent slurry concentration by decantation or centrifugation [1].

Correspondence: *Marney Cereda*, FCA/UNESP, Botucatu – SP, Brazil. E-mail: cereda@raizes-ong.org,br.

The relevance to use yam as a raw material for starch extraction in Brazil had been proposed by *Cereda* [3] as a possibility for the crop valorization.

Taro and yam accumulate non-starch polysaccharides (NSP) in their tubers. Characteristics of taro and yam starch have been tested. *Dufour* et al. [4] mentioned that 32 plants were tested for extraction and showed different starch contents and ease of extraction. All the yam and taro species tested by the authors were classified as having average starch content and being difficult to extract.

Moorthy [5] reports that for the genus *Dioscorea*, the mucilage increases viscosity, hinders the sieving processes and increases decantation times. Aqueous ammonia solution (0.03 M) was tested by the author to extract starch from several tuber crops by decantation. There was noticeable improvement in the yield of starch from *Dioscorea* and *Colocasia*, while yields decreased in sweet potato starch and remained almost the same with the other starches [5]. The author does not give any explanation why an ammonia solution can improve starch extraction of *Dioscorea alata, D. rotundata* and *D. esculenta.*

Research Paper

Other authors mention extraction at high temperatures [6], a treatment that may alter the starch properties.

An article published by *Chu* and *Ribeiro* [7] was aimed at studying NSP extraction from yam using oxalic acid and ammonium oxalate solutions, to eliminate the pectin from hemicelluloses polysaccharides of this tuber.

The purpose of this article was to evaluate the effects of four extraction methods in the characteristics of *D. alata* starch.

2 Materials and Methods

2.1 Materials

Pectinex was provided by Novozyme (Bagsvaerd, Denmark), HCl, NaOH, oxalic acid, ammonium oxalate were all purchased from Merck (PA, USA).

The *Dioscorea alata* tubers were purchased in the local market in their maximum physiologic development stage, corresponding to the commercial harvest which generally is with tubers of 10 to 11 months [5]. The moisture content of raw material was 76.1%.

2.2 Methods

2.2.1 Analytical methods

Acidity, pH, moisture (dry matter), nitrogen and fiber content were determined according to AOAC methods [8]. Starch content was analyzed by enzymatic hydrolysis [9], followed by determination of reducing sugars [10], with three repetitions for each sample.

2.2.2 Sample preparation

Tubers were washed, peeled and chopped into smaller pieces to facilitate grating. In the four treatments performed, distilled water and a kitchen blender were used. Chopped tubers were used for starch extraction in a ratio of 1:2 with water or other extraction solution. The decanted starch was dried. The different extraction tests were performed as follow:

Water (*control*): tuber pieces were triturated in water, the tuber:water ratio being 1:2. Then the triturated suspension was passed through a 0.250 mm sieve. The resulting starch slurry was kept in a cold chamber (2°C) for 8 h for decantation. The upper layer was discarded and the precipitated mass passed through a 0.075 mm sieve. The slurry was again allowed to stand for another 8 h and the supernatant liquid was discarded. The total time for setting was 16 h. The pH of the extracted starch corresponded to the natural of yam tubers.

Pectinase: pieces of tubers were triturated with pectinase solution at a 1:2 ratio (tuber:water). A Pectinex concentration of 8 mg/kg of tuber was used, according to [11]. The pH was not adjusted because the ground yam tubers had a pH very close to 5.6, the pH recommended by Novozyme. The ground material was agitated at 45°C for 2 h. The resulting starch slurry was kept in a cold chamber (2°C) for 8 h for decantation. The upper layer was discarded and the precipitate passed through a 0.075 mm sieve. The slurry was again allowed to stand for another 8 h and the supernatant liquid was discarded. The total time for setting was 16 h and the total time for starch extraction was 18 h.

Sodium hydroxide: small pieces of tubers were soaked for 1 h in an 0.03 M aqueous NaOH solution. Afterwards the solution was discarded and the tubers ground in a blender with an 0.03 M aqueous NaOH solution [12] (1:2 ratio tuber:solution). The pH of the slurry was close to 11.0. To avoid alteration of the starch fraction, the pH of the suspension was adjusted with 0.1 M HCl to about 6.0. The resulting starch slurry was kept in a cold chamber (2°C) for 8 h to achieve setting. The upper layer was discarded and the precipitate passed through a 0.075 mm sieve. The slurry was again allowed to stand for another 8 h and the supernatant liquid was discarded. The total setting time was 16 h and the total time for starch extraction was 17 h.

Oxalic acid/ammonium oxalate: these reagents were selected following a publication by *Chu* and *Ribeiro* [7]. A 10% solution was prepared, proportion of reagents being 1:1. Tubers were ground in a blender with the solution, ratio tuber:solution being 1:2. The slurry was first passed through a 0.250 mm sieve, afterwards through a 0.075 mm sieve. The pH of the slurry was 2.0 and remained at this level for almost 30 min. To avoid the alteration of the starch fraction, the suspension was neutralized with 0.1 M aqueous NaOH to approx. pH 6.0. The resulting starch slurry was kept in a cold chamber (2°C) for 8 h for setting. The upper layer was discarded and the precipitate passed through a 0.075 mm sieve. The slurry was again allowed to stand for 8 h and the supernatant liquid was discarded. The total time for setting was 16 h.

Drying: the decanted starch of the four treatments was partially dried in a cold chamber at 4–5°C overnight and the drying completed in an air-circulation oven at 35°C to a final moisture content of 12%.

Characterization of yam tubers: Acidity, pH and initial moisture content of the tubers were determined. Samples were sliced and dried in an oven with air circulation at 60°C, ground and stored for analysis.

Extraction evaluation: The starch recovered or starch yield in each treatment was expressed as: g starch (dry basis, d. b.)/100 g tuber. To calculate the recovery in starch extraction, moisture and starch content of the raw material, residual mass and starch were analyzed.

Starch granules measurements: measuring of yam starch granules were performed with an AXIOSKOPII-Zeiss light microscope (Oberkochen, Germany) (magnification 100 ×) using KS 300 software for image analysis, the method being adapted from *Schoch* and *Maywald* [13]. 100 counts were performed per slide, with 5 slides repetitions.

Starch apparent viscosity: Samples of each treatment were analyzed in duplicate at their pH values. Pasting properties were obtained using a Rapid Visco Analyser (RVA), series 4, (Newport Scientific, Warriewood, Australia) and Thermocline for Windows computer program. For every run 2.5 g of sample (corrected to 14% moisture) and 25 g of distilled water was used, following the operation programming s Standard 2 [14]. The viscosity was measured in RVA units [RVU], pasting temperature, maximum viscosity (peak), peak time, breakdown, final viscosity and setback were obtained from the graph.

3 Results and Discussion

The yam tuber had average moisture contents of 76.0%, the starch content in the dry matter was 80.0%.

Treatments with 0.03 M NaOH and pectinase did not reduce NSP viscosity, and the same difficulties in extraction were encountered as in the control treatment with water. The recovery was about 10% (g of dry starch per 100 g of fresh tuber) for the control, treatment with pectinase and with NaOH. Oxalic acid/ammonium oxalate (AO/AC) treatment gave a better recovery of 18%. This recovery was larger that the 16% obtained by *Moorthy* [5] with ammoniacal solution. Raw material, residual mass and starch were characterized.

Tab.1. Effect of treatment on the extraction rate of yam starch.

Treatments	% Starch recovered in raw material [100%]		
	Extracted	Retained in mass	Lost[1]
Water	43.64	0.39	55.97
NaOH	42.73	28.04	29.23
Pectinase	42.59	32.69	24.72
Oxalate	62.92	26.82	10.26

[1] by calculation.

NSP strongly increases the viscosity of the extraction water, hindering decantation of starch. Tab. 1 show that low extraction rates found for treatments with water, NaOH and pectinase may be explained mainly by the great losses of starch into the aqueous phase. These losses may be caused by the presence of the viscous water soluble NSP that carried starch granules over into the water. In the treatment with NaOH and pectinase, even if extraction rates were similar to control, the characteristic NSP viscosity of yam was reduced, explaining the lower losses in waste water.

Tab. 2 shows the effect of treatment on acidity and pH content. The yam tubers had an acidity of 6.24 mL of 1 M NaOH for 100 g of fresh weight (FW) with a 6.12 pH value. Starch extracted with water showed an acidity of 0.2 mL, thus a lower acidity than the raw material because tuber's acidity was removed with water. pH and acidity after treatment with pectinase were similar to those of water extraction. The neutralization of the slurry in the NaOH treatment results in a starch acidity and pH similar to control. Treatment with oxalic acid/ammonium oxalate showed a starch with high acidity.

Tab. 3 indicates nitrogen content of starch, residual mass and water (by calculation) for each treatment. The raw material presented 2.53 g nitrogen per 100 g dry matter, nitrogen contents decreased in the sequence of treatments with NaOH, control and pectinase. Treatment with OA/AO retained the highest nitrogen content, both in residual mass and starch and less nitrogen was lost in the water. This may be due to the loss of NSP, which enabled protein to be fixed on starch and residual mass, instead of being removed with water as in the other extractions.

Starches were subjected to granulometry analysis to verify if the particles size distribution was influenced by the treatments, because NSP may transfer granules of smaller size into the aqueous phase. Histograms for distribution of granules with smaller and larger diameters and of granule areas, respectively, are given in Figs. 1–3. These figures indicate that the distributions for smaller and larger diameters and granule area had a normal pattern for all treatments. Histograms show small variations for the size and area of granules in treatments.

Also Tab. 4 shows that the size distribution values of yam starch granules extracted with water follows a normal distribution pattern. With 511 measures, the smaller diameters vary from a minimum of 10.3 to a maximum of 45.8 μm, whereas the larger diameters varied from 13.52 to 66.10 μm, therefore granules from such extractions were slightly flat. The area varied from a minimum of 97.2 to a maximum of 2,231.4 m^2.

Tab. 2. Acidity and pH values in raw material, starch and in residual mass after treatments.

Samples	Water		NaOH		Pectinase		Oxalic Ac./Oxalate	
	pH	Acidity[1]	pH	Acidity[1]	pH	Acidity[1]	pH	Acidity[1]
Raw material	6.1	6.24	6.1	6.24	6.1	6.24	6.1	6.24
Starch	7.5	0.20	7.5	0.10	7.2	0.59	5.4	3.72
Fibers	8.0	4.31	7.8	0.49	6.5	2.59	5.3	4.54

[1] acidity expressed in % of NaOH (N/100g fresh weight).

Tab. 3. Effect of treatments on nitrogen content expressed in dry matter samples.

Samples	Nitrogen content in [%] dry matter			
	Water	NaOH	Pectinase	Oxalic Ac./Oxalate
Raw material	2.53	2.53	2.53	2.53
Starch	0.06	0.02	0.20	0.67
Residual mass	0.76	0.69	0.72	2.21
Water $^{-1}$	1.71	1.81	1.61	0.35

$^{-1}$ by calculation

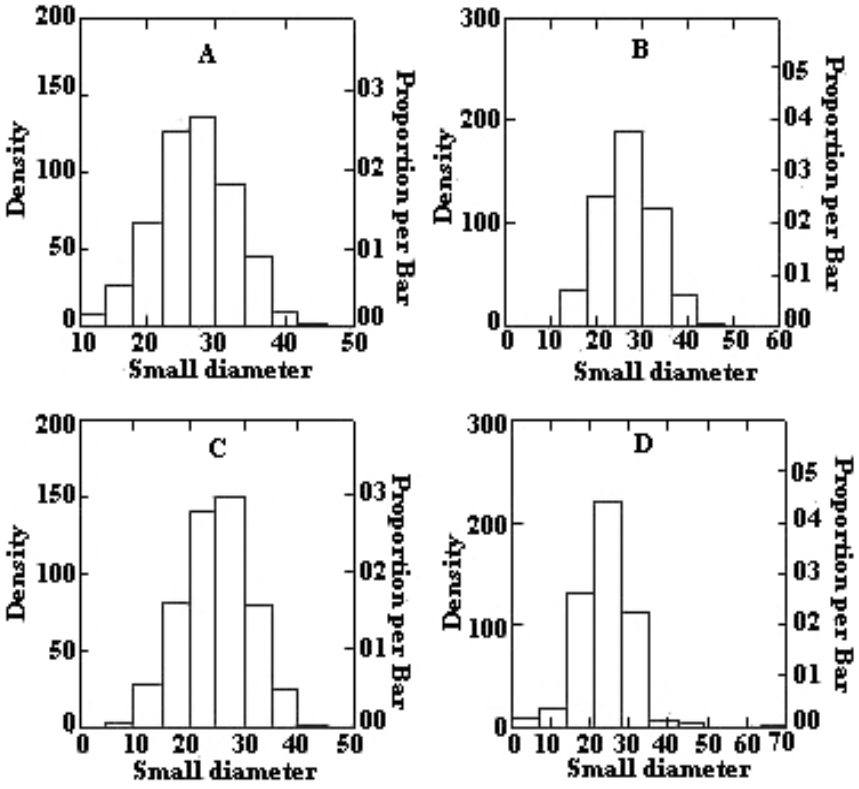

Fig. 1. Size distribution of smaller diameter granules of yam starch extracted with water (A), pectinase (C), NaOH (B) and oxalic acid/oxalate (D).

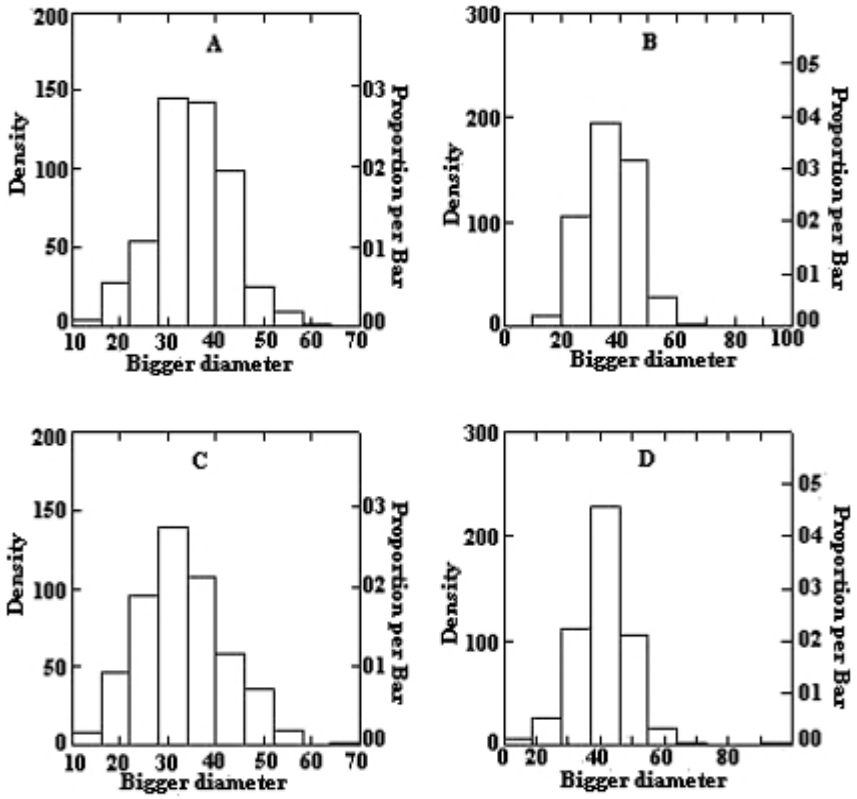

Fig. 2. Size distribution of larger diameter granules of yam starch extracted with water (A), pectinase (C), NaOH (B) and oxalic acid/oxalate (D).

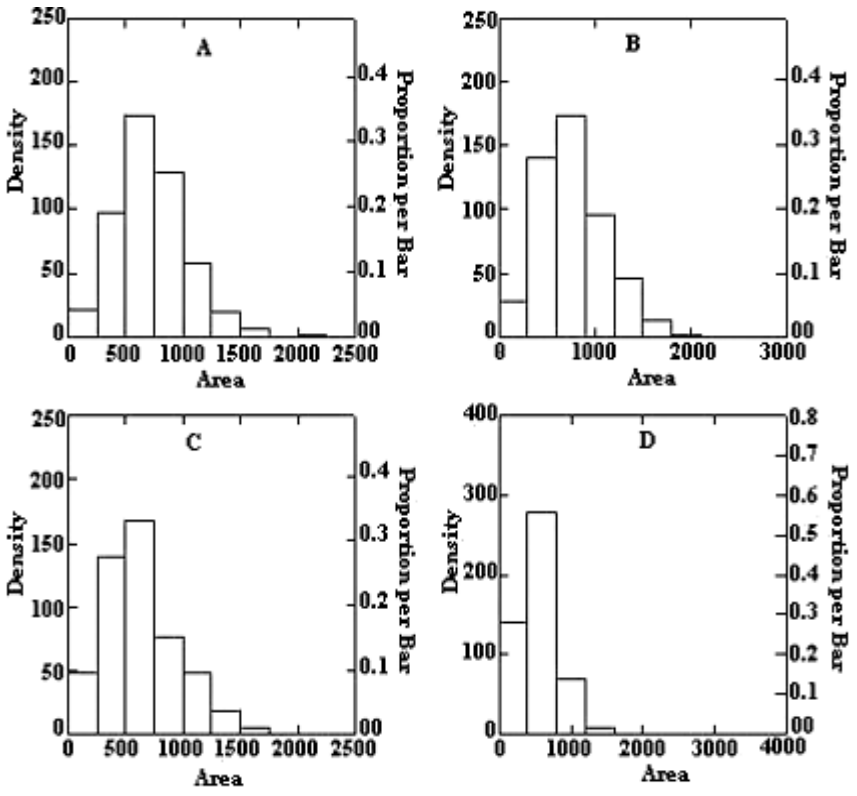

Fig. 3. Size distribution of granule area of yam starch granules extracted with water (A), pectinase (C), NaOH (B) and oxalic acid/oxalate (D).

Tab. 4. Variation of diameter [μm] of yam starch granules extracted with different treatments.

	Smaller diameter [μm]				Larger diameter [μm]			
	Water	**Pectinase**	**NaOH**	**OA/AO**	**Water**	**Pectinase**	**NaOH**	**OA/AO**
No. of measurements	511	503	506	500	511	503	506	500
Minimum	10.30	4.86	9.58	1.94	13.52	8.29	13.03	4.85
Maximum	45.80	51.44	40.95	67.68	66.10	92.22	67.82	87.07
Average	26.75	26.79	24.82	23.73	35.07	36.97	33.05	31.23
S. Deviation	5.747	6.042	6.105	6.401	8.068	9.184	9.29	8.97

The size distribution of yam granules extracted with pectinase also follows a normal distribution pattern (Figs. 1–3), with very similar average and median values. However, values were different, with greater variations in the extraction with pectinase than with water (Tab. 4).

Thus, it can be concluded that with pectinase a larger range of granules was extracted than with water, or rather, that during extraction with water more granules were lost, which may have been carried over to the aqueous phase by NSP. However, the area did not show a corresponding variation.

In case of the extraction with NaOH, larger and smaller diameter and area values for starch granules did not differ significantly from those from treatment with water. The 506 measurements were adequate with low variability.

The results for yam starch granules extracted with oxalic acid and ammonium oxalate were very different from the other treatments. Smaller granules showed a variation from 1.94 to 67.68 μm, these measurement values being smaller than those found with other treatments for the first one and higher with the second one. Larger diameters also showed lower minimum values, of 4.85 μm, whereas higher values were similar to those obtained with pectinase treatment, which were the highest. As a consequence, the variation of diameter for yam starch granules was more pronounced for extraction with oxalic acid/ammonium oxalate. This method of extraction enabled to recover a higher quantity of smaller granules than treatments with pectinase and NaOH.

An important parameter in determining quality and in indicating the uses of starches is its viscosity. Viscosity profiles of starch extracted with the different treatments are plotted in Fig. 4 and the pasting properties in Tab. 5. Viscosity may be considered a measure of the integrity of starch granules; those which underwent chemical and microbial damages show alterations in these values. Viscosity also measures internal associative forces of starch granules. With the aid of Tab. 5 it is possible to compare the effect of the four extraction methods on the RVA viscosity of the starch. Extraction with water was used as reference for testing alterations introduced by other extraction methods. It should also be taken into account that the treatments with water and with pectinase were the only ones that kept the natural pH of yam. However, pectinase extraction submitted starch to a higher temperature during more than half an hour.

Yam starch extracted with water has a viscographic behavior in agreement with literature data, where pastes are described with good resistance to mechanical disintegration (stability) during gelatinization and high setback values. Such a profile makes this starch suitable for use in

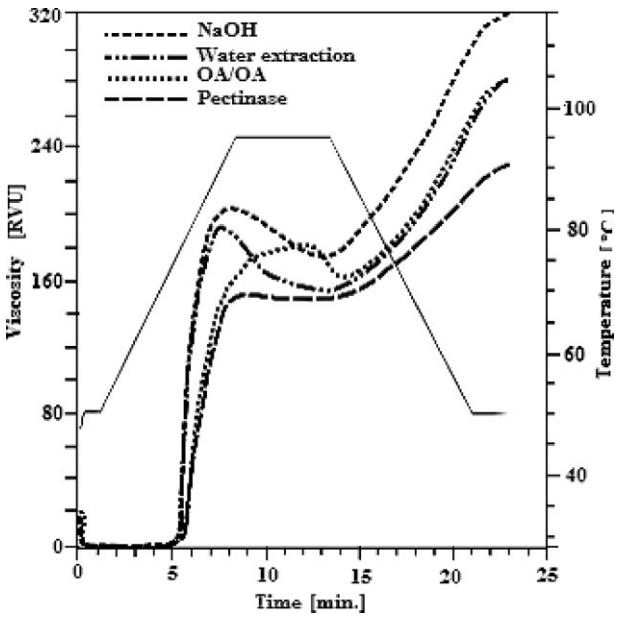

Fig. 4. RVA viscograms of yam starch extracted with water, NaOH, pectinase and oxalic acid/oxalate.

Tab. 5. Pasting properties (RVA) of yam starches extracted by different treatments.

	Pasting temperature [°C]	Peak time [min]	Viscosity [RVU]			
			Peak	Breakdown	Final	Setback
Water	75.5	7.5	193	39	283	130
Pectinase	78.4	9.0	152	8	230	87
NaOH	75.9	8.6	204	30	322	148
Oxalic ac/oxalate	77.6	12.3	182	19	282	119

heat-processed food, however, restricts its use in food to be kept refrigerated. For yam starch, *Dufour* et al. [4] found a high viscosity (Brabender), especially in acid environments and under sterilization, though low resistance to syneresis compared to other starchy tubers.

Our results agree with literature data although the extraction methods were different. The pasting profiles of starch extracted in the presence of chemicals, however, when compared to the curve obtained for the starch extracted only with water, showed interference in the viscosity pattern. The viscosity profile of starch obtained with water presented a rather sharp peak (193 RVU), showing that internal binding forces are very uniform. Starches obtained by treatments with NaOH and pectinase had marked differences compared to others treatments.

The alkali treatment, even with pH neutralization and successive washing, may have cleaved bonds in the inner structure of the granules and, consequently, the viscosity peak of 204 RVU was higher than that of control. Besides, this starch seemed more stable to viscosity breakdown, and presents a higher retrogradation tendency.

Treatment with pectinase reduced the starch's potential to expansion (lower viscosity values) in comparison to other treatments. Treatment with OA/AO affected the viscographic behavior of starches. The peak occurred after a longer time than that of starches obtained by other extraction treatments and show a slight decrease in viscosity. Alterations of viscosity in this sample may be associated to the acidity effect, which the granules were exposed to. However, with the cooling of pastes, viscosity values were very similar to control starch.

Gelatinization temperatures of yam starch vary slightly with treatments, from 75.5 to 78.4°C (Tab. 5), comparable or even lower than values mentioned in literature for *Dioscorea* by *Franco* et al. [15]. The lowest temperatures were found in starches extracted with water and sodium hydroxide, the highest temperatures in those treated with pectinase. Starch with higher pasting temperatures provided the lowest viscosity peaks and the highest resistance to rupture, evidencing internal binding forces more intense in granules.

Starch with the highest viscosity peaks showed the highest viscosity breakdown under heat and agitation, which is considered a normal behavior. The setback values observed were high for all treatments and followed the same profile of viscosity peak and viscosity breakdown.

4 Conclusions

Results will enable to obtain the following conclusions:

– Extraction of yam starch with ammonium oxalate/oxalic acid showed the best result, with instantaneous loss of mucilage viscosity upon triturating the tuber. Extraction was facilitated and considerably faster, providing a recovery of 18%, the highest among the tested methods.

– The raw material had 2.53 g of nitrogen per 100 g dry matter. Most of the nitrogen was eliminated in water in all treatments, probably carrying over starch and resulting in a lower recovery. An exception was the extraction with ammonium oxalate/oxalic acid, where nitrogen could be detected in the residual mass and in the extracted starch.

– The size for the smaller diameter of starch yam granules varied from 1.94 μm (extraction with OA/AO) to 10.30 μm (water). For the larger diameter, the variation was from 66.10 μm (water) to 92.22 μm (pectinase). The area of granules reflects these variations, and the extraction with OA/AO showed the two extremes, the lowest area of 4.72 μm^2 and the highest of 3,454.14 μm^2. Treatments influenced granulometry of starch showing that NSP may interfere in extraction or in decantation, so that in the treatment with loss of mucilage viscosity it was possible to find the smallest granules such as the biggest range of variation in granules size.

– Viscograms of extracted starch showed small differences among themselves in terms of shape and values, though they agree with literature, where pastes are described with good stability at higher temperatures and high setback. Pasting temperatures of yam starch varied with treatments, from 75 to 78°C.

– Treatment with sodium hydroxide showed the highest viscosity values, next to those with water. Treatment with pectinase has affected the internal granular structure of starch, generating a less pronounced viscosity peak and lower values. Extraction with OA/AO, of higher recovery, generated starch granules with a different behavior; however, upon finishing the programming of the viscosimeter, the starch recovers the same viscosity levels as that obtained by extraction with water.

Acknowledgements

Ana Teresa Burlamaqui Faraco Antonangelo is thanked for kindly correcting our English. The São Paulo Foundation for Research Supporting (FAPESP) is acknowledged for their support (Process 98/ 01649-9).

References

[1] O. Vilpoux: Produção de fécula a partir de biri (Canna edulis*)* na China, in: *Tecnologia, usos e potencialidades de tuberosas amiláceas Latino Americanas*, vol. 3, (Eds*.* M. P. Cereda, O. Vilpoux) Fundação Cargill, São Paulo, **2003** Chap. 9, p. 191–200 (Série Culturas de Tuberosas Latino Americanas).

[2] O. Vilpoux: Produção e uso de amido, in *Propriedades gerais do amido* (Ed. M. P. Cereda) vol. 1., Fundação Cargill, Sao Paulo, **2001**, Chap. 2, p. 21–56, (Série Culturas de Tuberosas Latino Americanas).

[3] M. P. Cereda: Importância, modo de consumo e perspectivas para raízes e tubérculos de hortícolas no Brasil, in Anais do 1° Simpósio Nacional sobre as culturas do inhame e do cará, Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, Venda Nova do Imigrante, **2001**.

[4] D. Dufour, J. J. Hurtano, J. Ruales, C. Mestres: Functional properties of starches from tropical roots and tubers: starch behaviours under different agro-industrial stress conditions, in *Proceedings of the 12 h International Society of Tuber and Root Crops*, 2000, Tsukuba, International Society of Tuber and Roots Crop, Tsukuba, **2002**, 21–24.

[5] S. N. Moorthy: Extraction of starches from tuber crops using ammonia. *Carbohydr. Polym.* **1991**, *16*, 391–398.

[6] A. R. El-Mahdy, L. A. El Sebauy: Preliminary Studies on the mucilages extracted from okra fruits, taro tubers, jew's mellow, leaves and fenugreek seeds. *Food Chem.* **1984**, *14*, 237–249.

[7] E. P. Chu, R. C. L. Figueredo-Ribeiro: Polissacarídeos hemicelulósicos de tubérculos de Dioscorea delicata R. Knut e D. Olfersiana KL, composição e alterações durante o desenvolvimento. *Hoehnea* **1986**, *13*, 87–94.

[8] Association of Official Analytical Chemists: *Official methods of analysis*, 13. ed. AOAC, Washington, **1989**.

[9] J. E. Rickard, K. R. Behn: Evaluation of acid and enzyme hydrolytic methods for determination of cassava starch. *J. Sci. Food Agric.* **1987**, *41*, 373–379.

[10] M. Somogyi: Determination of blood sugar. *J. Biol. Chem.* **1945**, *160*, 69–73.

[11] M. Leonel, M. P. Cereda: Avaliação da concentriação de pectinase no processo de hidrólise-sacarificação do farelo de mandioca para produçao de etanol. *Ciência e Tecnologia de Alimentos*, **2000**, *20*, 220–227.

[12] S. B. S. Sarmento: Alterações na fração amido durante o armazenamento de raízes de uma cultivar de mandioca (Manihot esculenta C.) de uso industrial. *Dissertation (Mestrado em Tecnologia de Alimentos)*, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, **1989**.

[13] T. J. Schoch, E. C. Maywald: Microscopic examination of modified starches. *Anal. Chem.* **1956**, *28*, 382–387.

[14] Newport Scientific: Operation manual for series 4: *Instructions Manual*, Warriewood, Australia, **1998**.

[15] C. M. L. Franco, E. R. Daiuto, I. M. Demiate, L. J. C. B. Carvalho, M. Leonel, M. P. Cereda, O. F. Vilpoux, S. B. S. Sarmento: *Propriedades gerais do amido*. Fundação Cargill, São Paolo. Vol. 1 (Série Culturas de tuberosas amiláceas latinoamericanas), **2001**.

(Received: May 26, 2004)
(Revised: November 16, 2004)
(Accepted: November 16, 2004)

