Research Article
Modification of the Fermentation Process for Sour Cassava Starch with Expansion Properties

[1]Figueroa Jorge A., [1]Cadena Edith M., [1]Rincón Cristian C. and [2]Salcedo Jairo G.
[1]Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Medellín, Colombia
[2]Facultad de Ingeniería, Universidad de Sucre, Sincelejo, Colombia

Abstract: The present research aimed to evaluate the effect of the manipulation of the process variables during the natural fermentation of the cassava native starch. In Colombia, the production process is carried out with a traditional and low-tech method in small companies called "rallanderías". Sour starch is a high added-value product that is obtained through submerged spontaneous fermentation with native starch, without control of processes variables and a product of heterogeneous quality. Thus, was evaluated the effect of the solid/liquid ratio (1:1, 1:2, 1:3 w/w) and the enrichment of the fermentation medium by adding glucose syrup and yeast extract (0.75% w/w) on the physicochemical properties of the medium, such as pH and titratable acidity, during the fermentation step. In addition, the quality of the fermented starch was evaluated with infrared spectroscopy (FT-IR), along with analysis of the paste viscosity behavior during the heating and cooling cycles with a viscoamylograph and the mass expansion capacity. The results showed a significant increase in the titratable acidity of the fermentation medium ($p<0.05$) that was linked to a pH decrease during the first 15 days of the fermentation. The infrared FT-IR spectra revealed substantial changes in the structural properties of the cassava starch resulting from the introduction or substitution of functional -OH and -COOH groups. The 1:1 solid/liquid ratio was the best treatment, with significant changes in the pasting properties, crystallinity index and spreading capacity of the cassava starch.

Keywords: Amylopectin, cassava, crystallinity index, fermentation, retrogradation

INTRODUCTION

In Colombia, sour cassava starch (*Manihot esculenta* Crantz) is traditionally obtained in small and medium-sized companies, as in some other South American countries such as Paraguay and Brazil. It is produced with spontaneous fermentation for approximately 30 days in warm-climate regions, which may be extended up to 45 days, depending on climatic conditions (Cadena *et al*., 2005). This product is widely used in the food industry because of its particular ability to expand dough during baking without the need for adding leavening additives. It has a high solubility index, a high swelling capacity, a lower viscosity peak and a lower tendency for retrogradation that potentiate its use in the development and formulation of traditional soft textured gluten-free products (Aplevicz, 2006; Marcon *et al*., 2009). The techno-functional characteristics of modified starches are the basis for specific applications (Salcedo-Mendoza *et al*., 2016).

The modification of starch involves changes in the structure through the introduction of a functional group and/or a controlled degradation and is driven by the biocatalytic action of amylolytic enzymes and oxidative reactions associated with the presence of organic acids, especially lactic acid produced during fermentation (Aquino *et al*., 2013). Spontaneous fermentation occurs when environmental conditions allow for the interaction of a microbial load and susceptible organic compounds rich in carbon and nitrogen (Diniz, 2006). Aplevicz (2006) and Diniz (2006) argued that the suitable fermentation of native starch involves an excess of water, with a supernatant layer that oscillates between 15 and 20 cm. However, the tendency to preserve artisan practices has meant that the fermentation process is carried out without the control of process variables where the solid/liquid ratio, despite being a fundamental factor at the beginning of the fermentation stage, has not been established as a parameter that needs to be controlled. In addition, because of the low level of industrialization, the sour starch productive sector faces environmental problems, highlighted by an excessive use of water with an average consumption of around 70 L/kg of starch, followed by inappropriate handling of the liquid residues with high values of

Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) (Reginatto *et al*., 2009).

In the fermentation, it has been determined that the presence of substrates is limited to the native starch granules because, during the native starch extraction process, water-soluble compounds such as vitamins and nitrogen compounds are removed (Aquino *et al*., 2013). The absence of nutrients is a critical factor in the fermentation process that influences the functional quality of sour starch; in addition, longer fermentation times are required for the initial production of oligosaccharides and nitrogen compounds with amylolytic bacteria and the generation of a synergistic effect for the consequent proliferation of lactic acidbacteria. Marcon *et al*. (2009) and Reginatto *et al*. (2009) reported a reduction in the fermentation time, without altering the filling properties or the expansion capacity of the starch after the incorporation of substrates such as ammonium sulfate and glucose syrup. Some studies have reported the effect of inclusion of substrates in the fermentation process using a 1:1 (Reginatto *et al*., 2009) or 1:4 starch/water ratio (Marcon, 2004), generates dissimilar results in both the organic acid production and the final quality of the sour starch. Thus, the present research aimed to evaluate the effect of the manipulation of the process variables during the natural fermentation of the cassava native starch by analyzing the behavior over time of the physicochemical properties of the fermentation medium, as well as changes in the structural characteristics and techno-functional properties of the modified granule.

MATERIALS AND METHODS

Modification of starch via fermentation: The native cassava starch (*Manihot esculenta* C.) was supplied by Almidones de Sucre S.A.S. The fermentation process was enhanced in 5.0 L capacity bioreactors and suspensions of native cassava starch were prepared with different solid/liquid ratios (1:1, 1:2 and 1:3); the liquid phase was pre-treated at a boiling temperature for 20 min in order to eliminate possible traces of chlorine. The glucose syrup and yeast extract were incorporated into the medium at a concentration of 0.75% w/w. Subsequently, the starch suspensions were subjected to a spontaneous fermentation process at room temperature for 30 days. Samples were extracted every 6 days and dried at 35°C for 10 h.

During the modification process of the cassava native starch, the behavior of the physicochemical properties, such as pH and titratable acidity of the fermentation medium, was evaluated. Measurements were taken in triplicate over 3 days and the acidity was expressed as lactic acid g/100 g of sample (AOAC, 2005).

FT-IR spectroscopy analysis: Pellets were prepared by mixing 20 mg of starch with KBr at a ratio of 1:5. IR spectra were obtained with a Fourier Transform Infrared Spectrometer (Thermo Scientific Reference Nicolet iS5 Transmission iD1) in the range of 500 to 4000/cm. Thirty two readings were taken at a resolution of 4/cm (Marcon *et al*., 2009). In addition, the Crystallinity Index (CI) was calculated based on the technique proposed by Smits *et al*. (1998), where the crystalline zones and amorphous areas of the starch granule are associated with the bands 1047 and 1022/cm, respectively.

Starch pasting properties: The filling properties were determined according to the method described by Salcedo-Mendoza *et al*. (2016) using a rheometer (Anton Paar, MCR 302, Austria) and a spindle for rheology of starches (Anton Paar, ST24-2D/2V, Austria). Two gram of starch were dissolved in 25 mL of distilled water. The suspensions were subjected to heating cycles and controlled cooling with continued stirring at 180 rpm. Initially, a temperature scan was performed at 50°C for a min, then at 95°C for 7.5 min, held at 95°C for 5.0 min, immediately cooled to 50°C for 7.5 min and finally held at 50°C for 2 min. The rates of ascent and descent were 7.5°C/min for each stage. The peak viscosity, final viscosity, breakdown viscosity and setback viscosity were obtained from the analysis of the viscoamylograph.

Expansion Capacity (EC): The starch expansion capacity was estimated in traditional bread products with a product formulation containing 33% starch, 8.5% butter, 6.5% sugar and 52% water with respect to a base of 100 g of coastal cheese. The different ingredients were mixed using a blender (SINMAG, SM 101) for 5 min at 500 rpm and then baked at 120°C for 300 min in an oven (PIRON, PF6004D). The expansion capacity was determined based on the displacement volume, using a 200 mL specimen and millet grains as reference "solid particles" (Aquino *et al*., 2013). The specific volume was expressed as the volume/weight ratio of the bakery product (cm^3/g).

Statistical analysis: A categorical multifactorial design was implemented, where the established factors were: Solid/Liquid ratio (S/L), substrate addition and fermentation time (Table 1). The results were analyzed using statistical tools such as Analysis of Variance (ANAVA) and multiple comparison test with a significance level of 5% using STATGRAPHICS software version XVI.I.

RESULTS AND DISCUSSION

Physicochemical properties: The solid/liquid ratio significantly influenced the pH variation and titratable acidity of the fermentation medium ($p<0.05$). The treatments with the 1:1 ratio (T1, T2) had the lowest pH values; however, no significant differences were seen

Table 1: Experiment design

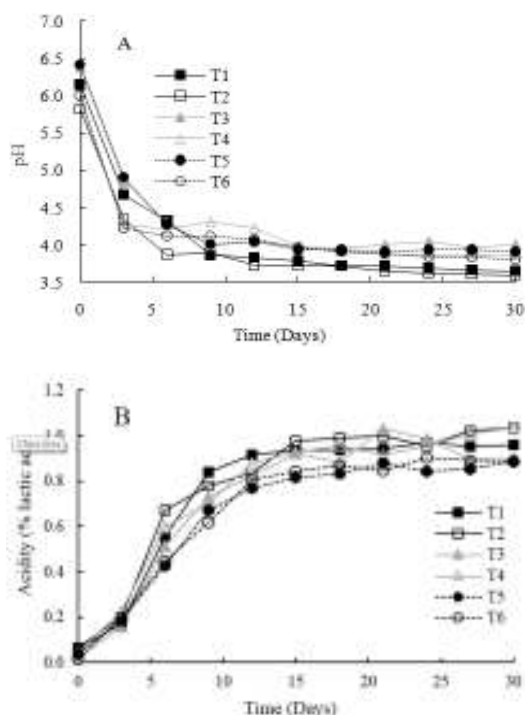| No.	Treatment	Ratio	Time (days)
T1	Fermented starch, without substrate added	1:1	30
T2	Fermented starch, with substrate added	1:1	30
T3	Fermented starch, without substrate added	1:2	30
T4	Fermented starch, with substrate added	1:2	30
T5	Fermented starch, without substrate added	1:3	30
T6	Fermented starch, with substrate added	1:3	30

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Fig. 1: Behavior of the physicochemical properties of the fermentation medium during the cassava sour starch production A) pH, B) Titratable acidity
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for the treatments with a 1:2 ratio (T3, T4) and the treatments with more water (T5, T6). Lacerda *et al.* (2005) argued that excess water can increase the dissolved oxygen concentration, slowing the biocatalytic activity of the aerotolerant anaerobic microbiota present in the initial fermentation phase, characterized by the production of reducing sugars and oxygen consumption.

The fermentation time had a significant effect on the pH and acidity ($p < 0.05$). The results evidenced a significant drop in the pH value during the first 6 days of fermentation, which continued to decrease slowly until 18 days, reaching equilibrium until the end of the process (Fig. 1A). A similar behavior was observed by Marcon (2004) and Reginatto *et al.* (2009), evaluating the process of sour starch production for 45 days with the addition of a carbon source. The authors argued that the initial fermentation stage is marked by a decrease in pH because of the biocatalytic activity of lactic acid bacteria, responsible for the conversion of reducing sugars to organic acids.

The results showed an accelerated increase of acidity during the first 9 days, remaining constant until the end of the fermentation (Fig. 1B). The acid amounts or concentrations determined in this period are similar to those reported by Demiate *et al.* (1999) and Reginatto *et al.* (2009), which were 0.036 to 0.813 g/100 g. In this study, the production of organic acids ranged from 0.023 to 3.422 g/100 g during the first 15 days of fermentation.

Treatments T2, T4 and T6 presented higher values of acidity, with a reduction of pH in comparison with the other treatments. This was possibly due to the incorporation of glucose and yeast extract in the fermentation process. Marcon *et al.* (2009) found a significant increase in the acidity of the medium with a consequent decrease in pH when glucose syrup was added at concentrations of 0.25 and 0.50% w/v. This same behavior was observed by Reginatto *et al.* (2009) when using ammonium chloride in the cassava native starch fermentation process. The authors argued that both carbon and nitrogen sources contribute to the increase in the production of organic acids.

FT-IR spectroscopy analysis: In the infrared spectroscopy analysis, absorbance peaks were observed in the range of 1000 to 1200/cm (Fig. 2A and 2B), related to the crystalline and amorphous regions, respectively, which form the molecular structure of the amylaceous material (Smits *et al.*, 1998). Absorbance peaks at 1010 and 1160/cm are possibly attributed to C-O, C-C or O-H functional groups, which are the most frequent chemical bonds found in amylose and amylopectin molecules (Van Soest *et al.*, 1995).

A characteristic peak of native starch is seen in the band length at 1646/cm because of the presence of bound water in the starch (Fang *et al.*, 2004). In the modified starches, this band was displaced by the possible incorporation of OH groups, which favor the ability of the starch granule to retain and absorb water (Marcon *et al.*, 2009). The treatment also increased the intensity of the band to 2920/cm, which is characteristic of vibrations in C-H groups that are possibly affected during fermentation. In addition, Van Soest *et al.* (1995) reported that peaks of 3300 and 2900/cm are attributed to molecular vibrations in O-H and C-H groups, which are abundant in polysaccharides. Comparing the FT-IR spectra suggests that starch O-H groups possibly participate in molecular interactions with -COOH groups in organic acids (Demiate *et al.*, 2000).
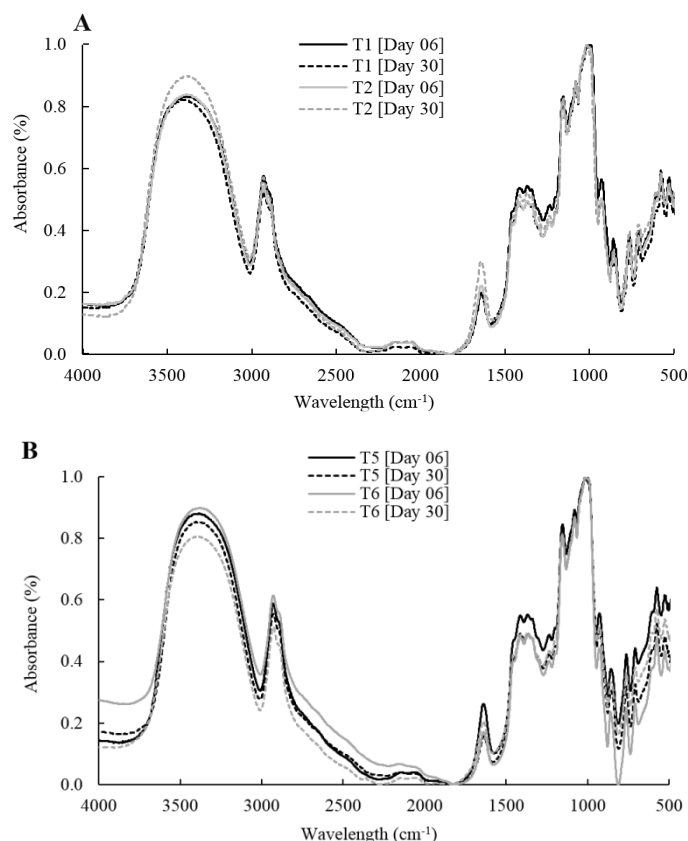
Fig. 2: Infrared Fourier Transform (FT-IR) spectra of cassava starches, A) Solid/liquid ratio 1:1, B) Solid/liquid ratio 1:3
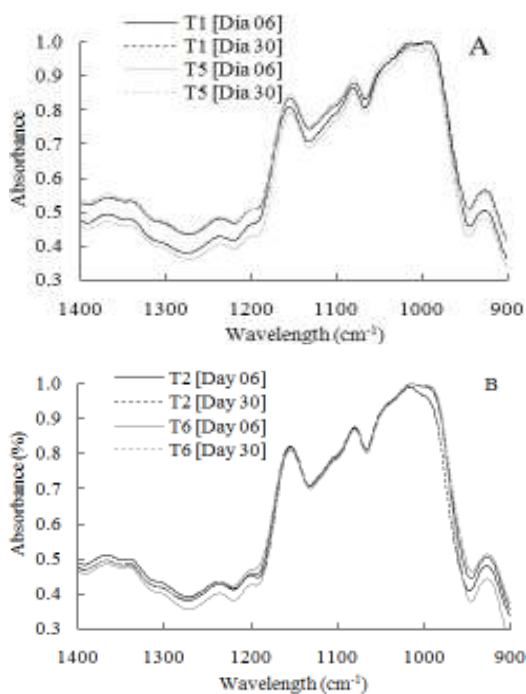   Prepared by researchers

Fig. 3: Infrared Fourier Transform (FT-IR) spectra of cassava starches in the 1400 to 900/cm range, A) No addition of substrates, B) With addition of substrates
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In order to determine the degree of sorption of the granule, the 1047/cm band was associated with the crystalline structure (Fig. 3A and 3B), while 1022/cm band was associated with the amorphous structure, which is sensitive to the hydrolysis processes of polymers. The results showed a shift of the bands in this region, associated with the significant increase in the crystallinity index -CI ($p < 0.05$) in the treated starches with respect to the native counterpart, possibly because of the amylolytic action of the lactic acid bacteria present in the fermentation medium. However, no differences were detected in the CI of the treated starches where substrates were incorporated into the medium (T2, T6), as compared to the established control (T1, T5). In addition, there was an increase in the intensity of the bands in the range of 1000 to 1057/cm, related to vibrations of the -COC- bonds present in the polysaccharide materials formed by the D-glucose units (Fang *et al*., 2004). These shifts of the spectra in the bands 3300, 2900, 1646 and 1057/cm, respectively suggest the possible substitution of functional groups in the chemical structure of the granules during the natural fermentation process.

Starch pasting properties: The natural fermentation process slightly decreased the initial paste formation

Table 2: Crystallinity index and filling properties of the cassava native and modified starch

Time	Treatment	CI	GT	PV	BD	SB
Day 0	Native	0.473	67.8	2670.1	1728.6	1065.6
Day 6	T1	0.481	65.8	2255.6	1504.0	902.0
	T2	0.482	65.9	2528.3	1672.4	891.8
	T5	0.484	65.7	2644.9	1837.8	982.3
	T6	0.485	65.0	2763.0	1893.1	1046.6
Day 12	T1	0.482	65.8	2152.2	1433.6	745.1
	T2	0.485	65.0	2327.4	1575.2	865.7
	T5	0.480	65.7	2334.0	1589.0	821.0
	T6	0.482	65.1	2559.5	1750.8	921.3
Day 18	T1	0.495	65.8	2056.5	1367.8	678.3
	T2	0.485	65.3	2229.0	1489.9	846.4
	T5	0.495	65.9	2226.5	1510.1	780.1
	T6	0.482	65.5	2226.3	1522.3	849.8
Day 24	T1	0.501	66.3	2072.0	1318.3	650.8
	T2	0.494	65.1	2087.0	1376.0	627.5
	T5	0.500	65.9	2286.5	1458.2	773.7
	T6	0.496	65.6	2215.0	1422.7	781.2
Day 30	T1	0.518	66.1	1836.5	1392.3	577.1
	T2	0.515	66.0	2082.4	1419.3	573.5
	T5	0.517	65.3	2240.5	1448.2	756.7
	T6	0.517	65.7	2189.8	1363.0	727.4

TG: Gel temperature (°C); PV: Peak viscosity (mPa.s); BD: Breakdown (mPa.s); SB: Setback (mPa.s); Prepared by researchers

temperature, relative to the native starch. However, no significant differences ($p > 0.05$) were found, which was associated with the solid/liquid ratio and incorporation of substrates (Table 2). This decrease in the gelatinization temperature may have been due to the weakening of intragranular forces as a result of the depolymerization of the granule by the biocatalytic action of amylolytic bacteria (Diniz, 2006). Similar results were reported by Vatanasuchart *et al*. (2005), Aplevicz (2006) and Alonso-Gómez *et al*. (2016), highlighting changes in the microstructure of the granule and increases in the degree of crystallinity.

The peak viscosity decreased significantly during the fermentation process, as a function of time and substrate incorporation ($p < 0.05$), possibly because of the increase in the ability of the granules to absorb and retain water (Alonso-Gómez *et al*., 2016), similar results to those found with the FT-IR analyzes. Vatanasuchart *et al*. (2005) also argued that the depolymerization of granules affects the swelling and solubility properties of the starch granule, causing a decrease in the viscosity peak. Similar results have been found in samples of starch under changing conditions of cassava cultivars and fermentation time (Aplevicz, 2006; Diniz, 2006; Rodrigues, 2012).

The difference between the maximum viscosity and the viscosity at the end of the heating period, known as "breakdown", allows for the evaluation of the resistance to deformation of the starch suspensions (Fig. 4A and 4B). The results showed an increase in the stability of the fermented starch paste, as compared to the native one, with the fermentation time. This reduction of the breakdown viscosity in the sour starches, possibly through the introduction of hydroxyl or carboxyl groups, which affected the structure of the granule, produced a greater resistance to deformation (Aplevicz, 2006; Alonso-Gómez *et al*., 2016).
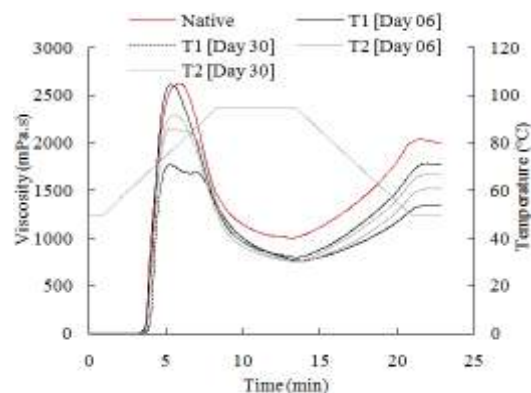
(a)
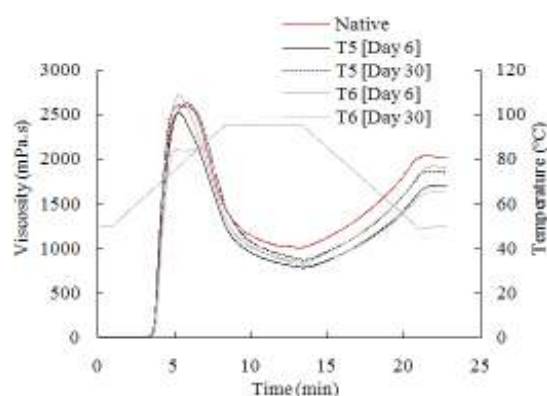
(b)

Fig. 4: Pasting properties in cassava starches, as a function of fermentation time; (a): Solid/liquid ratio 1:1; (b): Solid/liquid ratio 1:3.
Prepared by researchers

Because of the restoration of the hydrogen bonds between the amylose and amylopectin molecules, the

Table 3: Behavior of the titratable acidity, peak viscosity, crystallinity and expansion capacity in cassava sour starch

Treatment	Treatment	Native	Day 18	Day 30
T1	Acidity (% lactic ac.)	0.061	0.936	0.961
	CI (%)	0.473	0.495	0.520
	PV (mPa.s)	2670.100	2056.500	1836.500
	EC (cm^3/g)	2.124	3.672	3.761
T2	Acidity (% lactic ac.)	0.065	0.989	1.032
	CI (%)	0.473	0.485	0.515
	PV (mPa.s)	2670.100	2229.000	2082.400
	EC (cm^3/g)	2.119	3.721	3.703
T5	Acidity (% lactic ac.)	0.037	0.834	0.887
	CI (%)	0.473	0.495	0.510
	PV (mPa.s)	2670.100	2226.500	2189.800
	EC (cm^3/g)	2.108	3.255	3.638
T6	Acidity (% lactic ac.)	0.016	0.869	0.887
	CI (%)	0.473	0.482	0.517
	PV (mPa.s)	2670.100	2226.300	2240.500
	EC (cm^3/g)	2.116	3.640	3.621

Prepared by researchers

viscosity increased during the cooling period, leading to a gel-like consistency, which was higher in the native starch suspensions, related to the high tendency for retrogradation. The acid starches presented a lower tendency to retrogradation since the setback values decreased with the fermentation time, solid/liquid ratio and incorporation of substrates to the medium ($p < 0.05$). Similar results were found by Aplevicz (2006) and Rodrigues (2012) during the modification by spontaneous fermentation of native cassava starch. Vatanasuchart *et al*. (2005) argued that the decrease in the retrogradation tendency is a characteristic of starches hydrolyzed by fermentation, which enhances their incorporation in food matrices such as gluten-free baking products.

Expansion Capacity (EC): The expansion capacity of the sour starch increased significantly as a function of time and the solid/liquid ratio ($p < 0.05$). According to Marcon *et al*. (2009), the EC of starch depends on the degree of polymerization, number of carboxyl and hydroxyl groups, acidity, peak viscosity, density, type and conditions of the drying process of the granules. This agrees with the results obtained from the starch characterization, where fermented starches with marked differences in the acidity percentage, crystallinity index and peak viscosity showed a higher EC (T1, T2). However, no significant differences were detected for the EC in the baked product, in relation to the incorporation of substrates in the medium ($p > 0.05$).

The native starch presented a low EC, with respect to the fermented starches (Table 3). Vatanasuchart *et al*. (2005) pointed out that this difference may be associated with the depolymerization of the granule and a high CI because of the action of amylolytic bacteria during fermentation. Native starch does not undergo these changes and its EC is associated with the natural swelling power experienced by granulated materials in the presence of water.

The modified starches had an EC ranging from 2.116 to 2.761 cm^3/g. These results are lower than those reported by Diniz (2006), who evaluated the EC of citrus starch after 6 days of fermentation. However, they were superior to those reported by Marcon *et al*. (2009) and Reginatto *et al*. (2009), who used sour starch after 45 days of fermentation. These researchers suggested that the ingredients in the formulation, native starch provenance and fermentation time significantly influence the quality of the final product.

The results showed that some parameters do not have a correlation with the expansion properties, such as the gelatinization temperature, while others can be used as a means of prediction for standardizing the production process of sour starch according to the expectations of the baking industry. Also, Rodrigues (2012) argued that after fermentation, dextrins and -COOH groups are also present in cassava sour starch granules as well as OH groups in the amylose and amylopectin chains, which may alter fermented pellet expansion properties.

CONCLUSION

The time and solid/liquid ratio had a significant effect on the fermentation process of the cassava sour starch ($p < 0.05$). The introduction of OH and -COOH groups into the structure of the starch granules was corroborated with a Fourier Transform Infrared Spectroscopy (FT-IR) analysis. Likewise, an increase in the crystallinity index, associated with a decrease in the amorphous area during the degradation of polymer chains by the amylolytic microbiota present in the medium, was observed. The process carried out with a 1:1 solid/liquid ratio was the best treatment, significantly affecting the structural characteristics and filling properties, which correlated with an increase in the expansion properties of the fermented granule. The results obtained allowed some bases for standardization processes and improvement of the productive process and final quality of cassava starch for the baking industry.

ACKNOWLEDGMENT

CONFLICT OF INTEREST

The authors do not have a "Conflict of interest" for the research results.

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