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Effects of single and dual physical modifications on pinhão starch



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ABSTRACT

Pinhão starch was modified by annealing (ANN), heat-moisture (HMT) or sonication (SNT) treatments. The starch was also modified by a combination of these treatments (ANN-HMT, ANN-SNT, HMT-ANN, HMT-SNT, SNT-ANN, SNT-HMT). Whole starch and debranched starch fractions were analyzed by gel-permeation chromatography. Moreover, crystallinity, morphology, swelling power, solubility, pasting and gelatinization characteristics were evaluated. Native and single ANN and SNT-treated starches exhibited a CA-type crystalline structure while other modified starches showed an A-type structure. The relative crystallinity increased in ANN-treated starches and decreased in single HMT- and SNT-treated starches. The ANN, HMT and SNT did not provide visible cracks, notches or grooves to pinhão starch granule. SNT applied as second treatment was able to increase the peak viscosity of single ANN- and HMT-treated starches. HMT used alone or in dual modifications promoted the strongest effect on gelatinization temperatures and enthalpy.

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1. Introduction

Traditionally, starch has been obtained from corn, wheat, rice, potato and tapioca for food and non-food applications. Non-conventional starch sources, such as pinhão seed, have gained increased research interest due to their unique physicochemical characteristics and potential alternative uses. Pinhão seeds are from Paraná pine (*Araucaria angustifolia* Bert O. Ktze) grown in native forests of southern Latin America. The main interest in *Araucaria*'s tree is related to timber exploitation and their seeds are under-utilized. Considering that 68–72% of the seed is composed of starch, it is reasonable to take advantage of this characteristic to develop novel starch technologies that broaden its industrial applications and may prevent the clandestine timber exploitation of *Araucaria* (Bello-Pérez et al., 2006).

Native pinhão starch presents a C-type crystalline structure, 26–27% of amylose content, and high paste viscosity and retrogradation (Bello-Pérez et al., 2006; Pinto et al., 2012). The native starch properties can be modified by applying physical, chemical or enzymatic modifications. Physically processed starches can be

considered as a natural material that is safe. The most common physical modifications are heat-moisture treatment (HMT) and annealing treatment (ANN). HMT is carried out under limited moisture content (10–30%) and elevated temperatures (90–120 °C), while ANN treatment typically involves the presence of higher moisture content (50–60%) and relatively lower temperatures that are below the onset temperature of gelatinization (Zavareze & Dias, 2011). Sonication treatment (SNT) is another physical treatment that has been successfully used as physical modification method (Chan, Bhat, & Karim, 2010; Zuo, Knoerzer, Mawson, Kentish, & Ashokkumar, 2009). Ultrasound possesses audio frequency beyond what the human is able to hear (>20 kHz). It can be generated with either piezoelectric or magnetostrictive transducers that create high-energy vibrations. These vibrations are amplified and transferred to a sonotrode or probe, which is in direct contact with the fluid (Feng, Barbosa-Cánovas, & Weiss, 2011; Jambrak et al., 2010).

The effects of different temperature and moisture conditions during HMT on the physicochemical, pasting, crystallinity, thermal and morphological properties of pinhão starch were studied by Pinto et al. (2012). However, no reports regarding the characteristics of ANN- and SNT-treated pinhão starch are found in the literature. Modification of starches using dual ANN and SNT has recently received the attention of scientists. Dual HMT was performed by Klein et al. (2013) in rice, cassava and pinhão starches.

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The authors reported a change in the crystalline structure type of pinhão starch from C-type to A-type when the starch was subjected to HMT. Moreover, there was greater effect of HMT when 120 °C was used instead of 100 °C. These authors reported that the single and dual HMT-treated pinhão starches had lower relative crystallinity, swelling power, peak viscosity, and gelatinization enthalpy than to the native starch. The combined effects of ANN and HMT were studied by Chung, Hoover, and Liu (2009) in corn, pea, lentil and navy bean starches (Chung, Liu, & Hoover, 2010), and in waxy rice starch (Zeng, Ma, Kong, Gao, & Yu, 2015). In general, the ANN–HMT and HMT–ANN modifications resulted in similar effects on the structural and functional properties of these starches. Several studies have investigated the effect of physical modification of different starches using sonication treatment, including those from rice, potato, wheat and corn (Chan et al., 2010; Sujka & Jamroz, 2013; Zuo et al., 2009). In general, sonication treatment attacks mainly the amorphous regions of starch granule, producing microporous structures within the granule (Sujka & Jamroz, 2013).

This study aims to evaluate the amylose and amylopectin chains distribution, and the crystallinity, morphological, pasting and thermal properties of pinhão starch subjected to single (ANN, HMT, and SNT) and dual (ANN–HMT, ANN–SNT, HMT–ANN, HMT–SNT, SNT–ANN, and SNT–HMT) physical modifications.

2. Materials and methods

2.1. Material

Pinhão seeds (*A. angustifolia* Bert O. Ktze) were purchased from a popular market in the city of Pelotas, State of Rio Grande do Sul, Brazil. The pinhão starch was isolated using water as solvent and dried at 40 °C overnight according to the method described by Pinto et al. (2012). The obtained starch had a 99% purity. All chemicals used in this work were ACS grade.

2.2. Starch modifications

2.2.1. Annealing treatment (ANN)

Pinhão starch (200 g, dry basis) in excess of deionized water (800 mL) were placed in a 1 L glass container, covered with aluminum foil, and incubated (MLR-350; SANYO Electric Co., Ltd., Ora-Gun, GU, Japan) at 50 °C for 16 h. At the end of the incubation period, the starch suspension was filtered through Buchner funnel containing paper filter, and washed twice with water, before being oven dried at 40 °C overnight (Fisher Scientific Isotemp Standard Lab Incubator, 230 D). The resulting samples were then ground for further analyses.

2.2.2. Heat-moisture treatment (HMT)

The moisture content of isolated pinhão starch was determined and the amount of water to give 22% moisture content as added. Starch (300 g) was continuously stirred in a laboratory mixer (Cuisinart Stand Mixers, Model SM-55C, China) and the amount of distilled water needed for the starch to achieve 22% moisture content was gradually added and mixed. Subsequently, the starch samples were left to equilibrate at 4 °C overnight before being placed in sealed 1 L bottles and HMT by autoclaving at 100 °C for 1.0 h, and further oven dried at 40 °C overnight. The resulting samples were then ground for further analyses.

2.2.3. Sonication treatment (SNT)

Pinhão starch samples (100 g) were suspended in deionized water (400 mL) in 1 L beaker and subjected to sonication in an ultrasonic processor (Vibra-cell VC 505, Sonics and Material Inc.,

Newtown, Conn., USA) using a CV33 probe head for 90 min (30 s on and 5 s off pulses, 50% of amplitude frequency of 20 kHz). The sonicator horn head was 5 mm in diameter. The ultrasonic treatment was conducted in an ice bath to prevent thermal starch modification or gelatinization. After this process, the starch suspension was filtered through Buchner funnel containing paper filter, washed twice with water and oven dried at 40 °C overnight. The resulting samples were then ground for further analyses.

2.2.4. Dual modifications using sequential treatments

Annealed starch from Section 2.2.1 was further modified by heat-moisture treatment either with HMT as outlined in Section 2.2.2 (ANN–HMT) or SNT treatment as described in Section 2.2.3 (ANN–SNT). Similarly, heat-moisture-treated starch samples from Section 2.2.2 was further modified using the same annealing procedure in Section 2.2.1 (HMT–ANN) and by SNT treatment from Section 2.2.3 (HMT–SNT). Finally, SNT starch samples from Section 2.2.3 were also further modified by ANN (Section 2.2.1; SNT–ANN) and HMT treatment (Section 2.2.2; SNT–HMT).

2.3. Gel-permeation chromatography (GPC) on Sepharose CL-2B of whole starch

Samples were prepared as described by Bertoft, Piyachomkwan, Chatakanonda, and Sriroth (2008), with some modifications described by Pinto et al. (2015). Starch granules (7 mg) were dissolved in 90% DMSO (0.3 mL) by gently stirring at room temperature overnight, followed by dilution with warm water (1.0 mL). The starch components were fractionated on a Sepharose CL-2B column (1.6 × 32 cm) (Pharmacia, Uppsala, Sweden). A dissolved starch solution of 0.3 mL was eluted through the column with 0.01 M NaOH at a rate of 0.5 mL min⁻¹. Fractions of 1 mL were collected, and the carbohydrate content was determined by phenol-sulfuric acid reagent (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956). The maximum wavelength (λ_{\max}) of the starch/iodine complex was determined using 0.1 mL of iodine solution (0.01 M I₂/0.1 M KI) after the fractions had been neutralized with 0.01 M HCl (Morrison & Laignelet, 1983). All determinations were duplicated.

2.4. Analysis of debranched starch on Sepharose CL-6B

The starch samples (4 mg) were dissolved in 90% DMSO and debranched with pullulanase and isoamylases (Megazyme, Wicklow, Ireland), following the procedure as described by Bertoft et al. (2008). The chain length distribution of the debranched starch samples (1 mL) was determined by GPC using a Sepharose CL-6B column (1.6 × 90 cm) (Pharmacia, Uppsala, Sweden), eluted with 0.5 M NaOH at 1 L/min. Fractions of 1 mL were collected and every 1 mL fraction was analyzed for carbohydrates using the phenol-sulfuric acid reagent (DuBois et al., 1956). The amylose content was determined following the procedure by Bertoft et al. (2008). All determinations were triplicated.

2.5. Wide angle X-ray diffraction (WAXD) analyses

X-ray diffractograms of the starches were obtained with an X-ray diffractometer (Rigaku Powder Diffractometer, Rigaku Co., Tokyo, Japan) using a CuK α 1 radiation source from ($\lambda = 1.54 \text{ \AA}$) at 2 theta degree. The scanning region of the diffraction ranged from 5° to 30°, with a target voltage of 30 kV, a current of 30 mA, and a scan speed of 1°/min. The relative crystallinity of the starch granules was calculated following the equation $RC (\%) = [A_c / (A_c + A_a)] \times 100$, where A_c is the crystalline area and A_a is the amorphous area in the X-ray diffractogram.

2.6. Scanning electron microscopy (SEM)

A small quantity of each starch sample was spread directly on to the surface of the stub and coated with gold (20 nm) using a sputter coater (Desk V, New Jersey, USA). Starch morphology was examined using a scanning electron microscope (Jeol JSM-6610LV, New Jersey, USA) at an accelerating voltage of 10 kV.

2.7. Swelling power and solubility

The swelling power and solubility of the starches were determined as described by Leach, Mcwen, and Schoch (1959). All determinations were triplicated.

2.8. Pasting properties

The pasting properties of the starch samples were determined using a Rapid Visco Analyser (RVA – 4, Newport Scientific, Australia) with Thermocline software for Windows (Version 3). Starch (3.0 g, 14 g/100 g moisture content) was weighed directly into the RVA canister, and 25 mL of distilled water was added. The sample was heated to 50 °C and stirred at 160 rpm for 10 s for thorough dispersion. The slurry was held at 50 °C for up to 1 min, and then heated to 95 °C for 7.2 min and held at 95 °C for 5 min, and finally cooled to 50 °C over 7.4 min and then kept at 50 °C for 2 min. All determinations were duplicated.

2.9. Thermal properties

Thermal properties from native, single and dual modified pinhão starches were determined using differential scanning calorimeter (DSC-60, Shimadzu, Kyoto, Japan). Starch samples (approximately 2.5 mg) were weighed directly in an aluminum pan (Mettler, ME-27331) and distilled water was added (1:3 w/v). The pan was hermetically sealed and allowed to equilibrate for 1 h before analysis. An empty pan was used as a reference. The sample pans were then heated from 40 to 140 °C at the rate of 10 °C min⁻¹.

2.10. Statistical analyses

The RVA, swelling power and solubility results were subjected to analysis of variance (ANOVA), followed by post hoc Tukey's test at a 5% significance level.

3. Results and discussion

3.1. Amylose and amylopectin chains distribution

3.1.1. Molecular fractions as determined by GPC on Sepharose CL 2B

Native and single modified pinhão starches were eluted through a Sepharose CL-2B gel permeation column. The molecular weight distribution and the wavelength maxima of iodine complex (λ_{\max}) were recorded and are presented into Fig. 1. The fractions can be divided in high molecular weight fraction (HMF) (fractions 18–30), intermediate molecular weight fraction (fractions 30–40), and low molecular weight fraction (LMF) (fractions 40 and above). The relative abundance of HMF and LMF was similar between all treatments (data not shown). The materials eluted from the gel showed a lower λ_{\max} for HMF than LMF (Fig. 1A). There was an increase in the λ_{\max} of HMF of modified starches as compared to native pinhão starch, in the following order: HMT > ANN = SNT > Native. According to Vamadevan, Hoover, Bertoft, and Seetharaman (2014), the overlapping of amylopectin with intermediate components with similar size to those of smaller

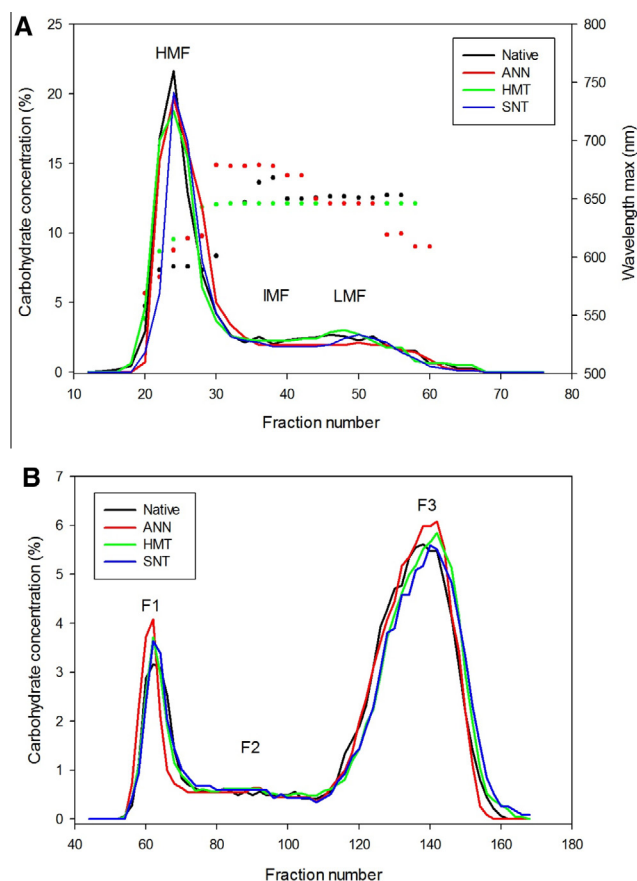


Fig. 1. (A) Fractionation of native and single-modified pinhão starches by gel permeation chromatography (GPC) using Sepharose CL 2B. Curves were divided in three fractions: high molecular weight fraction (HMF), intermediate molecular weight fraction (IMF) and low molecular weight fraction (LMF). Wavelength maxima (λ_{\max}) of the iodine–glucan complex are indicated as dots for each treatment following the same color legend than GPC curves. The λ_{\max} of ANN-treated and SNT-treated starches was the same. (B) Fractionation of debranched native and single-modified pinhão starches by GPC using Sepharose CL 6B. Curves were divided in fractions F1, F2 and F3, which represents long, medium and short chains, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

amylopectin molecules is indicated by high λ_{\max} . The λ_{\max} of IMF was higher in ANN- and SNT-treated starches, but similar between HMT and native starches (Fig. 1A). Zheng et al. (2013) who reported cleavage of α -1,6 linkages in amylopectin chains of sweet potato starch by ultrasound treatment, no α -1,6 cleavage was observed in pinhão starch. This behavior can probably be attributed to the higher sonication frequencies (25 and 80 kHz) used by Zheng et al. (2013) as compared to that used (20 kHz) in the present study. The molecular weight profiles curves for starches subjected to sequential treatments were very similar to those from single treatment (data not shown).

3.1.2. Molecular fractions as determined by debranching of starch

Three apparent fractions can be detected by GPC for native and modified pinhão starches after debranching with pullulanase and isoamylase (Fig. 1B). Fractions F1, F2 and F3 were comprised of long, intermediate, and short glucan chains, respectively. Fraction F3 has been related to amylopectin chains by Vamadevan et al. (2014). The native and the single ANN- and HMT-treated pinhão starches exhibited similar relative proportions of eluted starch fractions (data not shown). Kohyama and Sasaki (2006) reported that ANN had no effect on α -1,4 glycosidic linkages of starch.

Jiranuntakul, Puttanlek, Rungsardthong, Pancha-arnon, and Uttapap (2012) reported that starch chain distributions as well as unit chain distributions of the treated samples were not affected by HMT at 25% moisture content, 100 °C for 16 h; however, higher treatment temperatures (120 and 140 °C) can promote thermal degradation on amylose molecules. The use of 22% moisture content and 100 °C, for 1 h, in the present study was not sufficient to induce thermal degradation of starch molecules during HMT, since no differences were observed in the relative proportion of F1–F3 fractions compared to native pinhão starch. However, an increase in the proportion of F1 fraction was noted for all SNT based modifications (data not shown). This was probably due to the shearing gradient provided by the microbubbles during cavitation process of sonication. In ultrasound treatments, intense agitation of starch slurry was generated by sonication and localized spots of very high temperature and pressure were created by cavitation activities (Zhang, Niu, Eckhoff, & Feng, 2005). These conditions might lead to configurational modifications of the granular structure, which could be in the forms of diffuse erosion or pitting of the starch granules as observed by Degrois, Gallant, Baldo, and Guilbot (1974). Cavitation process has been shown to be capable of causing some breakage of C–C linkages, resulting in the formation of polysaccharides of long linear chains (Suslick, 1990). Also Czechowska-Biskup, Rokita, Lotfy, Ulanski, and Rosiak (2005) studied the effects of 360 kHz ultrasound on aqueous solutions of chitosan and starch, and reported that it was an efficient procedure for reduction of molecular weight of polysaccharides, which was caused by OH radicals (favor intermediary reactions) and mechanochemical (breaking the polymers chains) effects introduced by ultrasound.

3.2. Wide angle X-ray diffraction (WAXD) and relative crystallinity (RC)

The X-ray diffraction pattern and the relative crystallinity of native and modified pinhão starches are presented in Fig. 2. WAXD pattern of A-type starches present strong diffraction peaks at diffraction angle 2θ of 15° and 23°, and an unresolved doublet at diffraction angle 2θ of 17° and 18°. On the other hand, the WAXD pattern of B-type starches possess strong diffraction peak at diffraction angle 2θ of 17°, with small peaks at diffraction angles 2θ of 15°, 20°, 22° and 24°, and a typical peak at diffraction angle 2θ of 5.6°. C-type starch is a mixture of both A- and B-type crystalline structures (Cai, Cai, Man, Zhou, & Wei, 2014; Cairns, Bogracheva, Ringa, Hedley, & Morris, 1997; Gernat, Radosta, & Damaschun, 1990).

In general, the single and sequential modified pinhão starches showed a similar WAXD diffraction pattern to native starch. As shown in Fig. 2, the X-ray diffractograms of native pinhão starch displayed peaks at 2θ 15°, 17° and 23°, and another small peak at 5.7°, which are typical of CA-type crystalline structures. Cai et al. (2014) described C-type crystalline starch granule as a mixture of both A- and B-type structures, being further classified as CA-type (closer to A-type), C-type, or CB-type (closer to B-type), depending on the proportion of A- and B-type allomorphs. The single ANN treatment seems to have favored the formation of a more organized crystalline region in pinhão starch, since the peak at 2θ of 5.7° was higher in ANN-treated than in native pinhão starch (Fig. 2A). Wang, Jin, and Yu (2013) reported an increase in B-type peak intensity ($2\theta = 5.7$) after ANN. When ANN starch was subjected to sequential treatment with HMT or SNT, the peak at diffraction angle 2θ of 5.7° disappeared, causing the crystalline structure of starch to change from CA-type to A-type. Vermeylen, Goderis, and Delcour (2006) when studied HMT potato starch reported that there was some losing on initial diffraction peak at 5.7° and, triggered gradually its crystalline pattern from B-type to A-type when treatment temperatures increased.

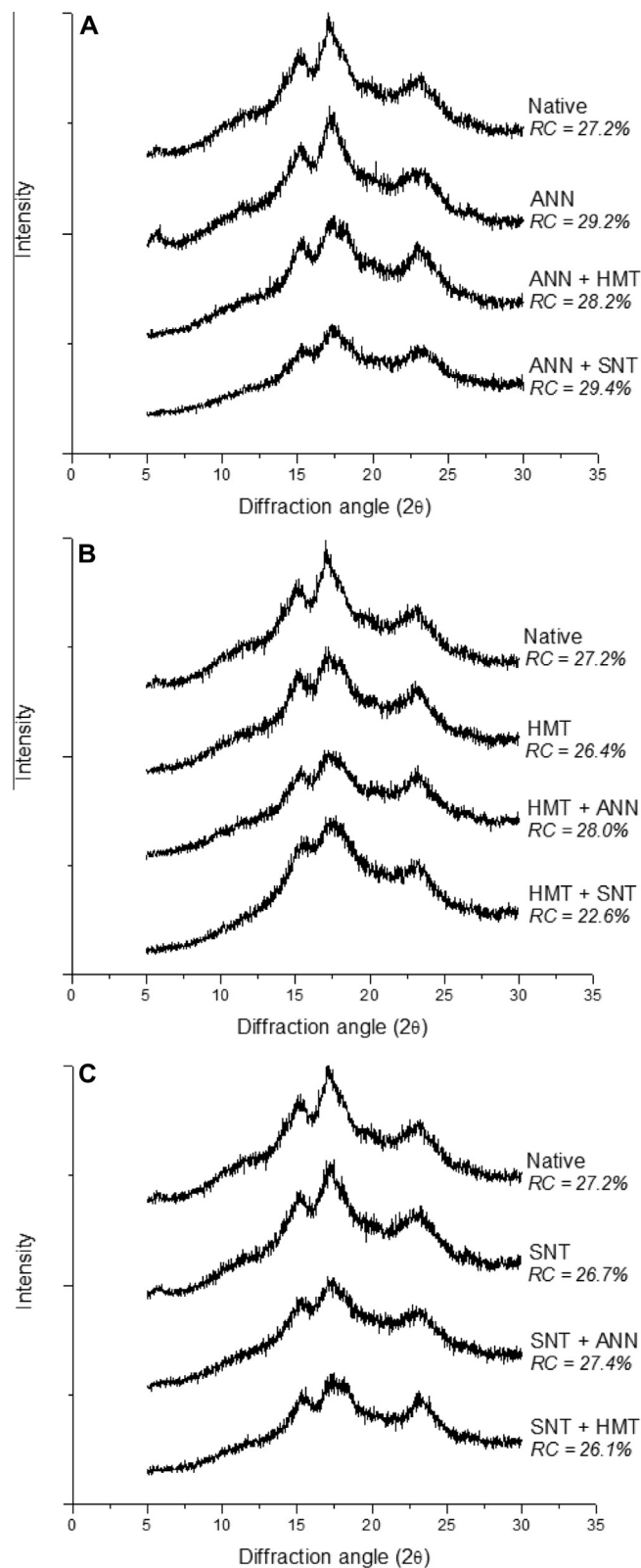


Fig. 2. X-ray diffractograms of native and modified pinhão starches.

Starch samples subjected to single ANN treatments possessed higher relative crystallinity than the native pinhão starch. On the other hand, HMT and SNT decreased the relative crystallinity of native pinhão starch (Fig. 2). This observation implies that ANN might have promoted the realignment of the starch chains within

the amorphous and crystalline regions of the granule (Zavareze & Dias, 2011), providing a more stable and crystalline structure to the granule. An increase in crystallinity as a function of ANN was also reported by Waduge, Hoover, Vasanthan, Gao, and Li (2006) in wheat and high-amylose barley starches, respectively. The decrease in the crystallinity of pinhão starch due to HMT, as observed in Fig. 2, was also observed by Klein et al. (2013). This could be attributed to the destabilization of the lamellar array of starch granules. Chung et al. (2009) reported a reduction in the relative crystallinity of corn starch after HMT, who attributed this to the disruption of amylopectin crystallites. Similar behaviors after SNT treatment (Fig. 2C) were observed by Bel Haaj, Magnin, Pétrier, and Boufi (2013) on normal and waxy maize starches. They reported a decrease in the crystallinity of ultrasonic treated starches, compared to the native starches.

Similar relative crystallinity was observed between starches modified by ANN–HMT and HMT–ANN (Fig. 2A and B). This observation is in accordance with those reported by Chung et al. (2009) on corn starch modified by ANN–HMT and HMT–ANN. ANN–SNT (Fig. 2A, ANN–SNT) did not affect the crystalline arrangement of starch granules provided by single ANN. The highest decrease in the relative crystallinity of native pinhão starch was observed with HMT–SNT starch (Fig. 2B). This behavior probably is due the combination of the modifications (HMT and SNT) that decreased the relative crystallinity when applied alone (Fig. 2B and C).

3.3. Scanning electron microscopy (SEM)

The scanning electron micrographs of native and single-modified pinhão starches are presented in Fig. 3A–J. The starch granules from all single treatments were round in shape, and for the most part the granules presented a flat surface on one side. The granules from native and single modified starches had smooth surfaces and similar size. Pinto et al. (2015) reported aggregation of pinhão starch granules after HMT with 25% moisture content and 110–120 °C. In the present study, the HMT was prepared with 22% moisture content and 100 °C, which were not enough to promote aggregation of starch granules (Fig. 3E). In agreement with the present study, Hoover and Vasanthan (1994) reported no change in granule morphology of wheat, oat, lentil and potato starches upon annealing. Sujka and Jamroz (2013) reported the presence of small visible cracks in the surface of corn, wheat and potato starch granules subjected to ultrasound. However, these authors did not detect visible cracks in rice starch granules subjected to the same ultrasound treatment. Zhu, Li, Chen, and Li (2012) reported notches and grooves in the surface of their potato starch granules after sonication treatment. However, in the present study, the single sonication conditions did not provide visible cracks, notches or grooves to pinhão starch granule (Fig. 3H).

The dual modifications caused changes on the starches granules surfaces (Fig. 3C, D, F, G, I and J). The changes that the annealing promoted on the starches granules when applied as the second treatment (HMT–ANN, SNT–ANN) were the most intense; these granules showed irregular surface and partial disintegration (Fig. 3F and I). The starches granules submitted to the HMT–SNT and SNT–HMT modifications had slight imperfections and non-smooth surface on their external structures (indicated by arrows in Fig. 3G and J). This characteristic can be due to the cavitation process occurred by microbubbles during sonication (Suslick, 1990) improved by HMT applied before or after.

3.4. Swelling power and solubility

The swelling power and solubility of native and modified pinhão starches are presented in Table 1. The single ANN starch presented similar swelling power and lower solubility compared

to native starch. All HMT samples, both from single and dual modification, presented lower swelling power and solubility than native starch. The lowest swelling power was observed with ANN–HMT and HMT–ANN starches. Single SNT treatment provided high swelling power to pinhão starch, with no effect on solubility as compared to native starch (Table 1). In general ANN reduces the swelling power and solubility of starches. The ANN conditions used facilitated the infusion of water into the amorphous layers (Jayakody & Hoover, 2008), but ANN starch seemed to not have retained the absorbed water during centrifugation. Pinto et al. (2012) observed a decrease in swelling power and solubility of pinhão starch as a function of HMT conditions, which is in agreement with the findings presented in Table 1. The decrease in swelling power of starches due to HMT has been attributed to increased crystallinity, amylose–lipid interactions, and/or interactions involving amylose–amylose and/or amylose–amylopectin chains (Chung et al., 2009; Hoover & Vasanthan, 1994). In the present study, it is more reasonable to attribute the reduced swelling power of HMT starches to amylose–amylose and/or amylose–amylopectin interactions, since there was a decrease in relative crystallinity of HMT starch compared to native starch, seeing the starch purity was 99%, and the residual lipids were very low.

Sujka and Jamroz (2013) reported an increase in swelling power and solubility of potato, wheat, corn, and rice starches subjected to sonication. The increase in swelling power has been attributed to the damage of the crystalline molecular structure of starch and bounding of water molecules to the free hydroxyl groups of amylose and amylopectin by hydrogen bonds (Singh, Singh, Kaur, Sodhi, & Singh Gill, 2003; Sujka & Jamroz, 2013). In the present study, the effects of OH radicals and mechanochemical, introduced by ultrasound (Czechowska-Biskup et al., 2005) can be considered to have influenced the higher swelling power of single SNT-treated starch as compared to native starch.

3.5. Pasting properties

The pasting properties of single and dual modified pinhão starches are presented in Table 2. Both ANN and HMT applied alone, increased the pasting temperature and final viscosity, while decreasing the peak viscosity and breakdown of pinhão starch. The single SNT increased the peak viscosity and breakdown of pinhão starch, with no changes on pasting temperature, setback and final viscosity. The increase in relative crystallinity (Fig. 2A) reflects the improvement on crystalline lamella (Vamadevan, Bertoft, Soldatov, & Seetharaman, 2013), and the bond strengthen provided by ANN (Adebowale, Henle, Schwarzenbolz, & Doert, 2009) can be attributed to the increasing in the temperature required to gelatinize starch granules after this treatment. Hoover and Vasanthan (1994) reported a decrease in peak viscosity and an increase in the final viscosity of potato and oat starches after ANN, suggesting that ANN increases the granular structure stability.

Previously, we showed that HMT increased pasting temperature, final viscosity and setback viscosity of pinhão starch, but reduced peak and breakdown viscosities, as compared to native pinhão starch (Pinto et al., 2012). These results are consistent with the data presented in Table 2. We hypothesized that HMT-treated pinhão starch possess a more stable structure when subjected to continuous heating and shearing, as compared to native starch. The decrease in breakdown promoted by HMT indicates that hydrothermal treatment improved the mechanical and thermal stability of starches (Watcharatewinkul, Puttanlek, Rungsardthong, & Uttapap, 2009). This stability can be attributing as a result of reorientation of starch granules molecules or chain (Sun, Dai, Nan, & Xiong, 2014) by strengthening of linkages between amylose and the amylopectin side chains (Zavareze & Dias, 2011).

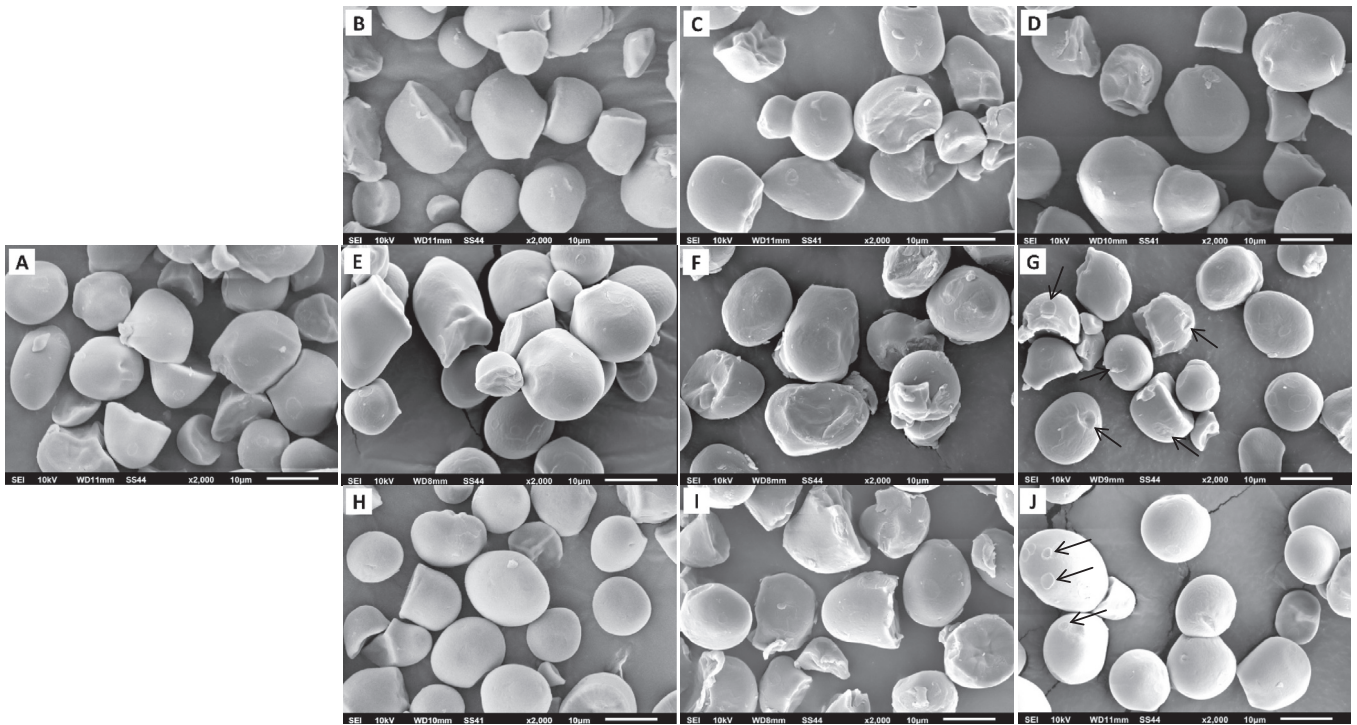


Fig. 3. Scanning electron micrographs of native, single and dual modified pinhão starches. (A) Native pinhão starch; (B) annealed (ANN) starch; (C) ANN–HMT starch; (D) ANN–SNT starch; (E) heat-moisture-treated (HMT) starch; (F) HMT–ANN starch; (G) HMT–SNT starch; (H) sonicated (SNT) starch; (I) SNT–ANN starch; (J) SNT–HMT starch.

Table 1
Swelling power and solubility of native and modified pinhão starches.

Treatment ^a	Swelling power (g/g)	Solubility (%)
Native	17.87 b	10.35 a
ANN	17.35 bc	7.48 b
ANN–HMT	13.34 g	3.59 c
ANN–SNT	16.68 cd	7.18 b
HMT	14.46 ef	3.51 c
HMT–ANN	13.83 fg	4.28 c
HMT–SNT	14.76 e	4.54 c
SNT	20.41 a	8.75 ab
SNT–ANN	16.16 d	4.26 c
SNT–HMT	15.27 e	3.59 c

^a Results are the means of three determinations. Values with different lowercase letters in the same column statistically differ ($p < 0.05$).

Regarding SNT-treated pinhão starch, the higher peak viscosity observed, as compared to native starch, may be related to the higher swelling power of the SNT-treated starch granules. The

cavitation process occurred by microbubbles during sonication (Suslick, 1990) provides high swelling power to SNT-treated starches, probably due some chains degradation (Czechowska-Biskup et al., 2005; Degrois et al., 1974) and/or weakening of linkages between amylose and the amylopectin side chains. Also, SNT-treated starch presented the highest breakdown (Table 2), which is indicative of a weaker physical structure of the granules, making them more susceptible to collapse while under thermal and shear treatments.

The starches samples from ANN–HMT and HMT–ANN presented similar peak viscosity, setback and final viscosity (Table 2). It corroborates with the similarity of chains distribution (Fig. 1), crystalline structure and relative crystallinity (Fig. 2), and swelling power and solubility (Table 1) between both ANN–HMT and HMT–ANN samples. However, ANN–HMT presented lower pasting temperature and higher breakdown than HMT–ANN. When SNT was used as the second treatment (ANN–SNT and HMT–SNT), it promoted an increase in the peak viscosity as compared to the ANN and HMT applied alone. This fact may be related

Table 2
Pasting properties of native and modified pinhão starches.

Treatment ^a	Pasting temperature (°C)	Peak viscosity (cP)	Breakdown (cP)	Setback (cP)	Final viscosity (cP)
Native	60.7 e	4761.0 b	2656.0 b	1675.5 cd	3861.5 e
ANN	65.0 c	3701.3 d	907.6 d	1885.0 c	4678.6 d
ANN–HMT	70.0 b	3290.0 ef	740.0 d	2868.5 b	5418.0 bc
ANN–SNT	64.5 c	4383.0 c	1487.0 c	1907.0 c	4803.0 d
HMT	70.2 b	3142.0 f	1402.0 c	3537.0 a	5277.0 c
HMT–ANN	74.5 a	3270.0 ef	402.5 e	2794.5 b	5662.0 ab
HMT–SNT	73.3 a	3578.0 de	739.5 d	3043.5 b	5882.0 a
SNT	60.8 e	5347.5 a	3058.5 a	1329.0 d	3618.5 e
SNT–ANN	62.6 d	4312.5 c	977.5 d	1880.0 c	5215.0 c
SNT–HMT	69.1 b	3915.0 d	873.5 d	2709.0 b	5750.5 a

^a Results are the means of three determinations. Values with different lowercase letters in the same column statistically differ ($p < 0.05$).

to the effects of cavitation promoted by SNT treatment. The HMT–SNT sample presented similar breakdown to single HMT-treated starch, suggesting that the SNT effect is minimized by using HMT starch. The HMT, used as both single or associated in dual treatments, showed higher effect on final viscosity than ANN, ANN–SNT, SNT and SNT–ANN.

3.6. Thermal properties

Thermal properties of native and modified pinhão starches were measured by DSC. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures of gelatinization, the temperature range of gelatinization (ΔT), and the enthalpy of gelatinization (ΔH) were determined and are presented in Table 3. There was an increase in T_o , T_p and T_c of all modified starches compared to native starch, except for SNT-treated pinhão starch. These results are in accordance with the data presented in Table 2, regarding pasting temperature measured by RVA. The increase in T_o , T_p and T_c has been attributed to structural changes within starch granule, which involves amylose–amylose and amylose–lipid interactions, repressing the mobility of starch chains within the amorphous lamellae (Hoover, 2010). On the other hand, lower temperatures after single SNT (Table 3) may be a result of the higher facility for water and heat transfer within the amorphous lamellae of starch granule. The slightly higher content of long chains (data not shown) observed in SNT-treated starch can be the responsible for its lower gelatinization temperatures than to the native starch. The ΔT decreased for all single- and dual-treated starches compared to native starch. Waduge et al. (2006) attributed lower ΔT to higher perfection of the crystalline structure and amylose–amylose and/or amylose–amylopectin interactions. Chung et al. (2009) suggested that HMT after ANN in corn starch may promote disruption of some of the crystallites that were perfected on ANN when compared to single HMT and native starch. This statement is partially supported by the increase in relative crystallinity of all modified starches, except HMT, SNT, HMT–SNT and SNT–HMT, which presented lower relative crystallinity than native starch (Fig. 2). These treatments, namely HMT, SNT, HMT–SNT and SNT–HMT displayed higher ΔT values as compared with other modified starches (Table 3), showing a strong correlation between ΔT and crystalline structure of starch granules.

Vamadevan et al. (2014) reported similar results regarding thermal properties of ANN-treated starches. The authors reported higher T_o and T_p but lower ΔT values in ANN-treated corn, waxy corn and Hylon V starches. However, the authors observed similar or higher ΔH in ANN-treated starches compared to the native counterparts. Chung et al. (2009) reported an increase in T_p and T_c but a decrease in ΔT values in ANN-treated corn starch compared to native corn starch. The authors, differently from the

observations of the present study, determined similar ΔH between ANN-treated starch and native starch. Klein et al. (2013) applied single and dual HMT to pinhão starch and reported an increase in T_o , T_p and T_c , and a decrease in ΔT and ΔH in single and dual HMT starches prepared at 100 °C, being in accordance with the results for HMT-treated starch verified in the present study. According to Sun, Han, Wang, and Xiong (2014), in HMT starches the amylose molecules are allowed to interact more freely with the amylopectin chains present in the branched crystalline regions, consequently reducing the mobility of the amylopectin chains. This interaction will promote the increasing of gelatinization temperatures.

Comparing ANN–HMT and HMT–ANN treatments, it is possible to observe high T_p and T_c in HMT–ANN-treated starch (Table 3), which is in accordance with the pasting temperature observed in Table 2. Chung et al. (2010) studied the effects of ANN–HMT and HMT–ANN on thermal properties of pea, lentil and navy bean starches. The authors also observed a higher effect of HMT–ANN than ANN–HMT, and reported that the increase in T_p and T_c reflect additional formation of crystallites resulting from amylose–amylose and/or amylose–amylopectin interactions on HMT. In the present study, the occurrence of amylose–amylose and/or amylose–amylopectin interactions that leads to additional formation of crystallites is supported by the increase in relative crystallinity of both ANN–HMT and HMT–ANN starches (Fig. 2).

4. Conclusion

To our knowledge, this is the first study that focuses on investigating the effects of single and dual ANN and SNT modifications on pinhão starch properties. Native and single ANN-treated pinhão starch presented a CA-type crystalline structure, while others modifications promoted the migration of crystalline structure from CA to A-type. The relative crystallinity decreased in starches subjected to HMT and SNT alone, as well as HMT–SNT and SNT–HMT starches. The ANN, HMT and SNT did not provide visible cracks, notches or grooves to pinhão starch granule. SNT-treated starch presented the highest breakdown; which indicates a weaker physical structure of the granules, making them more susceptible to collapse while under thermal and shear treatments. In general, HMT used as both single or dual modifications, had a stronger effect on gelatinization temperatures and enthalpy than ANN and SNT.

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Table 3
Thermal properties of native and modified pinhão starches.

Treatment	Gelatinisation temperatures ^a			ΔT (°C)	ΔH (J/g)
	T_o (°C)	T_p (°C)	T_c (°C)		
Native	54.25	60.15	68.22	14.67	11.60
ANN	61.87	64.99	69.71	7.84	7.20
ANN–HMT	65.39	67.88	73.37	7.98	3.61
ANN–SNT	60.61	63.86	68.70	8.31	10.31
HMT	64.89	69.69	76.07	11.18	2.84
HMT–ANN	64.35	69.28	74.73	9.78	4.88
HMT–SNT	58.93	67.82	72.56	13.63	2.16
SNT	53.17	58.36	65.18	12.01	11.73
SNT–ANN	59.96	63.43	69.43	9.40	7.43
SNT–HMT	61.39	66.07	71.33	9.94	2.12

^a T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, ΔT = gelatinisation temperature range, and ΔH = gelatinisation enthalpy.

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