



Technological properties of sour cassava starches: Effect of fermentation and drying processes

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ABSTRACT

Cassava sour starch is a naturally-fermented, sun-dried product. It allows obtaining less dense bakery products than the native starch, being an appreciated gluten-free ingredient. The aim of this work was to study the effect of fermentation and drying method on the physicochemical and technological characteristics of cassava sour starch and to identify the relationships among those distinctive features that may condition their potential food applications.

Natural fermentation following starch extraction could not be replaced by the use of a starter. Sour starches pastes showed lower apparent viscosities related to their lactic and/or butyric acid content, and a higher retrogradation tendency, mainly associated to sun-drying. The fermentation combined with the solar exposition represent a complex process that induce changes not easily observed on starch powders but clearly evidenced after gelatinization of the starch suspensions.

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a sturdy, perennial crop grown in many regions of Asia, Africa and South America. Cassava starch has low gelatinization temperature, which facilitates the cooking process and can be useful for products containing heat-labile ingredients. The high viscosity of the starch paste is also appreciated for food products that require a cohesive texture, like some gravies used in the Orient (Moorthy, 2004).

Besides the native (sweet) cassava starch, the sour or fermented starch is also commercially available, but despite sour starch is commonly used in the manufacture of traditional bakery products, its obtaining process is not well defined, so there is a considerable variation in the quality of the final products (Acosta, Villada, & Prieto, 2006).

The production of sour starch comprises the peeling, washing, and grinding of the roots, and the aqueous extraction of the starch. The resultant product is then subjected to a spontaneous anaerobic lactic fermentation, and the fermented starch is subsequently sunlight-dried (Dufour, Larssonneur, Alarcón, Brabet, & Chuzel, 1996). The organic acids produced during fermentation generate changes in the molecular weight and the surface morphology of the granules (García, Franco, Júnior, & Caliarí, 2016) and both, fermentation and sun-drying, confer

sour cassava starch specific functional properties, such as greater expansion during dough baking.

Cassava sour starch (known as *amido azedo*, *polvilho azedo*, or *almidón agrío*) is produced in Latin America, particularly in Brazil, Colombia and Paraguay, being a product of traditional rural industry. This kind of starch is used for obtaining industrially processed snacks, and for making cheese breads such as *pandebono* and *pan de yuca* (Colombia), and *pão de queijo* (Brazil). These bakery products do not undergo yeast fermentation as typically done for wheat-based bread, but the dough is instead baked immediately after kneading. Although the final product does not involve a protein-gluten network production (Dufour et al., 1996), cassava sour starch is able to produce higher volume bakery products than sweet (un-fermented) starch. This greater capacity of gas retention of the dough (Mestres, Rouau, Zakhia, & Brabet, 1996) represents a very attractive feature, mainly in the market of gluten-free baked goods.

The objective of the present work was to study the effect of fermentation and drying method on the physicochemical and technological characteristics of cassava sour starches and identify the features that may be distinctive for them.

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2. Materials and methods

2.1. Starch extraction and fermentation

Cassava plants (Rocha variety) were grown at the INTA Montecarlo farm (Misiones, Argentina). Starch was extracted from cassava roots following previously described procedure (Díaz, Dini, Viña, & García, 2016). In brief, roots were washed, sanitized with 250 ppm chlorine, hand peeled and cut. Water was added (2L per kg) and the mixture was processed with a domestic grinder, left for 24 h at 4 °C, and then filtered. The bagasse was discarded and the starch slurry was allowed to decant for 24 h at 4 °C. Part of the decanted cake was removed and oven-dried (GMX 9203A model, PEET LAB, USA) at 40 °C to obtain the extracted native starch (N). The other part was capped and left in contact with the decantation supernatant for 20 days at 20 °C for natural fermentation. The fermented cake was then divided into two equal parts: one of them was oven-dried at 40 °C (FO) for 48 h and the other one was sun-dried (FS) (20–25 °C for 5 days). The supernatant obtained from this fermentation process was used as a starter for a further fermentation to evaluate the possibility of standardizing the process and also dissociating the starch extraction from the fermentation. In this sense, a commercial native starch (CN) was assayed for fermentation in the same conditions as described above (20 °C for 20 days) using a 1:20 dilution of this supernatant in water (to include all the microorganisms that could be involved in the conversion of sweet to sour starch). For maintaining the water:starch ratio used in the natural fermentation, 2L of the diluted supernatant were used per 100 g of starch (considering 10% wb as the mean content of cassava starch separated in one extraction step). A sample of commercial native starch fermented with water (without starter) was simultaneously prepared. After the fermentation period, sour starches obtained using the starter (S) and without the starter (W) were oven-dried (SO and WO) or sun-dried (SS and WS), as previously described.

All the dried samples were grinded and sieved through a 60 mesh sieve.

The pH of the three fermentation supernatants (F, S and W), was registered along incubation period. Each fermentation process was carried out in duplicate.

For comparison, four commercially available cassava sour (fermented) starches were sieved (60 mesh) and analyzed, belonging to the Brazilian brands Amafil® (Amafil Ltda.), Beija Flor® (Huber Ltda.), Fritz & Frida® (Fröhlich Inc.), and Pinduca® (Pinduca Ltda.), henceforth referred to as: AMA (Amafil), BF (Beija Flor), Fritz (Fritz & Frida) and PIN (Pinduca).

2.2. Incubation supernatant analysis

Supernatants were stained with crystal violet and observed under the microscope. Isolation of the main microorganism distinguished (lactic acid bacteria (LAB), yeasts and molds) was performed in MRS (Difco, Detroit, USA), YGC (Merck, D-64271 Darmstadt, Germany) and Malt extract (Biokar, Beauvais, France) agar plates, respectively. All incubations were carried in aerobic conditions: 48 h at 30 °C for LAB and yeasts, and 5 days at 30 °C for fungus. Single colonies isolated in MRS and YGC were Gram stained. For mold screening, spores from the surface of each morphologically different colony obtained were scrapped off, extended and stained with lacto phenol cotton blue (Tiwari, Hoondal, & Tewari, 2009).

2.3. Starch characterization

2.3.1. Acidity and organic acid profile of native and fermented starches

Starch suspensions in distilled water (10% w/v) were mixed at room temperature for 30 min, centrifuged and the pH of the supernatant was measured with a pHmeter (Dufour et al., 1996).

Organic acid profile was analyzed according to Dufour et al. (1996)

with some modifications: starch samples (0.5 g) were extracted with 1.5 mL of H₂SO₄ 4.5 mM, vortexed for 1 min, and kept at 25 °C. Suspensions were homogenized again for 1 min after 15 and 30 min of extraction, and then centrifuged. Filtered samples (20 µL) were analyzed by HPLC-DAD (Waters Model 6000A LC, Milford, MA., USA) using an Aminex HPX-87H column (Bio-Rad, USA) and an isocratic elution mode with H₂SO₄ 4.5 mM as mobile phase. The flow rate was kept at 0.7 mL min⁻¹ and peaks were detected at 214 nm. The quantification of oxalic, citric, formic, succinic, malic, lactic, butyric, propionic, acetic and ascorbic acid was performed using analytical grade standards. Results were expressed as µg organic acid/g starch (ppm).

2.3.2. Amylose content and surface color

Amylose content (%) was determined spectrophotometrically at 600 nm according to Hoover and Ratnayake (2001). Starch color luminosity (L*) and chromaticity parameters (a* and b*) of the CIELab color space were obtained using a CR-400 Konica Minolta colorimeter (Osaka, Japan) (Díaz et al., 2016).

2.3.3. X-ray diffraction analysis

Starch powders were analyzed in a Philips 3020 Goniometer with PW 3710 Controller using Cu K α radiation ($\lambda = 1.5405 \text{ \AA}$) and Ni filter. The scan was performed at 40 kV and 20 mA for a 2θ range of 4–60°, with a step size of 0.04° and a collection time of 1 s at each step. For the determination of peaks area, baseline was drawn and subtracted using QualX 2.0 software (Altomare et al., 2015), applying a “filter” type background adjusted after 5 iterations. The crystallinity degree (CD) was calculated as the ratio between the absorption peaks area and the diffractogram total area, and expressed as percentage (%).

2.3.4. ATR-FTIR

Samples spectra were collected in the 4000–400 cm⁻¹ range by co-adding 64 scans with 4 cm⁻¹ spectral resolution on a Thermo Nicolet iS10 spectrometer with a diamond ATR accessory (Thermo Scientific, MA, USA). Starches were analyzed as dry powders pressed onto the crystal surface, as 50% (w/w) suspensions in deionized water (Warren, Gidley, & Flanagan, 2016), as fresh pastes (5% w/w) gelatinized at 90 °C for 20 min and cooled at room temperature, or as retrograded pastes (freshly prepared pastes stored at 4 °C for 48h). Starch suspensions and pastes were poured onto the crystal without applying any pressure. Background spectra were obtained following cleaning of the crystal with a mixture of ethanol and water. For each sample, at least nine FTIR spectra were registered.

Data was analyzed using the OMNIC software (version 8.3, Thermo Scientific, MA, USA). Spectra were baseline corrected and then deconvolved in the range of 955–1065 cm⁻¹ (within the fingerprint region of sugars, comprised between 1200 and 900 cm⁻¹); the assumed line shape was Lorentzian with a half-width of 19 cm⁻¹ (Sevenou, Hill, Farhat, & Mitchell, 2002). Three main bands were obtained after deconvolution of all samples, located around 1000, 1020 and 1045 cm⁻¹. The maximum IR absorbance of each band was recorded, and the absorbance ratios of the first-to-second and third-to-second bands were calculated.

2.3.5. Thermal analysis

Starches thermal properties were determined using a Q100 differential scanning calorimeter controlled by a TA 5000 module (TA Instruments, New Castle, DE, USA), according to Dini, Doporto, García, and Viña (2013). Thermal behavior was characterized by means of the onset (To), peak (Tp) and end temperature (Tc) (°C) together with the enthalpy (ΔH) of the process (area under the peak, J g⁻¹ of dry sample).

2.3.6. Rheological behavior

Starch aqueous suspensions (5% w/w) were gelatinized at 90 °C for 20 min, cooled at room temperature and analyzed in a Rheo Stress 600 ThermoHaake (Haake, Germany) rheometer with a plate-plate system

PP35 (gap size 1 mm) at controlled temperature (20 °C).

The obtained curves by rotational mode were mathematically modeled according to the Ostwald de Waele equation ($\tau = k\dot{\gamma}^n$, k : consistency coefficient; n : flow behavior index) (Díaz et al., 2016).

Viscoelastic behavior of starch pastes was studied by dynamic assays according to Díaz et al. (2016). The average of at least three records was reported.

2.3.7. Granule size distribution

Granules size distribution (expressed in % volume) was determined by Dynamic Light Scattering (DLS) with a particle size analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd., Worcestershire, U.K.), using refractive indices of 1.33 for water and 1.58 for starch according to Haaj, Magnin, and Boufi (2014). Measurements were performed in quadruplicate at room temperature.

2.4. Statistical analysis

Results were analyzed by a one way Analysis of Variance (ANOVA) using the Fisher's least significant difference (LSD) at a significance level of 5% ($p = 0.05$). For ANOVA and PCA analyses InfoStat software was used (Di Rienzo et al., 2011).

3. Results and discussion

3.1. Fermentation process and starch physicochemical characteristics

The pH profiles of the supernatants during fermentation of F, W and S starches are shown in Fig. 1. The differences observed in the curves during the first days, were related to the fermentation media and the amount of microorganisms present, while in the last days of fermentation, differences were mainly attributed to the type of microorganisms favored and/or the paths of fermentation followed in each case.

For F starches, the soluble components of cassava roots (amino acids, free sugars, etc.) are readily available at the initial stage. Nutrients and dissolved oxygen are rapidly consumed due to a fast proliferation of the microbiota, producing a pH drop of more than 1 unit in the first day of incubation (Fig. 1). After day 1, starch fermentation takes place, microbial proliferation rate decreases producing a slow change in the pH along the further 19 days, reaching a final value of 3.8 (the lowest from the three assayed methods).

For S starches, the low pH (4.6) at day 0 is due to the organic acids present in the starter (obtained from the supernatant of fermentation of the F starch). The starter represents a source of nutrients *per-se*, but there is also a high nutrient consumption at the beginning of the process, due to the relatively high amount of active microorganisms present. The pH is slightly increased over the first 3 days, probably related to the consumption of the organic acids as carbon source, and then

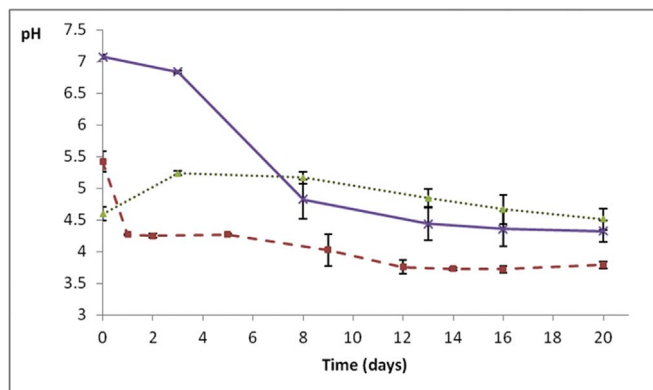


Fig. 1. pH profiles of the supernatants of starches naturally fermented F (---■---), fermented using a starter S (···▲···) and fermented in water W (—×—).

gradually decreases, finalizing in almost the same value it had at the beginning.

In the case of W starches, the medium is much poorer than that obtained after starch extraction from cassava roots, with starch as the only carbon source available. During the first days of fermentation (until day 3) microbial growth is limited, and the pH value drops just 0.3 units, starting at neutral pH. After day 3, the number of microorganisms capable of degrading starch is increased and the liberation of free sugars allows to a fast proliferation of the microbiota, reflected by a fall of 2 pH units until day 8. From then on, the pH is almost stabilized, reaching a final value of 4.3.

Lactic acid bacteria (LAB), yeasts and fungus have been distinguished as the main microorganisms present at the end of the three fermentation processes assayed (F, W and S).

LAB (mainly *Leuconostoc* and *Lactobacillus* species) and yeasts have been previously isolated from spontaneous fermentation of cassava starch (Lacerda et al., 2011; Lacerda et al., 2005). Mold has also been previously isolated from cassava starch fermentation supernatant, but no specific function in the process could be assigned (Cárdenas & de Buckle, 1980).

Despite no important differences were evidenced in the microscopic observation of the microbiota arising from the F, S and W fermentation processes, Fig. 1 shows that after day 8, pH kinetics follow the same behavior for W and S starches, while F starch is distinct, indicating differences in the paths of fermentation followed and/or the type of microorganisms favored during these processes.

Accordingly, as observed in Table 1, there is a clear difference in the organic acids profile among the fermented starches obtained from the roots (F) and those obtained from a commercial native starch (S and W), regardless of the drying method used. Starch acidity was mainly provided by lactic acid for F starches, while butyric acid is the principal organic acid present in W and S starches (Table 1). Besides butyric acid, W and S starches also showed small amounts of many other organic acids (Table 1). In contrast, only succinic and malic acids were detected on F starches, other than lactic acid (Table 1). It has been previously reported that lactic acid bacteria are able to produce formic, lactic, acetic, succinic, propionic and butyric acids (Özcelik, Kuley, & Özogul, 2016). Citric, oxalic and ascorbic acid were absent in all the samples analyzed.

Lactic acid resulted prevalent in commercial sour starches (Table 1), in agreement with the findings reported by Aquino, Gervin, and Amante (2016). Butyric acid was also found in two of the commercial sour starches analyzed, one of them (Fritz), with a concentration similar to that of lactic acid (Table 1). The presence of butyric acid was previously reported for commercial sour starches, but the concentrations found only reached up to 1450 ppm (Aquino et al., 2016). However, a marked input of lactic acid to the total acidity of the starch seems to be a common feature among naturally fermented starches.

F starches exhibited higher values of total acidity and lower pH of their aqueous suspensions than S and W starches. Among each fermentation process, sun dried starches exhibited lower acidity than the oven dried ones. Given that oven-drying temperature (40 °C) was higher than the sun-drying temperature (20–25 °C), this reduction in the organic acids content cannot be attributed to volatilization, but it can rather be related to a consumption of the lactic acid in a chemical reaction, as suggested in literature (Dufour et al., 1996). In the present work the same was observed for butyric acid, and particularly, for S starches, the effect of sun-drying resulted much more pronounced than that observed for F and W starches (Table 1).

A wide variability was observed in the total acidity of commercial sour starches, denoting that a high total acidity is not a specific trait related to the baking quality of sour cassava starches.

The quantification of amylose of sour and sweet samples showed values within the expected range for native cassava starches (15–25%) (Rolland-Sabaté et al., 2012), not differing the fermented ($p > 0.05$) from the respective native starches. Also, sour starch from the brand

Table 1
Acids content of starches (ppm).

	Lactic	Butyric	Acetic	Propionic	Formic	Succinic	Malic	Total	pH
FS	7013.8	–	–	–	–	53.3	207.7	7274.8	4.12
FO	8546.7	–	–	–	–	172.2	82.4	8801.2	3.87
N	84.9	439.8	93.9	–	–	48.6	34.2	701.4	5.42
SS	123.9	1774.0	38.4	–	–	101.9	119.2	2157.5	5.58
SO	192.7	3496.3	189.5	192.7	–	–	113.3	4184.5	4.87
WS	61.5	1754.2	28.2	137.7	29.7	–	73.8	2085.1	5.23
WO	170.2	1915.5	78.2	180.7	22.6	141.8	99.5	2608.3	4.91
NC	65.4	–	144	–	–	60.3	222.3	492	6.88
AMA	5917.8	1806.9	193.2	–	60.6	372.0	266.4	8616.9	3.52
BF	979.8	–	1764.9	–	220.6	93.3	97.8	3156.4	5.11
Fritz	4989.3	4978.5	504.1	657.4	48.1	234.6	319.9	11731.9	3.53
PIN	7220.2	–	290.4	–	113.8	212.2	345.2	8181.8	3.29

Note: Content of individual and total organic acids expressed as ppm of acid in the starch powder. The last column shows the pH of starch suspensions (10% w/v) in distilled water. N: native extracted starch. FS and FO: naturally fermented N starch, sun-dried (FS) and oven-dried (FO). NC: native commercial starch. SS, SO, WS and WO: NC starch fermented in aqueous medium with a starter (S) or only water (W), sun-dried (SS and WS) and oven-dried (SO and WO). AMA, BF, Fritz and PIN: commercial sour starches from brands Amafil, Beija Flor, Fritz& Frida and Pinduca, respectively.

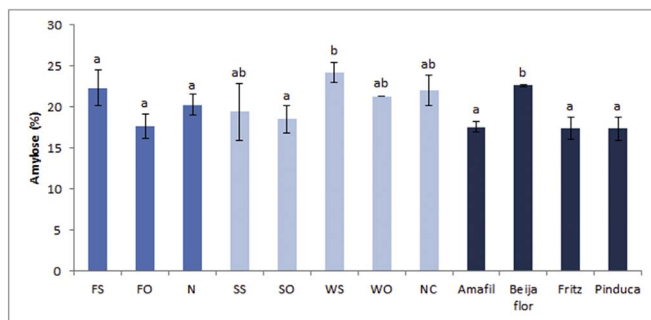


Fig. 2. Amylose content (% w/w) of native and fermented starches. Different letters above bars of the same color indicate significant differences ($p < 0.05$). N: native extracted starch. FS and FO: naturally fermented N starch, sun-dried (FS) and oven-dried (FO). NC: native commercial starch. SS, SO, WS and WO: NC starch fermented in aqueous medium with a starter (S) or only water (W), sun-dried (SS and WS) and oven-dried (SO and WO). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Beija Flor® considerably differed from the other commercial sour starches ($p < 0.05$) (Fig. 2), therefore the amylose content is not a feature that enables to distinguish fermented from sweet starches.

Regarding to the color, whiteness is considered a quality parameter of starches, with lighter tones associated to purer products. Cassava native starch is known to have a good white color which, at naked eye, seemed not to differ from that of fermented starches. The color parameters of the powders exhibited only slight differences among samples (Table 2), all of them with a high degree of luminosity (L^*) and low ΔE values associated to great whiteness of the samples.

Commercial sour starches exhibited the highest ΔE values, mainly determined by a greater contribution of the parameter b^* , related to the presence of a yellowish faint. In the case of F starches, natural fermentation reduced the b^* value with respect to the starting native starch, but sun-drying provided a higher b^* than oven-drying (Table 2). Therefore, this increment of b^* could be attributed to the starch oxidation due to light exposure. In agreement with this hypothesis, the commercial sour starch Beija Flor (BF) exhibited a significant higher b^* value compared to the other brands (Table 2), and was the one exhibiting the lowest total acidity among commercial samples (Table 1), which could be related to a higher length of exposure to sunlight (thus, a more extent reduction of organic acids due to photo-oxidation, as previously mentioned). Nevertheless, variations in the starting raw material or the fermentation and drying conditions should be taken into account for confirming this hypothesis.

Table 2
Color parameters of starches.

	L^*	a^*	b^*	ΔE
FS	98.42 ± 0.74 ^a	0.11 ± 0.04 ^b	1.94 ± 0.14 ^b	1.00 ± 0.58 ^a
FO	98.60 ± 1.03 ^a	0.05 ± 0.19 ^b	1.61 ± 0.21 ^a	1.35 ± 0.58 ^{ab}
N	98.69 ± 0.48 ^a	−0.07 ± 0.02 ^a	2.81 ± 0.09 ^c	1.62 ± 0.21 ^b
SS	97.88 ± 0.43 ^{ab}	0.07 ± 0.05 ^{ab}	2.01 ± 0.12 ^{ab}	1.04 ± 0.29 ^b
SO	98.26 ± 0.43 ^b	0.06 ± 0.02 ^a	2.07 ± 0.14 ^{bc}	0.87 ± 0.18 ^{ab}
WS	97.62 ± 0.98 ^a	0.09 ± 0.03 ^b	1.92 ± 0.12 ^a	0.87 ± 0.48 ^{ab}
WO	97.95 ± 0.65 ^{ab}	0.09 ± 0.03 ^{ab}	2.13 ± 0.17 ^{cd}	0.79 ± 0.36 ^a
NC	97.82 ± 1.01 ^{ab}	0.30 ± 0.03 ^c	2.24 ± 0.10 ^d	1.14 ± 0.20 ^b
AMA	98.19 ± 0.30 ^b	0.04 ± 0.02 ^b	4.19 ± 0.21 ^a	2.55 ± 0.16 ^b
BF	97.72 ± 0.50 ^a	−0.28 ± 0.03 ^a	4.97 ± 0.18 ^b	3.29 ± 0.14 ^c
Fritz	97.99 ± 0.53 ^b	0.03 ± 0.03 ^b	3.97 ± 0.20 ^a	2.32 ± 0.12 ^a
PIN	98.09 ± 0.39 ^{ab}	0.10 ± 0.02 ^c	4.10 ± 0.24 ^a	2.47 ± 0.16 ^{ab}

Note: Reported values correspond to the mean ± standard deviation. Groups of samples compared to each other are separated by horizontal lines. Different superscript letters within the same column indicate significant differences ($p < 0.05$). N: native extracted starch. FS and FO: naturally fermented N starch, sun-dried (FS) and oven-dried (FO). NC: native commercial starch. SS, SO, WS and WO: NC starch fermented in aqueous medium with a starter (S) or only water (W), sun-dried (SS and WS) and oven-dried (SO and WO). AMA, BF, Fritz and PIN: commercial sour starches from brands Amafil, Beija Flor, Fritz& Frida and Pinduca, respectively.

3.2. Starch structure and thermal properties

The DLS analysis of native and fermented starches showed that the native commercial starch exhibited a mono-modal distribution while all the other samples showed a bi-modal one. The peak of NC was centered at 15 μm , same as the first peak of the fermented starches derived from it (W and S); and the second peak that appeared in the fermented starches was placed at 138 μm for WO, SS and SO, and 120 μm for WS. For the native extracted starch and its derived fermented starches (FS and FO) the first peak was placed around 17 μm and the second one was at 138 μm for N and 158 μm for FS and FO (Suppl. Figure 1). Besides the shift produced by fermentation in the average size of the second peak, the fermentation processes increased the contribution of large-sized granules in the %volume, which might be related to an enhanced swelling due to a greater erosion of the granules produced by acids and enzymes action (Suppl. Fig. 1). This effect was particularly notorious for the commercial sour starches, which also exhibited the highest mean particle size for the second peak (182–240 μm), although showed different histograms, indicating that these products are not characterized by a specific granule size distribution.

The crystalline to amorphous ratio of the starch granules was studied by DRX and DSC and, at surface level, by ATR-FTIR.

Table 3
Crystallinity degree, thermal parameters and ATR peak ratios of starches.

Sample	XRD	Thermal parameters			Peak ratios	
	% Crystallinity	T _{onset} (°C)	T _{gelatinization} (°C)	Enthalpy (J g ⁻¹)	997:1018 cm ⁻¹	1047:1018 cm ⁻¹
FS	14.26 ± 0.31 ^a	60.5 ± 0.9 ^a	66.8 ± 0.4 ^a	14.1 ± 0.5 ^a	0.91 ± 0.05 ^b	0.56 ± 0.03 ^a
FO	16.28 ± 0.55 ^b	60.6 ± 0.9 ^a	67.2 ± 0.2 ^{ab}	13.8 ± 0.4 ^a	0.83 ± 0.06 ^a	0.61 ± 0.06 ^b
N	15.90 ± 0.59 ^b	60.8 ± 0.5 ^a	68.5 ± 0.7 ^b	15.4 ± 0.5 ^a	0.88 ± 0.04 ^b	0.55 ± 0.03 ^a
SS	15.15 ± 0.59 ^a	58.0 ± 0.6 ^a	66.1 ± 0.4 ^{bc}	16.5 ± 0.4 ^c	0.86 ± 0.06 ^a	0.60 ± 0.04 ^c
SO	15.48 ± 0.63 ^a	57.8 ± 0.0 ^a	65.4 ± 0.1 ^{ab}	15.5 ± 0.3 ^{abc}	0.87 ± 0.07 ^a	0.59 ± 0.02 ^{bc}
WS	16.12 ± 0.66 ^a	57.6 ± 0.0 ^a	66.2 ± 0.3 ^c	16.2 ± 0.6 ^{bc}	0.89 ± 0.04 ^a	0.57 ± 0.02 ^b
WO	15.42 ± 0.53 ^a	57.5 ± 0.2 ^a	66.5 ± 0.3 ^c	15.1 ± 0.7 ^a	0.88 ± 0.07 ^a	0.59 ± 0.05 ^{bc}
NC	17.73 ± 0.60 ^b	57.1 ± 0.7 ^a	65.4 ± 0.4 ^a	15.4 ± 0.3 ^{ab}	0.98 ± 0.04 ^b	0.51 ± 0.02 ^a
AMA	16.26 ± 0.57 ^a	62.8 ± 0.3 ^b	68.8 ± 0.1 ^b	15.5 ± 1.1 ^a	0.97 ± 0.03 ^b	0.54 ± 0.03 ^b
BF	16.28 ± 0.58 ^a	62.5 ± 0.7 ^{ab}	70.1 ± 0.2 ^c	17.0 ± 0.2 ^a	0.93 ± 0.09 ^b	0.57 ± 0.03 ^c
Fritz	15.11 ± 0.63 ^a	61.3 ± 0.2 ^a	68.0 ± 0.2 ^a	16.3 ± 1.6 ^a	0.83 ± 0.10 ^a	0.60 ± 0.04 ^d
PIN	16.37 ± 0.66 ^a	63.4 ± 0.3 ^b	70.2 ± 0.2 ^c	15.9 ± 0.5 ^a	0.96 ± 0.12 ^b	0.36 ± 0.01 ^a

Note: Reported values correspond to the mean ± standard deviation. Groups of samples compared to each other are separated by horizontal lines. Different superscript letters within the same column indicate significant differences ($p < 0.05$). N: native extracted starch. FS and FO: naturally fermented N starch, sun-dried (FS) and oven-dried (FO). NC: native commercial starch. SS, SO, WS and WO: NC starch fermented in aqueous medium with a starter (S) or only water (W), sun-dried (SS and WS) and oven-dried (SO and WO). AMA, BF, Fritz and PIN: commercial sour starches from brands Amafil, Beija Flor, Fritz&Frida and Pinduca, respectively.

Table 4
Rheological parameters of starch pastes.

Sample	Apparent viscosity at 500 s ⁻¹ (Pa s)	Consistency index, K (Pa s ⁿ)	Flow index, n	Thixotropy index (Pa s ⁻¹)
FS	0.289 ± 0.011 ^a	5.18 ± 0.26 ^a	0.5282 ± 0.0020 ^a	6288 ± 38 ^a
FO	0.319 ± 0.007 ^a	5.32 ± 0.12 ^a	0.5387 ± 0.0003 ^b	8717 ± 625 ^b
N	0.413 ± 0.011 ^b	6.62 ± 0.26 ^b	0.5451 ± 0.0018 ^c	15470 ± 297 ^c
SS	0.223 ± 0.011 ^c	3.47 ± 0.87 ^b	0.5526 ± 0.0320 ^{ab}	7744 ± 593 ^b
SO	0.378 ± 0.016 ^d	6.58 ± 0.14 ^c	0.5313 ± 0.0028 ^a	15460 ± 636 ^c
WS	0.105 ± 0.003 ^a	1.01 ± 0.08 ^a	0.6299 ± 0.0022 ^c	3626 ± 135 ^a
WO	0.191 ± 0.004 ^b	2.59 ± 0.18 ^b	0.5736 ± 0.0080 ^b	7332 ± 247 ^b
NC	0.428 ± 0.016 ^c	6.14 ± 0.34 ^c	0.5604 ± 0.0022 ^{ab}	25905 ± 346 ^d
AMA	0.068 ± 0.001 ^b	0.43 ± 0.01 ^b	0.7010 ± 0.0014 ^c	1231 ± 207 ^b
BF	0.081 ± 0.004 ^c	0.88 ± 0.01 ^c	0.6069 ± 0.0064 ^a	7843 ± 263 ^d
Fritz	0.119 ± 0.006 ^d	1.14 ± 0.07 ^d	0.6299 ± 0.0022 ^b	4616 ± 98 ^c
PIN	0.038 ± 0.002 ^a	0.17 ± 0.02 ^a	0.7619 ± 0.0073 ^d	355 ± 124 ^a

Note: Reported values correspond to the mean ± standard deviation. The correlation coefficient (r^2) obtained was 0.99 in all cases. Groups of samples compared to each other are separated by horizontal lines. Different superscript letters within the same column indicate significant differences ($p < 0.05$). N: native extracted starch. FS and FO: naturally fermented N starch, sun-dried (FS) and oven-dried (FO). NC: native commercial starch. SS, SO, WS and WO: NC starch fermented in aqueous medium with a starter (S) or only water (W), sun-dried (SS and WS) and oven-dried (SO and WO). AMA, BF, Fritz and PIN: commercial sour starches from brands Amafil, Beija Flor, Fritz&Frida and Pinduca, respectively.

By X-ray diffractograms, all samples exhibited a typical B-pattern, characteristic of tuber and roots starches. Crystallinity degrees of native and fermented starches are presented in Table 3. Starch fermentation resulted in granules with same or slightly less crystallinity ($p < 0.05$) than the starting native starches as previously reported for lactic acid bacteria fermented starches (Putri, Haryadi, Marseno, & Cahyanto, 2012), although not direct relation was observed with the drying method used (Table 3). The crystallinity of the commercial sour starches was within the range of that obtained for the starches fermented in the lab (Table 3), but did not exhibit values that allow differentiating them from sweet starches.

No marked differences were observed in the thermal properties of all the starches assayed (commercial and prepared samples), (Table 3). Fermented samples showed gelatinization temperatures close to their respective native starch and most of them with enthalpy changes rounding 15 J/g with small but significant ($p < 0.05$) variations among samples. Nevertheless, it is worth to mention that these differences would not represent a noticeable change in terms of energy needed for the industrial processing of bakery products. The lack of direct correlation between the DRX and DSC results could be associated to the complex nature of the process as well as the variations in the composition of the final products (organic acid, enzymes and remaining

microorganisms).

FTIR spectra of dry and hydrated starches resulted similar to those reported by Warren et al. (2016) for such samples (see Fig. 2A and B of supplementary material).

Dry starches profiles were almost identical for all the analyzed samples, even in the fingerprint region of sugars (900–1200 cm⁻¹), as previously observed by Demiate, Dupuy, Huvenne, Cereda, and Wosiacki (2000). Deconvolution of the spectra showed three main peaks located at 990, 1015 and 1045 cm⁻¹. The intensity of the bands around 1022 cm⁻¹ has been related to the amorphous regions of the granule outer layer, while the bands located at wavenumbers around 1000 cm⁻¹ and 1045 cm⁻¹ have been reported to be sensitive to the amounts of crystalline zones (Sevenou et al., 2002; van Soest, De Wit, Tournois, & Vliegert, 1994). The 1000 to 1022 cm⁻¹ and the 1045 to 1022 cm⁻¹ absorbance ratios have been used to predict the relative degree of crystallinity of starches (Bello-Pérez, Ottenhof, Agama-Acevedo, & Farhat, 2005; Sevenou et al., 2002; Warren et al., 2016). In the present work, no significant differences ($p > 0.05$) were observed in the absorbance ratios of 990:1015 and 1045:1015 cm⁻¹ for either of the analyzed samples in the dry state (data not shown), while hydrated samples exhibited slight differences in their FTIR-ATR spectra, particularly in the regions of 970–1040 cm⁻¹ and 1100 to 1140 cm⁻¹

(Fig. 2B of Supplementary material).

Deconvolution of hydrated starches showed peaks centered at 997, 1018 and 1047 cm^{-1} , similar to the values reported by van Soest et al. (1994) for potato starch aqueous suspensions. An inverse trend was observed for the 997:1018 cm^{-1} compared to the 1047:1018 cm^{-1} ratios for the sour starches obtained and the respective native ones, with no significant differences ($p > 0.05$) between fermented samples subjected to different drying methods (Table 3). The direct correlation of the 1000:1022 cm^{-1} and 1045:1022 cm^{-1} ratio to the crystallinity of acid hydrolyzed starches reported in previous works (Capron, Robert, Colonna, Brogly, & Planchot, 2007; Sevenou et al., 2002) was not observed in this case. This is probably related to the greater complexity of the fermentation process (which comprises enzymatic and acid hydrolysis plus chemical reactions) compared to the treatment with mineral acids only.

The peak ratio of 1000:1022 cm^{-1} has also been reported to increase with the hydration level, being starches with higher degree of crystallinity more sensitive to ratio changes (Capron et al., 2007; Warren et al., 2016). The 1000:1022 cm^{-1} peak of starches from different botanical origins in aqueous suspensions has been related to their gelatinization enthalpy, being higher ratios linked to higher values of ΔH (Warren et al., 2016). In the present work, no association was observed between the absorbance ratios of the 997:1018 cm^{-1} or the 1047:1018 cm^{-1} peaks and the ΔH of gelatinization, as result of the fermentation or drying methods applied (Table 3). Nevertheless, it is worth to mention that the changes observed in the ATR profiles of cassava starches subjected to different treatments are slight compared to those of starches from other plant sources (Warren et al., 2016). Evidently, the fermentation and drying processes are not producing considerable changes in the granule structure that might have a noticeable repercussion in the gelatinization enthalpy.

Commercial sour samples exhibited significant variations ($p < 0.05$) among their 997:1018 and 1047:1018 peak ratios, reflecting once again the variability of the products (Table 3). Furthermore, no specific peaks could be attributed to sour starches to be differentiated from the native ones.

3.3. Rheological behavior and ATR-FTIR of pastes

Gelatinized starch pastes exhibited a pseudoplastic behavior satisfactorily adjusted to the power law model (Table 4).

Fermentation processes (F, S and W) notably affected the rheological parameters of gelatinized pastes; in all cases, the viscosity, consistency index and flow behavior were decreased compared to the native starch pastes, exhibiting a minor thixotropic character (Table 4).

The viscosity decline observed for fermented starches with respect to the native ones could be attributed to the hydrolysis of the starch main molecules, especially those of short chain amylopectin, associated to both, the action of microbial amylases and the hydrolysis produced by the organic acids generated during fermentation. The lower apparent viscosities of sun-dried compared to oven-dried sour starches (Table 4) could be explained considering the partial depolymerization of the chains through UV-light catalyzed oxidation reactions (Vanier, El Halal, Dias, & da Rosa Zavareze, 2017). Particularly, Bertolini, Mestres, Colonna, and Raffi (2001) observed that UV irradiation on lactic acid treated cassava starch produces a further depolymerization than that due to the organic acid, and Zhu (2015) reported that both treatments reduce the viscosity and setback of pastes with little effect on the DSC gelatinization parameters.

Regarding to dynamic assays, the mechanical spectra of all pastes exhibited the viscoelastic character proper of gels, ($G' > G''$). Fermentation following extraction of the starch provided an enhanced elastic character to the pastes, which was evidenced by the higher G' values obtained for FS and FO compared to N starch pastes, while this enhancement of the G' was not observed for W and S compared to NC starch pastes (Fig. 3), reflecting the differences in the fermentation

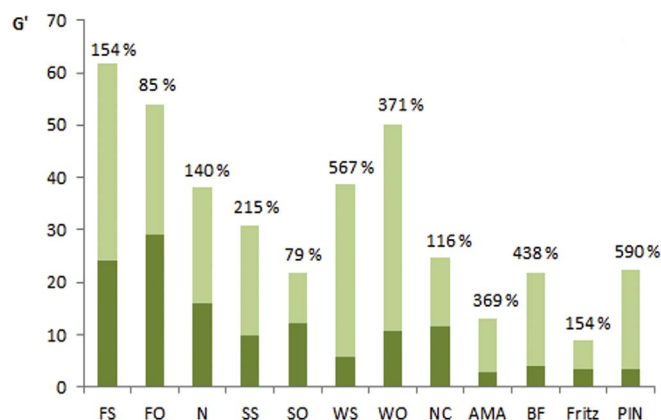


Fig. 3. Elastic modulus (G') of fresh (dark green bars) and retrograded (dark + light green bars) starch pastes. Percentage values above bars indicate the increments of G' after retrogradation. N: native extracted starch. FS and FO: naturally fermented N starch, sun-dried (FS) and oven-dried (FO). NC: native commercial starch. SS, SO, WS and WO: NC starch fermented in aqueous medium with a starter (S) or only water (W), sun-dried (SS and WS) and oven-dried (SO and WO). AMA, BF, Fritz and PIN: commercial sour starches from brands Amafil, Beija Flor, Fritz&Frida and Pinduca, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

paths and the resultant organic acids.

When comparing the G' value of the FS starch paste with those from the commercial sour starches, it is evident that the sun-drying process should lead to a much more pronounced reduction of G' of FS compared to FO pastes, thus reverting the increment of G' produced by the fermentation process (reflected in the higher G' of FO compared to N), (Fig. 3).

The four tested commercial sour starches (AMA, BF, Fritz and PIN) exhibited similar values of G' (and lower than those of all the other samples analyzed), but no homogeneity was observed in the degree of retrogradation (Fig. 3).

Regarding the samples fermented in the lab (F, S and W), all sun-dried starch pastes resulted more prone to retrogradation than the oven-dried ones (Fig. 3). Once again, it can be attributed to the hydrolysis and depolymerization derived from both fermentation and UV-radiation, where the resulting short chains could have a greater capacity to rearrange during refrigerated storage. Particularly, for S and W starches, sun-drying dramatically increased the retrogradation tendency compared to the respective oven-dried starch, but no direct relation could be established between the reduction of butyric acid by sun-drying (Table 1) and the extent of retrogradation of the pastes (Fig. 3).

ATR-FTIR spectra of freshly prepared and retrograded pastes were obtained (Supplementary Figs. 2C and D) and analyzed within the fingerprint region of sugars (1200–900 cm^{-1}). In all cases, deconvolution of the spectra showed peaks centered at 1000, 1023 and 1048 cm^{-1} from which the absorbance peaks ratios (1000:1023 and 1048:1023 cm^{-1}) were calculated. As previously mentioned, the absorbance ratios of 1047:1022 and 1022:995 cm^{-1} have been related to the order in more crystalline regions and the state of organization of the double helices localized inside crystallites, respectively (Wang, Li, Copeland, Niu, & Wang, 2015). In this sense, as a general trend, it was observed that the peak of retrograded pastes in the range of 980–1070 cm^{-1} gets slightly wider and the maximums of the three contributing peaks get more defined compared to the freshly prepared gelatinized pastes (Suppl. Fig. 2C and D). This widening reveals a reduction in the contribution of the 1023 cm^{-1} peak, related to the proportion of amorphous zones, and the increased definition of the peaks implies the increment of ordered structures in the paste.

In the PCA regarding starch pastes characteristics (peaks ratios, G' and G'' of fresh and stored pastes, viscosity and acidity of the starting

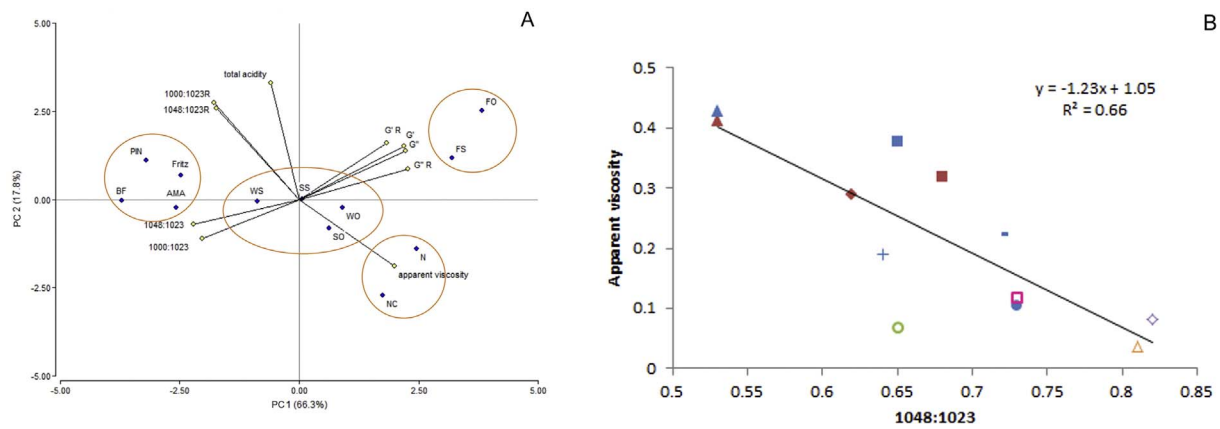


Fig. 4. A) PCA bi-plot (first and second components) of starch samples (blue dots) regarding paste characteristics (yellow dots). Samples were circled in orange according to their spatial closeness. B) Apparent viscosity vs 1048:1023 cm^{-1} peak ratio of refrigerated stored gelatinized pastes. Symbols: \blacklozenge FS, \blacksquare FO, \blacktriangle N, --SS , \blacksquare SO, \bullet WS, +WO , \blacktriangle NC, \circ AMA, \diamond BF, \square Fritz, \triangle PIN. N: native extracted starch. FS and FO: naturally fermented N starch, sun-dried (FS) and oven-dried (FO). NC: native commercial starch. SS, SO, WS and WO: NC starch fermented in aqueous medium with a starter (S) or only water (W), sun-dried (SS and WS) and oven-dried (SO and WO). AMA, BF, Fritz and PIN: commercial sour starches from brands Amafil, Beija Flor, Fritz&Frida and Pinduca, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

starch), the first two principal components (PC1 and PC2) explained the 84.1% of the total variance (Fig. 4A). Samples were grouped in four blocks: one including F starches, another of native starches, a third one gathering the commercial sour samples and the remaining samples (W and S starches) concentrated near the center of the graph. A negative correlation was observed between apparent viscosity and the 1048:1023 cm^{-1} ratio of the retrograded pastes (Fig. 4A). When plotting the apparent viscosity of the retrograded pastes vs the respective 1048:1023 cm^{-1} ratio, a linear negative correlation was observed ($r^2 = 0.66$) exhibiting the native starches the highest values of apparent viscosity with the lowest ratios, and the opposite for the commercial sour starches (Fig. 4B).

An inverse correlation between peak absorbance ratios of fresh and retrograded pastes and their G' and G'' values was also found (Fig. 4A). The elastic modulus (G') of fresh and retrograded pastes was plotted against the respective 1048:1023 cm^{-1} peak ratio (Fig. 5). For fresh pastes, lower 1048:1023 cm^{-1} ratios were associated to higher G' values (Fig. 5A), and despite no effect was observed regarding the fermentation or drying processes, this relation seems to be dependent on the starch origin (Fig. 5A). Conversely, a clear division was observed between native and fermented starches after refrigerated storage (Fig. 5B). Despite some pastes increased and other lowered their peak ratio after storage at 4 °C, all the fermented samples were scrolled to values of 1048:1023 cm^{-1} above 0.58 after retrogradation, while both native starches resulted in coincident peak ratios (0.53), significantly lower ($p < 0.05$) than the fermented starch pastes. Particularly, for the

starches fermented in this work, this result reflects a different re-organization of the hydrolyzed starch chains during refrigerated storage compared to the respective native starches but no direct relation with the fermentation or drying processes could be established.

4. Conclusions

Both fermentation and drying processes influenced the characteristics of the obtained products. The use of a starter did not cause the same results than the natural fermentation following starch extraction, which would indicate a staggered proliferation of the microorganisms involved during natural fermentation, conditioned by the available nutrients along the process. The differences in the microbiota and/or fermentation paths were evidenced in the particular organic acid profile of F with respect to the W and S starches. The similarity of the acid profiles between S and W starches is probably related to a nutritionally more restricted fermentation media.

No specific features were found that allowed to easily differentiate starch powders obtained from different fermentation conditions. Furthermore, broad differences were observed among commercial sour starch powders. However, after gelatinization, commercial sour starches did behave similarly but differed from the sun-dried fermented starches obtained in this work. This divergence could be attributed to a lower intensity of UV radiation in the region where this work was carried out, than the areas of production of the studied commercial sour starches (south of Brazil). Nevertheless, the exposure of all the

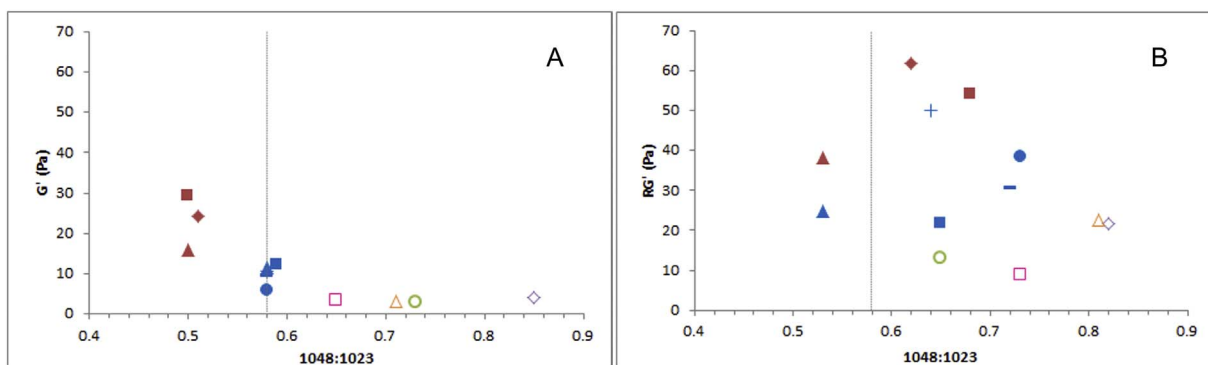


Fig. 5. Gelatinized starch pastes elastic moduli (G') as a function of ATR peak ratio (1048:1023 cm^{-1}) for: A) freshly prepared gelatinized pastes and B) pastes stored 48 h at 4 °C. Symbols: \blacklozenge FS, \blacksquare FO, \blacktriangle N, --SS , \blacksquare SO, \bullet WS, +WO , \blacktriangle NC, \circ AMA, \diamond BF, \square Fritz, \triangle PIN. Dotted vertical lines are for guidance. N: native extracted starch. FS and FO: naturally fermented N starch, sun-dried (FS) and oven-dried (FO). NC: native commercial starch. SS, SO, WS and WO: NC starch fermented in aqueous medium with a starter (S) or only water (W), sun-dried (SS and WS) and oven-dried (SO and WO). AMA, BF, Fritz and PIN: commercial sour starches from brands Amafil, Beija Flor, Fritz&Frida and Pinduca, respectively.

fermented samples to sun light led to a reduction in the organic acid content and a consequent increase in the pH of starch suspensions, regardless of the type of prevailing organic acid (lactic or butyric).

The rheological behavior of pastes reveals the hydrolysis and depolymerization of the starch chains occurring during fermentation. The decrease in the viscosity of freshly prepared pastes is associated to a greater retrogradation tendency during refrigerated storage, and related to the boost of the ATR peak ratios ($1048:1023\text{ cm}^{-1}$) of fermented starch pastes after refrigerated storage compared to the native ones. Also, an inverse correlation of retrograded pastes peak ratios to the viscosity of fresh pastes enabled differentiating native from fermented starches.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.lwt.2018.03.029>.

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