

In vitro digestibility and pasting properties of epichlorohydrin modified cassava starch

Cassava
starch

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Received 24 November 2015

Revised 12 February 2016

8 March 2016

Accepted 8 March 2016

Abstract

Purpose – The purpose of this study was to analyze the influence of epichlorohydrin (ECH) concentration and reaction time on the food-grade resistant starch production and its pasting properties by using native cassava starch of Misiones-Argentina origin.

Design/methodology/approach – Cassava starch was modified using ECH (0.30 and 0.15 per cent) during 4 or 8 h. Digestibility was evaluated by determining resistant starch as total dietary fiber. Pasting properties and the cross-linking degree were studied using a micro-viscoamylograph (Brabender).

Findings – Resistant starch content was not influenced by ECH concentration and reaction time. Cross-linking was detected at higher reaction times (8 h) and ECH concentrations (0.30 per cent), where a decrease in viscosity peaks by more than 80 per cent was observed. Both pasting temperature and breakdown were increased, whereas a decrease in retrogradation was detected.

Practical implications – Starches can be suitable for different food applications. This is because of the ability to modify its pasting properties and the invariability of the *in vitro* digestibility of cassava starch as a result of using ECH (at concentrations approved by local and regional legislation) and reaction times of 4 and 8 h.

Originality/value – Information related to the modification of cassava starch using ECH is scarce or not available nowadays in literature.

Keywords Cassava starch, Cross-linking, Epichlorohydrin, Pasting properties, Resistant starch

Paper type Research paper



The authors thank the National University of Misiones for a doctoral scholarship and the Fundación para el Desarrollo e Investigación Científica y Tecnológica for the use of their equipment and laboratories. JMP was supported partially by Universidad Nacional del Litoral (Santa Fe, Argentina), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina) and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, Argentina).

Nutrition & Food Science

Vol. 46 No. 4, 2016

pp. 517-528

© Emerald Group Publishing Limited

0034-6659

DOI 10.1108/NFS-11-2015-0151

Introduction

Native starches from several botanical origins, such as cereals, legumes, tubers, roots and fruits, are used as ingredients in the manufacturing process of food products. Starches can influence different food properties such as texture, consistency, moisture, gel formation, film-forming behavior, adhesion and homogeneity. Non-food applications of starches can be found in pharmaceutical, textile, adhesive and alcohol-based fuel industries. Currently, starches are used as substitutes in low-calorie foods, biodegradable packaging materials, thin films and thermoplastic materials (Biliaderis, 1998).

The use of native starches in the industry is restricted because of their limited range of physical and chemical properties suitable for certain types of technological processes. Also, some temperature, pH and pressure conditions (and their combinations) can reduce the use of native starches because of their low resistance to shear stresses, thermal decomposition, retrogradation and high degree of syneresis (Bello-Perez *et al.*, 2002; Kaur *et al.*, 2004; Amani *et al.*, 2005).

Modifications on native starch can improve its functional properties to obtain pastes with specific attributes that can withstand extreme processing conditions. These modifications can enhance the industrial application of starches and improve the price/value of manufactured products (López *et al.*, 2010). Methods used to modify starches are classified into four categories: chemical, physical, enzymatic and biological (BeMiller, 1997; Jobling, 2004; Tharanathan, 2005). Chemical modifications are the most used technique. Particular methods commonly found involve degradation by partial acid hydrolysis or enzymes and oxidation treatments, derivatization of functional groups introduced in the starch molecule by esterification, etherification, cross-linking and the combination of treatments (Wurzburg, 1986a; Singh *et al.*, 2007). Chemical and functional properties, obtained using previously mentioned modifications, depend on the source of the starch, reaction conditions (reagent concentration, reaction time, pH and the presence of catalysts), type of substituent, extent of substitution (degree of substitution) and distribution of the substituent on the starch molecule (Kavitha and BeMiller, 1998; Richardson *et al.*, 2000; Hirsch and Kokini, 2002; Wang and Wang, 2002).

From a nutritional point of view, starches are classified into three categories depending on their rate of enzymatic digestion in the small intestine: rapidly digestible starch, slowly digestible starch and resistant starch (RS) (Englyst *et al.*, 1992). Additionally, RS can be subdivided into five types (Homayouni *et al.*, 2014):

- (1) Type 1 corresponds to starch physically inaccessible because it is trapped in an indigestible matrix.
- (2) Type 2 represents the raw starch present in the granules inaccessible to enzymatic digestion.
- (3) Type 3 corresponds to the RS fraction composed of retrograded amylose formed during the cooling of the gelatinized starch.
- (4) Type 4 is obtained by chemical modifications of the starch (Englyst *et al.*, 1992; Woo and Seib, 2002; Cummings and Stephen, 2007).
- (5) Type 5 is defined as a linear polysaccharide of type poly- α -1,4-D-glucan (insoluble in water) that is not susceptible to degradation by α -amylases (Frohberg and Quanz, 2008).

The digestibility of starch varies according to its botanical origin, processing method, granule size, amylose–amylopectin ratio, crystal structure and the presence of amylose–lipid complexes (Ring *et al.*, 1988; Holm and Bjorck, 1992). RS is found naturally in foods such as legumes, potatoes, bananas, wheat, rice, etc. and is present in different proportions depending on the source of the starch. Moreover, processing and storage conditions can affect its content. This fact has attracted the interest of food technologists because of the possibility to increase the RS content using appropriate treatments (Sajilata *et al.*, 2006).

RS can be obtained by chemical, hydrothermal and enzymatic treatments (or a combination of hydrothermal and enzymatic treatments). The concentration RS depends on the method, nature of the reactants, reaction conditions (pH, temperature, time, etc.) and botanical origin of the native starch (Woo and Seib, 2002). These resistant starches, unlike those found in natural sources, are not modified during processing or storage (Holm and Bjorck, 1992).

Chemical modifications such as esterification, etherification and cross-linking can not only modify starch but also produce RS. RS Type 4 has been recognized as the most resistant to *in vitro* treatments using appropriate enzymes (Mun and Shin, 2006). Among the cross-linking-forming agents used to produce food-grade RS, the most common are sodium trimetaphosphate (STMP), sodium tripolyphosphate, phosphorous oxychloride (PClO_3) and epichlorohydrin (ECH) (Singh *et al.*, 2007).

The maximum amount of the cross-linking-forming agents to be used in food products is regulated by regional and local legislation (CAA, 2014). According to this code, 0.3 per cent ECH is the highest authorized concentration for food-grade uses.

Currently, RS gained great interest for its beneficial health effects. RS is associated with the prevention of colon cancer (probable similar effects to those of dietary fiber) and hypoglycemic properties. In addition, RS is considered a substrate for probiotic microorganisms for reducing the formation of gallstones, lowering cholesterol, inhibiting fat accumulation and increasing mineral absorption (Sajilata *et al.*, 2006). Finally, RS contributes positively to deal with health problems, such as type 2 diabetes, hyperlipidemia and obesity. As a consequence, RS can be used as a food ingredient to achieve nutritional and physical functionalities with stability during processing (Fuentes-Zaragoza *et al.*, 2010).

The objective of this study was to evaluate the influence of ECH concentration and reaction time on the production of food-grade RS and pasting properties using native cassava starch of Misiones-Argentina origin.

Materials and methods

Commercial native cassava starch (*Manihot esculenta* Crantz) produced by Cooperativa Agrícola Mixta Limitada de Montecarlo (Montecarlo, Misiones, Argentina) was used. The chemical composition (wet basis) of native cassava starch sample was: 11.60 per cent moisture, 0.75 per cent crude protein (nitrogen \times 5.75), 0.10 per cent fat, 0.10 per cent dietary fiber and 0.08 per cent ash. Total carbohydrates, obtained by difference, were 87.37 per cent.

Reagents used in this study were analytical grade: ECH (The Dow Chemical Company, Midland, MI, USA), ethyl alcohol, acetone, Na_2HPO_4 , NaH_2PO_4 , NaOH, HCl and a TDF-100A kit from Sigma-Aldrich, Inc (St. Louis, MO, USA).

Experimental design

A full factorial design with two factors at two levels and two or three replicates per condition was performed (Montgomery and Runger, 2014). This design enabled the evaluation of the effects of ECH concentration and the reaction time on the production of RS and the pasting properties. The levels used were 0.15 and 0.30 per cent of ECH concentration and 4 and 8 h of reaction time. Experimental condition codes were: NS (native starch), 1CE (0.15 per cent ECH, 4 h), 2CE (0.30 per cent ECH, 4 h), 3CE (0.15 per cent ECH, 8 h) and 4CE (0.30 per cent ECH, 8 h). Temperature and pH were kept constant.

Preparation of cross-linked starch with epichlorohydrin

Food-grade-ECH-modified cassava starch was obtained by following the technique of Gall *et al.* (1975) with modifications.

A slurry was prepared by adding 100 parts (dry basis) of native cassava starch to 150 parts of distilled water. The pH was adjusted to 11.0 using 2 per cent NaOH solution. An alkaline solution (0.8 per cent of NaOH) of ECH (0.15 or 0.30 per cent w/w on a dry basis) was added into the starch slurry with constant agitation using a magnetic stirrer. The reaction was held for 4 or 8 h at $30 \pm 2^\circ\text{C}$. Then, the reaction was stopped by adjusting the pH to 6.5 with 0.5 N HCl solution. The suspension was filtered using a Buschner funnel with a Whatman filter paper No.1 and washed twice with distilled water (350 mL each time). Samples were dried in a natural convection air oven at $50 \pm 1^\circ\text{C}$ for 72 h. Subsequently, samples were milled, passed through a 100-mesh sieve (149- μm opening) and stored in sealed bags for further analysis. Two replicates were obtained for each condition.

Resistant starch determination as a total dietary fiber

RS content (as total dietary fiber) of the native and modified cassava starch samples was determined according to the standard procedure (AOAC, 1997) using the TDF-100A kit (Sigma-Aldrich, Inc., St. Louis, MO, USA).

Because of the low protein and ash contents present in the non-digestible residue, no corrections were done. This method estimates the total dietary fiber content using a combined enzymatic–gravimetric technique. The procedure was carried out by dispersing 1 g of starch sample in 50 mL of 0.08 M phosphate buffer at pH = 6.0 in a 250 mL flask. Then, 100 μL of thermostable α -amylase was added. The flask containing the dispersion was covered with an aluminum foil and then heated in a water bath at 95°C for 15 min with manual stirring every 5 min. After being cooled at room temperature, the pH of the dispersion was adjusted to 4.0–4.6 by adding 0.325 M HCl solution. Finally, 100 μL of amyloglucosidase solution was added.

The covered flask containing the dispersion was heated again in a water bath at 60°C for 30 min with constant agitation. Then, four volumes of 95 per cent (v/v) ethanol were added (approximately 200 mL) to precipitate soluble polysaccharides. Finally, each flask was stored overnight at room temperature.

The precipitate was filtered using a Celite bed (0.5 g) on a sintered glass crucible (porosity G-2). The insoluble residue was rinsed with solutions of 78 per cent (v/v) alcohol, 95 per cent (v/v) alcohol and 95 per cent (v/v) acetone. The crucible with the residue was dried overnight in a natural convection air oven at $105 \pm 2^\circ\text{C}$. RS determinations were performed by duplicate.

RS content was determined as the residue remaining after drying and is expressed as percentage of the starch on dry basis [equation (1)]:

$$RS (\%) = \frac{P_{residue}}{P_{sample}} \times 100 \quad (1)$$

where $P_{residue}$ is the weight of the residue (g) and P_{sample} is the weight of the sample (g).

Starch pasting properties

Pasting properties of the native and modified starch samples were determined using a Brabender micro visco-Amylograph (Model 803202, Brabender GmbH & Co. KG, Duisburg, Germany) equipped with the software Viscograph Data correlation (version 4.2.0).

Samples of the starch slurries underwent controlled heating and cooling cycles, and the pasting parameters were obtained from the amylograph curves, showing the starch gelatinization process in a simple way.

A suspension of 100 mL (7 per cent dry basis), based on the starch samples, was prepared in distilled water. The pH of the suspension was adjusted to 5.5-6.0 with solutions of 0.1 N HCl and 0.1 N NaOH while maintaining constant agitation using a magnetic stirrer. The suspension was transferred into the bowl of the equipment, and the rotor speed was fixed at 75 rpm. Heating and cooling rates were adjusted to 3°C/min, and a cartridge of 350 cmg sensitivity was used. The suspension was heated from 50 to 95°C. At 95°C, the sample was held constant for 10 min and was then cooled to 50°C and held at the same temperature for 10 min.

The following measurements were taken from the amylograph curve:

- A: pasting temperature (the gelatinization onset temperature) (°C);
- B: pasting peak viscosity or high viscosity in Brabender units (BU);
- C: viscosity at the start of the first maintenance period at 95°C (BU);
- D: viscosity at the beginning of the cooling period at 95°C (BU);
- E: viscosity at the end of the cooling period at 50°C (BU);
- F: viscosity at the end of the second holding period at 50°C (BU);
- B-D: stability of pasta during cooking (breakdown; BU) obtained by subtracting the value of D from B; and
- D-E: retrogradation tendency (setback; BU) obtained by subtracting the value of E from D.

Degree of cross-linking

The degree of cross-linking (DC) [equation (2)] was quantitatively estimated from the amylograph curves following the method described by Chatakanonda *et al.* (2000):

$$DC (\%) = \frac{B_{NS} - B_{DC}}{B_{NS}} \times 100 \quad (2)$$

where B_{NS} is the peak viscosity B of native starch (BU) and B_{DC} is the peak viscosity B of cross-linked starch (BU).

Statistical analysis

Resistant starch content, the starch pasting properties and the DC were analyzed by using ANOVA, and, when the effect of the factors was significant ($p < 0.05$), the Tukey's honestly significant difference multiple ranks test was applied (confidence level of 95 per cent) (Montgomery and Runger, 2013). All statistical analysis was performed using the commercial statistics software Statgraphics Centurion XV (v. 2.15.06) (Statpoint Technologies, Inc., Warrenton, VA, USA).

Results and discussion*Resistant starch determination by the method of total dietary fiber*

Table I shows the RS content of native and modified cassava starches. The results show that the different treatments did not produce significant changes ($p < 0.05$) in the RS level compared with native starch.

Woo and Seib (2002) showed similar results by using 0.3 per cent of ECH and a reaction time of 1 h at pH 11.5 and 25°C. However, these authors found that when ECH concentrations were higher than values allowed in food, that is 1 and 2 per cent, the levels of RS in the modified wheat starch increased to 53 and 76 per cent, respectively. Aćkar *et al.* (2010) observed that the use of ECH concentrations of 0.1 and 0.3 per cent increased the digestibility of wheat starch (variety Golubica), whereas 0.5 per cent slightly decreased its digestibility. Nevertheless, in the variety Srpanjka, the use of 0.3 and 0.5 per cent of ECH caused an increase in the RS content.

The enzymatic degradation of cross-linking starch by α -amylase is influenced by the structural characteristics of the hydrogel network (Hamdi and Ponchel, 1999). Unlike other cross-linking agents (i.e. STMP and POCl₃), ECH is not a highly efficient cross-linking agent. Some researchers reported that ECH is a weak water-soluble, slow-acting compound, and, also, cross-linking is not homogeneous and predominate in the non-crystalline domain (Shiftan *et al.*, 2000; Singh *et al.*, 2007). The use of ECH at low levels approved for food-grade modified starch could result in structural changes that do not have a significant effect on *in vitro* digestibility, as reported by Aćkar *et al.* (2010).

Sample ^a	RS ^b (%)
NS	2.6 ± 0.04*
1CEa	2.7 ± 0.12*
2CEa	2.8 ± 0.01*
3CEa	2.8 ± 0.10*
4CEa	2.9 ± 0.03*
1CEb	2.5 ± 0.03*
2CEb	2.5 ± 0.07*
3CEb	2.6 ± 0.15*
4CEb	2.5 ± 0.05*

Table I.
Resistant starch
content of native and
modified-with-ECH
cassava starches

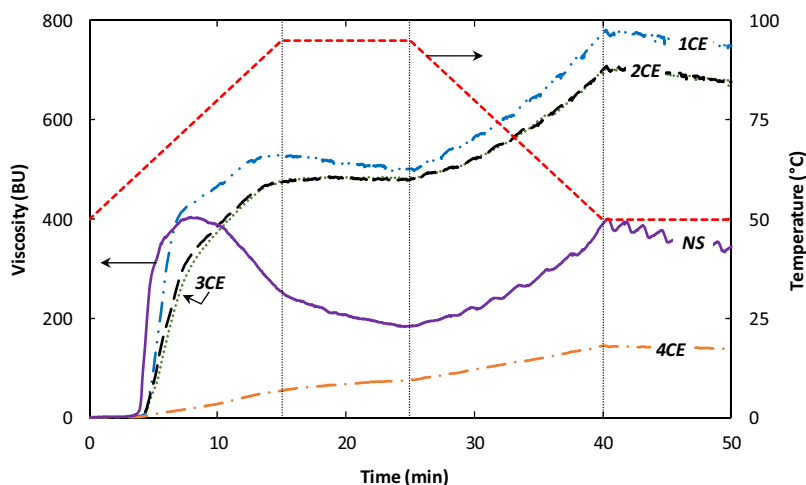
Notes: ^a Letters a and b correspond to repetitions of each treatment; NS = native starch; ICE: 0.15% ECH, 4 h; 2CE = 0.30% ECH, 4 h; 3CE = 0.15% ECH, 8 h; 4CE = 0.30% ECH, 8 h; ^b Values are means ± standard deviation of three replicates; * All values are not statistically different ($p < 0.05$)

Degree of cross-linking and pasting properties

Amylograms corresponding to the native and modified cassava starch are presented in Figure 1, and the representative points in the amylograms and the DC are shown in Table II.

Table II shows that treatments produced a significant increase in pasting temperatures of modified starches compared to the native samples. Similar results were found by Aćkar *et al.* (2010). The cross-linking process adds intra- and intermolecular bonds that stabilize the granule structure (Acquarone and Rao, 2003). However, these differences were not observed among the cross-linked samples. The factorial analysis also indicated that none of the individual factors (i.e. ECH concentration and reaction time) or their interactions produced significant differences on the pasting temperatures (Parameter A). This negligible incidence on pasting temperatures could be due to the short range of ECH concentration and reaction time used in this work.

The peak viscosity (Parameter B) indicates the highest viscosity obtained by the starch during gelatinization. Starches with high B have a high swelling power. The shape of the curves reflects the ability of the granules to swell freely before physical disintegration (Singh *et al.*, 2007). Treatments influenced significantly the values of B compared to native samples. Increments in B were found for conditions 1CE, 3CE and 2CE, where no formation of cross-linking (DC) was detected. A decrease in B was found for condition 4CE, where the value of DC was significantly higher than the values found in rest of conditions. These results are consistent with findings reported by other authors who showed that the DC influences the peak viscosity (Aćkar *et al.*, 2010; Hirsch and Kokini, 2002; Jyothi *et al.*, 2006). Also, low degrees of cross-linking caused an increase in B because of the ability of granules to swell without disintegrating. However, high levels of DC lead to a significant reduction in B because of a decrease in the swelling



Notes: NS = native starch; 1CE = starch treated with 0.15 per cent of ECH for 4 h; 2CE = starch treated with 0.30 per cent of ECH for 4 h; 3CE = starch treated with 0.15 per cent of ECH for 8 h; 4CE = starch treated with 0.15 per cent of ECH for 8 h; dashed line represents the temperature profile; BU = Brabender units

Figure 1. Amylograms of the cassava starch samples studied

Parameter	Sample				
	NS	1CE	2CE	3CE	4CE
A (°C)	62.3 ± 0.1 ^a	64.8 ± 0.7 ^{ab}	65.5 ± 0.9 ^{ab}	64.7 ± 2.1 ^{ab}	67.6 ± 1.5 ^b
B (BU)	419.5 ± 14.5 ^a	529.0 ± 28.9 ^b	487.0 ± 6.4 ^b	486.0 ± 9.9 ^b	75.0 ± 9.0 ^c
C (BU)	258.0 ± 3.0 ^a	526.0 ± 27.6 ^b	475.0 ± 9.2 ^c	474.0 ± 21.2 ^c	53.5 ± 8.5 ^d
D (BU)	186.5 ± 2.5 ^a	502.0 ± 33.2 ^b	484.0 ± 3.5 ^b	479.0 ± 8.5 ^b	75.5 ± 9.5 ^c
E (BU)	394.0 ± 3.0 ^a	775.0 ± 43.1 ^b	700.0 ± 17.7 ^c	700.0 ± 36.8 ^c	144.5 ± 13.5 ^d
F (BU)	345.5 ± 1.5 ^a	750.0 ± 37.5 ^b	676.0 ± 15.6 ^c	677.0 ± 36.1 ^c	139.5 ± 12.5 ^d
B-D (BU)	233.0 ± 12.0 ^a	28.0 ± 4.24 ^b	4.0 ± 2.83 ^c	7.0 ± 1.41 ^c	–
E-D (BU)	207.5 ± 0.5 ^a	274.0 ± 9.9 ^b	216.0 ± 14.1 ^a	221.0 ± 28.3 ^a	69.0 ± 4.0 ^c
DC (%)	–	ND	ND	ND	82.12

Notes: NS = native starch; 1CE = 0.15% ECH, 4 h; 2CE = 0.30% ECH, 4 h; 3CE = 0.15% ECH, 8 h; 4CE = 0.30% ECH, 8 h; BU = Brabender units; A = pasting temperature; B = peak viscosity; C = viscosity at the start of the first maintenance period at 95 °C; D = viscosity at the beginning of the cooling period at 95 °C; E = viscosity at the end of the cooling period at 50 °C; F = viscosity at the end of the second maintenance period at 50 °C; B-D = stability of the pasting during baking; E-D = retrogradation; DC = estimated % of cross-linking degree; ND = not detectable; values indicate the mean ± standard deviation of two replicates; values within the same row with different superscripts (a-d) are significantly different ($p < 0.05$)

Table II.
Pasting properties of native and modified-with-ECH cassava starches

power of the starch granules (Jyothi *et al.*, 2006; Kartha and Srivastava, 1985; Kurakake *et al.*, 2009). These results indicate that cassava starch may be used as viscosity agent in food products mainly because its thickening properties may be modified and managed by the DC and the extension of swelling.

Table II shows that an increase in reaction time, ECH concentration and their interactions produced a reduction in the values of B. This reduction may be due to the fact that ECH is a slow-acting cross-linking agent that penetrates into the granule and is distributed throughout the granule volume (Hirsch and Kokini, 2002). The effect of ECH on the cross-linking formation is benefited by increasing its concentration and reaction time, which is reflected in a decreased peak paste viscosity. Jyothi *et al.* (2006), using 0.75 and 1.0 per cent of ECH concentrations, also observed a decrease in peak paste viscosity of cassava starch. Also, similar results have been reported by Hirsch and Kokini (2002) using waxy maize starch and by Ačkar *et al.* (2010) using wheat starch. As a result of the cross-linking process, the swelling potential of starch granules is reduced (Singh *et al.*, 2007) because the starch chains in the amorphous regions have less mobility and fewer interactions with water molecules (Gunaratne and Corke, 2007). It is important to mention that the greater the DC the smaller the granule volume, which reduces the contact among the granules, resulting in reduced viscosity values (Hirsch and Kokini, 2002).

In general, starches with high degree of swelling are also less resistant to physical disintegration during cooking, and, therefore, they exhibit a significant decrease in viscosity after reaching the maximum value (as shown in native starch profile in Figure 1; Adebowale and Lawal, 2003).

Each treatment did not produce significant variations ($p < 0.05$) in viscosity at the end of cooking at 95 °C (Parameter C), after the maintenance period at 95 °C (Parameter D), at the end of the cooling period at 50 °C (Parameter E) and at the end of the second maintenance period at 50 °C (Parameter F; Figure 1 and Table II). This result indicates

that treatments contributed to increase the physical strength of the granules, thereby maintaining invariable the paste viscosity during cooking. The presence of cross-links may prevent the rupture of the swollen granule and minimize or prevent loss in viscosity (Wurzburg, 1986b). The treatments had a significant influence on the paste stability during cooking (Parameters B-D), showing an increased stability of the paste with values of B-D much lower than those obtained for native starch. Similar results were reported in literature using cross-linked wheat starch and normal and waxy rice starch (Aćkar *et al.*, 2010).

Samples under treatment 1CE have shown lower paste stability than the other samples. The rest of the treatments did not show significant differences with each other. This invariability indicates that the formation of cross-links, even at low levels, provides good strength to the pastes during heating and stability to the paste when cooked and cooled at 50 °C because it prevents disintegration of the granules.

The increase in ECH concentration and reaction time significantly influenced the amylograms, resulting in a decrease in the values of Parameters C, D, E, F and B-D (Figure 1 and Table II).

The increase in viscosity during the cooling period (Parameter E-D) can reflect the tendency of the starch to retrograde or to re-associate when the temperature of the paste decreases (Luo *et al.*, 2009).

The 1CE and 4CE treatments have shown a significant influence on the retrograde starch paste ($p < 0.05$) in comparison to the native samples. The first treatment produced an increase in the tendency of pastes to retrograde, whereas the second treatment caused a decrease in the retrograding tendency of samples. This effect is probably related to the peak viscosity and the DC because of their tendency to produce arrangements in the structure of the paste.

Xiao *et al.* (2011) found that low levels of cross-linking resulted in an increased retrogradation of corn and rice starches treated with 0.3 per cent of ECH, whereas high degrees of cross-linking resulted in a decrease in potato starch retrogradation.

The rest of the treatments presented no significant differences ($p < 0.05$) with respect to the native samples. Factorial analysis has shown that the reaction time, the ECH concentration and their interactions significantly influenced the studied parameters, resulting in a decrease in the retrogradation phenomenon as their levels increased. Similar results were found by Aćkar *et al.* (2010). Also, Kurakake *et al.* (2009) reported less setback in cross-linking waxy corn starch compared to native starch.

The general changes caused on starch samples by the treatments used in this work showed that cassava starches, that underwent a cross-linking process, could be used in industrial processes where minimum changes in viscosity during high-temperature treatments and in texture and appearance caused by retrogradation during storage are needed.

Conclusions

The influence of ECH concentration and reaction time on the production of food-grade RS and pasting properties using native cassava starch of Misiones-Argentina origin was studied. ECH at concentrations approved by local and regional legislation and reaction times of 4 and 8 h did not affect the *in vitro* digestibility of cassava starch evaluated by the formation of RS as a total dietary fiber.

The pasting properties of the starch samples were affected by ECH concentration and reaction time. All treatments produced an increment in the pasting temperature of cassava starch.

The increase in the concentration of ECH and the reaction time produced an increase in cross-linking, resulting in a decrease in the viscosity peak, an increment of the granule resistance to physical disintegration and less retrogradation of the paste. Also, low levels of cross-linking increased the peak viscosity and retrogradation.

Although these conditions were not able to affect *in vitro* digestibility of cassava starch, pasting properties were changed, making it suitable for various uses.

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