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Effect of added calcium hydroxide during corn nixtamalization on acrylamide content in tortilla chips

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A B S T R A C T

This study has analyzed the influence of lime ($Ca(OH)_2$) concentration used during nixtamalization process on the physicochemical properties of nixtamalized corn flours and its effect on the acrylamide content in tortilla chips prepared from them. Flours prepared from nixtamalized corn with 0.5 and 1.0 g/100 g of $Ca(OH)_2$ showed higher levels of insoluble fiber, lower concentrations of calcium and ash, and a pH close to neutral. The acrylamide content of tortilla chips was correlated with the physicochemical properties of the flours. The results showed that acrylamide content was mainly affected by the ash, calcium, soluble fiber concentration in flour, as well pH value ($p < 0.05$). In comparison with tortilla chips made of flours prepared from nixtamalized corn with lime concentration of 1.0 g/100 g, a reduction of 52 and 36% in acrylamide content in tortilla chips made with flours prepared from nixtamalized corn with lime concentrations of 1.5 and 2.0 g/100 g, was obtained. The results suggest that controlling the concentration of $Ca(OH)_2$ during nixtamalization process can be used as an effective strategy for reducing acrylamide formation in fried products produced from nixtamalized corn flour.

1. Introduction

The products made of nixtamalized corn flours like tamales, tostadas, tortilla and tortilla chips are widely consumed in Mexico and Guatemala and by people of Hispanic origin living in the United States (Méndez-Montealvo, García-Suárez, Paredes- López, & Bello-Pérez, 2008). The tortilla chips have the second place of consumption of salty snacks, behind only potato products. In 2010, the market of tortilla chips represented the first place in terms of sales volume in the area of sweet and savory snacks in the United States, with a value of US$ 6295 million (Market Indicator Report, 2011).

Nixtamalization is a process developed by the Mayans and Aztecs to convert corn into tortilla. It consists in the alkaline cooking of corn grains in a calcium hydroxide solution (0.3–2.0 g/100 g), followed by a steeping period of 14–16 h. After the steeping, the cooking liquor or nejayote is decanted, and the grain is slightly washed prior to milling to obtain the dough to make tortillas. This process is responsible for important physicochemical, nutritional and sensory characteristics of nixtamalized corn products (Pappa, de Palomo, & Bressani, 2010). The main objective for using of $Ca(OH)_2$ in nixtamalization process is the husking of corn grain to facilitate the milling as well as other effects, such as the increasing of niacin bioavailability and the calcium concentration, the reducing of dietary fiber, the loss of vitamins and carotenoids and decreasing of aflatoxins content (Braham & Bressani, 1966; Bressani, Paz y Paz, & Scrimshaw, 1958; Edwin et al., 2003; Serna-Saldivar, Rooney, & Greene, 1991, 1992).

On the other hand, the acrylamide, a neurotoxic compound (Spencer & Schaumburg, 1974) and possible human carcinogen (IARC, 1994), has attracted the attention of the scientific community because it have been found in high concentrations in thermally processed foods (Tareke, Rydberg, Karlsson, Eriksson, & Tornqvist, 2002). Its formation is closely related to Maillard reaction, and specifically with the presence of carbonyl compounds with groups

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capable of forming a Schiff Base with the asparagine amino acid (Hidalgo, Delgado, & Zamora, 2009; Mottram, Wedzicha, & Dobson, 2002; Stadler et al., 2002).

In addition to investigations about the mechanisms responsible for the formation of acrylamide in foods, various chemical agents have been tested for their effectiveness as food additives in reducing the formation of acrylamide on thermally processed foods. Thus, asparaginase, acids, divalent cations, phospholipids, lecithin, hydrocolloids, antioxidants, and amino acids, among other additives, have been proposed as effective mitigation agents in model systems and real foods (Anese, Quarta, & Frias, 2011; Kalita & Jayanty, 2012; Salazar, Arámbula-Villa, Hidalgo, & Zamora, 2012; Zamora, Delgado, & Hidalgo, 2011).

Among the above mentioned additives, the use of divalent cations has been reported as an easy, cheap and feasible approach for reducing acrylamide in foods (Tomoda, Hanaoka, Yasuda, Takayama, & Hiwatashi, 2004). In this context, the calcium content in corn grains increases along nixtamalization process, suggesting that its presence affects directly the acrylamide content generated during the frying of foods prepared from nixtamalized corn. Based on the health risks involved in the consumption of acrylamide, the development of alternatives aimed at reducing the levels of this compound in foods that are consumed by a large segment of the population are required. Thus, this study analyses the impact of the amount of lime ($Ca(OH)_2$) used in the corn grain nixtamalization, on acrylamide formation in tortilla chips.

2. Materials and methods

2.1. Materials

Labeled [2,2,3-$^2H_3$]acrylamide was purchase from Sigma–Aldrich (St. Louis, MO). All other chemicals were analytical grade and purchased from Sigma (St. Louis, MO) or Merck (Darmstadt, Germany). Soybean oil was obtained from local supermarkets in Querétaro, México.

2.2. Elaboration of nixtamalized corn flour and tortilla chips

Nixtamalized corn flour (NFC) was prepared with commercial corn variety named Pioneer 30P16 and commercial lime ($Ca(OH)_2$) (El Topo, Monterrey, N.L. Mexico), commonly used in the tortilla industry. This flour was prepared by cooking (8 kg) of whole corn kernels in a solution of 16 L of water with 40, 80, 120 and 160 g of $Ca(OH)_2$, corresponding to 0.5, 1.0, 1.5 and 2.0 g/100 g of lime relative to the corn weight used. The corn was boiled in an aluminum pan for 25 min and steeped for 16 h at room temperature (22 ± 1 °C). The steep liquor was removed. The cooked corn was washed with 16 L of water, then ground into corn dough (FUMASA, M100, Querétaro, Mexico), and finally dehydrated using a flash type dryer (Cinvestav-GAV, M2000, Querétaro, Mexico). The drying conditions were adjusted to have 250 °C inlet air temperature and 90 °C to the exhaust air to avoid burning the material. Before storage, the nixtamalized corn flour was milled using a hammer mill (PULVEX 200, México, D.F.) equipped with a 0.5 mm screen.

For tortilla chips elaboration, nixtamalized corn flour was rehydrated with enough water to provide fresh dough with proper consistency to make tortillas. The dough was shaped into thin disks (11 cm diameter and 1.0 mm thickness) using a commercial tortilla roll machine (Casa Herrera, México, D.F.). The dough shaped into tortillas were cooked on both sides for around 1.0 min by using an iron hot plate (270 ± 10 °C) named "comal". The resulting tortillas were cut into circular pieces with an average area of 10 cm^2. Tortilla pieces were fried in soybean oil at 180 °C for 30 and 45 s. The 30 s time was used because in preliminary tests, this time was found as the best time to produce tortilla chips. A frying time of 45 s was also assayed to analyze the effect of extended frying times on acrylamide formation. After frying, tortilla chips were cooled on a paper towel to remove superficial oil and the color, breaking force, oil absorption and acrylamide content determined.

2.3. Proximate analysis

The experimental nixtamalized corn flours were analyzed in triplicate, and the protein content, total fat, ash and pH values were determined using standard methods of the American Association of Cereal Chemists (AACC, 1997). Dietary fiber and calcium content were evaluated by the 991.43 and 985.35 methods of AOAC (1997), respectively.

2.4. Color determination in tortilla chip samples

Color changes were determined using a colorimeter MiniScan XE, model 45/0-L (Hunter Associates Laboratory, 11491 Sunset Hill Rd., Reston, Va., U.S.A.). Total color differences (ΔE) at the different periods of time were calculated from the determined CIELAB L^* a^* b^* values according to Hunter (1973): $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$; where L^* = brightness or lightness (100 = perfect white, to 0 = black); a^* = greenness/redness [negative (green) to positive (red)]; b^* = yellowness/blueness [negative (blue) to positive (yellow)]; ΔL^*, Δa^*, and Δb^* = absolute differences of the values between the reference tile (white porcelain) and sample values; ΔE = total difference between reference and sample color. The reference values (calibration) were: $L^* = 92.22$, $a^* = -0.82$ and $b^* = 0.62$.

2.5. Texture determination in tortilla chips

The fracture force of the tortilla chips was evaluated using the Texture Analyzer TA-XT2 (Texture Technologies Corp., N. Y.). Fracture force was evaluated in freshly prepared samples. The test was carried out using a 2.03 mm diameter stainless-steel probe and a platform accessory with a hollow cylindrical base with 33.5 and 10 mm external and internal diameters, respectively. The probe traveled at a velocity of 10 mm/s until it cracked the sample (distance 6 mm).

2.6. Acrylamide determination in tortilla chips

Acrylamide was analyzed as the stable 2-bromopropenamide derivative by gas chromatography–mass spectrometry (GC–MS) using a combination of the methods of Andrawes, Greenhouse, and Draney (1987) and Castle, Campos, and Gilbert (1991) as described previously (Salazar, Arámbula-Villa, Hidalgo, & Zamora, 2012). Tortilla chips were ground in a mortar lab and powered samples (~1.0 g) were successively weighed in centrifugal tubes, spiked with 20 L of internal standard solution (0.5 mg/mL of deuterium-labeled [2,2,3-$^2H_3$]acrylamide in acetonitrile), and stirred with 8 mL of distilled water and 10 mL of *n*-hexane at room temperature for 5 min. After centrifugation at 2000 x *g* for 10 min, organic phases were removed, and supernatants filtered. These extracts (3 mL) were treated with 0.45 g of potassium bromide, 200 L of sulfuric acid (10 mL/100 mL), and 300 μL of potassium bromated solution (0.1 mol/L). After 1 h in the dark at 4 °C, the bromination reaction was terminated by adding of 1 mol/L sodium thiosulfate until solutions became colorless, and solutions were extracted with 5 mL of ethyl acetate/hexane (4:1). Organic layers were recovered after centrifugation at 2000 × *g* for 10 min, and were dried with sodium sulfate and evaporated to dryness under nitrogen. Each sample was dissolved in 50 μL of ethyl acetate, treated with 25 μL of triethylamine, and analyzed by GC–MS. The ions monitored for the identification of the analyte, 2- bromopropenamide, were $[C_3H_4NO]^+ = 70$, $[C_3H_4^{79}BrNO]^+ = 149$, and $[C_3H_4^{81}BrNO]^+ = 151$, using m/z 149 for quantification. The ions monitored for the identification of the corresponding derivative 2-bromo[$^2H_2$]propenamide were $[C_3^2H_2H^{81}Br]^+ = 110$ and $[C_3^2H_2H_2^{81}BrNO]^+ = 153$, using m/z 153 for quantification.

GC–MS analyses were conducted with a Perkin Elmer GC Clarus 500 coupled with a Perkin Elmer Clarus 560 MSD (Mass Selective Detector-Quadrupole type). In most experiments, a 30 m × 0.32 mm i.d. × 0.25 μm Elite-5MS capillary column was used. Working conditions were as follows: carrier gas helium (1 mL/min at constant flow); injector, 250 °C; oven temperature: from 50 (1 min) to 240 °C at 5 °C/min and then to 300 °C at 10 °C/min; transfer line to MSD, 280 °C; ionization EI, 70 eV.

Quantification of acrylamide was carried out by preparing standard curves of this compound. Acrylamide content was directly proportional to the acrylamide/internal standard area ratio ($r = 0.999$, $p < 0.0001$). The coefficients of variation at the different concentrations were lower than 10%.

2.7. Statistical analysis

All results were expressed as mean ± SD values ($n = 3$). Statistical comparisons between two groups were made using Student's t-test. With several groups, one-way analysis of variance was used. When significant F values were obtained, group differences were evaluated by the Tukey test. All statistical procedures were carried out using the JMP 9.0 package (SAS Institute Inc., Cary, NC). The significance level was $p < 0.05$ unless otherwise indicated.

3. Results

3.1. Effect of calcium hydroxide on physicochemical properties of nixtamalized corn flour

Table 1 shows significant variations in the nutritional composition of the flours prepared from nixtamalized corn with different addition levels of $Ca(OH)_2$. Protein and fat content in flours were similar ($p < 0.05$). These results were in accordance to those reported previously for commercial and ecological nixtamalized corn flours (Campechano-Carrera et al., 2012; Palacios-Fonseca, Vázquez-Ramos, & Rodríguez-Garcia, 2009). Ash and calcium content were directly related to $Ca(OH)_2$ used ($r = 0.99$, $p < 0.05$) (Table 2). The ash content of the flours processed with 0.5 and 1.0 g/100 g $Ca(OH)_2$ ranged from 15.98 to 17.82 g/kg, whereas flours processed with 1.5 and 2.0 g/100 g had higher ash content (20.09 and 21.35 g/kg, respectively). It is well known that the addition of $Ca(OH)_2$ during nixtamalization process of corn grain increases the calcium content in the nixtamalized corn flour, compared to that found in raw corn (Bressani, Paz y Paz, & Scrimshaw, 1958; Serna-Saldivar et al., 1991; 1992). In this experiment, the flours produced from nixtamalized corn with 1.5 and 2.0 g/100 g $Ca(OH)_2$, registered high calcium content (2.67 and 3.18 g/kg, respectively). On the other hand, the total dietary fiber and insoluble dietary fiber were negatively correlated with the concentration of $Ca(OH)_2$ used in the corn grain nixtamalization (Table 2). The flours prepared from nixtamalized corn with 0.5 and 1.0 g/100 g $Ca(OH)_2$ showed higher total dietary fiber values (175.83–128.02 g/kg) in relation to that obtained from corn processed with 1.5 (116.02 g/kg) and 2.0 g/100 g (122.02 g/kg). Martínez-Flores, Martínez-Bustos, Figueroa and González-Hernández, 2002 and Campechano-Carrera et al., (2012) suggest that dietary fiber content in nixtamalized corn flours can be increased if pericarp retention is achieved during nixtamalization process. According to this, high levels of insoluble dietary fiber in flour produced from nixtamalized corn with 0.5 g/100 g $Ca(OH)_2$ were obtained (135.59 g/kg) as a consequence of a higher pericarp retention.

3.2. Effect of calcium hydroxide on acrylamide content in tortilla chips

Fig. 1 shows acrylamide content in tortilla chips, related to calcium content in flours prepared from nixtamalized corn with different addition levels of $Ca(OH)_2$. All flours induced the formation of acrylamide in the tortilla chips, but tortilla chips made of flours prepared from nixtamalized corn with 1.0 g/100 g $Ca(OH)_2$ always contained more acrylamide ($p < 0.05$) than those made of flours prepared from corn processed with higher levels of lime. These results were obtained with independence of the frying time. The only significant differences found were the amount of acrylamide produced (which increased with the frying time, as expected) and the percentage of acrylamide mitigation observed by the calcium content of flour (which decreased when the frying time increased). For samples fried for 30 s a significant reduction of 22, 53 and 36% on acrylamide content was obtained (flours prepared from nixtamalized corn with 0.5, 1.5 and 2.0 g/100 g $Ca(OH)_2$, respectively). While, samples fried for 45 s a significant reduction of 24 and 21% on acrylamide content was obtained (flours prepared from nixtamalized corn with 1.5 and 2.0 g/100 g $Ca(OH)_2$, respectively). No significant differences were obtained between tortilla chips fried 45 s and made of flours prepared from nixtamalized corn with 0.5 and 1.0 g/100 g $Ca(OH)_2$.

As the calcium concentration increased, higher pH values were registered in flours (Table 1). Thus, tortillas chips made of flours prepared from nixtamalized corn with $Ca(OH)_2$ concentrations of 1.5 and 2.0 g/100 g, presented the lower acrylamide levels. Besides, acrylamide content was negatively correlated with soluble dietary

Table 1
Chemical composition[a] of flours prepared from nixtamalized corn with different concentrations of lime ($Ca(OH)_2$).

Parameter (g/kg)	$Ca(OH)_2$ (g/100 g)			
	0.5	1.0	1.5	2.0
Ash	15.98 ± 0.12 a	17.82 ± 0.11 b	20.09 ± 0.15 c	21.35 ± 0.01 d
Fat	41.42 ± 1.17 a	44.97 ± 2.34 a	43.67 ± 2.46 a	43.78 ± 1.43 a
Protein	84.44 ± 1.20 a	83.15 ± 1.44 a	80.86 ± 3.62 a	83.14 ± 0.77 a
Total dietary fiber	175.83 ± 2.64 a	128.02 ± 4.13 b	116.02 ± 1.97 c	122.02 ± 1.94 bc
Insoluble dietary fiber	135.59 ± 1.55 a	97.11 ± 1.03 b	71.62 ± 3.85 c	71.92 ± 6.12 c
Soluble dietary fiber	40.23 ± 4.19 b	30.91 ± 3.10 c	45.03 ± 2.38 ab	50.11 ± 4.21 a
Calcium	0.87 ± 0.01 a	1.55 ± 0.02 b	2.67 ± 0.04 c	3.18 ± 0.03 d
pH (pH units)	6.39 ± 0.01 a	7.18 ± 0.06 b	8.50 ± 0.05 c	8.71 ± 0.01 d

Mean ± standard deviation. Means values followed of different letter, in the same row, are significantly different (Tukey, $p < 0.05$).
[a] Dry basis.

Table 2
Correlation coefficients[a] between lime ($Ca(OH)_2$) used in corn nixtamalization process, chemical composition of flours and acrylamide content in tortilla chips.

Parameter	L	F	P	TDF	IDF	SDF	A	pH	Ca	AT30s
F	ns									
P	ns	ns								
TDF	−0.81	−0.51	0.47							
IDF	−0.92	−0.46	0.48	0.96						
SDF	0.64	ns	ns	ns	−0.47					
A	0.99	ns	ns	−0.84	−0.94	0.64				
pH	0.97	ns	−0.44	−0.86	−0.96	0.64	0.99			
Ca	0.99	ns	ns	−0.82	−0.94	0.67	1.00	0.99		
AT30s	−0.56	ns	ns	ns	0.50	−0.77	−0.60	−0.67	−0.65	
AT45s	−0.53	ns	ns	ns	0.47	−0.78	−0.58	−0.60	−0.59	0.71

L = lime amount; F = fat; P = protein; TDF = total dietary fiber; IDF = insoluble dietary fiber; SDF = soluble dietary fiber; A = ash; pH = pH value; Ca = calcium content; AT30s = acrylamide content in tortilla chips fried 30s; AT45s = acrylamide content in tortilla chips fried 45s.
[a] Significant at $p < 0.05$; ns = no-significant.

fiber (Table 2). Soluble fiber mainly is composed of pectin, gum and fructoligosaccharides. These compounds have been suggested as ingredients capable of inhibiting the formation of acrylamide in food, and model systems (Zeng et al., 2010).

3.3. *Effect of calcium hydroxide on oil absorption, texture and color in tortilla chips*

Oil absorption, texture and color are considered the most important parameters of quality and acceptability of fried products. Fig. 2 shows the effect of $Ca(OH)_2$ concentration used in the corn nixtamalization and the frying time on the parameters of tortilla chips above mentioned. Regardless of the frying time, the tortilla chips with higher oil absorption (Fig. 2A) showed a lower fracture force (Fig. 2B), but no important changes were observed between treatments. The oil absorption values and fracture force obtained were similar to those described previously for commercial tortilla chips (Lujan-Acosta & Moreira, (1997). The color has been correlated with the acrylamide generation in thermally processed foods (Gökmen & Şenyuva, 2006; Lukac et al., 2007; Majcher & Jelen, 2007; Pedreschi et al., 2007). In this study, regardless of processing time, the acrylamide reduction was not related to a change in the appearance of the tortilla chips. In fact, tortilla chips made of flours prepared from nixtamalized corn with 1.0, 1.5 and 2.0 g/100 g $Ca(OH)_2$ did not show any change in color. Nevertheless, the highest change in color (Fig. 2C) corresponded to tortilla chips made of flours prepared from nixtamalized corn with 0.5 g/100 g $Ca(OH)_2$. This result can be explained based on the high insoluble fiber content in the flour. According to Palermo, Fiore, and Fogliano (2012), the fiber is capable of reduce the availability of water during frying process and thereby accelerate the Maillard reaction.

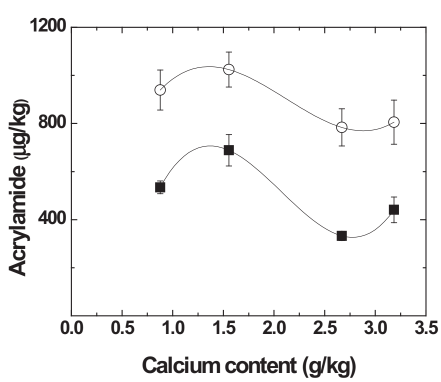

Fig. 1. Effect of calcium content in acrylamide levels of tortilla chip fried 30 (■) and 45 (○) seconds, prepared from nixtamalized corn flours.

4. Discussion

Many studies have tried to obtain processed food products with minimal sensorial changes and reduced acrylamide content. Gökmen and Senyuva (2007b) and Açar, Pollio, Di Monaco, Fogliano, and Gökmen (2012) applied successfully calcium chloride to reach acrylamide mitigation in potato strips and cookies. The results confirmed that divalent cations were capable of inhibiting the formation of acrylamide. Despite the promising efficiency of calcium in acrylamide reduction strategy in food, trials at large-scale have given variable results, strongly affecting the product quality, because, for example, the calcium hindered the rise of sweet biscuits and the products were unpalatable when tasted. For that reason, these approaches have not been commercialized yet (Kukurova, Ciesarova, Bednarikova, & Markova, 2009). The above mentioned is not a drawback for tortilla chips, because $Ca(OH)_2$ is used in large quantities during corn nixtamalization and represents an important factor in color, odor, flavor, shelf life and texture characteristics of all nixtamalized products. If the $Ca(OH)_2$ content is not sufficient to give the characteristic alkaline flavor, the tortillas

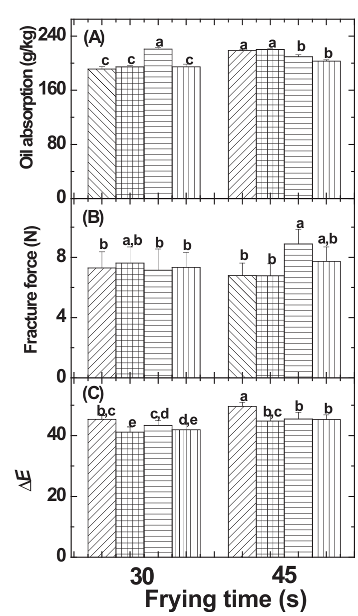

Fig. 2. Effect of added $Ca(OH)_2$ during corn nixtamalization process (0.5 g/100 g (diagonal striped bars), 1.0 g/100 g (crosshatched bars), 1.5 g/100 g (horizontal striped bars), 2.0 g/100 g (vertical striped bars)) on: oil absorption (A), fracture force (B), and color (C), in tortilla chips fried at 180 °C for 30 and 45 s. Bars with different letters, in the same panel, are significantly different (Tukey, $p < 0.05$).

and tortilla-based products are rejected by consumers (Méndez-Albores, Martínez-Morquecho, Moreno-Martínez, & Vázquez-Durán, 2012).

The flour composition influences on the acrylamide content in tortilla chips. The results obtained pointed out that the amount of calcium, soluble dietary fiber and pH value will determine the final amount of acrylamide. On one hand, an increase of calcium content in flours will decrease acrylamide content because the Schiff Base responsible for acrylamide formation will be inhibited. Calcium cation is able to prevent the formation of Schiff base, which is one of the key intermediate leading to acrylamide (Gökmen & Senyuva, 2007a). On the other hand, a decrease of calcium content in flours will increase the acrylamide and the insoluble fiber content, and will also produce a neutral pH value. Acrylamide formation occurs in an optimum pH values between 6 and 8 (Mestdagh et al., 2008; Rydberg et al., 2003), where the conversion of 3-aminopropionamide to acrylamide is favored (Zamora, Delgado, & Hidalgo, 2009).

Previous studies have reported significantly reduction in tortilla chips using a piquin pepper oleoresin (77%) and amaranth protein isolate (51%) (Salazar, Arámbula-Villa, Hidalgo, & Zamora, 2012; Salazar, Arámbula-Villa, Vázquez-Landaverde, Hidalgo, & Zamora, 2012). The acrylamide mitigation observed in tortilla chips under the employed conditions was more than acceptable (21–53%). However, the combination of $Ca(OH)_2$ addition during nixtamalization with other suggested pretreatments, such as the addition of asparaginase or proteins, or by the adjustment of heating conditions, as suggested in different studies (see, for example, Pedreschi, Kaack, & Granby, 2004; Kukurová, Morales, Bednáriková, and Ciesarová, 2009; Anese, Quarta & Frias, 2011, may produce further decreases in acrylamide content. Additional studies are needed to optimize the concentration of $Ca(OH)_2$ during nixtamalization in a way that in combination with these other treatments, the acrylamide content can be minimized.

Although a much more detailed sensory evaluation of tortilla chips prepared using different addition level of $Ca(OH)_2$ during nixtamalization is needed. Preliminary results showed that the products obtained were acceptable in terms of color, texture and oil absorption. Therefore, despite the lack of strategies for reducing acrylamide formation in products made from nixtamalized corn, variation of alkalinity during nixtamalization can be a simple and effective way to mitigate acrylamide formation during processing of tortilla chips and other maize-based foods thermally processed.

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