Factors influencing anthocyanin loss during nixtamalization of blue purple maize grain

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Revised: 9 October 2017 / Accepted: 15 October 2017 / Published online: 26 October 2017
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Abstract Blue purple maize grain loses a great amount of anthocyanins during the nixtamalization processing. The impact of the process factors on anthocyanins losses has not been studied in detail. The objectives of this work were to: (1) determine the step of the nixtamalization procedure where the greatest anthocyanin loss occurs and (2) study the effect of cooking time, alkali concentration, and sample size on anthocyanin losses (AL), and on the color of masa and tortilla from blue purple maize grain. Two cooking times were assayed (25 and 35 min), three alkali concentrations (0.5, 0.7, and 1.0% w/w), and two maize grain sample sizes (100 and 1000 g). Alkali concentration determines the time required to solubilize the maize grain pericarp, higher concentrations resulted in shorter maize grain pericarp solubilization times and this variable was related with AL. The greatest AL occurred during the grain cooking step, but an additional loss took place during the steeping of cooked grain. For the cooking time of 25 min, AL were of 38.3% during the cooking step, and 21.3%

during the steeping of cooked grain. The cooking time had no effect ($p > 0.05$) on AL in masa and tortilla whereas alkali concentration and sample size significantly affected it. The greater the concentration and size, the higher the AL. The color of masa and tortilla were affected by cooking time and alkali concentration. Increasing alkali concentration during the nixtamalization procedure, reduced the brightness and chroma of masa and tortilla and made these products seem darker and dull.

Keywords *Zea mays* · Anthocyanins · Nixtamalization · Color · Tortillas

Introduction

Consumers interest in products elaborated from non-conventional cereal grains, with high content of antioxidants has increased. Among the benefits attributed to an antioxidant-rich diet is the reduction of oxidative stress (Meyers et al. 2008), which is a cellular disturbance of the oxidant-pro-oxidant balance (favoring the former) that can lead to cellular damage (Kohen and Nyska 2002) and is linked to chronic degenerative diseases. Oxidative stress can be reduced or prevented by eating foods rich in antioxidants.

Colored cereal grains commonly have higher antioxidant capacity than non-colored grains, due to the presence of anthocyanins, which are potent antioxidants (Rice-Evans et al. 1996). Purple wheat grain has a higher antioxidant capacity than white grain (Liu et al. 2010) and bread elaborated with purple wheat varieties exhibited higher antioxidant capacity than that from white grain varieties (Li and Beta 2011).

Mexico is one of the main maize grain consumer for human purposes in the world. Its consumption per capita is

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107 k year⁻¹ (INEGI 2012) and around 90% are consumed as tortillas, which are made with white kernels that have less antioxidant content than colored kernels (De la Parra et al. 2007). An alternative to increase antioxidant consumption in the population would be to promote the consumption of colored tortilla, but at the same time, the antioxidant loss problem arising during the transformation of the kernel to tortilla (nixtamalization process) must be solved (Salinas et al. 2003; Lopez-Martinez et al. 2011).

Different factors of the nixtamalization process (time and cooking temperature, alkali concentration, and steeping time) on the masa and tortilla quality from white maize grain have been widely studied (Bedolla et al. 1983; Almeida-Domínguez et al. 1997; Sahai et al. 2001). However, similar information regarding these topics for blue purple grain, is limited. Works have mainly focused on evaluating the impact of traditional nixtamalization (Del Pozo-Insfran et al. 2006) or alternative methods such as extrusion (Zazueta-Morales et al. 2001), or fractionated nixtamalization (Cortés et al. 2006) on the masa and tortillas total anthocyanin content. The level of anthocyanin losses varies according to the nixtamalization method and the type of corn used, but reports show losses that range from 50 to > 70% (Zazueta-Morales et al. 2001; Salinas et al. 2003; Lopez-Martinez et al. 2011).

Factors such as the cooking time required to solubilize the pericarp of the maize grain during the nixtamalization process is considered as the end of the alkaline cooking time (Martínez-Herrera and Lachance 1979; Bedolla and Rooney 1982). However, in the nixtamalization of blue purple maize grain, it represents the time when the alkaline solution becomes in full contact with the aleurone layer, where the anthocyanins of the grain are concentrated (Salinas et al. 1999).

The effects of the factors participating in the traditional nixtamalization process on the anthocyanin stability have not been studied in detail, thus the objectives of this study were: (1) to evaluate the effect of the cooking time and the alkali concentration in: (a) the time required to solubilize kernel's pericarp, (b) anthocyanin content of masa and tortilla, (c) the color of masa and tortilla, and (2) to determine the loss of anthocyanins in the cooking and steeping stages of the blue purple maize grain.

Materials and methods

Vegetal material

A blue purple maize grain population belonging to Cónico race was used, which was grown in the region of Toluca, State of Mexico, with elevation 2600 m above sea level. This population has floury grain and a total anthocyanin

content of 643.4 mg equivalent of cyanidin 3-glucoside (CGE)/kg of dry sample.

Effect of the alkali concentration on the pericarp solubilization

One hundred grams of clean maize grain were placed in a 500 ml beaker with 200 ml of distilled water, calcium oxide (CaO) in concentrations of 0.5, 0.7, and 1.0% were added in relation to the kernel weight. Samples were placed on heating trays and taken to full boiling, which was reached at about 92 °C. At this point, the time required for the solubilization of the pericarp to begin was monitored. Samples were taken every minute, rubbing kernels between the thumb and the index fingers to observe the moment the pericarp was solubilized, according to Martínez-Herrera and Lachance (1979). A 1–3 classification scale was used, where: 1 = starts to solubilize when feeling slippery, 2 = pericarp slides off kernel using light friction with fingers, and 3 = solubilized pericarp.

Anthocyanins loss during cooking and steeping of blue purple maize grain

Samples of 100 g of clean blue purple maize grain were nixtamalized to evaluate the anthocyanin loss during the steps of cooking and steeping of cooked grain. Each sample was deposited in a 500 ml beaker; 200 ml of water were added plus CaO at 0.7% in weight regarding grain weight. The CaO concentration was selected considering the results of alkali concentration and time needed for pericarp solubilization. Cooking time was 25 min. The samples to quantify the anthocyanins were taken at the end of the cooking period and every hour during the steeping period until the fourth hour and at the end of the steeping period, which lasted 16 h. The temperature during the steeping of nixtamal was taken during the first 5 h, in order to relate the anthocyanin loss to this factor. In every case, a cooked grain (nixtamal) sample (20 g approximately) was rinsed with cold water to stop its cooking and to remove the excess of alkali. Samples were placed on paper towels to eliminate excess water, and then placed in aluminum boxes and dehydrated in the oven at 40 °C for 20 h. Later, the dried nixtamal was ground in a hammer mill with a 0.5 mm mesh to obtain the nixtamalized flour that was then placed in dark vials at – 20 °C until the anthocyanin content was analyzed. All nixtamalization and steeping treatments were carried out in duplicate.

Effect of batch size on anthocyanins loss during nixtamalization of blue purple grain

Batch size determines the speed at which the nixtamal cools down after the cooking and this could impact the AL. Therefore, an experiment was conducted in duplicate to determine the effect of the kernel sample size, during nixtamalization, on the loss of anthocyanins, using 100 and 1000 g grain samples. The alkali concentrations evaluated were 0.5, 0.7, and 1.0%; at a 2 to 1 water:grain relation and 25 min of nixtamalization time. The steeping period was 16 h. In the case of the 1000 g samples: the nixtamalization was carried out in 5 L pewter pots. Grains were cooked on a gas burner. During cooking, the pots were covered to reduce water loss due to evaporation. At the end of the steeping period, samples were rinsed with running water to eliminate excess lime and they were ground in a stone grinder to obtain masa. Portions of 10 g of masa of each treatment were dehydrated in a stove at 40 °C for 20 h, to be then ground in a hammer mill with a 0.5 mm mesh. The remaining masa was used to make tortillas, which were manually shaped and cooked on a steel tray.

Masa and tortilla color

The color was measured with a Hunter LabMiniScan XE Plus (Model 45/O-L) colorimeter, at a CIE L*a*b* scale with a D/65 brightener and a 10° angle. The following values were determined: L* (color brightness), a* and b* (chromaticity coordinates), and the Hue angle tone was calculated ($h = \arctan b^*/a^*$) as well as the Chroma color saturation index ($Cr = (a^2 + b^2)^{1/2}$). Masa and tortilla

from the nixtamalization size of 100 g were used for this determination.

Nixtamal, masa, and tortilla anthocyanin content

Nixtamal, masa, and tortilla anthocyanin content from each treatment was determined using the method described by Abdel-Aal and Hucl (1999), with modifications in the extraction solvent implemented by Moreno et al. (2005). This consisted of applying acidified methanol at 1% with trifluoroacetic acid (TFA) during the first extraction, and in the second, a mixture of methanol:acetic acid:water (10:1:9, v/v/v). A cyanidin 3-glucoside (Polyphenols, NW) standard curve was prepared to express total anthocyanin content in mg equivalent to cyanidin 3-glucoside (CGE)/kg of dry matter (DM). The anthocyanins loss for each product (nixtamal, masa and tortilla) was calculated taking into account the anthocyanin content in raw grain.

Statistical analysis

Data on the anthocyanin loss during the blue purple maize grain steeping were adjusted to a polynomial model. The results of the effect of cooking time and alkali concentration on anthocyanins were analyzed under a completely random model with a 2×3 factorial arrangement. Anthocyanin data from the two sample sizes used in the nixtamalization were analyzed under a completely random design and Tukey's mean comparison, $p = 0.05$, were carried out using the SAS system version 9.3 (SAS version 9.3 2011).

Table 1 Effect of the alkali concentration on the pericarp's solubilization in blue purple maize grain during the nixtamalization procedure

Alkali concentration (% w/w)	Cooking time (min)	Classification scale values ^a	Observations on pericarp
0.5	5	1	Starts to solubilize when feeling slippery
	10	2	Pericarp can slide off the kernel by slightly rubbing with the fingers
	15	3	Solubilized
	20	3	Solubilized
0.7	5	1	Starts to solubilize when feeling slippery
	10	2	Pericarp can slide off the kernel by slightly rubbing with the fingers
	15	3	Solubilized
	20	3	Solubilized
1.0	5	3	Solubilized
	10	3	Solubilized
	15	3	Solubilized
	20	3	Solubilized

^a1 = starts to solubilize when feeling slippery, 2 = pericarp slides off kernel using light friction with fingers, and 3 = solubilized pericarp

Results and discussion

Effect of the alkali concentration on the pericarp solubilization

The cooking time required for the pericarp to start solubilizing depended on the amount of alkali used. The shortest time for the pericarp's solubilization was 5 min of cooking with 1% of alkali concentration. In contrast, the treatment with 0.5% alkali, required 15 min (Table 1). These results agree with what was reported by González et al. (2004) for white maize grain pericarp, where the pericarp disintegration process was followed up by using FTIR (Fourier Transform Infrared Spectroscopy) methodology and confocal microscopy spectroscopy images.

Pericarp constitutes the blue purple maize grain anthocyanin protection against alkali during nixtamalization. With the lowest concentration used (0.5%), 15 min were needed for this structure to disintegrate while with the highest concentration (1.0%), only 5 min was required. Calcium oxide used as the alkali source, in contact with

water turns into calcium hydroxide, which solubility in water is very low and decreases when temperature increases (Guiomar et al. 1998; Zazueta et al. 2002). However, the pH is strongly alkaline, since for each $\text{Ca}(\text{OH})_2$ molecule that is dissociated, two OH^- ions are released into the medium, which confers calcium hydroxide as strong base characteristic (Halstead and Moore 1957).

The pH of the nejayote (alkali cooking water) at the end of the steeping period for 0.5, 0.7, and 1.0% alkali concentrations was 12.37 ± 0.02 , 13.06 ± 0.0 , and 13.24 ± 0.02 , respectively. These pH values are close to those determined at the beginning of the cooking period (12.45, 12.8, and 13.32), so even with the lowest alkali concentration used, the grain was exposed to strong alkaline conditions during cooking and steeping. Zazueta-Morales et al. (2001) used an alkali concentration of 0.12% and observed that the pH remained above 12 during the entire extrusion process. Small differences in the pH of the nejayote represent a large amount of OH^- ions in the solution (by the logarithmic scale of this variable) that the hemicellulose will hydrolyze, which constitutes the main component of the pericarp (57%) (González et al. 2004). These ions, due to the concentration gradient, will migrate to the interior of the maize grain. The migration speed of each ion will depend on its ionic charge and on the interactions established between said charge and grain components.

The accumulation kinetic of Ca^{++} ion during cooking maize grain step has been documented (Zazueta et al. 2002; Fernández-Muñoz et al. 2011). However, there are no studies of this kind with hydroxide ions. But, if the hydroxide ion migrates at the same speed as the calcium ion, then, from the first minutes of cooking, the grain anthocyanins concentrated in the aleurone layer would be degraded by alkaline pH.

Anthocyanin loss during the maize grain cooking and steeping stages

At the end of the 25-min cooking period of the blue purple maize grain, the nixtamal showed an anthocyanin content of 397.1 mg ECG/kg of MS (Fig. 1a). Based on the anthocyanin content in the raw kernel (643.4 ECG/kg), in this stage of the nixtamalization process the anthocyanin loss was 38.3%. The anthocyanin degradation during the cooking of blue purple maize grains was due to the highly alkaline pH of the cooking water in combination with the high temperature ($\sim 92^\circ\text{C}$). Of these two factors, the most detrimental for anthocyanins is the pH (Cortés et al. 2006).

The nixtamal cooled quickly during the first steeping hour, when its temperature dropped from 90 to 56°C .

Fig. 1 Nixtamal temperature and anthocyanin content (a) and anthocyanins losses of blue purple maize grain (b) during the steeping step of the nixtamalization process. ACNs:anthocyanins

Fig. 2 Effect of the cooking time and CaO concentration on the anthocyanin content in the dried masa from blue purple maize grain. The letters on the bars correspond to the Tukey means comparison ($p = 0.05$)

Later, the decrease was less pronounced, and after 4 h of steeping, it reached a temperature of 32.7 °C (Fig. 1a), and the anthocyanin content was 345.78 mg ECG/kg MS, hence the anthocyanin percentage loss during this steeping period was 12.9%. The nixtamal lost another 8.37% of anthocyanins, from the 4th to the 16th hour of steeping, to reach a final content of 316.82 mg ECG/kg of dry sample (Fig. 2b). The accumulated anthocyanins loss after this stage was approximately 60.0%. The loss of anthocyanins during the steeping of alkaline cooked grain fits well to a quadratic model.

Anthocyanins stability to temperature will depend on their chemical structure. Those containing an acyl radical in addition to the glucoside (acylated anthocyanin), will be more stable than those that only have the glucoside (non-acylated anthocyanin) (Castañeda-Ovando et al. 2009). Blue purple maize grain anthocyanins are mostly of the acylated type, with the malate radical as the most popular (Salinas-Moreno et al. 2012; Collison et al. 2015). However, during the nixtamalization process they degrade at the same or at an even higher rate, than the non-acylated type (Collison et al. 2015). There are no reports on anthocyanin degradation kinetics at a highly alkaline pH (11–13) and high temperature. Most works on degradation of these flavonoids by temperature have been carried out in fruits and vegetables, where pH is acid (Sadilova et al. 2006; Kara and Ercelebi 2013).

Effect of time and alkali concentration on the blue purple maize grain anthocyanins

At low and medium alkali concentration (0.5 and 0.7%), a significant effect ($p \leq 0.05$) of the nixtamalization time (cooking time) was observed on anthocyanin content. However, at high concentration (1%), no significant effect ($p > 0.05$) was detected (Fig. 2). With a cooking time of 25 min, the anthocyanin content of nixtamalized blue

Fig. 3 Effect of the sample size and alkali concentration (CaO) of nixtamalization process on anthocyanins loss of blue purple maize grain. The values within the dotted circles correspond to the loss of anthocyanins (AL) expressed as a percentage

purple grain was 389.7 ± 38.0 , 368.8 ± 10.6 , and 282.4 ± 11.8 mg equivalent to cyanidin 3-glucoside (CGE)/kg of dry matter for alkali concentrations of 0.5, 0.7 and 1.0%, respectively. The anthocyanin loss, considering the amount present in raw kernel, under these alkali concentrations was 39.4, 42.7, and 56.0%, respectively. With alternate nixtamalization methods, such as fractioned nixtamalization, Cortés et al. (2006) also reported an increase in the anthocyanin loss, when increasing the alkali concentrations (0.5–1.5%), losses were between 80.5 and 85.3%, for low and high concentrations, respectively. A direct relation between alkali concentration (0.1 and 0.3%) and anthocyanin loss (43.7 and 52.7%) was also found, by nixtamalizing blue purple maize grain through the extrusion process (Sánchez-Madrugal et al. 2014).

Effect of batch size on anthocyanins loss during nixtamalization of blue purple grain

Regardless of batch size used in the nixtamalization, when increasing the alkali amount, the anthocyanin loss increases. However, major losses occurred with the 1000 g batch size (Fig. 3) in relation to that of 100 g, and are attributed to the fact that the elevated temperature was maintained for a longer time in the first case, and the combination of high pH and temperature is highly detrimental to anthocyanins. Del Pozo-Insfran et al. (2006) nixtamalized blue corn in 3 kg sample lots with and alkali concentration of 1.0% with relation to kernel weight. The anthocyanin content in the nixtamal was 160 mg/kg of dry weight, and the anthocyanin loss was close to 50%.

According to our results, the masa-tortilla industry could reduce the anthocyanins loss during the nixtamalization of blue purple maize grain; if they drain the alkaline water in which the grain was cooked and add cold water to cool down the temperature of the system. With this action, both

Fig. 4 Color parameters in masa and tortilla as influenced by nixtamalization time and CaO concentration, during the nixtamalization process of blue purple maize grain. Bars with same letters in each color parameters are not different ($p > 0.05$). Capital letters comparison correspond to masa; lowercase letters comparison belongs to tortilla

pH and temperature are diminished and their negative effect on anthocyanins degradation are attenuated.

Effect of time and alkali concentration on the masa and tortilla color

Alkali concentration and nixtamalization time affected the color parameters of masa and tortilla. With 25 min of cooking time, the brightness (L) was reduced when the alkali concentration was increased, which means that both the masa as well as the tortilla obtained with 1.0% of CaO were darker than with 0.5% (Fig. 4). Similar results of the

effect of the alkali concentration on the color of the nixtamalized products have been reported by Collison et al. (2015) for maize grain fried products with anthocyanin pigments.

The Hue angle color (h°) did not show a defined behavior, while the chroma (Cr) value in the masa decreased when increasing the CaO concentration, a similar pattern was not observed in the tortilla; except in the 0.5 concentration with 35 min cooking time. In the alkali concentrations of 0.5 and 0.7%, and in the 25 and 35 min cooking times, the tone angle (hue) in Fig. 4 was within the same significance group. The results in the masa color are

Fig. 5 Digital photos of blue purple maize tortillas obtained with different concentrations of alkali during nixtamalization, with a cooking time of 25 min

similar to those reported by Sánchez-Madrigal et al. (2014) for nixtamalized flour produced through extrusion.

The green-yellowish tone of the masa and tortilla increased when increasing the alkali, as did the chroma value, which made tortillas look visually darker within this same tone. This color behavior happened with both nixtamalization time lengths used. Figure 5 shows the visual aspect of the tortillas obtained for a 25 min nixtamalization time and the different amounts of alkali used. It can be observed that those with 1.0% alkali look darker than those with 0.5%. This visual assessment is backed by the brightness and chroma values obtained in the tortillas of both treatments (Fig. 4). However, the anthocyanin content in the tortillas with 1.0% alkali was lower than that of the tortillas with 0.5% alkali (Fig. 2), hence the color intensity does not indicate a higher anthocyanin content.

Conclusion

The concentration of alkali during the nixtamalization process affects the time required for the solubilization of the pericarp, the higher concentration the shorter the time, and the greater loss of anthocyanins. During the nixtamalization process, it is in the cooking stage in which the largest loss of anthocyanins occurs, since only approximately one-third of the total loss occurred during the steeping of cooked grain (nixtamal). During the steeping of nixtamal, its cooling rate affects the loss of anthocyanins, and in turn, this variable is affected by the batch size of the nixtamalized grain. The larger batch size results in slower speed, and greater loss of anthocyanins. As for the parameters of the tortilla color, the brightness and the chroma were reduced to the extent the alkali concentration was increased. The information generated in this work could be useful to design a traditional nixtamalization method for blue purple maize grain that minimizes the loss of anthocyanins and allows to obtain masa and tortilla with acceptable color characteristics.

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