RESEARCH ARTICLE

Influence of enzyme active and inactive soy flours on cassava and corn starch properties

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The aim of this work was to study the influence of enzyme active and inactive soy flours on the properties of cassava and corn starches. Four starch/soy flour composites were evaluated: cassava/active soy flour (Cas/AS), cassava/inactive soy flour (Cas/IS), corn/active soy flour (Corn/AS) and corn/inactive soy flour (Corn/IS). Starch gelatinization occurred at 58.67°C for Cas and at 64.19°C for corn; gelatinization occurred at higher temperatures when soy flours were present, while ΔH diminished. The presence of AS reduced 80% the retrogradation enthalpy of Cas and 40% that of corn. Cas presented lower pasting temperature than corn starch (67.8 and 76.8°C, respectively) and higher peak viscosity (427.9 and 232.8 BU, respectively). The pasting properties of both starches were drastically reduced by soy flours, and this effect was more noticeable in Cas; AS had higher effect than IS. X-ray diffraction pattern of retrograded samples showed that both starches recrystallisation (mainly that of Cas) was reduced when AS was added. Tan δ values decreased with AS addition to corn, but they increased when added to Cas. The images obtained using confocal laser scanning microscopy (CLSM) showed that IS was distributed as large aggregates, whereas AS distribution was more homogeneous, especially when incorporated to Cas. These results show that cassava starch interacts specifically with active soy flour (AS, mainly in native state). The delaying effect of AS on cassava starch retrogradation was clearly shown. This finding could be useful in obtaining gluten-free breads of high quality and low retrogradation rate.

Keywords:
Cassava starch / Corn starch / Proteins / Soy flour

1 Introduction

People with celiac disease are unable to tolerate gluten protein present in wheat, rye, barley and hybrids such as triticale. Gluten is a protein fraction present in most cereals, and is responsible for the elastic and extensible properties required to produce good quality breads. Nevertheless, gluten free bread lacks this viscoelastic protein network able to retain the air incorporated during mixing and produced by yeast during proofing. For this reason, gluten free baked goods, in general, are of low overall organoleptic quality and are very prone to stale.

Several gluten free formulations found in the literature were used to obtain breads of varying properties [1–7]. Among the raw materials most commonly employed, rice flour, cassava and corn starches are probably the most important ones in gluten free bread production [2, 6, 8, 9].

It is known that the water amount present in gluten free formulations plays an important role on dough properties and bread quality. In a previous work [10], gluten free breads of varying quality were obtained using high water amount (from 110 to 210%, flour basis). The new goal was

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Abbreviations: AS, active soy flour; **BD,** breakdown; **Cas,** cassava starch; **CLSM,** confocal laser scanning microscopy; **Corn,** corn starch; **d-AS,** defatted active soy flour; **d-IS,** defatted inactive soy flour; **IS,** inactive soy flour; **PV,** peak viscosity; **RVA,** rapid viscoanalyser; **SB,** setback; **WHC,** water hydration capacity

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to produce high quality gluten free bread with a lower water incorporation. In another approach to the study of gluten free bread formulations, Ribotta et al. [2] assessed the effect of heating (160°C for 3 min) active soy flour (AS) on the quality of low-water gluten free breads made of rice flour and cassava starch (65% water, flour basis), before breadmaking. They obtained better results with active than with inactive soy flour (IS), and they attributed this effect to both enzyme inactivation and protein aggregation during heating. Thus, the study of the effect of soy addition to both cassava and corn starches was proposed to gain a deeper insight into the behaviour of the gluten free system containing low water amount. Therefore, the aim of this work was to study the influence of enzyme active and ISs on the properties of cassava and corn starches.

2 Materials and methods

2.1 Materials

Micronized active (36.4% proteins, 19.8% lipids, 4.7% ash, 2.8% crude fibre, 30.3% carbohydrates, 6.9% moisture) and inactive (36.7% proteins, 22.1% lipids, 4.5% ash, 2.3% crude fibre, 29.9% carbohydrates, 4.5% moisture) soy flours were supplied by Argensoja S.A. (Bahía Blanca, Argentina). Native cassava starch (0.2% protein, 0.01% lipids, 0.09% ash, 0.2% crude fibre, 86.6% carbohydrates, 12.9% moisture; 16.4% AM) and native corn starch (0.5% protein, 0.02% lipids, 0.01% ash, 0.4% crude fibre, 88.7% starch, 10.4% moisture; 17.3% AM) were provided by Nora's Skills (Buenos Aires, Argentina). To study the interactions between corn or cassava starches and active or inactive soy flour, four blends were used: cassava/active soy flour (Cas/AS); cassava/inactive soy flour (Cas/IS); corn/active soy flour (Corn/AS), and corn starch/inactive soy flour (Corn/IS). To obtain defatted soy flours (with final lipid content $<4\%$), flour was extracted 24 h with *n*-hexane at room temperature (flour/hexane 1:100 w/v); during this period, solvent was changed three times.

In order to keep the ingredients ratio present in gluten free bread formula [2], corn or cassava starches were mixed with soy flour in 90:10 ratio, respectively.

2.2 Water hydration capacity

Water hydration capacity (WHC) was determined according to AACC approved method 88-04 [11]. Briefly, ~0.5 g of starch, flour or starch/flour blend were mixed with 1 mL of distilled water and thoroughly stirred. Samples were then centrifuged (1000 × *g*, 20 min) and the supernatant was discarded. The amount of retained water (g) was calculated by difference and expressed as grams of water retained by each gram of sample. Results are the average of three replicates.

2.3 Syneresis

Syneresis of the blends described above and of single starches and flours used as controls, was determined. The composites were made by mixing 50 g of flour/starch blend with 40 mL of distilled water. A portion of this composite (~0.5 g) was put into centrifuge tubes and centrifuged at 16 000 × *g* for 20 min at room temperature. The sample weight was obtained before centrifugation and after free water separation. The water separated was expressed as percentage of the total water in the mixture. Results are the average of three replicates.

2.4 DSC

To assess thermal behaviour of starch/flour blends at the same starch/soy flour/water ratio used in breadmaking [2], four soy flours were used: full fat AS, defatted AS (d-AS), full fat IS, and defatted IS (d-IS). Each flour was combined with both starches, determining eight samples. The objective was to evaluate the effect of lipid presence (constituting ca. 20% of flour) on the thermal properties of composites.

DSC measurements of starches and composites were performed using a DSC 823 (Mettler-Toledo, Switzerland). For gelatinization analysis, 50 g of starch/soy flour blends (or 50 g of starch) were mixed with 40 mL of distilled water, allowed to rest for 30 min, and, finally, approximately 40 mg of sample were weighed into 100 μL hermetic aluminium pans and immediately analysed in the DSC. After analysis, all pans were stored for 7 days at 4°C to allow starch retrogradation, and re-analysed (30°C to 120°C) in order to study AP retrogradation.

DSC was calibrated with Indium, and an empty pan was used as reference. Pans were sealed and then heated from 30 to 120°C at a rate of 5°C/min. Onset temperature (T_o), peak temperature (T_p), gelatinization (ΔH_{gel}) and retrogradation (ΔH_{ret}) enthalpies were calculated. Samples were evaluated at least in duplicate.

2.5 RVA

Pasting properties of starches and starch/flour composites were measured on a Rapid Visco Analyser (RVA-4), using the RVA General Pasting Method (Newport Scientific Pty. Ltd., Australia). Three grams of sample (dry basis) were transferred into a canister and approximately 25.0 ± 0.1 mL distilled water were added. The slurry was heated to 50°C while being stirred at 160 rpm for thorough dispersion of ingredients. The slurry was held at 50°C for 1 min, and then heated to 95°C at a heating rate

of 9.4°C/min and a stirring rate of 960 rpm. It was held at 95°C for 2.5 min, and finally cooled to 50°C at a cooling rate of 11.8°C/min.

Pasting temperature (T_p), peak viscosity (PV), final viscosity (FV), breakdown (BD) and setback (SB) were obtained from the pasting curve. Samples were assessed in duplicate.

2.6 XRD

Twenty-five grams of starch or starch/flour blends were mixed with 25 mL of distilled water and put in a water bath at 97°C for 1 h, with manual stirring every 5 min the first half hour, and every 15 min the second. This process led to a complete gelatinization (verified by DSC assays). The gels thus obtained were stored for 7 days at 4°C. After this period, they were freeze-dried and milled. XRD data of these powders were obtained using a Philips PW3020 (Eindhoven, Netherlands) diffractometer. The X-ray patterns were taken with Cu Kα radiation ($\lambda = 0.154$ nm) and the X-ray tube (Philips PW3830) was operated at 40 Kv and 30 mA. The scanning region of the diffraction angle (2θ) was 2–40° with scanning speed 0.005°/s. Crystalline and amorphous areas were quantified using a PeakFit v4 for Win32 Software (AISN Software Inc.). Crystalline peaks were analysed as pseudo-Voigt form and the amorphous background as Gaussian form peaks. A total area for crystalline phase and another for amorphous phase were obtained, and relative crystallinity was determined.

2.7 Rheology

Fifty grams of the starch/soy flour blend were mixed with 40 mL of distilled water. The composites were allowed to rest for 15 min at room temperature. Samples were analysed with a Rheoplus rheometer (Anton Paar, Germany) using a plate–plate geometry, with 2 mm gap. Sample was placed between the plates and the excess was carefully trimmed. The edges were covered with Vaseline to prevent sample dehydration during the test. Sample was allowed to rest for 5 min before the analysis in order to allow relaxation of residual stresses.

Frequency sweeps from 0.1 to 10 Hz were performed at 0.1% strain, which guaranteed deformation conditions within the linear viscoelastic region. Temperature was kept constant at 25°C. Results are the average of three replicates.

2.8 CLSM

Fifty grams of starch/soy flour blend were mixed with 40 mL of distilled water. The composites thus obtained were allowed to rest for 15 min. A solution of Rhodamine B and FITC (0.1 and 1% w/v, respectively, in dimethylformamide) was then added to allow simultaneous observation of proteins (red/yellow) and starch (green). Samples were observed using an inverted spectral confocal microscope Nikon Eclipse C1si (Nikon Inc., Tokyo, Japan), in standard mode. Excitation was made with 488 lines of Argon laser and He-Ne laser 543 nm, both with 10% power. Emission filters were 515/30 and 305/75.

2.9 Statistical analyses

The data obtained were statistically treated by variance analysis, while means were compared by the Fisher LSD test at a significance level of 0.05. Correlation between variables was determined using Pearson's coefficient. These tests were carried out using INFOSTAT statistical software (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina).

3 Results and discussion

3.1 Effects of soy flour on starch properties

While WHC is a measure of the ability of a material to absorb/adsorb water, syneresis is related to phase separation under certain conditions, e.g. centrifugation. The difference between WHC of the two starches was not significant ($p > 0.05$). This parameter was high for soy flours and AS showed the highest ($p < 0.05$; Table 1). Inactivation process of soy flour causes protein denaturation and unfolding, while it is assumed that the proteins present in AS are mainly in a native state. Unfolded polypeptides may expose hydrophobic groups otherwise buried in the protein interior. In an aqueous medium, these proteins may aggregate and absorb less water than native proteins, which present high water affinity. As expected,

Table 1. Water hydration capacity (WHC) and syneresis of studied samples

Sample	WHC (g water/g solid)	Syneresis (%)
Cas	1.23 ± 0.04^{ab}	11.63 ± 1.33^{b}
Cas/AS	1.28 ± 0.01^{ab}	16.82 ± 1.13^{c}
Cas/IS	1.46 ± 0.07^{d}	12.38 ± 0.27^{b}
Corn	1.20 ± 0.09^{a}	12.28 ± 1.02^{b}
Corn/AS	1.34 ± 0.03^{bc}	7.18 ± 0.55^{a}
Corn/IS	1.42 ± 0.06^{cd}	9.18 ± 0.53^{ab}
AS	3.61 ± 0.01^{f}	59.58 ± 2.84^{d}
IS	2.82 ± 0.02^{e}	66.96 ± 3.30^{e}

AS, active soy flour; Cas, cassava starch; Corn, corn starch; IS, inactive soy flour.
Values followed by different letters in the same column are significantly different ($p < 0.05$).

WHC of starches increased when soy flour was added, with the exception of Cas/AS composite, which did not show significant differences to Cas. This unexpected result could be related to a specific interaction between cassava starch and native soy proteins which prevents starch and/or protein water absorption.

Syneresis was also higher for soy flour, and it was highest for IS; corn and cassava starches showed no significant differences in this parameter. AS addition increased Cas syneresis, while soy flour incorporation to Corn diminished syneresis values.

Syneresis and WHC showed a significant correlation ($r = 0.94, p < 0.001$), which indicates that as higher water amount was absorbed by the sample higher water amount was available to be liberated during centrifugation (this water was assumed to be weakly retained by flour or flour/starch blends).

3.2 DSC

The gelatinization process is affected by many factors, such as water availability, lipid, the presence of salts and sugars, temperature, conditions under which raw material was obtained, among others. Gelatinization parameters of starch and starch/soy flour composites are shown in Table 2. The results obtained for Cas are in agreement with previous work [12, 13], and the same was true for corn starch [14, 15]. AS presented two endotherms, the first occurred between 85 and 93°C, with a ΔH value of 0.5 J/g; and the second took place between 106.5 and 116.7°C with a ΔH of 3.1 J/g, corresponding to the denaturation of β-conglycinin and glycinin, respectively. No endotherm was observed for IS.

The presence of soy flour produced a shift of the endotherm towards higher temperatures for both starches. The delay in peak temperatures could be related to the interaction between material leached out of the granules and soy protein, between the granule surface and protein [15], and also to lower water availability for starch gelatinization [16, 17]. Defatted soy flour shifted Cas gelatinization endotherm towards higher temperatures when compared to full-fat soy flour. Cassava granules show a weak granular structure (see RVA results), losing their structure to a higher degree when compared to corn starch. In this regard, starch chains are more available to interact with exogenous material. In addition, defatted soy flours show higher protein ratio which may interact with starch and/or water. These interactions could be responsible for the delay in gelatinization peak.

Starch gelatinization enthalpy was also modified by soy flour incorporation, which led to lower transition enthalpies. When soy flour was added to Cas, ΔH diminished, irrespective to the type of soy flour used. On the other hand, only IS produced a significant decrease on this parameter when added to Corn. Several authors have suggested that in a starch–water system, ΔH decreases as the amount of water decreases [18–21]; this could be due to a restriction in molecular disordering of starch chains during gelatinization. Some solutes are known to impede granule swelling; there might be restrictive effects to the extent of conformational disordering of starch chains on gelatinization of starch, as inferred by the reduced enthalpy values observed in their presence. This influence of solutes may result from competition for water between starch and solutes (depression of a_w), starch–solute interactions, and an 'anti-plasticization' mechanism by the solute–water co-solvent [22]. However, other authors [14] found an increase in ΔH of starch gelatinization in the presence of solutes and attributed this finding to higher energy being needed to disrupt starch structure with lower moisture contents. García et al. [23] reported that

Table 2. Gelatinization and retrogradation parameters for studied samples

	Gelatinization			Retrogradation		
Sample	ΔH (J/g starch)	T_o (°C)	T_p (°C)	ΔH (J/g starch)	T_o (°C)	T_p (°C)
Cas	11.72 ± 0.60^{e}	58.37 ± 0.15^{a}	64.81 ± 0.19^{a}	3.46 ± 0.38^{c}	40.64 ± 0.37^{a}	58.27 ± 0.93^{d}
Cas/AS	10.18 ± 0.05^{cd}	59.91 ± 0.65^{ab}	66.83 ± 0.02^{b}	0.69 ± 0.09^{a}	45.20 ± 2.05^{c}	57.51 ± 0.01^{cd}
Cas/d-AS	10.29 ± 0.03^{cd}	60.46 ± 0.00^{b}	68.29 ± 0.10^{cd}	0.56 ± 0.00^{a}	46.24 ± 1.36^{c}	59.15 ± 0.25^{d}
Cas/IS	10.64 ± 0.14^{d}	60.27 ± 0.20^{b}	67.19 ± 0.23^{bc}	4.58 ± 0.09^{d}	42.49 ± 0.19^{ab}	54.16 ± 0.97^{a}
Cas/d-IS	10.51 ± 1.09^{cd}	62.46 ± 2.30^{c}	69.17 ± 1.56^{de}	4.53 ± 0.28^{d}	42.58 ± 0.04^{b}	54.49 ± 1.81^{ab}
Corn	9.66 ± 0.27^{bc}	64.19 ± 0.01^{c}	69.89 ± 0.01^{e}	4.34 ± 0.99^{d}	42.52 ± 0.65^{ab}	57.58 ± 2.85^{cd}
Corn/AS	8.98 ± 0.23^{b}	66.66 ± 0.16^{d}	71.84 ± 0.20^{f}	2.54 ± 0.23^{b}	42.99 ± 0.02^{b}	56.84 ± 1.00^{bcd}
Corn/d-AS	9.11 ± 0.03^{b}	67.62 ± 0.05^{d}	72.82 ± 0.14^{f}	2.97 ± 0.12^{bc}	43.23 ± 0.95^{b}	57.26 ± 0.07^{cd}
Corn/IS	7.90 ± 0.34^{a}	66.90 ± 0.17^{d}	72.04 ± 0.25^{f}	4.61 ± 0.00^{d}	42.83 ± 0.60^{b}	53.73 ± 0.55^{a}
Corn/d-IS	7.99 ± 0.09^{a}	67.94 ± 0.06^{d}	73.00 ± 0.37^{f}	4.45 ± 0.15^{d}	43.78 ± 0.05^{b}	55.06 ± 0.33^{abc}

AS, active soy flour; Cas, cassava starch; Corn, corn starch; d-AS, defatted active soy flour; d-IS, defatted inactive soy flour; IS, inactive soy flour; T_o, onset temperature; T_p, peak temperature; ΔH: transition enthalpy.
Values followed by different letters in the same column are significantly different ($p < 0.05$).

higher water contents led to lower gelatinization enthalpy assuming that the measured enthalpy was the result of a balance between endothermic melting (double helices disorganization and the packing arrangement of helices) and exothermic effects such as hydration.

Soy flour presence had different effects on AP retrogradation, depending on which starch and flour were combined (Table 2). Thus, AS diminished ∼80% retrogradation enthalpy of Cas, while corn retrogradation was diminished ∼40%. On the contrary, IS augmented Cas AP retrogradation, and did not affect corn retrogradation process.

Using fluorescence microscopy, Ribotta and Rosell [24] found that, after gelatinization, corn starch produced a gel that displayed a continuous phase formed by swollen starch granules tightly interacting, while no-starch granules were identified on cassava gel, presenting a homogeneous network-like structure. Cas granular structure was completely disrupted with a dispersed phase formed by continuous polymer dispersion (AM/AP). For this reason, proteins present in AS may easily interact with Cas during gelatinization process, below protein denaturation temperature (∼85°C). This interaction may explain the lower retrogradation enthalpies during storage. On the other hand, proteins present in IS closely interact among themselves, which makes protein/starch interactions difficult. Under such conditions, the effect of these proteins on starch retrogradation may be lower when compared to that of AS proteins. These observations are in agreement with the SB values found in RVA (see below).

From Table 2, it is evident that lipid presence did not significantly affect starch gelatinization or AP retrogradation. In agreement with this observation, Eliasson and Ljunger [25], when studying the effect of different lipids and surfactants such as cetyltrimethylammonium bromide, saturated and unsaturated monoglycerides, lecithin, soy bean oil and sodium cholate on AP retrogradation, found that cetyltrimethylammonium bromide, followed by lecithin were the additives which led to minimum retrogradation, while soy bean oil did not affect the retrogradation process.

3.3 RVA

Viscosity behaviour during heating from 30 to 95°C reflects starch capacity to absorb water and swell as the slurry is heated; this results in a concomitant increase in viscosity. Paste viscosity increases to the point where the number of intact swollen granules is maximum, reaching the PV, which is indicative of its water binding capacity [26]. As temperature increases, the granules continue to absorb water until they break up and viscosity decreases. This decrease is called BD. When gelatinized starch cools down, AM retrogrades and the result is an increase in viscosity or SB.

As shown in Table 3, PV was higher in Cas than in Corn, while the latter showed a higher pasting temperature and FV. Tuber and root starches are known to show a sharper rise in viscosity during cooking, and have higher PV than common cereal starches [27]. High PV is related to water absorption capacity which, in turn, depends on the packing arrangements of the starch crystallites and/or interactions between starch components in the amorphous regions. The low pasting temperature and the rapid increase in viscosity during Cas starch heating are indicative of a weaker granule structure and better water binding properties than corn starch. Wickramasinghe et al. [28] found similar T_p values for Cas starch, although they found lower peak viscosities. Sriroth et al. [29] found similar Cas pasting properties, and besides reported differences in starch properties when comparing different cultivars and roots with different harvest times. This could explain the differences found in the literature on Cas starch pasting and swelling properties [13, 30]. Corn pasting parameters are

Table 3. Pasting parameters for studied samples

Sample	T_p (°C)	PV (BU)	FV (BU)	BD (BU)	SB (BU)
Cas	67.8 ± 0.0^{a}	427.9 ± 1.6^{h}	237.5 ± 7.4^{g}	283.4 ± 1.7^{i}	92.6 ± 7.4^{e}
Cas/AS	68.6 ± 0.0^{bc}	167.5 ± 1.2^{c}	88.9 ± 0.6^{a}	110.3 ± 1.8^{f}	31.6 ± 0.1^{a}
Cas/d-AS	69.1 ± 0.6^{c}	165.9 ± 2.7^{c}	80.8 ± 0.9^{a}	113.8 ± 3.1^{f}	28.7 ± 1.4^{a}
Cas/IS	68.3 ± 0.6^{ab}	386.1 ± 0.3^{g}	207.7 ± 0.1^{e}	246.2 ± 1.9^{h}	67.8 ± 1.5^{b}
Cas/d-IS	68.6 ± 0.0^{bc}	366.0 ± 1.6^{f}	198.0 ± 2.7^{d}	232.2 ± 0.7^{g}	64.2 ± 1.8^{b}
Corn	76.8 ± 0.1^{d}	232.8 ± 0.2^{e}	247.6 ± 1.1^{h}	76.5 ± 0.2^{d}	91.2 ± 0.7^{de}
Corn/AS	76.7 ± 0.0^{d}	141.3 ± 0.7^{b}	188.6 ± 0.0^{c}	49.3 ± 0.7^{b}	96.5 ± 0.0^{e}
Corn/d-AS	76.3 ± 0.5^{d}	133.3 ± 5.8^{a}	176.4 ± 9.3^{b}	34.3 ± 3.2^{a}	77.4 ± 6.8^{c}
Corn/IS	76.3 ± 0.6^{d}	199.9 ± 2.3^{d}	216.3 ± 1.2^{ef}	87.9 ± 0.4^{e}	104.2 ± 1.5^{f}
Corn/d-IS	76.8 ± 0.0^{d}	196.9 ± 2.4^{d}	218.2 ± 0.4^{f}	63.0 ± 3.9^{c}	84.3 ± 1.8^{cd}

AS, active soy flour; Cas, cassava starch; Corn, corn starch; d-AS, defatted active soy flour; d-IS, defatted inactive soy flour; FV, final viscosity; IS, inactive soy flour; PV, peak viscosity; T_p, pasting temperature.
Values followed by different letters in the same column are significantly different ($p < 0.05$).

in agreement with previously reported results [31], although these parameters are highly dependent on assay conditions and the variety of corn used.

Breakdown of starch granules, related to the ability of the starches to withstand heating and shear stress, is an important factor in many processes. Usually high BD values are associated to high-peak viscosities [32]. Correspondingly, in this study a significant correlation between PV and BD was found ($r = -0.83$; $p < 0.05$).

Proteins may affect the gelatinization process in different ways depending on their ability to retain water and their interaction capacity with the starch molecules and granules surface [24]. Soy flour addition had a dramatic effect on pasting properties of both starches, although it was greater on Cas starch. This decrease in viscosity is partly attributed to the dilution of starch. Nevertheless, in both cases, AS had a larger impact than IS. As observed in Table 3, pasting temperature of Cas was increased by soy flour addition. Cas PV was reduced 60.9% by AS addition, while this reduction was only about 9.8% when IS was added. Similarly, AS addition decreased Corn PV in a 39.3% and IS reduced this value in a 14.1%. Following the same trend, Cas BD was reduced 61.2% by AS, and 13.1% by IS; whereas Corn BD decreased only 23.8% and 12.6% with AS and IS incorporation, respectively. Reduction percentage of RVA parameters for both starches when IS was added corresponds almost exactly to starch dilution effect (90% starch + 10% soy flour). Finally, SB was reduced 65.4 and 26.9% when AS and IS were incorporated to Cas, respectively.

In general, defatted soy flours reduced RVA parameters to a higher extent than full-fat flours, which may be related to the higher protein content present in the former.

Summing up, cassava starch presented a weak granular structure, exposing AM/AP chains which increase the surface for interaction with soy proteins. Besides, as mentioned above, proteins present in IS are assumed to be unfolded, favouring protein/protein interaction, while most of the proteins present in AS are in a native state and are able to interact with starch.

3.4 XRD

So far, results indicated that soy proteins interact with starch during gelatinization and that this interaction delays AP retrogradation. To corroborate this finding, X-ray diffractometry of composites was carried out. Figure 1 shows diffractograms for gelatinized and retrograded composites. All curves presented diffraction peaks corresponding to a B-type structure (2θ: 14, 15, 17, 20, 22, 24 and 26°), as is typical for retrograded starches [33], although intensities varied from one sample to another. For Cas, addition of AS produced a drastic crystallinity reduction confirming that it did interfere during AP retrogradation process. On the other hand, Cas/IS showed a similar diffraction profile to Cas alone. For Corn samples, the same trend was observed, with a crystallinity reduction with AS addition, although this effect was less pronounced than when it was added to Cas.

Relative crystallinity formed during retrogradation was greatly reduced when AS was added, and that this effect was observed when it was added to both starches, although more markedly when added to Cas (crystallinity was reduced from 25.43% for Cas to 11.59% for Cas/AS and 25.08% for Cas/IS; and from 23.75% for Corn to 21.38% for Corn/AS and increased to 25.45% for Corn/IS).

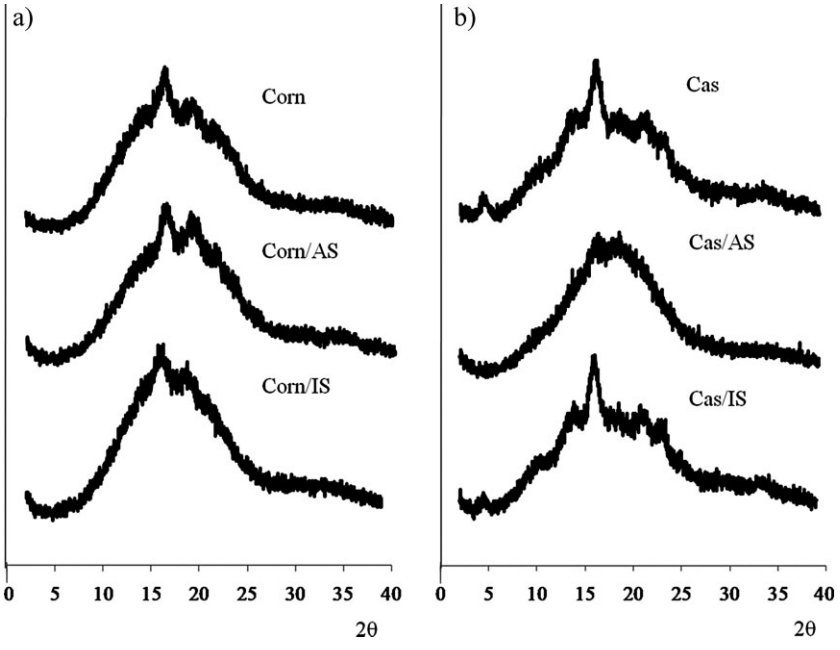

Figure 1. X-ray diffractograms for gelatinized and retrograded starch/soy flour composites. (a) Corn, corn starch, (b) Cas, cassava starch. AS, active soy flour; IS, inactive soy flour.

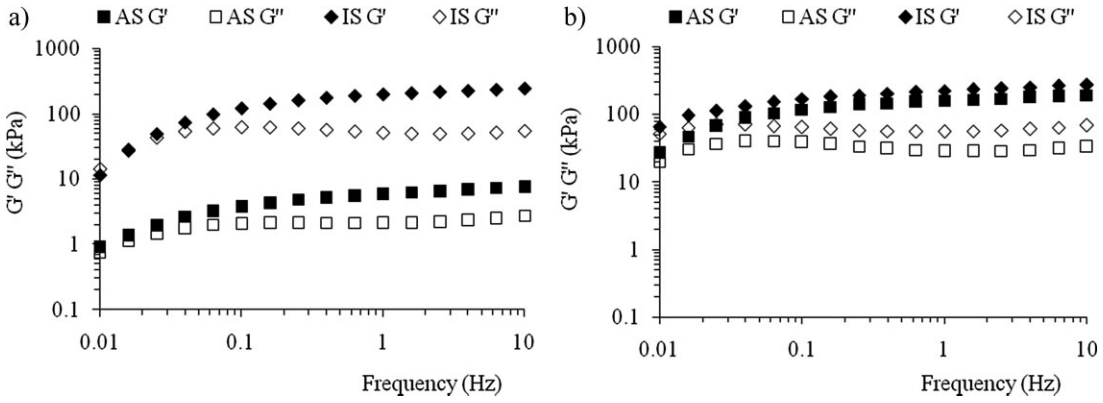

Figure 2. *G′* and *G″* as a function of frequency. (a) Cassava based composites; (b) Corn based composites. AS, active soy flour; IS, inactive soy flour.

This result is in agreement with those of DSC and SB, evidencing a clear reduction in Cas retrogradation when AS was incorporated.

3.5 Rheological properties

The frequency sweep shows how the viscous and elastic behaviour of the material changes with the rate of application of strain or stress. In this test, frequency is increased while the amplitude of the input signal (stress and strain) is held constant [34]. Figure 2 illustrates changes in elastic (*G′*) and viscous (*G″*) moduli as a function of frequency for starch–soy flour mixtures. The measurement of the starches alone was not possible since a rapid separation of water was observed. Both moduli were frequency-dependent; samples presented a rheological behaviour corresponding to a concentrated solution or a weak gel, where a modulus crossing over at low frequencies was observed, with higher *G″* at low frequencies, and an increase in *G′* at higher frequencies.

Table 4 presents *G′*, *G″* and tan δ at 1 Hz for all samples. Cas/AS exhibited the highest tan δ and the lowest *G′* values (Cas/AS *G′* value at 1 Hz was 25–37 times lower than other samples). It has been suggested that water can act either as inert filler reducing the dynamic properties proportionally to moisture content, or as a lubricant enhancing the relaxation phenomena [35]. The higher syneresis value of Cas/AS blend may indicate that there is more water acting as a lubricant, thus reducing *G′* values (Tables 1 and 4).

Other authors, working with starch-based cakes added with soy and gluten proteins found that the presence of proteins, mainly those of soy, significantly diminished tan δ value [36].

For Cas samples, tan δ values were higher when AS was added, while for corn samples tan δ was higher when IS was present: thus, the effect of AS on the rheological properties of starch was highly dependent on starch nature. It has been postulated that different starches may interact with exogenous proteins through starch associated proteins [37]. Starch granule associated proteins are those naturally found on the surface of the granule or as an integral part of the granule structure. They are different from grain or tuber reserve proteins, and are mainly enzymes associated to starch synthesis, with a MW between 5 and 149 kDa [38]. Yoshino et al. [39] found this type of proteins in several starches, among them cassava and corn. Ryan and Brewer [37], working with wheat starch and soy proteins, concluded that granule surface associated proteins could serve to anchor binding proteins

Table 4. *G′*, *G″* and tan δ values at 1 Hz for starch/soy flour composites

Sample	*G′* (kPa)	*G″* (kPa)	tan δ
Cas/AS	5.92 ± 1.50^{a}	2.11 ± 0.6^{a}	0.355 ± 0.018^{c}
Cas/IS	200.33 ± 11.50^{b}	50.83 ± 3.26^{c}	0.254 ± 0.005^{b}
Corn/AS	158.50 ± 65.81^{b}	28.37 ± 9.52^{b}	0.183 ± 0.016^{a}
Corn/IS	224.00 ± 4.10^{b}	55.60 ± 4.38^{c}	0.251 ± 0.027^{b}

AS, active soy flour; Cas, cassava starch; Corn, corn starch; IS, inactive soy flour.
Values followed by different letters in the same column are significantly different ($p < 0.05$).

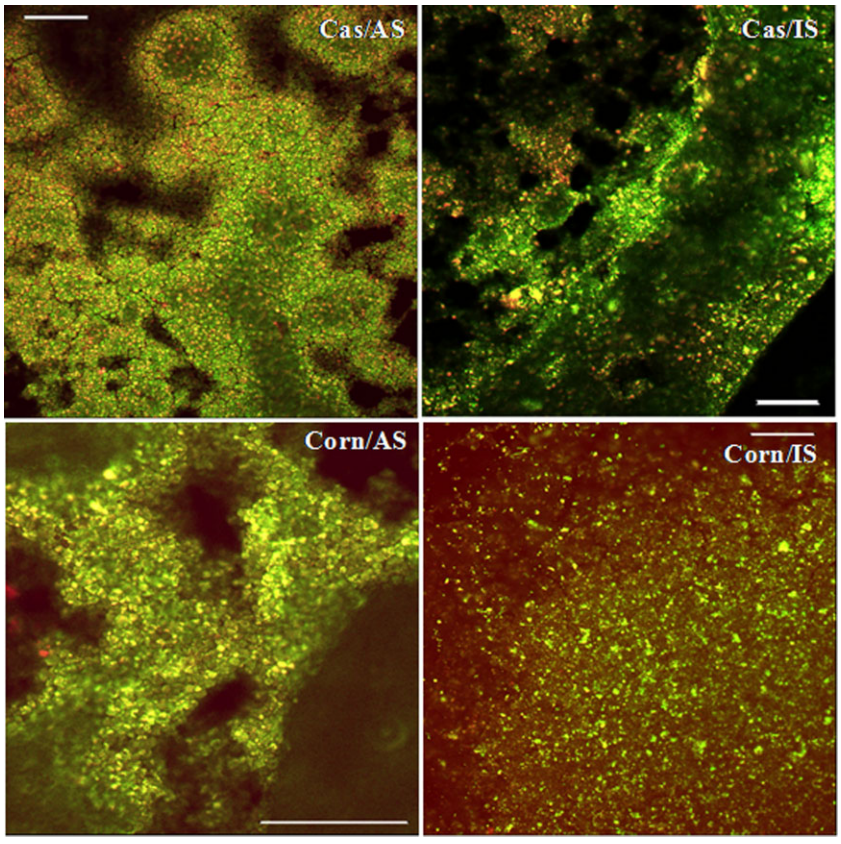

Figure 3. Images of starch/soy flour composites obtained using confocal laser scanning microscope. Red/yellow: proteins; green: starch. Bar: 200 μm.

to the starch granule surface, and maintained this interaction against desorption. Although it is known that starch associated proteins, present in low amounts (0.25–0.6% in cereal starches and 0.1% in cassava starch), notably affect starch physical properties, they have not been extensively studied, with the exception of those found in wheat. Besides, their chemical nature, and therefore the properties of starch surface, is variable according to the botanical origin. If the starch associated proteins of both starches differ in their nature, they may interact differently with exogenous material. On the other hand, denatured soy proteins have a surface with a more hydrophobic character than native proteins. Thus, starch/soy protein interaction is highly dependent on the nature of both types of proteins.

These results show that cassava starch and AS proteins interact not only during gelatinization, when AM/AP chains are exposed, but also under conditions where starch is in a native state.

3.6 CLSM

This technique was used to evaluate protein distribution in relation to starch. Figure 3 shows micrographs of starch/soy flour composites. According to the results exposed, for both starches, IS protein was present as large aggregates, while AS protein distribution was more homogeneous. Besides, in Cas/AS composites, protein distribution was even more homogeneous when compared to that of Corn/AS. Moore et al. [3] used confocal laser scanning microscopy to assess protein distribution of different sources in gluten-free dough made from rice, corn and potato starches. These authors found that soy proteins were distributed as large aggregates between the starch granules.

4 Conclusions

Results show that cassava starch and AS specifically interact between them, and that this interaction occurs mainly with soy proteins rather than with soy lipids, considering that lipid absence produced only slight changes in the composite behaviour. This interaction may occur: (i) under native conditions, where soy proteins may interact with starch – possibly through starch associated components – modifying composite rheological properties and protein distribution relative to starch fraction, and (ii) under heating conditions, where, during gelatinization, cassava starch exposes AM/AP chains more than corn starch, leaving these chains free to interact with soy proteins modifying pasting parameters and, mainly, the retrogradation process. The inhibitory effect of AS on

cassava starch retrogradation was clearly shown. This finding could be useful for obtaining gluten-free breads of high quality and low retrogradation rate.

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