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Oxidation of fermented cassava starch using hydrogen peroxide

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ABSTRACT

The effects of oxidation with hydrogen peroxide (H_2O_2) on the carbonyl and carboxyl content, pasting, expansion and textural properties of oven-dried fermented cassava starch were investigated. A 2^3 factorial design with three central points and six axial points was used to investigate the effect of H_2O_2 concentration, pH and temperature on the oxidation of fermented cassava starch; oven-dried and sun-dried fermented cassava starches were used as controls. The conditions of oxidation that presented the carbonyl and carboxyl contents of starch and specific volume and hardness of biscuits similar to the sun-dried fermented cassava starch were observed at the central point of the experimental design (1.5 g/100 g H_2O_2, pH 5.0 and 32.5 °C). The sun-drying of the fermented cassava starch increased the carboxyl and carbonyl content, specific volume as well as decreased the biscuit hardness when compared to the oven-dried starch. The oxidation with H_2O_2 provides a fermented cassava starch with characteristics similar to the sun-dried fermented cassava starch and produces more homogeneous products.

1. Introduction

Native starch structure can be modified by chemical, physical and enzymatic methods. Oxidation is an important modification applied to starches. Because of its low viscosity, high stability, clarity and film-forming and binding properties, oxidised starch has been widely used in many industries, particularly in the paper, textile, laundry finishing and binding materials industries to provide surface sizing and coating properties (Kuakpetoon & Wang, 2006; Sanchez-Rivera, Garcìa-Suàrez, Valle, Gutierrez-Meraz, & Bello-Pèrez, 2005). Several methods have been applied to prepare oxidised starch; these include the use of hydrogen peroxide, ambient oxygen, ozone, bromine, chromic acid, permanganate, nitrogen dioxide, ultraviolet radiation and hypochlorite oxidation (Lawal, Adebowale, Ogunsanwo, Barba, & Ilo, 2005; Vatanasuchart, Naivikul, Charoenrein, & Sriroth, 2005). Hydrogen peroxide has been used in commercial practice to a much lesser extent. Unlike sodium hypochlorite, hydrogen peroxide creates no harmful byproduct. Hydrogen peroxide decomposes inevitably to oxygen and water. This chemical is therefore considered more environmentally friendly and is preferred especially when a chlorine-free process is desired (Sangseethong, Termvejsayanon, & Sriroth, 2010). However, the oxidation reaction of starch using hydrogen peroxide requires a long reaction time, high temperature or high pH level (Hage & Lienke, 2006).

The main reason starch is chemically treated before commercial use is to split the long glucose chains of the polymer molecules to reduce the high viscosity of unmodified starch solutions and thereby increase and obtain the maximum possible amounts of starch in technical applications. These include, in addition to oxidation, acid hydrolysis and enzymatic modification (Tolvanen, Maki-Arvela, Sorokin, Salmi, & Murzin, 2009). Numerous oxidation methods are known, e.g., wet, semi-dry and dry oxidations (Tomasik & Schilling, 2004). The oxidation of starch allows carboxyl and carbonyl groups to substitute hydroxyl groups on the polymer backbone. The number of carboxyl and carbonyl groups on oxidised starch indicates the level of oxidation; this oxidation primarily occurs at the hydroxyl groups at the C2, C3 and C6 positions in a D-glucopyranosyl unit (Kuakpetoon & Wang, 2001, 2008). The desired properties of oxidised starch are mainly lower viscosity and improved starch paste stability. According to Kuakpetoon and Wang (2001), oxidation occurs mainly in the amorphous regions, as no changes were observed in the X-ray patterns and intensities of the oxidised products.

Cassava starch has a great number of applications in industry, such as in food, paper, and adhesives; however, only a small portion of starch is used in its native state, and most is destined to be modified by chemical agents. Fermented cassava starch is modified by a natural fermentation process, producing organic acids, mainly lactic acid (Dias, Elias, Oliveira, & Helbig, 2007). Cassava starch is

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extracted from roots and naturally fermented in tanks for a period of approximately 30 days, always in the presence of excess water, which forms a 5-cm layer above the bed of starch. After this period of predominant lactic fermentation, the moist acid starch is sun-dried for 1 or 2 days, depending on the season (Demiate, Dupuy, Huvenne, Cereda, & Wosiacki, 2000). Martínez-Bustos, Amaya-Llano, Carbajal-Arteaga, Chang, and Zazueta-Morales (2007), who used hydrogen peroxide as a catalyst, observed a change in crystallinity in cassava starch modified with organic acids (lactic, citric and acetic) with no change in the morphology of the granules.

During the oxidation of starch, the reaction pH, oxidant concentration, temperature and starch origin influence the type and amount of functional groups formed in the starch molecules (Sangseethong, Lertphanich, & Sriroth, 2009). It has been shown that the use of different levels of hydrogen peroxide the reaction yields oxidised products with different pasting, expansion and textural properties. Relatively few studies on starch oxidation by hydrogen peroxide are available in the literature, and reports on the oxidation of fermented cassava starch were not found. The objective of this study was to evaluate the effects of oxidative modification with hydrogen peroxide on the pasting properties, carbonyl and carboxyl contents as well as expansion and textural properties of fermented cassava starch.

2. Materials and methods

2.1. Materials

Cassava starch naturally fermented for 30 days was collected from industrial facilities in Brazil (*Indústria e Comércio de Polvilho Azedo Universo LTDA*). Wet starch was placed in ∼12-m^3 concrete tanks and covered with a 0.20-m layer of water. The tank was drained after 30 days, and the resulting material (containing 46% moisture) was removed, packed in plastic bags and stored at −18 °C.

2.2. Removal of the water-soluble fraction

As the oxidation reaction is influenced by the consumption of chlorine by organic acids, saccharides and soluble nitrogen compounds present in fermented cassava starch, the water-soluble fraction was removed. These compounds have easier access to the reagent because they are highly reactive, soluble molecules. The water-soluble fraction was removed by dispersing 216 g (dry basis (d.b.)) of fermented cassava starch in 500 mL of distilled water with constant stirring for 10 min. The starch was filtered twice in a Buchner funnel with medium porosity filter paper, resulting in 1000 mL of soluble fraction. The samples were then washed with distilled water (800 mL) and re-filtered. The control efficiency of the operation in removing the water-soluble fraction was obtained at values of acidity below 0.9 meq NaOH/100 g starch (d.b.) and ash content less than 0.01% (d.b.). The water-soluble fraction (1000 mL) was stored in flasks for later use.

2.3. Oxidation of fermented cassava starch

Fermented cassava starch lacking a water-soluble fraction was treated with an oxidant in a glass reactor. The starch (200 g (d.b.)) was resuspended in 300 mL of distilled water, heated to different temperatures and subjected to hydrogen peroxide treatment; the pH was adjusted with hydrochloric acid (0.1 N) and sodium hydroxide (0.1 N). After 50 min, samples of approximately 65 g starch (d.b.) were withdrawn from the reactor, filtered through medium porosity filter paper in a Buchner funnel, washed with 1200 mL of distilled water, resuspended in 600 mL of distilled water and re-filtered. The water-soluble fraction was added to the oxidised starch and was dried in a forced air oven at 42 °C until the starch retained only about 12% moisture. The amount of water-soluble fraction added to the oxidised starch was proportional to the amount of starch withdrawn (1000 mL/216 g starch (d.b.)). A 2^3 factorial design with three central points and six axial points was used to investigate the effect of hydrogen peroxide concentration, pH and temperature (Table 1) on the oxidation of fermented cassava starch lacking a water-soluble fraction. As controls, oven-dried and sun-dried fermented cassava starches were used. The fermented cassava starch was dried in a forced air oven at 42 °C until about 12% moisture in plastic trays up to 0.5 mm in thickness. For sun-drying, the fermented cassava starch was layered in plastic trays up to 0.5 mm in thickness and exposed to sunlight until about 12% moisture.

Table 1
A 2^3 factorial design with three central points and six axial points.

Variables	Levels				
	−∝	−1	0	+1	+∝
C (g/100 g)[a]	0.12	0.70	1.50	2.30	2.89
pH	1.64	3.00	5.00	7.00	8.36
T (°C)	20.0	25.0	32.5	40.0	45.0

[a] C: Concentration of hydrogen peroxide (g H_2O_2/100 g cassava starch, d.b.).

2.4. Carbonyl content

The carbonyl content was determined as described by Smith (1967). Dry starch (4 g) was resuspended in distilled water (100 mL) and heated in a boiling water bath for 30 min with continuous stirring until completely gelatinised and then stored at 40 °C. The pH was adjusted to 3.2 with 0.1 mol/L HCl; hydroxylamine chloride solution (15 mL) was then added to the solution (prepared by dissolving 25 g of reagent grade hydroxylamine chloride in water, adding 100 mL of 0.5 mol/L NaOH and bringing the final volume to 500 mL). Samples were covered with plastic film and put into a 38 °C oven for 4 h and rapidly titrated to pH 3.2 with 0.1 mol/L HCl. The carbonyl content was expressed as the quantity of carbonyl groups per 100 glucose units (CO/100 GU), as calculated by Eq. (1):

$$\mathrm{CO}/100\quad\mathrm{GU} = \frac{(Vb - Vs)\times M \times 0.028 \times 100}{W} \tag{1}$$

where *Vb* is the volume of HCl used for the blank (mL), *Vs* is the volume of HCl required for the sample (mL), *M* is the molarity of HCl and *W* is the sample weight (d.b.).

2.5. Carboxyl content

The carboxyl content was determined as previously described (Parovuori, Hamunen, Forssell, Autio, & Poutanen, 1995). Dry starch (5 g) was resuspended in distilled water (25 mL), stirred for 30 min and centrifuged. The residue was washed with distilled water, resuspended in 300 mL of distilled water and heated in a boiling water bath with continuous stirring for 30 min to achieve complete gelatinisation. The heated samples were then titrated to pH 8.2 with 0.01 mol/L NaOH. The carboxyl content was expressed as the quantity of carboxyl groups per 100 glucose units (COOH/100 GU), as calculated by Eq. (2):

$$\mathrm{COOH}/100\quad\mathrm{GU} = \frac{(Vs - Vb)\times M \times 0.045 \times 100}{W} \tag{2}$$

where *Vs* is the volume of NaOH required for the sample (mL), *Vb* is the volume of NaOH used to test the blank (mL), *M* is the molarity of NaOH and *W* is the sample weight (d.b.).

Table 2
Content of carbonyl and carboxyl groups in oven-dried fermented cassava starch oxidised with hydrogen peroxide.

Treatment	C (g/100 g)[a]	pH	T (°C)	Carbonyl content (CO/100 GU)	Carboxyl content (COOH/100 GU)	Sum (CO + COOH)
1	0.70	3.00	25.0	0.031 ± 0.003	0.065 ± 0.002	0.096
2	2.30	3.00	25.0	0.048 ± 0.005	0.067 ± 0.005	0.115
3	0.70	7.00	25.0	0.032 ± 0.005	0.051 ± 0.012	0.083
4	2.30	7.00	25.0	0.032 ± 0.009	0.060 ± 0.004	0.092
5	0.70	3.00	40.0	0.038 ± 0.004	0.051 ± 0.006	0.089
6	2.30	3.00	40.0	0.039 ± 0.006	0.038 ± 0.006	0.077
7	0.70	7.00	40.0	0.032 ± 0.004	0.040 ± 0.007	0.072
8	2.30	7.00	40.0	0.038 ± 0.005	0.040 ± 0.004	0.078
9	1.50	5.00	32.5	0.040 ± 0.003	0.064 ± 0.002	0.109
10	1.50	5.00	32.5	0.084 ± 0.002	0.064 ± 0.003	0.148
11	1.50	5.00	32.5	0.074 ± 0.004	0.054 ± 0.003	0.128
12	0.12	5.00	32.5	0.046 ± 0.010	0.034 ± 0.005	0.080
13	2.89	5.00	32.5	0.062 ± 0.005	0.036 ± 0.004	0.098
14	1.50	1.64	32.5	0.038 ± 0.005	0.049 ± 0.002	0.087
15	1.50	8.36	32.5	0.054 ± 0.003	0.048 ± 0.002	0.102
16	1.50	5.00	20.0	0.038 ± 0.006	0.041 ± 0.003	0.079
17	1.50	5.00	45.0	0.056 ± 0.004	0.049 ± 0.006	0.105
Oven-dried fermented cassava starch				0.044 ± 0.008	0.037 ± 0.007	0.081
Sun-dried fermented cassava starch				0.071 ± 0.006	0.065 ± 0.008	0.136

[a] C: Concentration of hydrogen peroxide (g H_2O_2/100 g cassava starch, d.b.). GU: glucose.

2.6. Pasting properties

Pasting properties were determined using a Rapid Visco Analyser (RVA-4, Newport Scientific, Australia) and the Thermocline for Windows programme (version 1.10); viscosity was expressed in RVU (Rapid Visco Units). Starch (2.5 g, 14 g/100 g moisture basis) was weighed directly in an RVA canister, and 25 mL of distilled water was added. The sample was held at 50 °C for 1 min, heated to 95 °C within 3.5 min, held at 95 °C for 2.5 min, then cooled to 50 °C within 4 min and finally held at 50 °C for 2 min. The rotating speed was held constant at 960 rpm for 10 s and then maintained at 160 rpm for the duration of the process. To prevent the activity of α-amylases, 100 μmol of $AgNO_3$/g starch (d.b.) was added to the sample. The recorded parameters included peak viscosity, holding viscosity and final viscosity.

2.7. Gel hardness

The gel hardness was analysed with a Texture Analyser (TA.XT2, Stable Micro Systems) according to the method used by Hormdok and Noomhorm (2007), with some modifications. After taking RVA measurements, the gelatinised mixture in the canister was held at 12 °C for 24 h, allowing the formation of a solid gel. The canister was sealed with parafilm to prevent moisture loss during storage. The gels were punctured at a rate of 3.0 mm/s to a distance of 10.0 mm using a stainless steel cylindrical probe (P/20, 20 mm diameter). The peak force measured was reported as gel hardness. The results were expressed in gf (gram-force) units.

2.8. Expansion properties

Expansion properties were evaluated indirectly using the “biscuit test” according to the method of Cereda (1983), with some modifications. In brief, biscuit dough was made with the following formulation: 100 g starch, 25 g hydrogenated vegetable oil, 3 g salt and 100 g water. Twenty-five grams of water was added to the starch and mixed for 1 min in a dough mixer. A boiling mixture containing the hydrogenated vegetable fat, salt and 25 g of the water was then added and mixed for a further 3 min; the remaining water (50 g) was added within the first minute. Using a plastic bag, 4.0 ± 0.1-g portions of the dough were weighed, placed on an aluminium baking tray and baked for 20 min in an electric oven (Fisher, Brazil) at 180 °C ± 10 °C. The specific volume was evaluated by the millet seed displacement method and expressed in mL/g.

2.9. Biscuit hardness

The biscuit hardness was analysed with a Texture Analyser (TA.XT2, Stable Micro Systems) according to the method described by Tyagi, Manikantan, Oberoi, and Kaur (2007), with some modifications. Biscuits were cut at a rate of 2.0 mm/s up to a distance of 50.0 mm using a stainless steel knife/guillotine blade probe (P/20, 20 mm diameter). The results were expressed in gf (gram-force) units.

2.10. Statistical analysis

Statistical significance was evaluated using the response surface method, where the response function, $f(x)$, could be written as $y_i = f(x_1, x_2, x_3)$. The results of each trial for each response were evaluated using multiple-regression analysis to develop mathematical models containing linear and quadratic terms. The interaction between the variables was evaluated using the Statistics 6.0 software at a confidence level of 90% for analysis of variance tests.

3. Results and discussion

3.1. Carbonyl and carboxyl contents

The carbonyl and carboxyl contents and their sum, which represents the total oxidation of starch, are presented in Table 2. The pH, temperature and hydrogen peroxide concentration had no significant effect on the carbonyl and carboxyl contents and their sum and, thus, do not present a regression model for this variable. The carbonyl and carboxyl contents of fermented cassava starch oxidised with hydrogen peroxide at different reaction conditions were higher when compared with those of the oven-dried fermented cassava starch but were lower when compared to those of the sun-dried fermented cassava starch (Table 2). The highest values of the sum of carbonyl and carboxyl contents were observed at the central point of the experimental design with a hydrogen peroxide concentration of 1.5 g/100 g, pH 5.0 and temperature of 32.5 °C (treatment 9, 10 and 11). The reaction mechanisms between hydrogen peroxide and starch are very complex and may change when changing the reaction conditions, the oxidant and the type of starch, becoming more efficient at alkaline pH (Tolvanen et al., 2009). The oxidation of potato starch with hydrogen peroxide under both alkaline and acid reaction conditions using copper, iron and tungsten as catalysts was studied by Parovuori et al. (1995). The objective was to introduce carboxyl and carbonyl groups into the constituent glucose molecules of starch. They concluded that the depolymerisation of oxidised starch was more intense under acidic conditions than under alkaline conditions, as was the modification of the starch.

According to Tolvanen et al. (2009), hydrogen peroxide spontaneously decomposes to oxygen and water, and the rate of decomposition is dependent on the temperature, concentration and impurities. The peroxide is broken down into a hydroxide ion and a hydroxyl free radical. Moreover, radical interactions can lead to the recombination of hydrogen peroxide. These authors reported that the decomposition of hydrogen peroxide is highly dependent on the pH and temperature of the reaction. El-Sheikh, Ramadan, and El-Shafie (2010) reported that in oxidised rice starch with hydrogen peroxide, the carbonyl and carboxyl contents increased by increasing the temperature of oxidation. These authors suggested that it is likely that during the later stages of the reaction, some of the oxidised starch is highly degraded and, therefore, becomes soluble in water; moreover, it is washed out along with its high content of carbonyl and/or carboxyl groups.

3.2. *Pasting properties*

The pasting properties represent the intensity of the alterations to which the starch was submitted. The gelatinisation of starch granules caused by hydrothermal action, followed by mechanical action, led to a structural breakdown and, consequently, to a loss of granule integrity. The intensity of this effect depends on the origin of the starch, the temperature, the mechanical shear and the chemical agents present during gelatinisation. The results of peak, holding, and final viscosity of fermented cassava starch are presented in Table 3. The determination coefficients for the peak, holding, and final viscosities were 0.91, 0.96 and 0.93, respectively, at a 90% confidence level. The quadratic mathematical model describing the response surface of the peak viscosity was defined by equation 3. Linear mathematical models that describe the response surface for the holding and final viscosities were defined by Eqs. (4) and (5).

The hydrogen peroxide concentration and pH had a negative effect on peak viscosity, and the temperature had a positive effect. The interaction between hydrogen peroxide concentration and pH also had significant effect on the peak viscosity. The lower values of peak viscosity of fermented cassava starch were presented in treatments 9, 10 and 15 (Table 3). The highest values of peak viscosity were obtained when the fermented cassava starch was oxidised at low pH or with low hydrogen peroxide concentration and high pH (Fig. 1a–c). Tavares, Zanatta, Zavareze, Helbig, and Dias (2010) reported that oxidation with hydrogen peroxide reduced the viscosity of rice flour. These authors reported that the reduction in pasting viscosity became higher with an increase in the concentration of hydrogen peroxide. The effect of oxidation on the peak viscosity is dependent on the conditions and intensity of the reaction and on the starch source. According to Martínez-Bustos et al. (2007), carbonyl and carboxyl groups are more voluminous than hydroxyl groups and tend to keep the amylose chains separated, thus delaying retrogradation. The sun-dried fermented cassava starch showed a lower peak viscosity compared to the oven-dried fermented cassava starch, which was non-oxidised with hydrogen peroxide due to the oxidation promoted by UV light during sun-drying (Table 3).

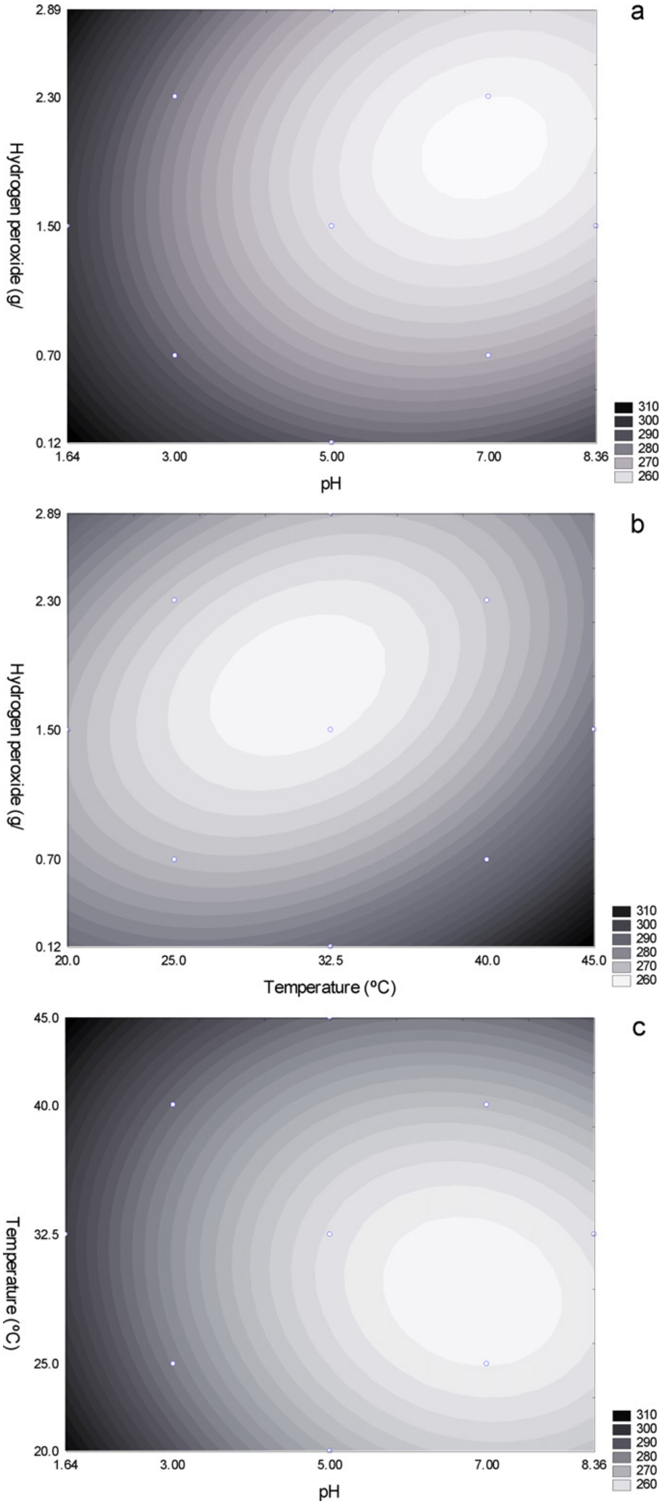

Fig. 1. Contour curve for the peak viscosity of fermented cassava starch oxidised with hydrogen peroxide as a function of hydrogen peroxide concentration and pH (a), hydrogen peroxide concentration and temperature (b), and temperature and pH (c).

The pH, temperature and the interaction between the two had significant effects on the holding viscosity. The pH had a positive effect, and the temperature had a negative effect, i.e., when the temperature was high and the pH low, the holding viscosity showed the lowest values (Table 3). The conditions that interfere with the oxidation reaction can be effectively evaluated by analysing the

Table 3
Pasting properties of oven-dried fermented cassava starch oxidised with hydrogen peroxide.

Treatment	C (g/100 g)[a]	pH	T (°C)	Peak viscosity (RVU)	Holding viscosity (RVU)	Final viscosity (RVU)
1	0.70	3.00	25.0	288.4 ± 4.7	74.8 ± 2.6	77.3 ± 2.8
2	2.30	3.00	25.0	291.8 ± 5.2	82.0 ± 3.5	98.6 ± 3.5
3	0.70	7.00	25.0	265.3 ± 3.8	103.8 ± 3.5	133.1 ± 4.5
4	2.30	7.00	25.0	263.8 ± 2.3	100.0 ± 3.1	132.9 ± 3.1
5	0.70	3.00	40.0	297.2 ± 5.2	54.2 ± 2.3	71.3 ± 3.6
6	2.30	3.00	40.0	290.2 ± 4.0	54.2 ± 2.5	66.8 ± 3.0
7	0.70	7.00	40.0	285.8 ± 2.2	108.1 ± 2.5	132.6 ± 3.7
8	2.30	7.00	40.0	263.8 ± 6.3	100.3 ± 4.6	125.7 ± 3.4
9	1.50	5.00	32.5	258.3 ± 1.9	92.2 ± 2.8	111.1 ± 2.6
10	1.50	5.00	32.5	259.2 ± 4.3	89.0 ± 3.5	106.5 ± 3.2
11	1.50	5.00	32.5	262.6 ± 4.8	86.3 ± 3.5	98.4 ± 2.8
12	0.12	5.00	32.5	285.0 ± 3.9	94.9 ± 3.4	112.4 ± 3.6
13	2.89	5.00	32.5	260.1 ± 3.1	88.5 ± 3.4	109.7 ± 2.3
14	1.50	1.64	32.5	287.6 ± 5.4	82.3 ± 4.7	100.6 ± 4.6
15	1.50	8.36	32.5	256.9 ± 0.9	105.8 ± 0.1	138.3 ± 1.3
16	1.50	5.00	20.0	264.8 ± 5.3	100.8 ± 4.7	121.8 ± 4.3
17	1.50	5.00	45.0	278.8 ± 1.1	82.4 ± 1.5	99.4 ± 1.2
Oven-dried fermented cassava starch				279.0 ± 5.2	96.3 ± 2.3	140.7 ± 3.3
Sun-dried fermented cassava starch				263.3 ± 3.2	71.4 ± 1.7	99.2 ± 1.9

[a] C: Concentration of hydrogen peroxide (g H_2O_2/100 g cassava starch, d.b.).

holding viscosity, as this variable is sufficiently sensitive to measure small changes in starch granules caused by oxidation. This result is an indication that the starch granules subjected to an oxidation with hydrogen peroxide are very sensitive to the maintenance of high temperatures by mechanical action.

Oxidation with hydrogen peroxide reduced the final viscosity of fermented cassava starch. The final viscosity was higher at neutral or slightly alkaline pH; thus, the pH had a positive effect on the final viscosity. Sun-drying also reduced the final viscosity of fermented cassava starch (Table 3). A reduction in final viscosity due to the introduction of carbonyl and carboxyl groups has been reported by several authors (Kuakpetoon & Wang, 2001; Li & Vasanthan, 2003; Martínez-Bustos et al., 2007) in regular corn, potato, pea and cassava starch granules during hypochlorite oxidation. The oxidation of starch mainly causes the scission of glucosidic linkages and the oxidation of hydroxyl groups to carbonyl and carboxyl groups. The scission of the glucosidic linkage results in the depolymerisation of amylose and amylopectin, thereby decreasing the swelling power and paste viscosity. According to Xie, Liu, and Cui (2005), oxidised starches exhibit the same X-ray diffraction patterns as native starches; this finding suggests that oxidation occurs mainly in the amorphous phases of the granules.

$$PV = 259.5 - 5.1X_1 - 10.3X_2 + 3.8\,X_3 + 6.2X_1^2 + 6.1X_2^2 + 5.9X_3^2 - 2.5X_1X_2 - 3.9X_1X_3 \quad (3)$$

$$HV = 85.9 + 18.4X_2 - 5.5\,X_3 + 6.6\,X_2X_3 \quad (4)$$

$$FV = 104.9 + 26.3X_2 - 5.7X_3 \quad (5)$$

where X_1 is the hydrogen peroxide concentration (g/100 g); X_2 is the pH; X_3 is the reaction temperature (°C); PV is the peak viscosity; HV is the holding viscosity; and FV is the final viscosity.

3.3. Gel hardness

The results of gel hardness tests on fermented cassava starch oxidised with hydrogen peroxide are presented in Table 4. The determination coefficient for the gel hardness was 0.82 at a 90%

Table 4
Gel hardness, specific volume and biscuit hardness of oven-dried fermented cassava starch oxidised with hydrogen peroxide.

Treatment	C (g/100 g)[a]	pH	T (°C)	Gel hardness (gf)	Specific volume (mL/g)	Biscuit hardness (gf)
1	0.70	3.00	25.0	93.5 ± 6.2	9.73 ± 0.28	13104 ± 986
2	2.30	3.00	25.0	78.6 ± 5.2	12.95 ± 0.45	4651 ± 1321
3	0.70	7.00	25.0	96.6 ± 5.3	5.26 ± 0.38	>25,000
4	2.30	7.00	25.0	89.3 ± 2.5	3.68 ± 0.26	>25,000
5	0.70	3.00	40.0	65.6 ± 4.2	9.72 ± 0.41	3996 ± 598
6	2.30	3.00	40.0	52.6 ± 2.8	11.74 ± 0.35	3393 ± 683
7	0.70	7.00	40.0	104.4 ± 4.9	11.11 ± 0.45	4715 ± 1208
8	2.30	7.00	40.0	120.1 ± 6.2	5.81 ± 0.23	>25,000
9	1.50	5.00	32.5	101.3 ± 6.9	13.49 ± 0.22	1513 ± 162
10	1.50	5.00	32.5	88.4 ± 5.3	11.13 ± 0.63	4288 ± 777
11	1.50	5.00	32.5	98.8 ± 6.4	13.00 ± 0.33	2011 ± 598
12	0.12	5.00	32.5	86.9 ± 5.5	11.88 ± 0.67	2433 ± 655
13	2.89	5.00	32.5	97.2 ± 5.7	9.29 ± 0.49	3611 ± 996
14	1.50	1.64	32.5	79.9 ± 3.7	8.29 ± 0.34	5630 ± 1286
15	1.50	8.36	32.5	111.8 ± 6.4	5.78 ± 0.25	>25,000
16	1.50	5.00	20.0	113.2 ± 5.9	11.34 ± 0.49	2125 ± 270
17	1.50	5.00	45.0	91.8 ± 3.6	9.21 ± 0.61	10,686 ± 835
Oven-dried fermented cassava starch				90.2 ± 2.3	2.65 ± 0.30	>25,000
Sun-dried fermented cassava starch				99.9 ± 1.5	13.54 ± 0.45	2431 ± 302

[a] C: Concentration of hydrogen peroxide (g H_2O_2/100 g cassava starch, d.b.).

confidence level. A linear mathematical model describing the response surface for the gel hardness was defined by Eq. (6).

The factor with the strongest influence on gel hardness was the pH and its interaction with temperature. The highest gel hardness values were presented in treatments 7, 8, 9, 15 and 16 (Table 4). The fermented cassava starch oxidised with 2.30 g/100 g of hydrogen peroxide, pH 3.00 and 40 °C (treatment 6) presented lower gel hardness. These results are consistent with those of Parovuori et al. (1995), who reported that the oxidation of potato starch with hydrogen peroxide at low pH, where there was a higher degree of oxidation, the gel hardness of potato starch decreased. The differences in gelation can be explained not only by the intensity of oxidation through the introduction of carbonyl and carboxyl groups and depolymerisation but also by different effects of hydrogen peroxide upon changing the reaction conditions, which act on different sites of the starch units' basic components; this reaction generates a diversity of products that interact with each other due to gelatinisation.

Sun-dried fermented cassava starch presented higher gel hardness compared to non-oxidised oven-dried fermented cassava starch (Table 4). The increase observed in the gel hardness of sun-dried starch may be from increased hydrogen bonding due to the formation of carbonyl and carboxyl groups and a reduction in the size of amylose molecules due to oxidation promoted by UV light (sun-drying), as amylose molecules with intermediate weight had a greater ability to retrograde and form gels.

$$GH = 89.9 + 15.0X_2 + 11.6X_2X_3 \qquad (6)$$

where X_1 is the hydrogen peroxide concentration (g/100 g); X_2 is the pH; X_3 is the reaction temperature (°C); and GH is the gel hardness.

3.4. Expansion properties

Baking expansion might involve the reassociation of size-reduced starch molecules of amylose and/or amylopectin in amorphous regions (Bertolini et al., 1998; Vatanasuchart et al., 2005). The specific volumes of biscuits made with oven-dried fermented cassava starch oxidised with hydrogen peroxide are presented in Table 4. The quadratic mathematical model describing the response surface for the specific volume was defined by Eq. (7); the determination coefficient for the specific volume was 0.80 at a 90% confidence level.

$$SV = 12.56 - 1.65X_2 - 0.77X_1^2 - 2.03\,X_2^2 - 0.88X_3^2 - 1.52X_1X_2 + 1.15X_2X_3 \qquad (7)$$

where X_1 is the hydrogen peroxide concentration (g/100 g); X_2 is the pH; X_3 is the reaction temperature (°C); and SV is the specific volume.

Oxidation with hydrogen peroxide promoted the expansion property of fermented cassava starch. The highest specific volumes of biscuits made from fermented cassava starch were exhibited under the conditions of oxidation at the central point of the experimental design (Fig. 2a–c). Despite the oxidation at extreme levels of pH, through the quantification of carbonyl and carboxyl functional groups, the sites where oxidation occurred or the secondary products formed most likely did not allow a suitable arrangement that would allow high expansion.

According to Tavares et al. (2010), who studied the oxidation of high-, medium- and low-amylose rice flours with hydrogen peroxide, reported that only the low-amylose rice flour showed the capacity for baking expansion. The expansion of biscuits is directly related to the structure of starch granules; this structure varies depending on the source and the cultivar of the plant.

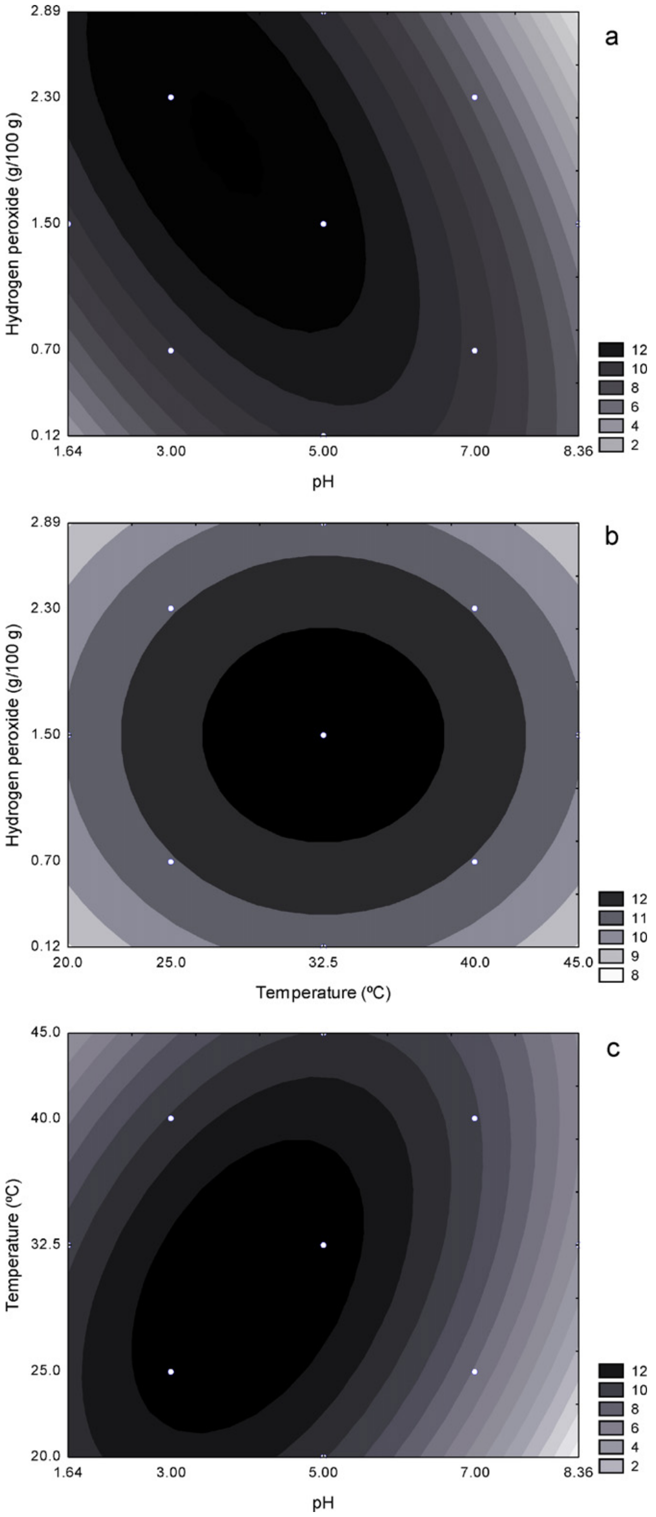

Fig. 2. Contour curve for the specific volume of biscuits made from fermented cassava starch oxidised with hydrogen peroxide as a function of hydrogen peroxide concentration and pH (a), hydrogen peroxide concentration and temperature (b), and temperature and pH (c).

The non-oxidised sun-dried fermented cassava starch also showed high expansion, indicating that sun-drying also promotes expansion during baking (Table 4). Bertolini, Mestres, Lourdin, Della Valle, and Colonna (2001) reported that sunlight, particularly some ultraviolet waves, as well as acid fermentation, are essential in developing the expansion capacity of cassava starch. However, exposure to only solar radiation already allows for the modification of the expansion properties, as reported by Tomasik and Zaranyika (1995); it was found that the irradiation of potato starch in solid form with ultraviolet light caused hydrolysis and oxidation of starch with reduced paste viscosity. Vatanasuchart et al. (2005) found that lactic acidification combined with UV energy resulted in a partial depolymerisation of cassava starch molecules.

3.5. Biscuit hardness

The biscuit hardness values of oven-dried fermented cassava starch oxidised with hydrogen peroxide are shown in Table 4. Oxidation with hydrogen peroxide reduced the hardness of biscuits made from oven-dried fermented cassava starch due to further expansion promoted by oxidation, except for treatments 3, 4, 8 and 15 (Table 4). Sun-drying also reduced the hardness of biscuits made from fermented cassava starch to a value of 2431 gf.

The biscuits made from treatments 3, 4, 8 and 15, in which the pH was ≥7.0, presented hardness values higher than 25,000 gf. These biscuits with high hardness exhibited low expansion (Table 4). In these treatments in which the biscuit hardnesses were higher than 25,000 gf, which is the maximum reading of the Texture Analyzer, it was not possible to obtain a statistical model for the biscuit hardness response. However, hardness is an important parameter in the quality of biscuits because the presence of a high specific volume does not guarantee an appropriate texture of biscuits. High hardness values are usually derived from biscuits with low specific volumes; however, biscuits that exhibit large expansion and do not have crispy crusts do not break easily and, thus, show high hardness values. Biscuits that exhibit large expansion usually develop holes in their crust and interior structures with large cells or that are completely hollow.

4. Conclusions

The expansion of fermented cassava starch can be obtained by sun-drying or oxidising with hydrogen peroxide. However, the use of oxidants has several advantages over sun-drying because it better controls the process parameters, is not dependent on climate conditions and produces more homogeneous products. Furthermore, the use of hydrogen peroxide as an oxidant is less polluting and decomposes easily in the heat. Sun-drying increased the content of carboxyls and carbonyls, increased the gel hardness, promoted the expansion of the fermented cassava starch and decreased biscuit hardness. The properties of fermented cassava starch oxidised with hydrogen peroxide were influenced by the oxidant concentration, pH and temperature. At the central point of the experimental design, with a hydrogen peroxide concentration of 1.5 g/100 g, pH 5.0 and 32.5 °C, were observed the highest values of the sum of carbonyl and carboxyl contents, where the greatest expansion occurred. Oxidation with hydrogen peroxide at neutral or slightly alkaline pH levels reduced the expansion of cassava. Other studies are required to obtain the expansion properties of starches from other botanical sources that do not exhibit this property by fermentation followed by sun-drying.

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