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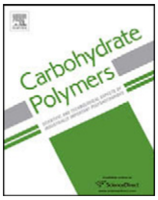

# Pasting, expansion and textural properties of fermented cassava starch oxidised with sodium hypochlorite

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ABSTRACT

This study investigated the effects of oxidation with sodium hypochlorite on the content of carbonyl and carboxyl as well as pasting, expansion and textural properties of fermented cassava starch. A $2^3$ factorial design with three central points and six axial points was used to investigate the effect of active chlorine concentration, pH and temperature during the oxidation of fermented cassava starch; oven-dried and sun-dried fermented cassava starches were used as controls. Sun-dried fermented cassava starch had a higher content of carboxyls and carbonyls, higher expansion and lower biscuit hardness compared to the oven-dried starch. Interestingly, the effect of oxidation was increased when the fermented cassava starch was oxidised with sodium hypochlorite. The highest values for the sum of carbonyl and carboxyl occurred at pH between neutral to alkaline, at high active chlorine concentrations and high temperatures. Oxidative treatment with sodium hypochlorite increases the expansion of starch and reduces biscuit hardness.

## 1. Introduction

Starch has received increasing attention because of its beneficial use in various food and industrial applications. Starch significantly contributes to the textural properties of many foods and is widely used as a thickener, colloidal stabiliser, gelling agent, bulking agent and water retention agent (Singh, Kaur, & McCarthy, 2007).

Although some characteristics of native starch are undesirable, only small modifications are required to drastically change its behaviour and properties. Chemical modifications result in changes to the structure of the glucose units in starch. Some of the principal reasons for starch modification are to change cooking characteristics, increase freeze–thaw and process stability, decrease retrogradation and gelling properties, improve film formation properties or render the polymer electrostatically charged (Bemiller, 1997). Oxidised starch is obtained by the reaction of an oxidising agent with the free hydroxyl groups in the glucose monomer, resulting in the formation of carbonyl and/or carboxyl groups. The oxidation process normally causes depolymerisation of starch molecules by scission of glycosidic linkages (Richardson & Gorton, 2003).

Several oxidising reagents can be used to oxidise starch, including periodate (Kanth et al., 2006; Tang, Du, & Fan, 2003; Zhang et al., 2007), sodium hypochlorite (Kuakpetoon & Wang, 2001; Kuakpetoon & Wang, 2008; Wang & Wang, 2003), hydrogen peroxide (Dias, Elias, Oliveira, & Helbig, 2007; Tavares, Zanatta, Zavareze, Helbig, & Dias, 2010), sodium bisulphite (Neves, Pereira, Zavareze, Dias, & Elias, 2010), peracetic acid, sulphur dioxide, potassium permanganate and ammonium persulphate (Singh et al., 2007). Of these, sodium hypochlorite is the most commonly used oxidising agent. During starch oxidation, hypochlorite could be consumed by three possible mechanisms: lipid oxidation, depolymerisation of amylose and amylopectin, and formation of carboxyl and carbonyl groups (Kuakpetoon & Wang, 2006). Several researchers have reported that sunlight, particularly certain UV wavelengths, as well as lactic acid fermentation are essential for the expansion ability of cassava starch during baking (Bertolini, Mestres, Lourdin, Della Valle, & Colonna, 2001).

Fermentation of starch is completely dependent on climate conditions: freshly extracted cassava starch is naturally fermented for 30–40 days and then dried by the sun and wind to obtain fermented starch (Gomes, Silva, & Ricardo, 2005). This fermented starch is more soluble and easily swells in water, creating a less viscous paste than that made from non-fermented cassava starch. According to Dias et al. (2007), the fermentation process itself causes changes in the starch that help to oxidise it. Demiate, Dupuy, Huvenne, Cereda, and Wosiacki (2000) also reported that dough made from

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sun-dried fermented cassava starch produces highly expanded bread-like products. They further showed that the extrusion process used to obtain high specific volume biscuits and bread-like foods is not necessary when cassava sour starch is used.

There is currently a need in the food industry for starches that possess specific rheological characteristics to allow for a diverse array of products. Native starch works as a good texture stabiliser and regulator in food systems, but has inherent limitations that prevent its use in certain industrial food applications, such as low shear resistance, low thermal resistance, low thermal decomposition and high tendency towards retrogradation. Starch modification or alteration of the physical and chemical features of native starch improves its functional characteristics and tailors starch for specific food applications. Oxidised starch can be used in batters and breading for coating various foodstuff, in confectionery products as binders and film formers and in dairy products as texturisers (Singh et al., 2007). However, native cassava starch lacks the pasting and expansion properties desirable for baking. Thus, the development of starches and flours with better expansion properties can lead to new ingredients for preparing gluten-free biscuits, cheese bread and other bakery products. Relatively little research has been done to investigate the effects of oxidation on the properties of fermented cassava starch. The objective of this study was to evaluate the effects of sodium hypochlorite oxidation on the pasting properties, content of carbonyl and carboxyl groups 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 to remove the water-soluble fraction was obtained with 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

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

| Variables | Levels | | | | |
|---|---|---|---|---|---|
| | −α | −1 | 0 | +1 | +α |
| C (g/100 g)[a] | 0.16 | 0.50 | 1.00 | 1.50 | 1.84 |
| pH | 2.50 | 3.60 | 5.20 | 6.80 | 7.90 |
| T (°C) | 20.0 | 25.0 | 32.5 | 40.0 | 45.0 |

[a] C: concentration of active chlorine (g Cl/100 g cassava starch, d.b.).

Fermented cassava starch lacking the water-soluble fraction was treated with an oxidant in a glass reactor. The starch (200 g (d.b.)) was resuspended in 500 mL of distilled water, heated to different temperatures and subjected to sodium hypochlorite 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 active chlorine concentration, pH and temperature (Table 1) on the oxidation of fermented cassava starch lacking the water-soluble fraction. As controls, oven-dried and sun-dried fermented cassava starches (dried for 8 h) were layered in plastic trays up to 0.5 mm thickness.

### 2.4. Carbonyl content

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 up 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. Carbonyl content was expressed as the quantity of carbonyl groups per 100 glucose units (CO/100 GU), as calculated by Equation 1:

$$\mathrm{CO}/100\,\mathrm{GU} = \frac{(V_b - V_s) \times M \times 0.028 \times 100}{W} \tag{1}$$

where $V_b$ is the volume of HCl used for the blank (mL), $V_s$ 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

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. Carboxyl content was expressed as the quantity of carboxyl groups per 100 glucose units (COOH/100 GU), as calculated by Eq (2):

$$\mathrm{COOH}/100\,\mathrm{GU} = \frac{(V_s - V_b) \times M \times 0.045 \times 100}{W} \tag{2}$$

where $V_s$ is the volume of NaOH required for the sample (mL), $V_b$ 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.).

### 2.6. Pasting properties

Pasting properties were determined using the Rapid Visco Analyser (RVA-4, Newport Scientific, Australia) and Thermocline for

**Table 2**
Content of carbonyl and carboxyl groups in oven-dried fermented cassava starch oxidised with sodium hypochlorite.

| Treatment | C (g/100 g)[a] | pH | T (°C) | Carbonyl content (CO/100 GU) | Carboxyl content (COOH/100 GU) | Sum (CO + COOH) |
|---|---|---|---|---|---|---|
| 1 | 0.50 | 3.60 | 25.0 | 0.024 ± 0.005 | 0.045 ± 0.010 | 0.069 |
| 2 | 1.50 | 3.60 | 25.0 | 0.127 ± 0.012 | 0.115 ± 0.012 | 0.242 |
| 3 | 0.50 | 6.80 | 25.0 | 0.070 ± 0.008 | 0.077 ± 0.007 | 0.185 |
| 4 | 1.50 | 6.80 | 25.0 | 0.267 ± 0.010 | 0.249 ± 0.015 | 0.516 |
| 5 | 0.50 | 3.60 | 40.0 | 0.064 ± 0.010 | 0.061 ± 0.012 | 0.125 |
| 6 | 1.50 | 3.60 | 40.0 | 0.192 ± 0.025 | 0.136 ± 0.008 | 0.328 |
| 7 | 0.50 | 6.80 | 40.0 | 0.093 ± 0.070 | 0.116 ± 0.008 | 0.209 |
| 8 | 1.50 | 6.80 | 40.0 | 0.248 ± 0.012 | 0.295 ± 0.012 | 0.543 |
| 9 | 1.00 | 5.20 | 32.5 | 0.117 ± 0.005 | 0.075 ± 0.020 | 0.192 |
| 10 | 1.00 | 5.20 | 32.5 | 0.120 ± 0.014 | 0.079 ± 0.009 | 0.199 |
| 11 | 1.00 | 5.20 | 32.5 | 0.105 ± 0.010 | 0.076 ± 0.005 | 0.181 |
| 12 | 0.16 | 5.20 | 32.5 | 0.099 ± 0.012 | 0.026 ± 0.008 | 0.125 |
| 13 | 1.84 | 5.20 | 32.5 | 0.186 ± 0.006 | 0.113 ± 0.008 | 0.299 |
| 14 | 1.00 | 2.50 | 32.5 | 0.072 ± 0.009 | 0.037 ± 0.007 | 0.109 |
| 15 | 1.00 | 7.90 | 32.5 | 0.152 ± 0.012 | 0.184 ± 0.005 | 0.336 |
| 16 | 1.00 | 5.20 | 20.0 | 0.073 ± 0.008 | 0.031 ± 0.006 | 0.104 |
| 17 | 1.00 | 5.20 | 45.0 | 0.136 ± 0.020 | 0.112 ± 0.017 | 0.248 |
| 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 active chlorine (g Cl/100 g cassava starch, d.b.). GU: glucose.

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. Recorded parameters included peak viscosity, holding viscosity and final viscosity.

### 2.7. Gel hardness

Gel hardness was analysed with the Texture Analyser (TA.XT2, Stable Micro Systems). After taking RVA measurements, the gelatinised mixture in the canister remained 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. 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). Results were expressed in gf (gram-force) units.

### 2.8. Expansion properties

Expansion properties were evaluated indirectly using the “biscuit test”. 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 also 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 ± 10 °C. The specific volume was evaluated by the millet seed displacement method and expressed in mL/g.

### 2.9. Biscuit hardness

Biscuit hardness was analysed with a Texture Analyser (TA.XT2, Stable Micro Systems). 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). 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. Interaction between the variables was evaluated using the Statistics 6.0 software at a confidence level of 90% for analysis of variance tests.

**Table 3**
Response surface regression models for the physicochemical properties of fermented cassava starch oxidised with sodium hypochlorite.

| Parameters[a] | Equation | $R^2$ |
|---|---|---|
| CO | $y_i = 0.1158 + 0.0534X_1 - 0.0271X_2 + 0.0157X_3 + 0.0129X_1^2 + 0.0151X_1X_2 - 0.0126X_2X_3$ | 0.86 |
| COOH | $y_i = 0.0732 + 0.0471X_1 + 0.0459X_2 + 0.0189X_3 + 0.0092X_1^2 + 0.0237X_2^2 + 0.0099X_3^2 + 0.0258X_1X_2 + 0.0060X_2X_3$ | 0.85 |
| CO + COOH | $y_i = 0.1858 + 0.0977X_1 + 0.0784X_2 + 0.0319X_3 + 0.0275X_2^2 + 0.0111X_3^2 + 0.0361X_1X_2 - 0.014X_2X_3$ | 0.86 |
| PV | $y_i = 210.1 - 57.9X_1 - 18.6X_2$ | 0.65 |
| HV | $y_i = 37.2 - 32.1X_1 - 13.7X_2 + 14.6X_1X_2$ | 0.95 |
| FV | $y_i = 47.9 - 42.1X_1 - 13.1X_2$ | 0.85 |
| SV | $y_i = 9.39 - 1.57X_1 - 0.54X_2 - 0.48X_3 - 0.33X_1X_3 - 0.93X_2X_3$ | 0.71 |
| BH | $y_i = 7808 + 1023X_1 + 788X_3 - 661X_1X_2 + 1679X_2X_3$ | 0.94 |

[a] $y_i$: function of response; $X_1$: active chlorine concentration (g/100 g); $X_2$: pH; $X_3$: reaction temperature (°C); CO: carbonyl content; COOH: carboxyl content; CO + COOH: sum of carbonyl and carboxyl groups; PV: peak viscosity; HV: holding viscosity; FV: final viscosity, SV: specific volume; BH: biscuit hardness; $R^2$: coefficient of determination; $p \leq 0.10$.

## 3. Results and discussion

### 3.1. Carbonyl and carboxyl contents

The oxidant concentration, pH and reaction temperature are important variables in the starch modification process. Changes that occur in the fermented cassava starch during sun drying are low compared to those from other chemical modifications. Table 2 lists the sum of carbonyl and carboxyl groups within the starch, which represents total oxidation state.

Sun drying promotes oxidation of fermented cassava starch, as the sun-dried fermented cassava starch had a higher sum of carboxyl and carbonyl groups compared to that of oven-dried fermented cassava starch. Interestingly, oxidation was increased when the fermented cassava starch was oxidised with sodium hypochlorite (Table 2). These results showed that starch samples modified with sodium hypochlorite had a higher content of carbonyl and carboxyl groups than did unmodified (oven-dried fermented cassava starch) samples. However, the starch was also oxidised at low active chlorine concentrations and at low temperatures (see Table 2, treatment 1 results). This increase in carbonyl and carboxyl content after oxidative treatment had also been previously observed by other labs (Takizawa, Silva, Konkel, & Demiate, 2004; Wang & Wang, 2003). The high carbonyl and carboxyl content observed in the modified starch may be directly related to molecular fragmentation during oxidative treatment.

Determination coefficients for the carbonyl and carboxyl groups were 0.86 and 0.85, respectively; the sum of the two functional groups was 0.86 at 90% confidence. Thus, the quadratic mathematical models describing the response surface for the content of carbonyls and carboxyls as well as the sum of the two groups are defined by the equations presented in Table 3.

The highest content of carbonyl and carboxyl groups (0.543) was observed at an active chlorine concentration of 1.5 g/100 g, pH 6.8 and temperature of 40 °C (treatment 8). Fig. 1 shows the contour curve for the sum of carbonyl and carboxyl groups in fermented cassava starch treated with sodium hypochlorite as a function of active chlorine concentration, pH and temperature. It appears that the highest carbonyl and carboxyl values occurred between pH 6.8 and 7.9, at an active chlorine concentration of 1.50 g/100 g to 1.84 g/100 g and temperatures between 40 °C and 45 °C (Fig. 1a–c). Therefore, reactions taking place at neutral to alkaline pH displayed the highest oxidation rates. According to Sangseethong, Lertphanich, and Sriroth (2009), the type and amount of functional groups on starch molecules formed during oxidation depend on the reaction time, temperature and pH. These authors reported that oxidation of starch with sodium hypochlorite occurs faster at neutral pH, while the reaction rate decreases with increases in acidity or alkalinity. Alkaline conditions favour the formation of carboxyl groups while acidic conditions favour the formation of carbonyl groups.

According to Sangseethong, Termvejsayanon, and Sriroth (2010), carboxyls were the major functional group produced during the oxidation of cassava starch with sodium hypochlorite. In fact, the carboxyl content progressively increased with reaction time, while only a minor amount of carbonyl groups was formed and essentially remained unchanged with reaction time. Wurzburg (1986) reported that hypochlorite oxidation of starch performed under alkaline conditions actually favours the formation of carboxyl groups. Parovuori et al. (1995) studied the oxidation of potato starch with hydrogen peroxide under both alkaline and acidic conditions to introduce carboxyl and carbonyl groups into the constituent glucose molecules of starch. These authors concluded that depolymerisation of oxidised starch was more intense under acidic conditions than under alkaline conditions, as was modification of the starch.

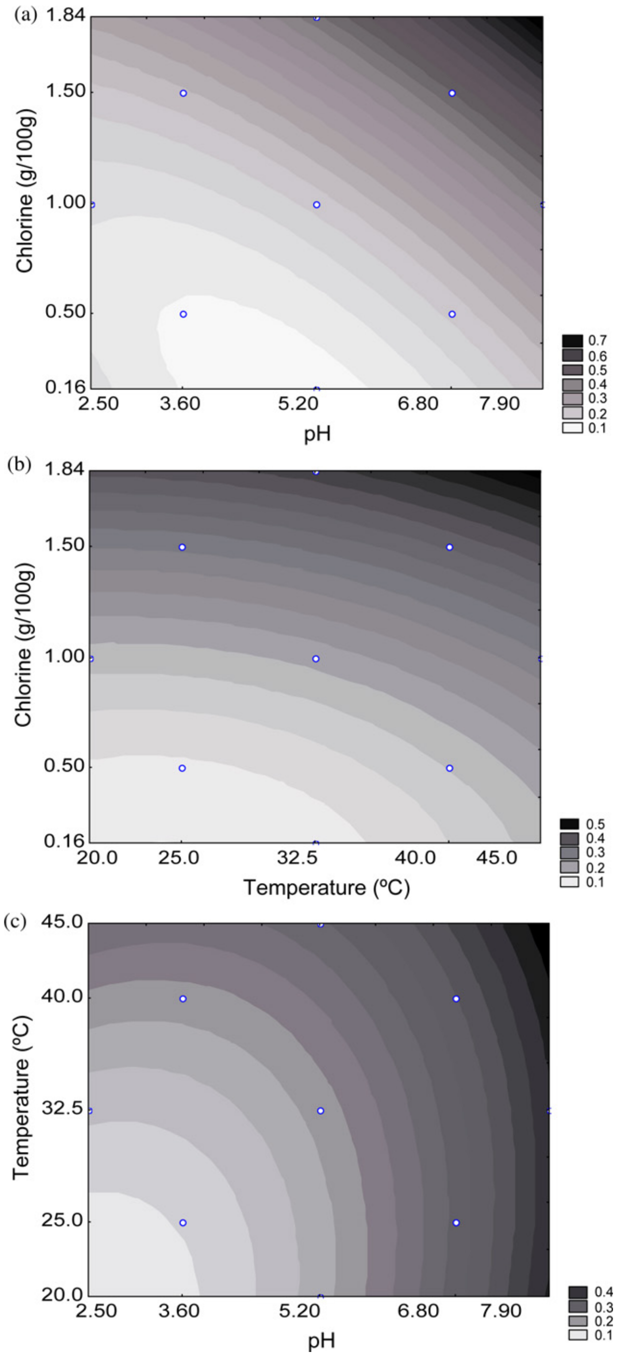

**Fig. 1.** Contour curve for the sum of carbonyl (CO) and carboxyl (COOH) groups in fermented cassava starch oxidised with sodium hypochlorite as a function of active chlorine concentration and pH (a), active chlorine concentration and temperature (b), and temperature and pH (c).

### 3.2. Pasting properties

Pasting properties are very representative of the intensity of changes that occur during starch modification. Peak viscosity reflects the molecular degradation of starch, while gelatinisation reflects granule integrity. Heat treatment followed by mechanical action on starch granules leads to structural breaks and, consequently, loss of granule integrity. The intensity of this break depends on the type of starch, temperature and the shear mechanical and chemical agents used during gelatinisation. Table 4 lists the results of peak, holding and final viscosities.

**Table 4**
Pasting properties of oven-dried fermented cassava starch oxidised with sodium hypochlorite.

| Treatment | C (g/100 g)[a] | pH | T (°C) | Peak viscosity (RVU) | Holding viscosity (RVU) | Final viscosity (RVU) |
|---|---|---|---|---|---|---|
| 1 | 0.50 | 3.60 | 25.0 | 243.5 ± 2.3 | 99.50 ± 2.5 | 118.1 ± 2.1 |
| 2 | 1.50 | 3.60 | 25.0 | 148.0 ± 1.7 | 3.50 ± 0.7 | 4.1 ± 1.2 |
| 3 | 0.50 | 6.80 | 25.0 | 240.7 ± 4.1 | 40.4 ± 1.9 | 66.5 ± 2.4 |
| 4 | 1.50 | 6.80 | 25.0 | 101.4 ± 0.4 | 4.1 ± 0.1 | 5.2 ± 0.2 |
| 5 | 0.50 | 3.60 | 40.0 | 262.6 ± 2.5 | 94.1 ± 4.2 | 117.5 ± 5.6 |
| 6 | 1.50 | 3.60 | 40.0 | 174.8 ± 3.2 | 3.3 ± 0.1 | 4.3 ± 0.5 |
| 7 | 0.50 | 6.80 | 40.0 | 239.6 ± 3.9 | 39.8 ± 3.0 | 57.9 ± 3.5 |
| 8 | 1.50 | 6.80 | 40.0 | 98.7 ± 4.3 | 6.4 ± 1.2 | 9.5 ± 1.1 |
| 9 | 1.00 | 5.20 | 32.5 | 245.0 ± 0.4 | 19.4 ± 1.3 | 21.5 ± 2.1 |
| 10 | 1.00 | 5.20 | 32.5 | 282.6 ± 4.9 | 48.6 ± 2.6 | 59.8 ± 3.2 |
| 11 | 1.00 | 5.20 | 32.5 | 274.1 ± 5.4 | 50.6 ± 1.9 | 62.5 ± 3.4 |
| 12 | 0.16 | 5.20 | 32.5 | 244.3 ± 3.4 | 112.5 ± 0.8 | 144.8 ± 2.2 |
| 13 | 1.84 | 5.20 | 32.5 | 224.9 ± 6.3 | 13.4 ± 1.7 | 16.4 ± 2.3 |
| 14 | 1.00 | 2.50 | 32.5 | 274.8 ± 3.1 | 105.3 ± 3.5 | 135.0 ± 5.6 |
| 15 | 1.00 | 7.90 | 32.5 | 205.4 ± 5.7 | 57.2 ± 3.5 | 79.1 ± 4.4 |
| 16 | 1.00 | 5.20 | 20.0 | 291.8 ± 4.1 | 109.8 ± 4.0 | 126.9 ± 2.5 |
| 17 | 1.00 | 5.20 | 45.0 | 243.2 ± 5.3 | 22.9 ± 2.1 | 29.2 ± 1.4 |
| 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 active chlorine (g Cl/100 g cassava starch, d.b.).

The peak viscosity of fermented cassava starch oxidised with sodium hypochlorite ranged from 98.7 RVU (treatment 8) to 291.8 RVU (treatment 16). In treatments of high active chlorine concentrations and high pH, the peak viscosity decreased more drastically than in treatments where these factors were low, indicating a greater degradation of starch. Sun drying as well as harsh treatment with sodium hypochlorite promoted a decrease in peak viscosity. The peak viscosity for oven-dried fermented cassava starch was 279.0 RVU and that for sun-dried fermented cassava starch was 263.3 RVU. Therefore, the sun drying promotes an overall decrease in the peak viscosity. In treatments with low intensity oxidation, low active chlorine concentrations and low pH, the peak viscosity was high (Table 4), indicating that even a small level of oxidation could promote molecular rearrangement of the granule surface and of low molecular weight components without disrupting glycosidic bonds. This leads to the formation of intermediate compounds that increase the resistance of starch granules during heating primarily because the carbonyl and carboxyl groups introduced into the starch molecule promote granule swelling (Kuakpetoon & Wang, 2001). Sanchez-Rivera, Garcìa-Suàrez, Valle, Gutierrez-Meraz, and Bello-Pèrez (2005) studied the effect of sodium hypochlorite oxidation on the properties of banana starch and reported an increase in peak viscosity at low active chlorine concentrations and reduction in peak viscosity at high active chlorine concentrations compared to those observed for native starch.

Holding viscosity (at a constant temperature) and mechanical action are related to the behaviour of starch during processing. High temperatures and mechanical action can disrupt granule structure, and consequently, reduce viscosity. Holding viscosity of fermented cassava starch oxidised with sodium hypochlorite ranged from 3.3 RVU (treatment 6) to 112.5 RVU (treatment 12). In general, holding viscosity of starch oxidised with sodium hypochlorite was significantly affected by the factors in our study, showing the lowest values at high active chlorine concentrations (treatments 2, 4, 6, 8 and 13). Sun drying also promoted a reduction in holding viscosity compared to that of oven-dried fermented cassava starch (Table 4).

Oxidation with sodium hypochlorite and sun drying promoted a reduction in the final viscosity of fermented cassava starch. This decrease in final viscosity due to oxidation can be attributed to depolymerisation of amylose molecules. Wang and Wang (2003) reported that both amylose and amylopectin were degraded by sodium hypochlorite oxidation but amylose was more susceptible to oxidation. In addition, Martínez-Bustos, Amaya-Llano, Carbajal-Arteaga, Chang, and Zazueta-Morales (2007) studied the effect of various organic acids (lactic, acetic and citric acid) as well as oxidation with hydrogen peroxide of starches and found that oxidation decreased the final and holding viscosities in oxidised potato and cassava starches.

Linear mathematical models that describe the response surface for the peak, holding and final viscosities were defined by the equations listed in Table 3. Determination coefficients for the peak, holding, and final viscosities were 0.65, 0.95 and 0.85, respectively, at 90% confidence.

### 3.3. Gel hardness

Textural properties of gels depend on the constituents of starch and amylose, the volume and deformation of the granules and the interaction between the continuous and dispersed phases (Choi & Kerr, 2003). Hardness is a measure of texture that corresponds to the force applied in order to cause deformation of a sample, measured in texturometers. Increases in gel firmness are related to retrogradation of the amylose and amylopectin components of starch (Karim et al., 2008).

Gel hardness results are listed in Table 5. Oxidative treatment with sodium hypochlorite differentially affected the gel hardness of oven-dried fermented cassava starch. Oven-dried fermented cassava starch oxidised with sodium hypochlorite presented higher gel hardness compared to non-oxidised starch, except at high active chlorine concentration, high pH and high temperature (treatment 8), which showed a weaker gel with lower hardness. The behaviour of the gels and the intensity of oxidation are related to changes in the internal structure of starch. Under low intensity oxidative treatments, there is an increase in gel hardness related to the formation of functional groups at certain sites, increasing gel resistance. The resistance of the gel decreases with increases in the intensity of oxidation, primarily due to depolymerisation or molecular rearrangement. The increase observed in gel hardness in starch oxidised with sodium hypochlorite may be from increased hydrogen bonding due to the formation of carbonyl and carboxyl groups and reduction in size of amylose molecules, as amylose molecules with intermediate weight had a greater ability to retrograde and form gels.

Statistical analyses showed that for gel hardness, the effects of the variables studied and the interaction between them were not significant at 90% confidence. Therefore, gel hardness is not a significant parameter for a regression model.

**Table 5**
Gel hardness, specific volume and biscuit hardness of oven-dried fermented cassava starch oxidised with sodium hypochlorite.

| Treatments | C (g/100 g)[a] | pH | T (°C) | Gel hardness (gf) | Specific volume (mL/g) | Biscuit hardness (gf) |
|---|---|---|---|---|---|---|
| 1 | 0.50 | 3.60 | 25.0 | 186.1 ± 10.2 | 9.27 ± 0.70 | 6656 ± 820 |
| 2 | 1.50 | 3.60 | 25.0 | 261.6 ± 16.5 | 8.83 ± 0.70 | 10534 ± 973 |
| 3 | 0.50 | 6.80 | 25.0 | 120.8 ± 2.6 | 12.61 ± 0.56 | 4841 ± 831 |
| 4 | 1.50 | 6.80 | 25.0 | 126.6 ± 6.2 | 7.04 ± 0.51 | 5220 ± 504 |
| 5 | 0.50 | 3.60 | 40.0 | 182.8 ± 3.9 | 12.21 ± 0.34 | 5385 ± 976 |
| 6 | 1.50 | 3.60 | 40.0 | 200.0 ± 24.9 | 7.69 ± 0.40 | 8241 ± 780 |
| 7 | 0.50 | 6.80 | 40.0 | 241.6 ± 19.7 | 8.01 ± 0.49 | 9432 ± 869 |
| 8 | 1.50 | 6.80 | 40.0 | 70.7 ± 6.5 | 6.00 ± 0.40 | 10500 ± 850 |
| 9 | 1.00 | 5.20 | 32.5 | 86.4 ± 3.5 | 10.50 ± 0.55 | 8435 ± 750 |
| 10 | 1.00 | 5.20 | 32.5 | 98.8 ± 5.6 | 10.60 ± 0.42 | 7721 ± 891 |
| 11 | 1.00 | 5.20 | 32.5 | 95.3 ± 5.4 | 10.56 ± 0.47 | 8920 ± 850 |
| 12 | 0.16 | 5.20 | 32.5 | 177.5 ± 9.6 | 9.38 ± 0.51 | 19250 ± 1530 |
| 13 | 1.84 | 5.20 | 32.5 | 326.7 ± 22.5 | 8.70 ± 0.50 | 6439 ± 876 |
| 14 | 1.00 | 2.50 | 32.5 | 178.6 ± 8.2 | 10.99 ± 0.47 | 6411 ± 455 |
| 15 | 1.00 | 7.90 | 32.5 | 385.7 ± 20.6 | 8.49 ± 0.58 | 9520 ± 781 |
| 16 | 1.00 | 5.20 | 20.0 | 186.3 ± 3.3 | 10.22 ± 0.47 | 6230 ± 530 |
| 17 | 1.00 | 5.20 | 45.0 | 319.9 ± 24.3 | 6.80 ± 0.48 | 9347 ± 824 |
| 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 active chlorine (g Cl/100 g cassava starch, d.b.).

### 3.4. Expansion properties

Table 5 shows the specific volumes of biscuits made with oven-dried fermented cassava starch oxidised with sodium hypochlorite. The linear mathematical model that described the response surface for specific volume was defined by the equation presented in Table 3; the determination coefficient for the specific volume was 0.71 at 90% confidence.

Oxidative treatment with sodium hypochlorite increased the expansion capacity of fermented cassava starch. The highest specific volumes were observed within an optimum oxidation range. Oven-dried fermented cassava starch had the lowest specific volume (2.65 mL/g), indicating that sun drying or sodium hypochlorite oxidation is necessary for increasing expansion properties (Table 5). The effects of fermentation and hydrogen peroxide oxidation on the expansion properties of cassava starch and cornstarch were studied by Dias et al. (2007). These authors reported that oxidised cassava starch had improved expansion properties with exposure to sunlight and hydrogen peroxide, obtaining specific volumes of 15.04 mL/g at 50 days of fermentation; however, no increases in expansion properties were observed for cornstarch (average specific volume of 2.27 mL/g).

The highest specific volumes were obtained at active chlorine concentrations between 0.16 g/100 g to 1.00 g/100 g, pH 2.50 to 5.20 (Fig. 2a) and temperatures of 25.0 °C to 32.5 °C (Fig. 2b). Fig. 2c shows that the highest specific volumes of biscuits made with sodium hypochlorite oxidised, fermented cassava starch were at high temperatures and low pH or low temperatures and high pH. Increases in active chlorine concentration and pH reduced the expansion capacity of biscuits oxidised with sodium hypochlorite (Table 5). It is possible that this negative effect on the expansion capacity is due to excessive oxidation and increased starch degradation (from breaking glycosidic linkages and increased depolymerisation), which affects the ability of the starch to form internal alveolar structures responsible for biscuit expansion.

Shirai et al. (2007) studied the effects of oxidation with hydrogen peroxide on starches from different sources (potato, sweet potato, cassava, maize and waxy maize). They found that only cassava starch and waxy cornstarch displayed increased expansion properties with values of 9.2 mL/g and 15.5 mL/g, respectively. Expansion during baking may occur from the reassociation of size-reduced amylose and/or amylopectin starch molecules into amorphous regions (Vatanasuchart, Naivikul, Charoenrein, & Sriroth, 2005).

Our results are consistent with those from Demiate et al. (2000) who also obtained similar specific volumes of ~10 mL/g from sodium hypochlorite oxidised native cassava starch biscuits. Differences observed in the expansion property of biscuits oxidised at different active chlorine concentrations, pH and temperature showed that oxidation is responsible for affecting the expansion property of fermented cassava starch. Therefore, factors that interfere with the oxidation process are a viable way to alter the production of artificially dried, fermented cassava starch and provide high quality products for the food industry. Non-oxidised sun-dried fermented cassava starch displayed a high expansion property (13.54 mL/g), indicating that sun drying also promotes expansion during baking. This result is also consistent with that reported by Mestres, Boungou, Akissoe, and Zakhia (2000).

Interestingly, the highest specific volume was not observed with the highest sum value of the carbonyl and carboxyl groups. This behaviour is likely due to the balance between the levels of carbonyl and carboxyl groups required to obtain maximum biscuit expansion.

### 3.5. Biscuit hardness

Biscuit hardness was evaluated by measuring the maximum force required to break the biscuit into two symmetrical parts. Table 5 shows the results of biscuit hardness made with oven-dried fermented cassava starch oxidised with sodium hypochlorite. The linear mathematical model that described the response surface for biscuit hardness was defined by the equation in Table 3; the determination coefficient for the specific hardness was 0.94 at 90% confidence.

Oxidative treatment with sodium hypochlorite reduced biscuit hardness when compared to non-oxidised, oven-dried samples (Table 5). The highest values of biscuit hardness occurred at high pH and low active chlorine concentrations and at high pH and high temperature. Biscuits made with sun-dried fermented cassava starch had lower hardness (2431 gf) compared to those made with oven-dried fermented cassava starch oxidised with sodium hypochlorite (Table 5).

Biscuits made with non-oxidised, oven-dried fermented cassava starch had the highest hardness (>25,000) and lowest specific volume (2.65 mL/g), indicating an inverse relationship between biscuit hardness and specific volume. This relationship was also observed

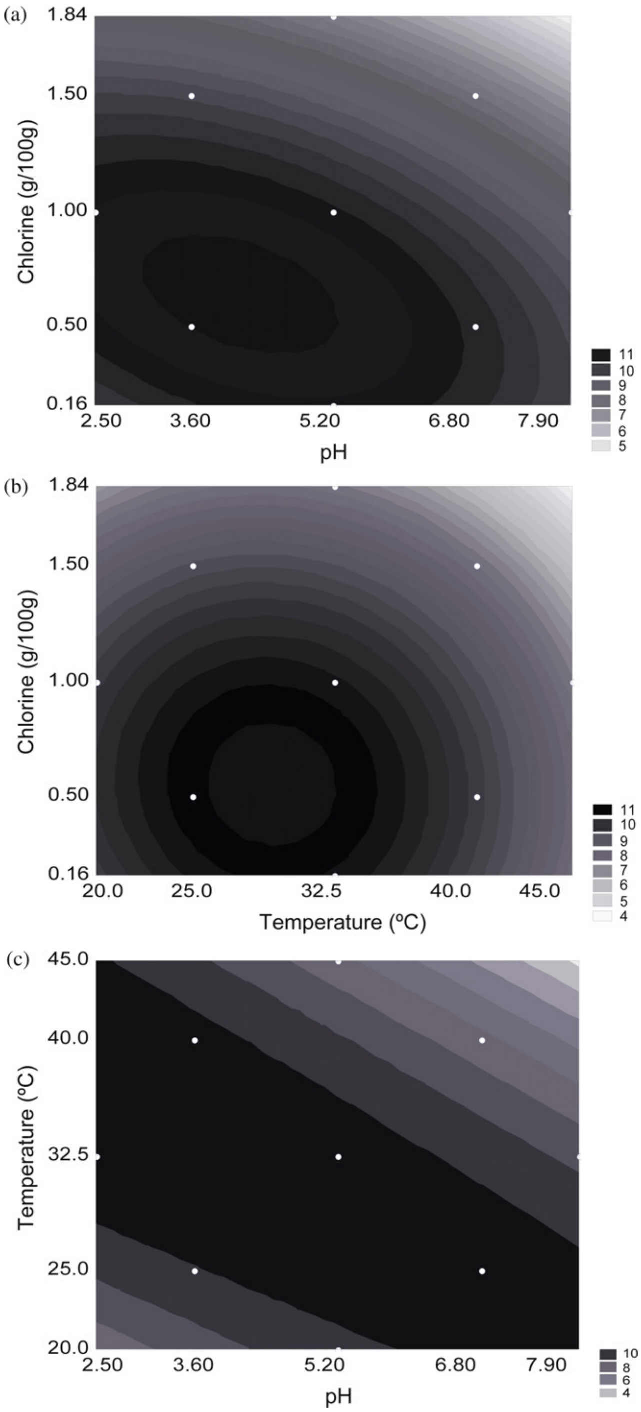

**Fig. 2.** Contour curve for the specific volume of biscuits made from fermented cassava starch oxidised with sodium hypochlorite as a function of active chlorine concentration and pH (a), active chlorine concentration and temperature (b), and temperature and pH (c).

in sun-dried fermented cassava starch, which had the lowest biscuit hardness and highest specific volume (Table 5).

## 4. Conclusions

Sun-dried fermented cassava starch had a higher content of carboxyl and carbonyl groups, higher expansion property and lower biscuit hardness compared to those of oven-dried fermented cassava starch. However, these oxidation effects were more pronounced when the fermented cassava starch was oxidised with sodium hypochlorite. The highest sum values for carbonyl and carboxyl groups occurred between a neutral to alkaline pH, at high active chlorine concentrations and at high temperatures. Pasting properties of fermented cassava starch ranged with the intensity of sodium hypochlorite oxidation. Furthermore, oxidative treatment with sodium hypochlorite increased the expansion property and reduced biscuit hardness. Therefore, the properties of fermented cassava starch were influenced by the intensity of sodium hypochlorite oxidation and ranged with active chlorine concentration, pH, and temperature. Expansion due to the addition of oxidants has several advantages over sun drying because it better controls process parameters, is not dependent on climate conditions and produces more homogeneous products.

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