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Evaluation of the effects of single and dual hydrothermal treatments on the properties of Carioca bean (*Phaseolus vulgaris* L.) starch

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Abstract

Different modified starches have been developed for many applications in the food industry. The present work investigated the effects of two physical modifications, annealing (ANN) and heat-moisture treatment (HMT), as well as dual modification (ANN+HMT and HMT+ANN), on the physicochemical properties of Carioca bean starch (*Phaseolus vulgaris* L.). The Differential Scanning Calorimetry results showed that after annealing and HMT, the bean starch became more stable during heating due to the higher temperature of gelatinisation. The pasting properties changed, and the ANN treatment reduced the peak viscosity and increased the bean starch pasting temperatures. Relative crystallinity decreased with the physical treatments, and the starch granules did not show different morphologies. Syneresis analysis showed that the physical modifications of the bean starches resulted in higher water release as compared to the native starch. Therefore, these starches are not suitable as ingredients in refrigerated or frozen foods but could be used as ingredients for dehydrated foods such as soups.

Introduction

The main methods used to produce physically modified starches involve different combinations of temperature/moisture, pressure, shear and irradiation. Another method involves mechanical friction to change the size of the starch granules. Physical modifications are frequently preferred because they do not involve any chemical treatment, hence less harmful to human health and the environment (Jacobs and Delcour, 1998; Ashogbon *et al.*, 2014). Starches are physically modified (heat-moisture treatment, annealing, high pressure treatment, *etc.*) to increase their range of applications in food and non-food fields, for example to promote changes from native starches into cold water-soluble starches or low-crystallinity starches. Hydrothermal treatments, such as annealing and heat-moisture treatment, modify the physicochemical properties of starch without destroying its granular structure thereby improving crystallinity and facilitating interactions between starch chains (Wu *et al.*, 2010; Varatharajan *et al.*, 2010; Ashogbon *et al.*, 2014).

Annealing (ANN) is a physical treatment of starch granules in the presence of heat and water, and its effects are dependent on the botanical source. In general, annealing involves increased molecular mobility without gelatinisation, as well as the promotion of physical reorganisation inside the granules (Simsek *et al.*, 2012; Ashogbon *et al.*, 2014). The main changes in the physicochemical properties of granules caused by annealing include decreased solubility (a narrowing of the thermal transition interval with an increase in the gelatinisation temperature and enthalpy change) and increased paste stability and enzymatic susceptibility (Wang *et al.*, 2013). Heat-moisture treatment (HMT) is a physical modification used at low moisture levels (10-30%) and high temperatures (90-120°C) for periods ranging from 15 minutes to 16 hours (Zavareze *et al.*, 2012). HMT influences the enzymatic digestibility, crystallinity, and thermal and pasting properties of

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starches (Jiranuntakul *et al.*, 2011; Zavareze *et al.*, 2012; Lee *et al.*, 2012). The main difference between ANN and HMT is that ANN involves starch treatment in excess water and temperatures between those of glass transition and the gelatinisation of starch (Tester and Debon, 2000), whereas HMT is performed at low moisture levels and high temperatures, generally over 100°C (Ashogbon *et al.*, 2014).

The combined effect of ANN and HMT on the structure and physical properties of corn starch has been previously reported, and the results indicated that the changes were more pronounced under dual modification compared to single treatment. Decreased amylose content and swelling factor as well as increased amylose leaching was reported (Chung *et al.*, 2009; Ashogbon *et al.*, 2014). The aim of the present work was to investigate the effect of physical modifications (ANN, HMT and dual modification) on the physicochemical properties of *Carioca* bean starch (*Phaseolus vulgaris* L.) in an attempt to find the potential uses for the starches with new technological properties.

Materials and methods

Materials

The starch, in its native form (sample a), was obtained from *Carioca* beans (*Phaseolus vulgaris* L.) that were acquired in the local retail market (Ponta Grossa, PR, Brazil). The starch (< 1% protein; <0.2% fat) extraction was performed as described by Betancur *et al.* (2001).

Hydrothermal treatment

Annealing (ANN)

For annealing, the starch samples were suspended in water (1:4 starch:water, w/v), and incubated at 50°C for 24 h in a water bath (Chung *et al.*, 2010).

Heat–moisture treatment (HMT)

The HMT was performed according to Andrade *et al.* (2014) with slight modifications, which consisted of weighing 100 g starch in glass containers and adjusting the moisture to 30% with water. The glass containers were sealed and kept for 24 h at room temperature (20ºC) for equilibration. The samples were then thermally treated in an autoclave at 120°C for 15 min. The containers were subsequently opened, and the samples dried in an oven (45°C) to achieve constant moisture of 10%.

Dual modification

The dual modification of the starch was performed in two ways: the first sample of doubly-modified starch was obtained by annealing, followed by heat-moisture treatment (ANN + HMT).The second sample was obtained by performing heat-moisture treatment first and then annealing (HMT+ ANN).

Characterisation of the starches

Colour parameters

A MiniScan EZ 4500L (HunterLab, USA) was used to determine the colour parameters of the starch. This colorimeter is based on the CIE colour space version (CIELab). Calibration was performed on white and black colour prior to sample analysis. The colour was expressed as a combination of three parameters: L*, brightness ranging from 0 (black) to 100 (white); a* ranging from positive (red) to negative (green); and b*, ranging from positive (yellow) to negative (blue) (Falade *et al.*, 2012).

Differential Scanning Calorimetry (DSC)

In this analysis, 2 mg starch (dry basis) was weighed in aluminium crucibles and distilled water was added (1:4 starch:water, w/v), moistening the starch, before left for 1 h to equilibrate. The crucibles were closed with proper lids to prevent water loss during heating. The DSC device was a TA Q-200 (TA Instruments, New Castle, USA) which was programmed for heating at 10°C/min, from 30 to 100°C. The equipment was calibrated with indium standard (99.99%, melting point of 156.6°C, ΔH = 28.56 J/g). A similar sealed aluminium crucible (empty) was used as reference material. Endothermic curves, with the corresponding onset, peak and conclusion temperatures, were obtained, as well as the transition enthalpies (J/g) (Vatanasuchart *et al.*, 2005).

Pasting properties

The pasting properties of the starch samples were determined using RVA-4 equipment (Newport Scientific Pvt. Ltd., Narabeen, Australia). A suspension of 2.5 g (8% moisture) starch in 28 g distilled water underwent a controlled heating and cooling cycle under constant stirring. It was held at 50°C for 1 min, heated from 50 to 95°C at 6°C/min, held at 95°C for 5 min, cooled to 50°C at 6°C/min, and held at 50°C for 2 min (Franco *et al.*, 2002).

Syneresis

The starch samples were suspended in deionised water (8%, w/w), gelatinised, and kept at 95°C under agitation for 10 min. After reaching room temperature, the paste was divided into three portions of 50 g, and frozen at -18°C in hermetic plastic cups. The pastes were submitted to three freeze-thaw cycles; they

were kept in the freezer for 72 h and thawed at 45°C for three hours (Wosiacki *et al.*, 1985; Demiate *et al.*, 2001). The amount of water liberated from the pastes under reduced pressure (-490 mmHg) was measured gravimetrically after thawing and expressed as liberated water in relation to the initial weight.

X-ray diffraction

The X-ray diffractograms were collected using a Rigaku Ultima IV instrument (Rigaku, Tokyo, Japan) with CuKα radiation (λ = 1.544 Å) at 40 kV and 20 mA (Beninca *et al.*, 2008). The analysis was performed at 20°C in a 2 θ angle range of 7-30° with a measuring period of 5 s/2θ. The relative crystallinity was quantitatively estimated as the ratio of the crystalline area to the total area (Nara and Komiya, 1983) between 7° and 30° (2θ) using Origin software (Version 8.0, Microcal Inc., Northampton, MA, USA).

Scanning Electron Microscopy

The morphology of the starch granules was examined using a scanning electron microscope (Tescan, VEGA 3, Kohoutovice, Czech Republic). All the samples were coated with gold, and examined in the scanning electron microscope under an acceleration voltage of 25 kV and magnification of 1,500×. The diameters of the granules were calculated using ImageJ software (ImageJ 1.47 for Windows).

Statistical analysis

The experimental data were evaluated for homogeneity of variance using the Brown-Forsythe test ($p > 0.05$ was considered parametric). The parametric data were then evaluated by Analysis of Variance (ANOVA), complemented with the mean comparison Fisher's LSD test. A value of $p < 0.05$ was considered significant. The statistical analyses were performed using Statistica software, version 7.0 (Statsoft, Tulsa OK, USA).

Results and discussion

Colour parameters

The starches modified by annealing and heat moisture treatment presented similar L* values (89.71-93.93; $p > 0.05$) without significant difference. There was an increase in the a* values (0.72-1.31, $p = 0.05$) for the HMT samples. A significant difference ($p < 0.05$) for the b* parameter (yellow) was also found, with a tendency to yellow for the HMT-modified samples (5.03, 5.13 and 5.29 for HMT, ANN+HMT and HMT+ANN, respectively) (Table 1). The same behaviour was reported by Lorlowhakarn and Naivikul (2006) in their study of the HMT modification of rice flour. The increase in the a* and b* parameters might have occurred due to the Maillard reaction of the starches in the modification period.

Differential Scanning Calorimetry (DSC)

The changes in the onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and gelatinisation enthalpy (ΔH_{gel}) were determined and the results are shown in Table 1. Changes resulting from hydrothermal treatments are dependent on the moisture level during the treatment, the temperature and time, the starch source and the amylose content (Tester and Debon, 2000; Ashogbon *et al.*, 2014; Andrade *et al.*, 2014).

Table 1 shows that after ANN and HMT, the gelatinisation endotherms shifted towards higher temperatures which indicated that the bean starches became more stable during heating. After ANN and HMT, changes are observed in the physicochemical properties of starch granules that are related to amylose-amylose and amylose-amylopectin interactions, resulting in a rearrangement of the crystalline structure (Trung *et al.*, 2017). Increased peak temperatures and a reduction in gelatinisation enthalpy were noted, mainly after HMT, which was

Table 1. DSC gelatinisation properties and colour parameters of bean starches.

Samples	DSC gelatinisation				Colour parameters		
	T_0/°C	T_p/°C	T_C/°C	ΔH_{gel}/J g^{-1}	L*	a*	b*
Native	66.1 ± 0.2^{e}	73.5 ± 0.3^{c}	79.2 ± 0.2^{c}	7.0 ± 0.5^{b}	93.5 ± 0.5	0.8 ± 0.2^{b}	4.2 ± 0.3^{b}
ANN	71.7 ± 0.1^{d}	75.4 ± 0.2^{d}	81.9 ± 0.1^{d}	7.8 ± 0.3^{a}	92.9 ± 0.5	0.7 ± 0.1^{b}	3.9 ± 0.2^{b}
HMT	88.3 ± 0.1^{b}	92.8 ± 0.1^{a}	95.9 ± 0.2^{a}	2.5 ± 0.3^{e}	93.0 ± 2.6	1.3 ± 0.5^{a}	5.0 ± 0.4^{a}
ANN+HMT	83.4 ± 0.5^{c}	91.4 ± 0.1^{c}	93.2 ± 0.1^{c}	5.4 ± 0.2^{c}	90.9 ± 0.8	1.1 ± 0.0^{ab}	5.1 ± 0.1a
HMT+ANN	89.2 ± 0.6^{a}	91.9 ± 0.5^{b}	95.8 ± 0.5^{b}	2.8 ± 0.4^{d}	89.7 ± 2.3	1.3 ± 0.1^{a}	5.3 ± 0.1^{a}
p(B-F)*	0.52	0.71	0.58	0.90	0.61	0.58	0.54
p(ANOVA)**	<0.05	<0.05	<0.05	<0.05	0.08	0.05	<0.05

T_o, onset temperature; T_p, peak temperature; T_C, endset or conclusion temperature; ΔH_{gel}, gelatinisation enthalpy.
*Probability value from Brown-Forsythe test for homogeneity of variance.
**Probability value from one-way ANOVA.
***Different letters in the same column represent significant difference according to Fisher's LSD test ($p < 0.05$).

related to the physical reorganisation of molecules (Tester and Debon, 2000). The ANN treatment alone increased the gelatinisation enthalpy when compared with the native starch, which was in line with other studies (Wang *et al.*, 2017). The same behaviour was described by Liu *et al.* (2009) who studied the effects of ANN on waxy and regular corn starches and found increased values for onset and peak temperatures, as well as for gelatinisation enthalpy after annealing at 50°C.

Pasting properties (RVA)

The annealing treatment slightly reduced the peak viscosity and increased the pasting temperature of the bean starch (Figure1 and Table 2). Previous studies reported that this treatment reduced the leaching of amylose from starch granules (Tester and Debon, 2000; Chung *et al.*, 2010; Zavareze and Dias, 2011). As a consequence, there is a decrease in the peak viscosity and the gelatinisation temperature. On the other hand, annealing increased the final viscosity of the bean starch. Similar results were reported for sorghum starch (Adebowale *et al.*, 2005) which indicated that when a starch suspension was cooled, the starch molecules were aggregated into larger units, increasing the final viscosity. Our results were also in line with those of Gomes *et al.* (2005) with a low setback viscosity of the modified starches.

When the starch was subjected to the treatments, including dual modification, there was a significant reduction in the viscosities (setback and breakdown) which was in accordance with previous studies (Chung *et al.*, 2009; Zavareze *et al.*, 2010). As a consequence, there was a decrease in the peak viscosity and the gelatinisation temperature. These changes occurred due to the structural rearrangement inside the granules after treatment; the higher the moisture content, the greater the changes in the starch properties (Adebowale *et al.*, 2005; Lawal *et al.*, 2005). Similar values were reported by Watcharatewinku *et al.* (2009) (canna starch and 25% moisture content); Varatharajan *et al.* (2010) (potato starch and 24% moisture content); and Pinto *et al.* (2012) (pinhão starch and 25% moisture content).

Syneresis

Syneresis is the water release from starch pastes. It is usually seen as a negative behaviour because it is related to the deterioration and reduced quality of a product (Ribotta *et al.*, 2007). The syneresis results of the native starch, ANN starch, HMT starch, ANN+HMT starch and HMT+ANN starch are shown in Table 2.

Syneresis did not change considerably after three successive freeze-thaw cycles. However, the native bean starch released higher amounts of water when compared with starches from other botanical sources, such as cassava, which liberated 1.5%, 1.0% and 3.5% during the first, second and third freeze-thaw cycles (Demiate *et al.*, 2011); rice starch (38.1%, 52.7% and 55.8%) (Charoenrien *et al.*, 2012); and potato starch (23.0%, 28.0% and 30.0%) (Zhang *et al.*, 2013). When the bean starch was subjected to physical modifications, an increase in syneresis was detected, irrespective of which modification was performed, or whether it was single or dual.

However, dual modification promoted significantly higher water release as compared to single modification due to greater alterations in the physicochemical properties of the starches. Thus, both the native and physically modified bean starches in the present work were not suitable to be used as ingredients in refrigerated or frozen products because of their low freeze-thaw stability. Some authors have already studied the syneresis of pastes from physically modified starches. Yadav *et al.* (2013) showed that

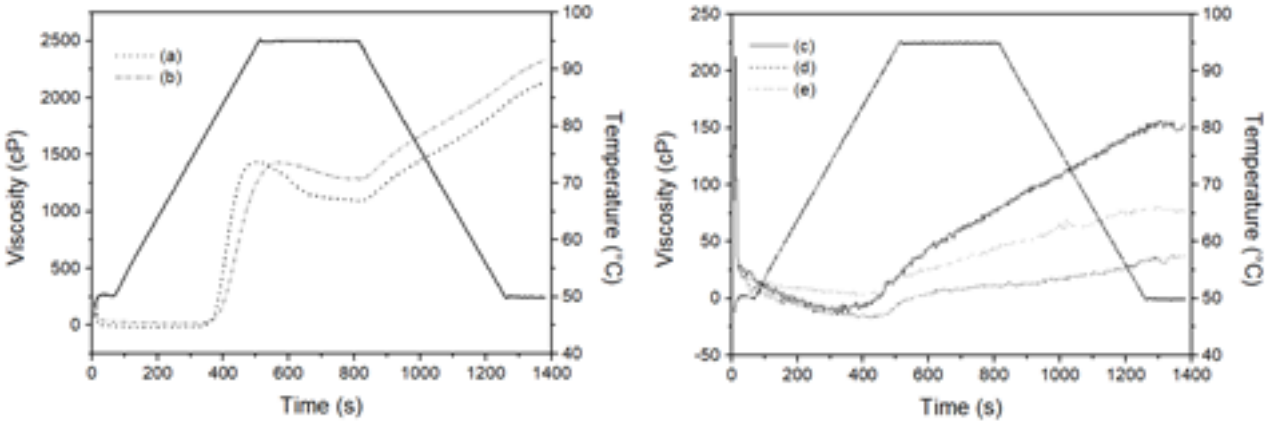

Figure 1. RVA curves of (a) native starch, (b) ANN starch, (c) HMT starch, (d) ANN+HMT starch. and (e) HMT+ANN starch.

Table 2. Pasting properties of bean starches and percentage of syneresis of bean starches.

Sample	Pasting properties					Percentage of syneresis		
	T_p/°C	V_p/cP	V_f/cP	Breakdown/ cP	Setback/cP	First cycle	Second cycle	Third cycle
Native	80.5 ± 0.1[b]	1436.7 ± 1.5[a]	2134.0 ± 2.0[b]	342.7 ± 2.5[a]	1040.3 ± 2.1a	66.7 ± 1.6[d]	68.2 ± 1.1[c]	68.9 ± 0.6[c]
ANN	82.7 ± 0.6[a]	1431.7 ± 3.5[b]	2330.0 ± 2.6[a]	143.7 ± 2.1[b]	1041.7 ± 3.2a	73.0 ± 1.8[c]	71.1 ± 1.0[bc]	73.5 ± 2.1[b]
HMT	-	37.7 ± 1.5[c]	99.7 ± 2.5[c]	2.0 ± 0.0[c]	64.3 ± 3.5b	75.5 ± 1.0[bc]	74.7 ± 3.3[ab]	75.9 ± 1.7[b]
ANN+HMT	-	13.7 ± 0.6[c]	37.0 ± 2.0[e]	2.7 ± 0.6[c]	27.7 ± 1.5d	79.4 ± 3.4[a]	78.5 ± 2.5[a]	80.3 ± 1.8[a]
HMT+ANN	-	41.0 ± 1.0[c]	76.7 ± 3.5[d]	-1.3 ± 0.6[d]	35.4 ± 0.6[c]	78.4 ± 2.1[ab]	75.1 ± 2.4[ab]	79.8 ± 0.8[a]
p(B-F)*	0.47	0.35	0.94	0.28	0.65	0.66	0.86	0.81
p(ANOVA)**	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

T_p, pasting temperature; V_p, peak viscosity; V_f, final viscosity.

*Probability value from Brown-Forsythe test for homogeneity of variance.

**Probability value from one-factor ANOVA.

***Different letters in the same column represent significant difference according to Fisher's LSD test ($p < 0.05$).

the HMT of Indian water chestnut starch produced a reduction in syneresis values after five freeze-thaw cycles, while annealing enhanced syneresis compared with the native starch counterpart. Simsek *et al.* (2012) found that annealing caused no significant differences in syneresis for black and pinto bean starches. Balasubramanian *et al.* (2011) reported that the HMT of pearl millet starch produced higher water release as compared to native starch.

X-ray diffraction

The X-ray diffraction of starches (Figure 2) was used to evaluate the behaviour of their crystalline regions: three main patterns are well established, A, B or C. All the samples presented the same behaviour, showing three peaks in the diffraction angles 2θ, which are characteristic of C-type (15°, 18° and 23°) and typical of legume starches (Zobel, 1988; Corradini *et al.*, 2005; Oladebeye *et al.*, 2013). The relative crystallinities of the bean starches were 25.6, 23.1, 22.4, 21.9 and 23.6%, for the native starch (a); ANN starch (b); HMT starch (c); ANN+HMT starch (d); and HMT+ANN starch (e) samples, respectively. The crystallinity decreased after modifications because of the partial disruption of the amylopectin crystallites. The low values for relative crystallinity in relation to the HTM treatment were evidenced by the reduced gelatinisation enthalpy, as shown in Table 1 (Chung *et al.*, 2009; Wang *et al.*, 2017; Chen *et al.*, 2017).

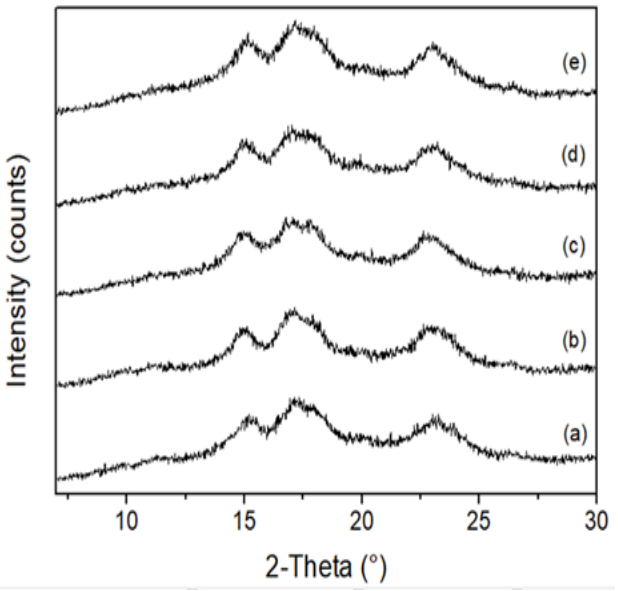

Figure 2. X-ray diffraction of (a) native starch, (b) ANN starch, (c) HMT starch, (d) ANN+HMT starch, and (e) HMT+ANN starch.

Scanning Electron Microscopy

As reported by Lawall *et al.* (2005) and Vanier *et al.* (2012), native bean starch granules have oval to spherical shapes, heterogeneous sizes and smooth surfaces without visible fissures (Figure 3a). The hydrothermal treatments performed in the present work did not promote morphological changes in the starch granules (Figure 3). As reported in a relevant review (Jacobs and Delcour, 1998), most of the published works upon hydrothermally treated starches did not find morphological evidence related to these physical treatments.

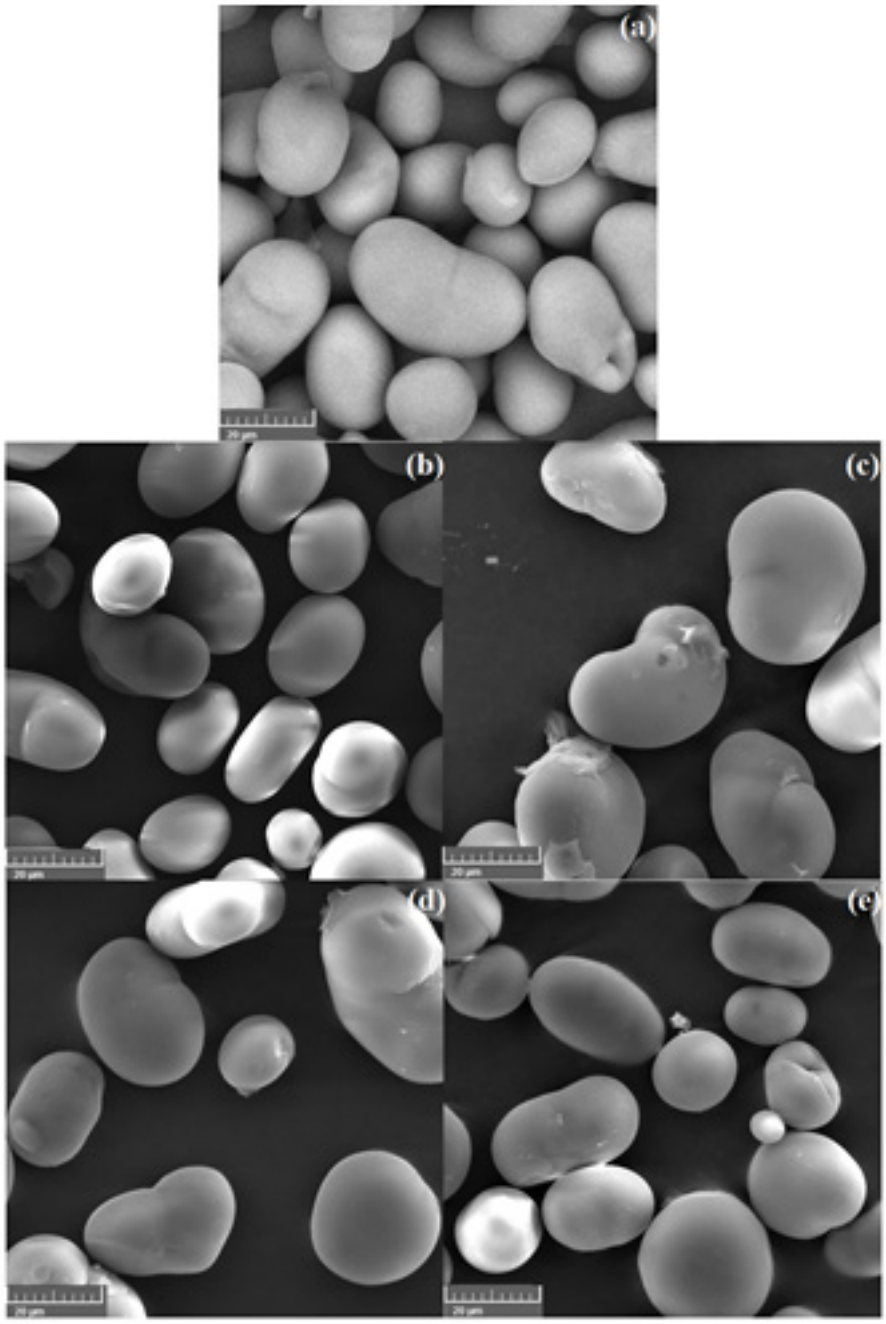

Figure 3. Electronic microscopy of (a) native starch, (b) ANN starch, (c) HMT starch, (d) ANN+HMT starch, and (e) HMT+ANN starch at 1,500× magnification.

The average diameters of the starch granules were calculated using the ImageJ software. The calculated diameters for the native starch (a); ANN starch (b); HMT starch (c); ANN+HMT starch (d); and HMT+ANN starch (e) samples were 24.5, 26.6, 26.7, 28.8 and 29.2 μm , respectively, without significant differences between these values ($p > 0.05$).

Conclusion

Physical modifications can be used to change starch properties and improve their suitability for different industrial applications. In the present work, ANN and HMT resulted in higher thermal stability, as shown by the DSC analysis, and lower viscosity of the bean starch. On the other hand, the physically modified bean starches were not suitable for use as ingredients in frozen foods because of their low freeze-thaw stability. The low viscosity of both the HMT and dual-modified bean starches produced in the present work makes them useful as ingredients in food preparations designed to be consumed hot, such as dehydrated soups.

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