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Partial characterization of banana starches oxidized by different levels of sodium hypochlorite

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Abstract

Chemical, physical, physicochemical and functional properties of oxidized banana starch by different levels of sodium hypochlorite (0.25–2.0% active chlorine) were studied. The content of carboxyl and carbonyl groups in the oxidized starches increased when chlorine concentration increased, however the level of carboxyl groups was higher than carbonyl groups because carbonyl groups were formed during the reaction were transformed to carboxyl groups. The L^* factor was used to characterize the whiteness of the oxidized starches. When chlorine concentration increased the whitness increased too, but at the highest chlorine concentration the L^* values were not different ($\alpha = 0.05$). The oxidation carried out with a chlorine concentration between 0.25 and 1.0% produced starches with a viscosity peak higher than its native counterpart, however when the active chlorine concentration increased (1.25–2.0%) an inverse pattern was obtained; high chlorine concentration cleaved the starch chains and the peak viscosity decreased. The gelatinization temperature (T_p) of oxidized starches was higher than its native counterpart, but at the highest active chlorine concentration a decreased in the T_p value was obtained. The enthalpy values of the oxidized starches were lower than the native starch and in general those values decreased when active chlorine concentration increased. These results agree with those obtained from viscoamylography since starch degradation is carried out with high chlorine concentration. The swelling power of the oxidized starches measured at low temperatures was lower than its native counterpart and the water solubility index increased when temperature and chlorine concentration increased.

1. Introduction

Banana is a climacteric fruit and, in México, is consumed when the fruit is ripe. For this reason, high quantities of fruit are lost during their commercialization due to poor post-harvest handling. Starch is the principal component of green bananas that is changed during ripening (Lii, Chang, & Young, 1982). Starch is deposited in the form of partially crystalline granules, whose morphology, chemical composition, and supermolecular structure are characteristics of each particular plant species. Native starches have been used since ancient times as a raw material to prepare different products. Utilization of native starches has some drawbacks because the process conditions (e.g. temperature, pH, pressure) reduce its use in industrial applications. Native starch has a low shear stress resistance, low descomposition, high retrogradation, and syneresis. These shortcomings may be overcome by starch modification. The starch structure can be modified by chemical, physical and enzymatic methods. A chemical modified starch is obtained when native starch is treatment with reactive specific to change some of its properties. This definition includes hydroxypropylated, cross-linked, and acetylated starches (Van de Bij, 1976).

Oxidized starch is widely used in industries such as paper, textile and food industries to provide surface sizing and coating properties (Kuakpetoon & Wang, 2001). The main outlets for oxidized starch are in the paper and textile industries, its applications in the food industry is increasing because of its low viscosity, high stability, clarity, film forming and binding properties.

Oxidized starch is produced by reacting starch with a specific amount of oxidizing reagent under controlled temperature and pH (Wang & Wang, 2003). The sodium hypochlorite is the oldest and most popular commercial

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oxidant. Hydroxyl groups of starch molecules are first oxidized to carbonyl groups and then to carboxyl groups. Therefore, the numbers of carboxyl and carbonyl groups on oxidized starch indicate the level of oxidation, which takes place primarily at the hydroxyl groups of C-2, C-3, and C6-position on a D-glucopyranosyl unit (Kuakpetoon & Wang, 2001; Thomas & Atwell, 1999). The factors affecting hypochlorite oxidation include pH, temperature, hypochlorite concentration, starch molecular structure, and starch origin (Chattopadhyay, Singhal, & Kulkarni, 1997; Rutenberg & Solarek, 1984). The objective of this study was to evaluate the effect of hypochlorite concentration on some physicochemical and functional properties of oxidized banana starches.

2. Materials and methods

2.1. Starch isolation

Unripe bananas (*Musa paradisiaca*) were purchased in the local market of Cuautla, Morelos, México. The starch was isolated using a pilot scale procedure (Flores-Gorosquera et al., 2004). The powder was ground to pass a US No. 100 sieve and stored at room temperature (25 °C) in a glass container.

2.2. Preparation of oxidized starches

A starch slurry was prepared according to Wang and Wang (2003) with slight modifications, by adding distilled water to 200 g starch (dry basis) to final weight of 500 g in a 1-L reaction vessel. The starch slurry was maintained at 35 °C by occasionally turning off the heating power and the pH was adjusted to 9.5 with 2 N NaOH. Sodium hypochlorite with 0.25% active chlorine w/v was slowly added into the starch slurry over 30 min while maintaining the pH at 9.5 with 1 N H_2SO_4. After the addition of NaOCl solution, the pH of the slurry was maintained at 9.5 with 1 N NaOH for an additional 50 min. The slurry was then adjusted to pH 7.0 with 1 N H_2SO_4, passed to 1-L flask to precipitate and decant the reaction mix and wash with several volume deionized water and dry in a convection oven at 50 °C for 48 h. The same procedure was applied to banana starch with different active chlorine concentrations (0.50, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0% w/v).

2.3. Carbonyl content

The carbonyl content was determined by following the titrimetric method of Smith (1967). Four grams of starch sample was suspended in 100 mL distilled water in a 500 mL flask. The suspension was gelatinized in a boiling water bath for 20 min, cooled to 40 °C, adjusted to pH 3.2 with 0.1 N HCl, and then mixed with 15 mL of hydroxylamine reagent. The flask was stoppered and placed in a 40 °C water bath for 4 h with slow stirring. The excess hydroxylamine was determined by rapidly titrating the reaction mixture to pH 3.2 with standardized 0.1 N HCl. A blank determination with only hydroxylamine reagent was performed in the same manner. The hydroxylamine reagent was prepared by first dissolving 25 g hydroxylamine hydrochloride in 100 mL of 0.5 N NaOH before the final volume was adjusted to 500 mL with distilled water. Carbonyl content was calculated as follows:

$$\%\,\text{Carbonyl Content} = \frac{[(\text{Blank} - \text{Sample})\,\text{mL} \times \text{Acid Normality} \times 0.028 \times 100]}{\text{Sample weight}\,(g, \text{dry basis})}$$

2.4. Carboxyl content

The carboxyl content of oxidized starch was determined according to the modified procedure of Chattopadhyay et al. (1997). About 2 g of starch sample was mixed with 25 mL of 0.1 N HCl, and the slurry was stirred occasionally for 30 min with a magnetic stirrer. The slurry was then filtered (under vacuum) through a 150 mL medium porosity fritted glass funnel and washed with 400 mL of distilled water. The starch cake was then carefully transferred into a 500 mL beaker, and the volume was adjusted to 300 mL with distilled water. The starch slurry was heated in a boiling water bath with continuous stirring for 15 min to ensure complete gelatinization. The hot starch dispersion was then adjusted to 450 mL with distilled water and titrated to pH 8.3 with standardized 0.01 N NaOH. A blank test was performed with unmodified starch. Carboxyl content was calculated as follows:

$$\frac{\text{Milliequivalents of Acidity}}{100\text{ g Starch}} = \frac{[(\text{Sample} - \text{Blank})\text{ mL} \times \text{N NaOH} \times 100]}{\text{Sample weigth }(g, \text{dry basis})}$$

$$\%\text{ Carboxyl content} = \frac{[\text{Milliequivalents of acidity}]}{100\text{ g starch}} \times 0.045$$

2.5. Color evaluation

Color evaluation was performed on the surface of the banana oxidized starch. The color was measured four times for each oxidized starch. Universal Colorimeter (Milton Roy, mod. Color Mate) color analyzer consisting of ilumination/D65, 10° viewing, was used. The chroma meter was first calibrated with a white tile. The L^* factor was obtained.

2.6. Microviscoamylography

To determine the viscosity (Brabender Units, BU) profile of native and oxidized starches pastes, the technique proposed by the AACC (2000), was used. Starch dispersions with 10% (w/v) of total solids were prepared; 100 mL of dispersion (sample) were transferred to the bowl of the microviscoamylograph (Brabender OHG, Duisburg, Germany). The machine was programmed to run a heating–cooking–cooling cycle that began at 30 °C and then increased to 95 °C, staying at this temperature for 10 min, then cooled to 40 °C and also maintained at this temperature for 10 min. A heating rate of 2.5 °C/min was used along the whole cycle with a speed of agitation of 125 rpm.

2.7. Thermal analysis

The thermal properties of native and oxidized starches of banana, temperature and enthalpy of gelatinization, were assessed by a Differential Scanning Calorimeter (DSC, TA Instrument, model 2010, New Castle, USA) previously calibrated with indium. The gelatinization of the starches was evaluated by the method proposed by Paredes-López, Bello-Pérez, & López, (1994). Two mg sample (dry basis) was weighed on an aluminum pan, adding 7 μL of deionized water. The pan was sealed tightly and then it was allowed to stand for 1 h before carrying out the analysis. An empty aluminum pan was used as a reference. The sample was subjected to a heating program over a range of temperature from 10 to 120 °C and a heating rate of 10 °C/min. The gelatinization or peak temperature (T_p) and the transition enthalpy (ΔH), were obtained directly from the analysis of the software TA Instruments OS/2 version 2.1.

2.8. Swelling and solubility

The swelling power (SP) and water solubility index (WSI) were measured by the technique proposed by Tecante & Doublier, (1999). Native and oxidized starches dispersions with 5% of total solids on dry basis were prepared, a kinetics of heating was followed using the microviscoamylograph bowl as heating devise, starting at room temperature with a heating rate of 1.5 °C/min until 95 °C maintaining the sample isothermal for 10 min, and then cooling to 40 °C at the same rate (1.5 °C/min). In the course of cooking of the starches, samples were taken at different temperatures (50, 70, 80, 95 °C) using tubes with deionized water, in such a way that the final concentration was 0.5% (w/v) of solids. The slurries were centrifuged at 2500 rpm for 25 min. The supernatant was decanted carefully, and thee volume was measured; aliquots were used to estimate the total carbohydrates (Dubois et al., 1956). The precipitate was used to determine the moisture content (24 h at 100 °C). The swelling power of the granules was calculated from the mass of the centrifuged residual and the mass of the dry residual. Water solubility index was calculated using the following equation:

$$\%S = (m_{ss}/m_{DS}) \times 100$$

where m_{ss} is the mass of starch in the supernatant, calculated as the product of concentration of total sugars in the supernatant times the volume of supernatant, and m_{DS} is the mass of dry starch en the aliquot, calculated as the product of ratio of dry to wet mass of the residue times the volume of dilution assuming a density of 1000 kg/m^3.

Swelling power was calculated according to the equation:

$$G = m_R/m_{RH}$$

where m_R is the mass of wet residue and m_{RH} is the mass of dry residue. Values reported are the mean of four measurements.

2.9. Statistical analysis

One way analysis of variance (ANOVA) at the significace level of 5% ($\alpha = 0.05$) was applied to the obtained results using the statistical program Sigma-stat, version 2.1 (Fox, Shotton, & Urlich, 1995), and when statistical differences were found, the test of multiple comparison of Tukey was applied (Montgomery, 1976).

3. Results and discussion

3.1. Carbonyl and carboxyl group contents

Carbonyl groups in oxidized banana starches were detected in those modified starches treated with 1.0% of active chlorine and above (Table 1). The carbonyl groups content increased when active chlorine concentration increased in the reactive solution and the values were significantly different ($\alpha = 0.05$). However, at the concentration of active chlorine of 0.5 and 0.75% carboxyl groups were detected. This shows that carbonyl groups were

Table 1
Carboxyl and carbonyl group content of oxidized banana starch at different active chlorine concentrations

Active chlorine concentration (%)	Carbonyl (%)	Carboxyl (%)
0.25	n.d	n.d
0.50	n.d	0.013 ± 0.003^a
0.75	n.d	0.021 ± 0.002^b
1.0	0.015 ± 0.001^a	0.040 ± 0.001^c
1.25	0.021 ± 0.001^b	0.045 ± 0.001^d
1.50	0.032 ± 0.002^c	0.050 ± 0.001^e
1.75	0.036 ± 0.001^d	0.061 ± 0.002^e
2.0	0.048 ± 0.003^e	0.076 ± 0.003^f

n.d = no detected. Mean values of six (carbonyls) and four (carboxyls) measurements ± standard error. Same letters inside each column indicate that there are not significant differences ($\alpha = 0.05$).

formed during the reaction, but they were transformed to carboxyl groups. This pattern is because during the oxidation reaction, hydroxyl groups (OH) of glucose are initially oxidised to carbonyl groups and thereafter to carboxyl groups (Wang & Wang, 2003). The carbonyl groups level in oxidized banana starches with 1.0–1.5%, were lower than those reported for normal and waxy corn starches (Wang & Wang, 2003); however, the oxidized banana starch with 2.0% of active chlorine, showed higher carbonyl groups than that of normal corn starch and was similar to waxy corn starch. At low active chlorine concentrations, the OH groups of phenols compounds presented in banana starch can react with the chlorine, for this reason carbonyl groups were low in oxidized banana starches. The modified banana starches were whiter than its native counterpart, because in an oxidation reaction some pigments and proteins are firstly oxidized before the glucose units (Rutenberg & Solarek, 1984) eliminating those compounds and producing a whiter starch.

The oxidation grade in a modified starch is determined by carboxyl groups concentration. On alkaline conditions (pH 9.5), they are produced in higher concentration than carbonyl groups (Rutenberg & Solarek, 1984). The carboxyl groups had a similar pattern than carbonyl groups, because they were increased when active chlorine concentration increased as well, and the values were statistically different ($\alpha = 0.05$). In the case of modified banana starch with 0.25% of active chlorine neither carbonyl and carboxyl groups were detected, indicating that this chlorine concentration is not enough to oxidize banana starch. The carboxyl groups level in modified banana starches were lower than those obtained in oxidized corn starches (Wang & Wang, 2003). The presence of phenol compounds in banana starch can react with the chlorine, eliminating the pigments and producing starch with a white color in comparison with native banana starch that has a cream color.

3.2. Color evaluation

An important physical property of oxidized starches is the whiteness, characteristic that in many applications is very important. The L^* factor is the better parameter that characterizes the whiteness of the oxidized starches (Table 2). The L^* values increased when active chlorine concentration increased as well, although at the highest chlorine concentration tested, there were not statistical differences ($\alpha = 0.05$). The highest L^* values obtained were near at 100%, that is the maximum value for this parameter, indicating a white material.

Table 2
Lightness factor (L*) readings of oxidized starches of banana at different active chlorine concentrations

Active chlorine concentration (%)	L^*
0.25	82.40 ± 0.39^{a}
0.50	86.79 ± 0.45^{b}
0.75	87.65 ± 0.21^{c}
1.0	88.76 ± 0.29^{d}
1.25	92.02 ± 0.13^{e}
1.50	93.87 ± 0.11^{f}
1.75	95.85 ± 0.40^{g}
2.0	96.74 ± 0.04^{g}

Mean values of two measurements ± standard error. Same letters inside each column indicate that there are not significant differences ($\alpha = 0.05$).

3.3. Pasting property

The oxidized starches with 0.25, 0.50, 0.75 and 1.0% of active chlorine, presented a viscosity peak that was higher than its native counterpart (Fig. 1). This behavior is because the carbonyl and carboxyl groups introduced to the starch molecule promote swelling of the granules (Kuakpetoon & Wang, 2001). These oxidized starches during the cooling step showed higher viscosity values than during the heating step and the values increased when the active chlorine concentration increased (1.0%), this pattern is due to amylose chains were solubilized during the heating step forming a network that had a more compact structure, that in the case of 1.0% of active chlorine we think is a protective effect of the carbonyl and carboxyl groups where the solubilized amylose chains were longer and produced a more compact structure. When the active chlorine concentration increased (1.25, 1.50, 1.75 and 2.0%) an inverse pattern was obtained, the viscosity decreased when chlorine concentration increased. This is probably because at concentrations higher than 1.25%, a cleavage of the starch chains is produced due to high chlorine concentration, and the peak viscosity decreased. In the case of the oxidized starches prepared with the highest concentration of active chlorine, the effect of the cleavage of the starch chains was more notorius, because viscosity did not increase during the cooling step, pattern due to the network formation of the short chains aligned and therefore minor interactions are produced and the gel structure has a softer consistency. In the same sense, the presence of carbonyl and carboxyl groups impede associations among starch chains and for this reason a true gel structure is not obtained (Adebowale, Afolabi, & Lawal, 2002).

3.4. Thermal properties

The gelatinization temperature (T_p) of oxidized starches was higher than its native counterpart (Table 3). However, at the highest active chlorine concentration a decrease in the T_p value was obtained. The oxidation procedure of the starches stabilizes that are desorganize at higher temperature. It is important to mention that the T_p is an average temperature and the granules have a range of temperature where they can become desorganized, for this reason, generally the T_p value of the oxidized starches did not change with the chlorine concentration. In the case of enthalpy of gelatinization, this parameter gives more information about the starch chain organization. The enthalpy values of the oxidized starches

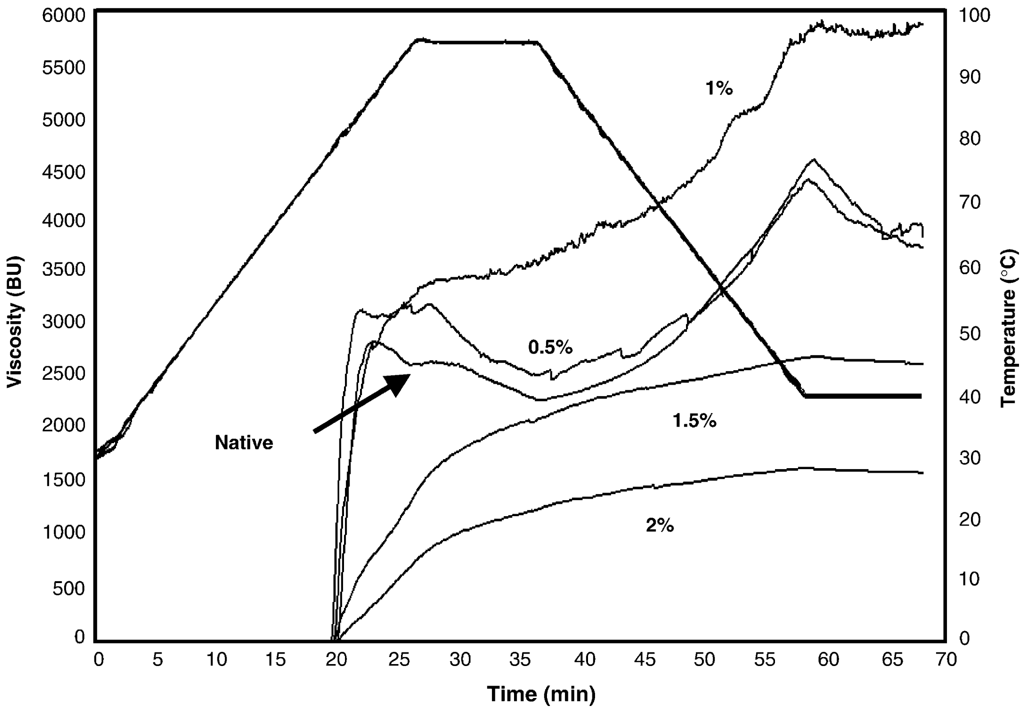

Fig. 1. Micro Viscoamylography pasting curves of unmodified and hypochlorite-oxidized starches of banana ‘Macho’ at different active chlorine concentrations.

were lower than the native starch and in general those decreased when active chlorine concentration increased. The starches prepared with 0.25 and 0.5% of active chlorine were not different ($\alpha = 0.05$), to the highest enthalpy values determined in the samples analyzed. This pattern might be due that the carboxyl groups introduced in starch molecules, stabilize the structure and the energy necessary to carry out the starch gelatinization increases. The samples oxidized with 0.75, 1.0 and 1.25% of chlorine were not different ($\alpha = 0.05$). However, at the highest concentration of active chlorine lowest enthalpy values were obtained. These values agree with those obtained with viscoamylography, since the starches prepared with the highest active chlorine concentration had the lowest viscosity values. The low enthalpy values show that the starch degradation is carried at high active chlorine concentration.

3.5. Swelling power

The Swelling power (SP) of the oxidized banana starch at different concentrations of active chlorine, measured at 70 °C was lower than its native counterpart (Fig. 2). In this first step of heating the amount of water captured for the native starch was higher than the oxidized starches, however when chlorine concentration increased the SP value was lower, this is because the high chlorine concentration produced depolymerization of the amylopectin and the starch lost the ability to hold the absorbed water (Wang & Wang, 2003). Hypochlorite oxidation is also highly effective for weakening the internal structure of starch granules, thereby making starch more soluble causing a decrease in the SP (Adebowale et al., 2002).

When temperature increased (80 °C) the starch oxidized with the highest chlorine concentrations had lower SP than that oxidized with low chlorine concentrations, however these latter did not show differences with the native banana starch. At this temperature some amylose molecules are solubilized from starch granule and contribute to increase SP values, but for those starches oxidized at low chlorine concentrations depolymerization amylose was not carried out, not contributing to increase the SP. In the case of starches oxidized with higher chlorine concentrations the effect was higher, because the SP values were the lowest determined in the test. The effect of amylopectin

Table 3
Temperature and enthalpy of gelatinization of oxidized banana starch prepared with different concentrations of active chlorine

Active chlorine concentration (%)	T_p (°C)	ΔH (J/g)
0	69.94 ± 0.96^a	10.13 ± 0.20^a
0.25	76.53 ± 0.21^b	13.22 ± 0.29^b
0.50	76.8 ± 0.57^b	13.29 ± 0.68^b
0.75	76.36 ± 0.9^b	9.04 ± 0.23^c
1.0	76.42 ± 0.24^b	9.04 ± 0.23^c
1.25	76.0 ± 0.41^b	9.81 ± 0.59^b
1.50	75.45 ± 0.22^b	8.26 ± 0.39^d
1.75	75.74 ± 0.15^b	7.26 ± 0.44^e
2.0	73.72 ± 0.36^c	3.31 ± 0.52^f

Mean values of three measurements ± standard error. Values with the same letters inside each column indicate that there are not significant differences ($\alpha = 0.05$).

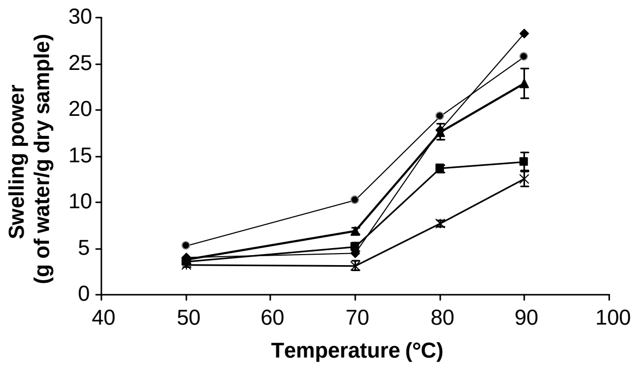

Fig. 2. Swelling power of oxidized banana starch prepared at different active chlorine concentrations (▲ 0%, ■ 0.75%, Ж 1%, ● 1.5 and ♦ 2%)
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depolymerization was better observed at this temperature. At the highest temperature assessed (95 °C) the SP value for the oxidized banana starch at the lowest chlorine concentration was higher than the native starch and in those modified with high chlorine concentration. The effect of the modification is more pronounced in this step, because the modified starches with low chlorine concentration had the effect of partial amylose depolymerization that has the ability of holding more water molecules, but in the case of those modified starches with higher chlorine concentration higher amylopectin depolymerization was produced contributing at low water captation. The amorphous lamella was suggested to be responsible for holding the absorbed water in addition to the crystalline lamella (Wang & Wang, 2003).

3.6. Water solubility index

The Water solubility index (WSI) increased when both temperature and chlorine concentration increased (Fig. 3). At these temperatures amylose was solubilized from starch granules, but in the case of oxidized starches the chlorine concentration produced a starch chain depolymerization (amylose and amylopectin) that was higher when concentration increased, explaining the high concentration of the solubilized carbohydrate. These results agree with our previous results of pasting and thermal analysis. Similar pattern was reported by Wang & Wang (2003) for common and waxy corn starch oxidized at different chlorine concentration; however, their values were lower than those determined in this study, for this reason we believe that the oxidized banana starch might be used for 'new' applications. The effect of temperature on swelling reveals that each type of starch swells differently, indicating differences in the molecular organization within the granules (Adebowale et al., 2001).

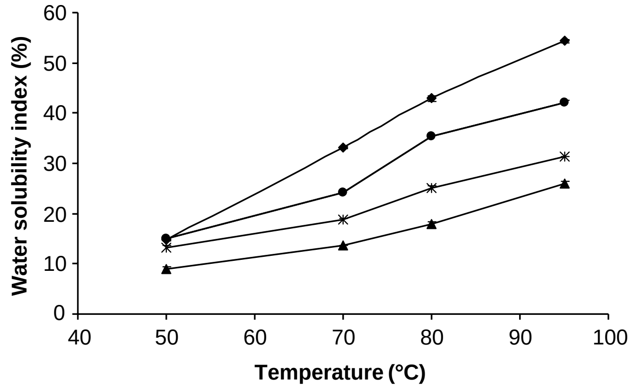

Fig. 3. Water solubility index of oxidized banana starch prepared at different active chlorine concentratios (▲ 0%, Ж 0.5%, ● 1.25%, ■ 2%).

4. Conclusions

Oxidized banana starch presented higher whiteness than its native counterpart. Pasting properties measured by the viscoamylographic test, showed that banana oxidized starches with low chlorine concentration had higher viscosity peak, but when chorine concentration increased the viscosity decreased. High chlorine concentration produced starch depolymerization. Gelatinization temperature (T_p) of oxidized starches was higher than the native; however with high chlorine concentration T_p decreased. The enthalpy values of the oxidized starches were lower than the native starch and in general those decreased when active chlorine concentration increased. These values agree with those found by the viscoamylographic test. The functional properties studied (swelling power and water solubility index) were affected by the temperature and the chlorine concentration. A starch isolated from non-conventional source can be used for produced oxidized starches with particular characteristics for diverse applications.

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