LWT - Food Science and Technology 58 (2014) 21–27

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Characterization of starch nanoparticles obtained from *Araucaria angustifolia* seeds by acid hydrolysis and ultrasound

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ARTICLE INFO

Article history:
Received 29 November 2013
Received in revised form
2 March 2014
Accepted 13 March 2014

Keywords:
Starch
Hydrolysis
Ultrasound
Polysaccharide
Nanoparticle

ABSTRACT

Native starch (NS) extracted from the seeds of *pinhão* (*Araucaria angustifolia*) was modified by two methods: acid hydrolysis (AH) and ultrasound (US). The three starch samples were subjected to spray drying and characterized. Chemical composition and rheological characteristics were evaluated and morphology was analyzed by scanning electron microscopy. The starch modified by US and AH achieved nanometric size, with mean particle size of about 453 and 22 nm, respectively. Besides the modified starch reached nanometric size, the three starch preparations were significantly different in four characteristics: starch and amylose content, the percentage of syneresis and colorimetric data. The AH sample differed from the others in terms of solubility (more soluble), hygroscopicity (more hygroscopic) and paste clarity (more translucent). Modified starch nanoparticles obtained by AH and US can be useful for development of novel biocomposites with improved properties to be employed as coating materials or films.

1. Introduction

Starch is widely distributed in plants as a storage carbohydrate and is particularly abundant in cereal grains (40–90 g/100 g dry solids), vegetables (30–50 g/100 g dry solids), tubers (65–85 g/100 g dry solids) and immature fruits (40–70 g/100 g dry solids) (Lajolo & Menezes, 2006). Structurally, starch is a homopolysaccharide composed by amylose and amylopectin chains. Amylose consists of D-glucose units joined by glycosidic α-1,4 bonds, resulting in a linear chain, while amylopectin consists of D-glucose units linked through α-1,4 and α-1,6 bonds, forming a branched structure. The proportions in which these structures appear depend on the botanical source, differences among cultivars of the same species and, even on the degree of maturity of the plant (Eliasson, 2004; Tester, Karkalas, & Qi, 2004). Native starch granules contain between 15 and 45 g/100 g of crystalline material with typical X-ray diffraction patterns. These correspond to two polyforms (A or B) or an intermediate form (C), and their classification is based on variations in the water content and the packaging configuration of double helices (Imberty, Buleón, Tran, & Pérez, 1991).

The market for starches is constantly growing, leading to a continuous search for products with specific features that meet industry requirements. The production of modified starch is an alternative that has been developed in order to overcome one or more limitations of native starches, and thus increase the utility of this polymer in industrial applications (Jimenez, Fabra, Talens, & Chiralt, 2012; Zambrano & Camargo, 2001). The modification by acid hydrolysis has been used to modify the structure of the starch granules and produce "soluble starch" (Murphy, 2000). The differences between the ratio and extent of acid hydrolysis of starches have been attributed to differences in granule size, extent of interactions of starch amorphous and crystalline regions of the granule, extent of phosphorylation, number of α-1,6 bonds, amylose–lipid complexes and extent of distribution of the α-1,6 bonds between the amorphous and crystalline domains (Jayakody & Hoover, 2002).

The ultrasound technique is a very effective method for physical disruption of cellular structures, which enables the extraction of intracellular materials, such as starch contained in the cellular matrix (Suslick et al., 1999). The exposure of polymer solutions to

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http://dx.doi.org/10.1016/j.lwt.2014.03.015
0023-6438/

high intensity ultrasonic radiation appears to result in the reducing of molar mass as a first effect. The hydrolysis occurs preferably near the middle of the chain, without causing important changes in the chemical structure (Madras & Kumar, 2000; Price & Smith, 1993).

Araucaria angustifolia belongs to the Araucariaciae family and is the only native Brazilian conifer species with economic importance (Zandavalli, Dillemburg, & Souza, 2004). The Araucaria Forest is distributed through Brazil (mainly in the Southern region), Chile, Argentina and Paraguay. The exploitation of this tree is over a hundred years, due to the quality of its wood, which is used by the furniture, building, and ship mast industries. Due to irrational extraction, Araucaria is endangered and currently is under environmental protection (Danner, Zanette, & Ribeiro, 2012). The seed of *A. angustifolia*, namely *pinhão*, is considered a source of starch, dietary fiber and magnesium, and its intake produces a low glycemic index (Cordenunsi et al., 2004). However, reports on nutritional and technological aspects of *pinhão* are scarce in the scientific literature.

The perception for nanotechnology applications in the food industry has become more apparent in recent years (Neethirajan & Jayas, 2011). Starch, being a biodegradable natural polymer, is a good candidate for the formation of nanocrystals or nanoparticles. Recent studies have shown that they could be used as fillers to improve mechanical and barrier properties of biocomposites (Le Corre, Bras, & Dufresne, 2010; Song, Thio, & Deng, 2011). *Pinhão* is primarily composed of starch and can be considered as a new source of this polysaccharide (Thys, Noreña, Marczak, Aires, & Cladera-Olivera, 2010). The aim of this study was to produce nanoparticles from *pinhão* starch, by applying ultrasound and acid hydrolysis, and to characterize the obtained nanoparticles.

2. Materials and methods

2.1. Materials

Pinhão seeds used in this work were obtained in Nova Petrópolis (Southern Brazil), harvested in 2011 and 2012. The seeds were selected by visual inspection, washed with tap water and subsequently stored at −18 °C in polyethylene bags.

2.2. Preparation of starch samples

The seeds were manually peeled and sliced. Water was added to a ratio of 1:2 (mL/mL) and the mixture was grinded. The obtained solution was passed through two sieves (0.250 and 0.074 mm) and collected in a recipient that was maintained at 4–5 °C for 40 min. After the precipitation of starch, the supernatant was discarded and the precipitate was washed with distilled water (the amount of water corresponded to the supernatant). This process was repeated four times. The starch obtained by precipitation was subjected to drying in an spray-drier (1.0 LABMAQ LM, São Carlos, Brazil) using inlet temperature of 90 °C, outlet temperature of 55 °C and a flow rate of 0.61 L/min.

For starch treatment by ultrasound, a solution containing 10 g of starch in 500 mL of distilled water was prepared. This mixture was subjected to a probe-type ultrasound (Unique OF S500, São Carlos, Brazil) using a power of 100 W. The sample was exposed to 30 cycles of sonication for 1 min, followed by 1 min stopped to allow the cooling of the sample in the ice bath. The resulting sample was subjected to spray drying as described above.

Starch treatment by acid hydrolysis was performed with a solution prepared with 1.1 g starch diluted in 500 mL of 2 mL/100 mL HCl, maintained for up to 50 days at 22 °C and stirring daily. After the stipulated time, samples were washed 5 times with distilled water at 4–5 °C. The material obtained was subjected to spray drying, as described above.

2.3. Characterization of native starch and starch nanoparticles

The particle size distribution of native starch was determined using the equipment Cilas 1064 Particle Size Analyzer (Cilas, Madison, USA). The size and polydispersity (PDI) of the nanoparticles was determined by dynamic light scattering (DLS; BI-200M goniometer, Brookhaven Instruments, Holtsville, USA). The XRD patterns were obtained on Siemens D-5000 X-ray diffractometer.

The chemical composition was determined according to the AOAC (1990) methodologies. The moisture content was measured considering the weight loss of samples subjected to heating at 105 °C (protocol 945.15). The measurement of water activity was performed by method number 978.18, using the equipment Aqualab S37E (Decagon Devices, Pullman, USA). The determination of crude fiber was determined based on organic insoluble residue insoluble in the samples, after acid and alkaline digestion, using the protocol 962.09. The methodology used to determine the ash content was based on weight loss of the material subjected to the burning furnace at the temperature of 550 °C (protocol 923.03). The procedure used to determine lipid was based on weight loss of the material subjected to extraction in a Soxhlet extractor with petroleum ether. The amount of protein was determined using the Kjeldal method (protocol 2055), which determines the total nitrogenous in the samples.

The determination of starch was conducted according to the AOAC (1990) methodology, based on the Lane–Eynon method (protocol 923-09). The amylose content of the samples was determined by the colorimetric method of McGrance, Cornell, and Rix (1998). The determination of reducing sugars was performed using the 3,5-dinitrolsalycilic acid method (Chaplin, 1986). The method of Eastman and Moore (1984) modified by Spada, Marczak, Tessaro, and Noreña (2012) was used to measure the percentual of solubility. The hygroscopicity was measured based on the method developed by Cai and Corke (2000), and the method proposed by Singh, Sandhu, and Kaur (2004) was used to determine syneresis and paste clarity. Colorimetric analysis was performed by direct reading in the equipment Chroma Meter CR-400 (Minolta, Osaka, Japan), using the CIELab system; the parameters L^* a^* b^* were used to describe the *Chroma* and *Hue* values (Fante & Noreña, 2012).

The morphological analysis of the native and modified starch samples by scanning electron microscopy followed the method developed by Thys et al. (2008), using the microscope JEOL JSM-6060 (JEOL, Tokyo, Japan) operating at 10 kV.

2.4. Statistical analysis

The analyzes were performed in triplicate and expressed as means ± standard error of measurement (s.e.m.). The statistical evaluations were conducted in SAS 9.3 for comparison of means (Tukey test) and Origin 5.0 software for analysis of crystallinity index.

3. Results and discussion

3.1. Particle size of native and modified starch

The average diameter of native starch was 15.34 μm, and the starch particles were mostly in the range of 12–28 μm. This value was similar to that found by Spada et al. 2012 (15.01 μm) and also in agreement with the range of average diameter of 10–25 μm

described by Bello-Pérez et al. (2006). Thys et al. (2008) also obtained similar results, reaching values ranging 7–20 μm.

The application of the two methods proposed in this work resulted in starch nanoparticles. The acid-modified starch had an average diameter of 21.8 nm with mean polydispersity of 0.202. Ultrasound-treated starch resulted in an average diameter of 454.3 nm and the polydispersity was 0.380. These values of polydispersity indicate a narrow size distribution and homogeneity of the nanoparticles. The samples differ significantly in both parameters analyzed, showing that the samples subjected to acid hydrolysis resulted in particles with more homogeneous size. The values of the average diameter of the sample subjected to acid hydrolysis and ultrasound represent a size reduction of about 700 and 35-fold, respectively, when compared to native starch. The higher size reduction observed for acid hydrolysis is probably associated to the extended treatment time, since starch fragments are successively released from the surface of the granule resulting in a small particle size.

3.2. Chemical characterization of starch samples

The composition of native starch and starch nanoparticles obtained by acid hydrolysis and ultrasound are shown in Table 1.

The recovery ratio of the samples ranged from 67 to 78 g/100 g for acid-modified and native starch, respectively. A significant difference was observed among samples (Table 1). This fact can be explained by the hydrolysis reactions occurring during starch modification, resulting in the release of smaller molecules, which are solubilized in water and therefore would be discarded with the supernatant during centrifugation. Previous data for native *pinhão* starch indicate variable results. Bello-Pérez et al. (2006) found a starch content of approximately 86 g/100 g, while Henríquez et al. (2008) reported that the sample contained 77.2 g/100 g, a value similar to that observed in this study. Thys et al. (2010) determined a content of 69 g/100 g, similar to the values found in starch modified by acid hydrolysis and ultrasound.

Regarding the amylose content, the values obtained in this work for the native starch and starch modified by ultrasound were similar to the results described in the literature. Bello-Pérez et al. (2006), Stahl et al. (2007) and Thys et al. (2008) found values between 22 and 26 g/100 g. However, the amylose amount was significantly reduced in starch that underwent the acid hydrolysis process. This decrease can be attributed to the fact that the hydrolysis reaction is initiated in the amorphous region of the granule, where amylose is located (Jayakody & Hoover, 2002), and combined with the long reaction time could result a low amylose value.

Table 1
Physical–chemical properties of native *pinhão* starch and starch nanoparticles obtained by acid hydrolysis and ultrasound processing.

	Native starch	Acid-modified starch	Ultrasound
Starch (g/100 g)	$78.09^{a} \pm 0.26$	$67.03^{c} \pm 0.26$	$72.63^{b} \pm 0.31$
Amylose (%)	$20.72^{a} \pm 0.21$	$5.2^{b} \pm 0.78$	$23.05^{a} \pm 0.57$
Protein (g/100 g)	$0.39^{a} \pm 0.04$	$0.36^{a} \pm 0.12$	$0.39^{a} \pm 0.06$
Lipids (g/100 g)	$0.55^{a} \pm 0.03$	$0.53^{a} \pm 0.02$	$0.54^{a} \pm 0.04$
Reducing sugars (mg/ml)	traces	traces	traces
Crude fiber (g/100 g)	$0.15^{a} \pm 0.04$	$0.13^{a} \pm 0.02$	$0.12^{a} \pm 0.02$
Ash (g/100 g)	$0.001^{a} \pm 0.0005$	$0.001^{a} \pm 0.0004$	$0.001^{a} \pm 0.0002$
Moisture (g/100 g)	$7.52^{a} \pm 0.11$	$7.29^{a} \pm 0.02$	$7.39^{a} \pm 0.26$
Water activity	$0.26^{a} \pm 0.01$	$0.29^{a} \pm 0.03$	$0.24^{a} \pm 0.001$
Solubility (%)	$3.27^{a} \pm 0.58$	$16.90^{b} \pm 0.95$	$1.43^{a} \pm 0.25$
Hygroscopicity (g H_2O/100 g)	$32.90^{a} \pm 0.60$	$39.62^{b} \pm 1.05$	$35.82^{a} \pm 0.15$

Values are the average of three replicates ± s.e.m. Same superscript letters in the same line do not differ by Tukey's test ($P > 0.05$).

The amounts of lipids and protein in the starch samples extracted from *pinhão* seeds were considerable lower when compared to the raw seeds. A decrease of approximately 60% and 89% in lipid and protein content was observed in comparison to the raw seeds (Cladera-Olivera, Marczak, Noreña, & Pettermann, 2009; Cordenunsi et al., 2004). The values for lipid concentration were similar to those obtained by Thys et al. (2008), which found 0.47 g/100 g for the lipid content of water-extracted *pinhão* starch. According to Buléon, Colonna, Planchot, and Balls (1998), lipids, proteins, enzymes, amino acids and nucleic acids are the components often removable by the starch extraction procedures. Thus, a similar rationale can be done for the reduced amounts of reducing sugars, crude fiber and ash of starch samples as compared with the amounts reported in the raw seeds (Cordenunsi et al., 2004).

Henríquez et al. (2008) reported moisture of native *pinhão* starch of 10.84 ± 0.03 g/100 g, obtained by drying in an oven at 60 °C for 1 h and Spada et al. (2012) found values of 6.23 ± 2.09 g/100 g in the form of lyophilized microcapsules. Thus, it can be seen that the results obtained in this study are within an expected range (Table 1), and the moisture of the samples depends on the type of drying to which the *pinhão* starch is subjected.

The a_w values obtained for starch samples were in the range corresponding to the higher food stability, i.e., the range from 0.2 to 0.4 (Chirife & Buera, 1994). The a_w values achieved in this work for native and modified starches did not differ significantly. Cladera-Olivera et al. (2009) reported water activity of 0.305 ± 0.03 for *pinhão* flour dehydrated in hot air at 70 °C.

3.3. Physical and rheological properties

The results shown in Fig. 1 represent the percentage of exuded water (syneresis) during 120 h of storage at 4 °C. Despite the significant differences by Tukey's test, the syneresis of native starch and nanoparticles of ultrasound-treated starch showed similar values. However, syneresis of acid-modified starch nanoparticles showed higher values, which may be due to the long exposure time during treatment (50 days in acid solution). This treatment may result in a three-dimensional network formed by short amylose and amylopectin molecules, generating a weaker structure and thus fewer sites at which the water molecules might be entrapped.

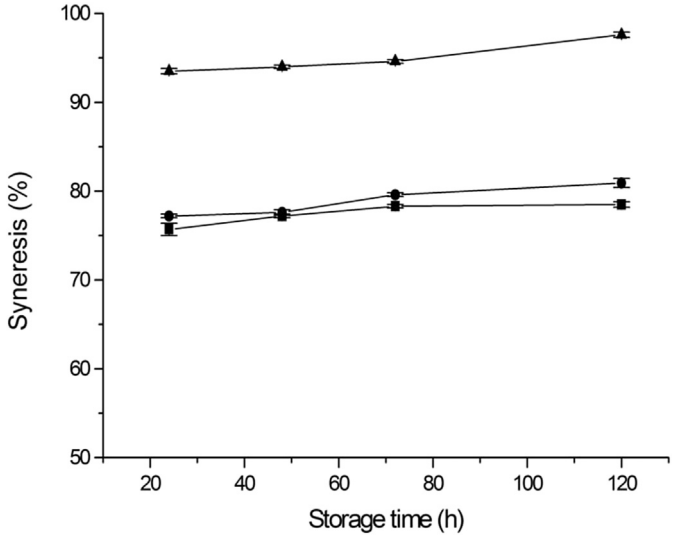

Fig. 1. Syneresis (%) of native starch (■) and nano starch obtained by ultrasound (●) or acid hydrolysis (▲). Values are the average of three independent experiments ± s.e.m.

The syneresis of the samples was almost constant during the storage time of 120 h, as can be visualized in Fig. 1.

The solubility of the starch molecules that underwent ultrasound processing showed no significant difference in relation to native starch (Table 1). Both samples have similar amylose content, which has a direct relationship with the solubility of starch molecules. The lower value observed for ultrasound-treated starch could be related to a partial assembly of amylose molecules induced by cavitation forces, reducing the solubility in relation to the untreated sample. This finding was also observed by Izidoro et al. (2007) for green banana starch. The high solubility index found in starch molecules subjected to acid hydrolysis may be associated to the increased degree of hydrolysis, which results in the significant increase in solubility of the samples (Loksuwan, 2007).

Similar hygroscopicity was observed for native starch and starch modified by ultrasound (Table 1). Only the samples from acid hydrolysis differed significantly, showing higher hygroscopicity values. This can be associated to the extensive hydrolysis of glycosidic linkages, yielding shorter molecules such as monosaccharides, disaccharides and small oligosaccharides, which are more hygroscopic than the polysaccharide due to the larger number free hydroxyl groups (Pérez & Berefoot, 2010).

The percentage transmittance of starch gels decreased significantly with storage time of the samples kept at 4 °C (Fig. 2). This decrease of paste clarity was also observed by several authors when analyzed starches from different botanical sources, including chickpea starch after 4 days of storage (Singh & Kaur, 2004), hollow olluco and mashua starches (Yamani, 2010), and green banana starch (Izidoro et al., 2007). According to these authors, this reduction in paste clarity can be related to the rearrangement of the amylose and amylopectin molecules, which would cause greater light absorption.

The starch subjected to ultrasound showed superior paste clarity as compared to native starch after 48 h storage, despite their similar amylose contents. The paste clarity can be explained not only by amylose amount, but also by smaller chains of this molecule, a characteristic that suggests easier alignment of linear chains (Karam, Grosmann, Silva, Ferrero, & Zaritzky, 2005). After 72 h storage, the sample became more turbid, which can be explained by the low stability of the amylose in solution, causing the association of linear chains through hydrogen bonds (Karam et al., 2005). The paste clarity of the starch modified by acid hydrolysis was the highest among the three samples, which can be associated to the lower amylose content of this sample. According to Takizawa, Silva, Konkel, and Demiate (2004), the solubility of starch is related to paste clarity, i.e., the more soluble the starch, more transparency has the paste. This observation agrees with the results of this work, because the molecules of acid hydrolyzed starch showed the higher solubility and increased transparency. In contrast, native starch and ultrasound-treated samples showed less solubility and higher turbidity.

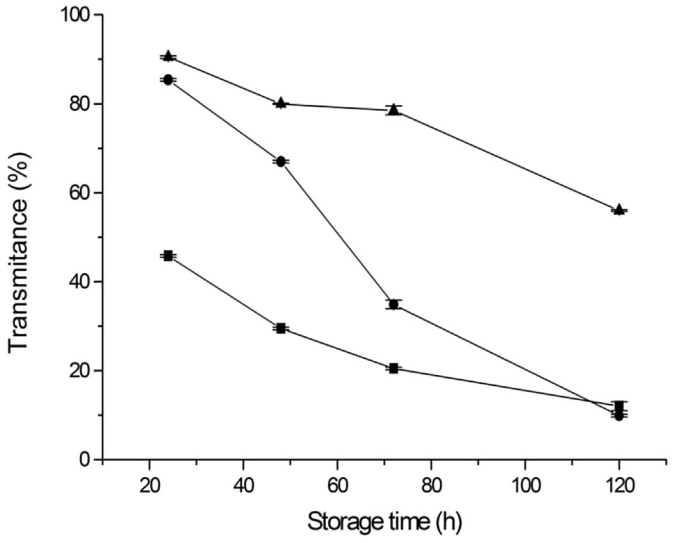

Fig. 2. Paste clarity of starch samples represented as transmittance (%) of native and nano starch gels versus storage time (h). Native starch (■) and modified nano starches obtained by ultrasound (●) and acid hydrolysis (▲). Values are the average of three independent experiments ± s.e.m.

3.4. Colorimetry

The colorimetric analysis of the starch samples showed values with significant differences among all parameters (Table 2), although these differences can be considered small when considering the standard interval used for these parameters. The brightness (L*) values of three samples analyzed approached to the white color, similar to L* = 92.03 observed by Henríquez et al. (2008) for isolated starch from pinhão (*Araucaria araucana* of Chile). The differences may be explained by the type of drying used. Starch exposed to the heat for a long time may result in darker samples than that obtained in this study. Cladera-Olivera et al. (2009) found L* = 88.18 for raw *pinhão* flour, indicating that there was a whitening of the extracted starch samples. This may be due to elimination of most of the other constituents, such as protein, fiber, sugars and minerals from the seed during the extraction process.

Although the samples differ significantly by Tukey's test, this difference was not visually noticeable because the major components often responsible for the color change (sugars) were eliminated during the extraction process.

Acid modified starch caused significant increase in the a* and b* values, and a similar trend was found for Pramodrao and Riar (2014) in starches during dry heat modification using ionic gums and dry heating. In general, the color of starch obtained by acid hydrolysis and ultrasound processing were among the red-yellow color (a* and b* parameters).

Chroma value changed upon process, decreasing color purity by ultrasound processing and this way lose saturation, but increasing through acid hydrolysis. In addition, the initial hue angle of native starch was 92.3° (Table 2), which represents a color in the very slightly green-predominantly yellow region (hue angle between 90° and 180°). However, hue angle decreased with the process (81.3° and 83.5°, which correspond to acid-modified and ultrasound, respectively) and their values shifted from the second to the first color quadrant shifting towards the slightly more reddish yellow region (hue angle less than 90°). Henríquez et al. (2008) reported that the Hue angle and the Chroma values were respectively 80 and 4.0 for the starch from *A. araucana* pinhão.

3.5. X-ray diffraction

The X-ray diffraction patterns obtained for the three samples (native starch and modified by acid hydrolysis and ultrasound)

Table 2
Colorimetric data of native *pinhão* starch and starch nanoparticles obtained by acid hydrolysis and ultrasound processing.

Parameter	Native starch	Acid-modified	Ultrasound
L*	$95.48^{a} \pm 0.15$	$94.98^{b} \pm 0.05$	$94.69^{c} \pm 0.07$
a*	$-0.08^{c} \pm 0.01$	$0.27^{a} \pm 0.03$	$0.14^{b} \pm 0.02$
b*	$1.54^{b} \pm 0.01$	$1.77^{a} \pm 0.06$	$1.22^{c} \pm 0.01$
Hue	$92.9^{a} \pm 0.8$	$81.3^{c} \pm 1.1$	$83.5^{b} \pm 0.5$
Chroma	$1.54^{b} \pm 0.01$	$1.79^{a} \pm 0.07$	$1.23^{c} \pm 0.02$

Values are the average of three replicates ± s.e.m. Superscript letters in the same line do not differ by Tukey's test ($P > 0.05$).

were very similar, as can be seen in Fig. 3. All starches showed the type A polymorph with predominant peaks appearing as one doublet at 2θ about 18° and a singlet at 22°. The starch subjected either to acid hydrolysis or ultrasound had similar patterns to that observed for native starch, suggesting that these procedures had no effect on the repeat distance of crystalline and amorphous lamellae of starch granules (Zheng, Han, & Bhatty, 1999). According to Salgado, Guerra, Andrade, and Oliveira (2005), the preservation of the crystallinity index indicates the maintenance of strong internal connections of the molecules and higher degree of association between the starch chains. On contrast, the crystalline structure of native tapioca starch was destructed by ultrasonic treatment. X-ray diffractometry showed that the crystalline structure was transformed into amorphous structure (Manchun, Nunthanid, Limmatvapirat, & Sriamornsak, 2012).

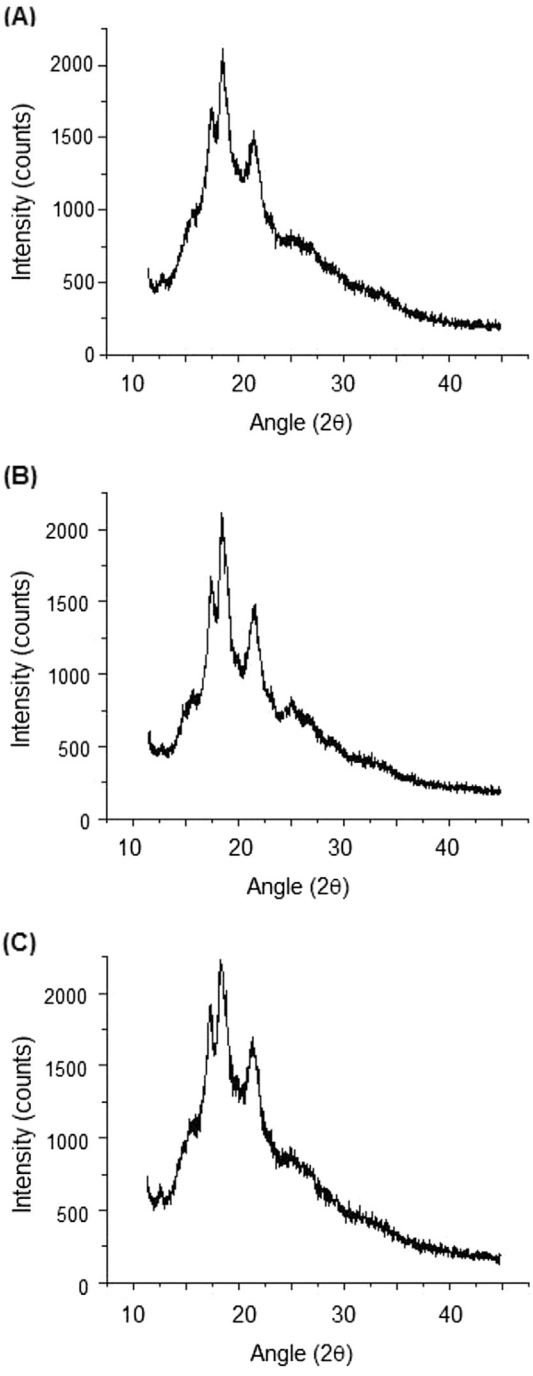

Fig. 3. X-ray diffraction of native starch (A) and modified nano starches obtained by ultrasound (B) and acid hydrolysis (C).

3.6. Electron microscopy

Through the images obtained by scanning electron microscopy, it was possible to evaluate the morphological changes in native and modified starch granules. The molecules of native starch had smooth surface without the presence of pores, but with some depressions (Fig. 4A), which are justified due to contraction of the particles during the drying and cooling that occur in the process of spray drying (Thies, 1995). Other authors who also worked with starch extracted from pinhão observed similar images (Bello-Pérez et al., 2006; Thys et al., 2008).

On contrast, the pinhão starch that underwent the acid hydrolysis process showed a rough appearance, with the extensive presence of pores in the surface (Fig. 4C). The surface erosion of starch after hydrolysis indicates that there was hydrolysis of the amorphous parts, mostly amylose, at the surface of the granules (Atichokudomchai, Shobngob, & Varavinit, 2000). Through the SEM images it can be concluded that this process of hydrolysis did not affect all of the granules uniformly; while some remained smooth, others had very irregular surface, which can affect their functional and physicochemical properties. It is also noteworthy the size reduction of the sample as compared to native starch samples, reaching nanometric size.

The starch subjected to ultrasound presented some irregularities in the surface, but in lower intensity than those subjected to acid hydrolysis. Some particles have depth-concave geometry (Fig. 4F). Furthermore, it can be seen that the granules exposed to sonication lean to cluster, which confirms the above hypothesis that the amylase molecules would have agglomerated during processing, thereby decreasing the solubility of the sample despite the similar content to native starch. Jambrak et al. (2010) explain this fact due to the rupture of hydrogen bonds, making possible novel connections and links between the polymers. Those researchers also found that disruption of the starch chains could be induced by increasing the intensity of ultrasound application. This effect may have occurred in this study, because the frequency used by them (24 kHz) was similar to that used in this study (20 kHz).

Previous reports on starch nanoparticles derived from rice, cassava and corn indicates that nanosized starches can be useful as fillers to improve mechanical and barrier properties of biocomposites (Chivrac, Pollet, & Avérous, 2009; Le Corre et al., 2010). However, development of nanostructures from *pinhão* starch has not been previously described in the literature. *Pinhão* starch presents some specific characteristics (Thys et al., 2008), which could be exploited as an alternative polysaccharide in nanocomposite development. The starch nanoparticles, for their different properties from those of native starch granules, could be utilized in new applications, such as development of novel biocomposites with improved properties.

4. Conclusion

The methods used to modify the starch extracted from the raw seeds of *pinhão* were effective, resulting in nanometric particles. The chemical composition of the three samples differed significantly only in relation to content of starch and amylose. The ultrasound-processed starch was more similar to native starch, presenting difference only in the syneresis. The starch modified by acid hydrolysis showed the greatest differences, being more soluble, more translucent and more hygroscopic among the samples.

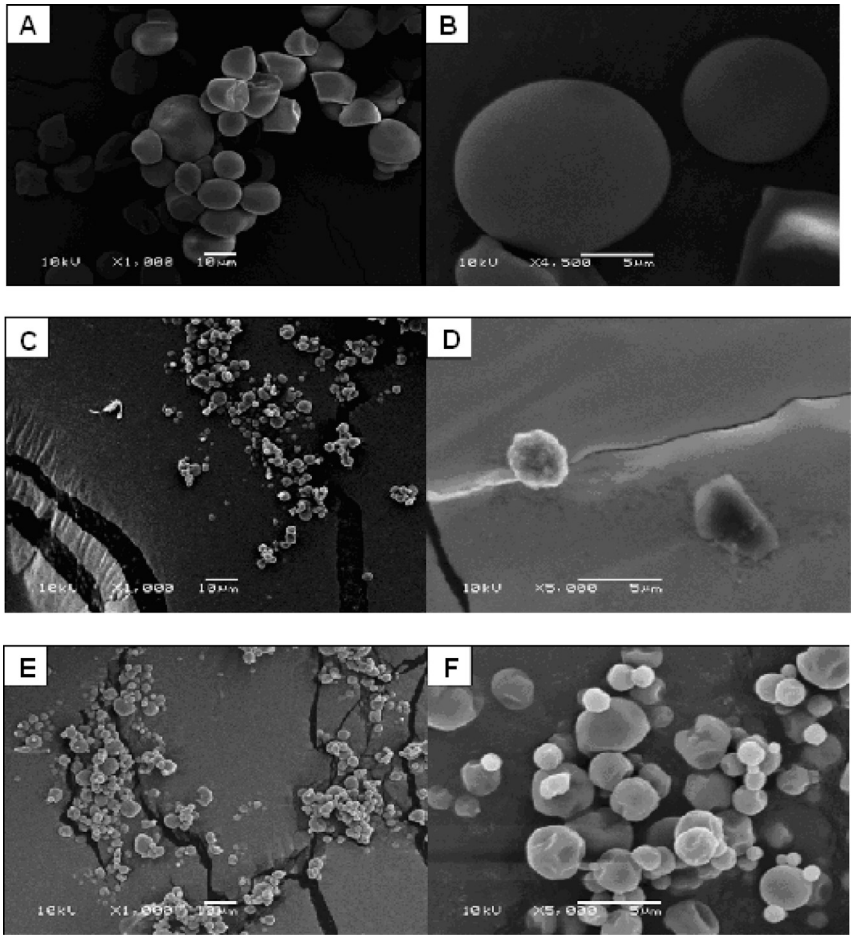

Fig. 4. Micrographs obtained by scanning electron microscopy of native *pinhão* starch (A,B) and starch subjected to acid hydrolysis (C,D) or ultrasound (E,F).

The greater solubility and reduced turbidity are interesting from a commercial standpoint, indicating that pinhão starch nanoparticles could be useful for development of coating materials or films.

Acknowledgments

This work was supported by the Brazilian agencies CNPq (grant 305693/2009-3) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

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