Color, phenolic composition and antioxidant activity of blue tortillas from Mexican maize races

Viridiana Hernández-Martínez*, Yolanda Salinas-Moreno**, José Luis Ramírez-Díaz**, Gricelda Vázquez-Carrillo***, Aurelio Domínguez-López**** and Antonio Gerardo Ramírez-Romero**

*Departamento de Ingeniería Agroindustrial, Universidad Autónoma Chapingo, Chapingo, México; **Programa de maíz, Campo Experimental ‘Centro-Altos de Jalisco’, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Tepatitlán, México; ***INIFAP, Campo Experimental Valle de México, Texcoco, México; ****Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México, Campus Universitario ‘El Cerrillo’, Toluca, México; **Departamento de Biotecnología, Universidad Autónoma Metropolitana, Unidad Iztapalapa, México, D. F., México

ABSTRACT
Cereal products made from pigmented grains are attractive to consumers. We determined the variability in color, phenolic composition and antioxidant activity (AA) of blue tortillas made from 18 landraces belonging to three blue/purple grained Mexican maize races – Chalqueño (Chal), Elotes Cónicos (Ec) and Bolita (Bol). The color of the tortillas produced ranged from yellow-green to blue-green. Ec tortillas were the only true blue type. Total anthocyanin content (TAC) varied between 3.67 and 1.11 mg equivalents of cyanidin 3-glucoside/kg dry weight (DW). Total soluble phenolics (TSP) were between 9.42 and 5.16 mg equivalents of gallic acid/kg DW. AA was highly correlated (p < 0.01) with TAC, but not with TSP. Blue Ec tortillas had the highest AA, and Bol tortillas had the lowest AA. Color parameters, TSP and TAC allowed the appropriate grouping of blue tortillas according to the maize race they belong to and were made from.

INTRODUCTION
Maize tortillas remain a mainstay of the Mexican and Central American diet. Per capita consumption in Mexico is about 86 kg/year (INEGI, 2012. http://www.inegi.org.mx/est/contenidos/proyEctos/encuestas/hogares/locales/enigh/enigh2010/ncv/default.aspx. Accessed 12 September 2012). Commonly, tortillas are made from white maize, but in some regions blue maize is also used. There is a growing consumer interest to purchase foods with a high content of antioxidants, which has been driven by scientific and media reports about the possible correlation between diets rich in bioactive compounds and a lower incidence of chronic degenerative diseases (Wootton-Beard & Ryan, 2011). Blue/purple maize grains have more antioxidants than white grains (De La Parra, Serna-Saldivar, & Liu, 2007; Mora-Rochin et al., 2010) due to the presence of anthocyanins, which are powerful antioxidants (Rice-Evans, Miller, & Paganga, 1996). The beneficial use of blue maize grain in the preparation of tortillas and snacks has been previously reported (Zazueta-Mora, Martínez-Bustos, Jacobo-Valenzuela, Ortega-Paczka, & Arellano-Vázquez, 2003). This results in the antioxidant content of blue maize tortillas to decline to levels that are similar to (De La Parra et al., 2007; Salinas-Moreno, Martínez-Bustos, Soto-Hernández, Ortega-Paczka, & Arellano-Vázquez, 2003). Nonetheless, high losses (56–70%) of anthocyanins occur during the processing of maize grain into tortillas (De La Parra et al., 2007; Salinas-Moreno, Martínez-Bustos, Soto-Hernández, Ortega-Paczka, & Arellano-Vázquez, 2003). This results in the antioxidant content of blue maize tortillas to decline to levels that are similar to (De La Parra et al., 2007; Mora-Rochin et al., 2010) or only slightly higher than (Aguayo-Rojas et al. 2012) white tortillas. However, these studies have been undertaken with few grain samples that represent just a small part of maize genetic and environmental diversity.

Color is often an important trait that consumers use to select food products. For blue tortillas, it is important to determine color objectively, in order to define values for...
color parameters. Food manufacturers need information that guide them in selecting grain from appropriate maize varieties or hybrids that after processing maintain an attractive color in final products. There is little published information on the color of tortillas prepared from pigmented maize grains that they could use. For nixtamalized maize flour (NMF) prepared with blue maize, Sánchez-Madrigal et al. (2015) reported the effect of calcium source and elaboration process (traditional or extrusion) on its color. A bluish tone was observed in extruded maize flours but not in NMFs. Collison, Yang, Dykes, Murray, and Awika (2015) studied the relationship between pigmented maize grains (red, blue/red, blue and purple) and the color of tortilla chips, and found that maize samples with blue/red grain color produced chips that retained their brilliance color.

The very large genetic diversity of maize in Mexico has been classified into broad races that have numerous landraces with different grain colors (Sánchez, Goodman, & Stuber, 2000). One of the most common grain colors is blue/purple and landraces with this color can be used to prepare blue tortillas. Recently, we reported on the physical characteristics and the variability in anthocyanin content found in the blue/purple grain of 18 landraces from three maize races with this grain color (Salinas-Moreno, Pérez-Alonso, Vázquez-Carrillo, Aragón-Cuevas, & Velázquez-Cardelas, 2012). We found high variability in grain anthocyanin content among these landraces and races. This variability can be used to develop nixtamalized products with attractive colors for consumers and with outstanding antioxidant content. Thus, the objective of this study was to determine the color, phenolic composition and antioxidant activity (AA) of tortillas prepared from 18 blue/purple grained landraces belonging to three major races of Mexican maize.

**Material and methods**

**Plant material**

We used the same 18 maize landraces with blue/purple grain that were used in the previous study by Salinas-Moreno et al. (2012). The landraces came from three Mexican races: Chalquéño (Chal), Elotes Cónicos (Ec) and Bolita (Bol). The criteria used to select the landraces for this study were (a) their use in elaboration of blue tortillas, (b) a contrasting anthocyanin content in their grains (high: Ec and Chal; low: Bol) and (c) their grain hardness. Six landraces were obtained for each race, with floury (Chal and Ec) and vitreous (Bol) blue/purple grains. The landraces belonging to Chal and Ec races were obtained at locations in the states of Mexico and the Federal District, located at elevations between 2250 and 2700 meters above sea level (masl). Landraces of the Bol race were obtained from locations in the states of Oaxaca, Mexico, grown at elevations of 1460–1700 masl. All maize landraces were grown during 2010 under rainfed conditions. Samples of 2.0 kg of clean grain were kept in plastic bags and stored at −20°C until their analysis. Recognized curators of the maize germplasm bank of Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) had previously classified each maize landrace from within the different maize races (Aragón et al., 2006). A white-maize-grained hybrid (H-40), with vitreous endosperm that is commonly used for commercial tortilla preparations, was used as a check.

**Nixtamalization and tortilla preparation**

Nixtamalization (the process of cooking and soaking maize grain in limewater) was performed using the method described by Vázquez-Carrillo, García-Lara, Salinas-Moreno, Bergvinson, and Palacios-Rojas (2011) which consisted of using 100 g of maize grain and adding 200 mL of water and 0.7 g of food-grade lime Ca(OH)₂. Cooking time varied with grain hardness, estimated by the flotation index (Secretaría de Economía, 2002). Chal and Ec maize landraces were cooked for 25 min, Bol landraces, which have vitreous grain, were also cooked for 25 min, to avoid effects of cooking time on phenolic composition. The cooking time for sample of H-40 maize was 45 min. It has a vitreous endosperm, as Bol landraces; however, its grain has a higher hardness. All nixtamalized samples were steeped in lime water for 12 h. The nixtamal (alkaline-cooked grain) was washed with 200 mL of water and milled in a stone mill to obtain masa (dough). Portions of masa (20 g) were molded in a hand press in disks of 11 cm diameter and then cooked for up to 1.5 min on a hot plate (200–270°C).

**Tortilla color**

Color was measured in freshly made tortillas on the face opposite to the blister, after they reached room temperature. The blister forms during the final stage of tortilla cooking. The tortilla face where the blister forms is thinner than the opposite side. The following color variables were obtained using a Hunter Lab colorimeter (Mini Scan XE Plus 45/0-L, Reston, VA, USA): lightness ($L^*$), $a^*$ (positive values = red color; negative values = green color) and $b^*$ (positive values = yellow color; negative values = blue color). Measurements were taken at three points on the tortilla surface, and three tortillas were used for each sample. The $a^*$ and $b^*$ values were used to calculate the parameters of hue angle ($H^* = \tan^{-1} b^*/a^*$) and chroma ($C^* = \sqrt{(a^*)^2 + (b^*)^2}$).

**Extraction of soluble phenolics**

Small pieces of tortilla were placed on aluminum trays and dehydrated in a stove at 40°C for 24 h. Then, the samples were ground in a mill (MF 10.2 Basic, IKA Laboratory Equipment, Wilmington, NC, USA) with a 0.5 mm mesh sieve to obtain the flour. One gram of tortilla flour was blended with 20 mL of acidified methanol (99% methanol and 1% trifluoroacetic acid, v/v). The sample was placed in a sonicator bath (Ultrasonic Bath, Branson 2510, Branson Ultrasonics, Danbury, CT, USA) for 15 min and then refrigerated at 4°C for 2 h. This sample was centrifuged at 2200 g (Universal 32, Hettich Zentrifugen, Germany) for 15 min. The supernatant was removed and adjusted to 20 mL with the extraction solvent, and stored at −20°C until used. This extract was utilized to quantify total anthocyanin content (TAC) and total soluble phenolics (TSP) and for the evaluation of AA.
Total anthocyanin content

The spectrophotometric method of Abdel-Aal and Hucl (1999) was used. Quantification was performed using a cyanidin 3-glucoside (CG) (Polyphenols, NW) calibration curve and the results were expressed as milligrams of CG equivalents (CGE)/kilogram dry weight (DW).

Total soluble phenolics

The Folin–Ciocalteu (FC) method (Singleton & Rossi, 1995) was used: 125 μL of FC reagent–water (1:1) was placed in assay tubes to react with 100-μL aliquots of the phenolic extract for 6 min. Afterward, the extract was neutralized using 1.25 mL of saturated Na₂CO₃, and its volume was adjusted to 3 mL with distilled water. Mixtures were shaken on a vortex and left in the dark for 90 min to achieve stabilization. Centrifugation (Hermle Z200 equipment, Labortechnik, Germany) at 15,300 × g for 10 min removed turbidity, and absorbance was determined in a Spectrophotometer Lambda 25 UV/vis (PerkinElmer, Waltham, MA, USA) at 760 nm. A standard curve was prepared with gallic acid to express TSP in milligrams of gallic acid equivalents (GAE) per kilogram DW.

Antioxidant activity

DPPH method

The free radical method (Wu et al., 2006) was used at 20°C with a 60 μM solution of 1,1-diphenyl-2-picryl-hidrazil (DPPH) (Sigma-Aldrich, St. Louis, MO, USA) in 80% methanol. Aliquots of 200 μL of extract reacted with 2.8 mL of DPPH in quartz cells, and absorbance was monitored every 5 min over a 30-min period at 515 nm, using an 80% methanol solution as blank. The percentage of reduced DPPH was

\[
\% \text{DPPH} = \frac{(A_0 - A_t)}{A_0} \times 100
\]

where \(A_0\) and \(A_t\) were blank and sample absorbance, respectively.

ABTS (TEAC) method

An aqueous 7 mM solution of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was combined (50:50) with a 2.45 mM solution of potassium persulfate and stored in the dark for 16 h to allow free radical generation. The mixture was diluted with phosphate buffer solution at 75 mM (pH = 7.4) until absorbance was 0.7 at 734 nm (Re et al., 1999). Aliquots of 200 μL of phenolic extracts reacted with 2.8 mL of ABTS–potassium persulfate solution in spectrophotometer cells, and absorbance was measured each minute. The percentage of reduced ABTS was calculated as

\[
\% \text{ABTS} = \frac{(A_0 - A_t) \times 100}{A_0}
\]

A curve was prepared using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at different concentrations (50, 150, 200, 250, and 300 μM) to express antioxidant capacity in Trolox equivalents.

Statistical analysis

Color, phenolic composition and AA of the tortillas were analyzed using a completely randomized design. A one-way analysis of variance and means comparison tests (Tukey, α = 0.05) were performed. Correlations between variables were determined using Pearson’s correlation test. Principal component analysis (PCA) was used as an exploratory tool to observe the existence of natural groupings. PCA is a multivariate method that allows the representation of the original dataset with a set of new orthogonal variables called principal components (PCs) generated as linear combinations of the original variables. The coordinates of the samples in the new reference system are called scores while the coefficients of the linear combination describe each PC. Original variables were color, phenolic content and AA, and PCA was conducted using the Varimax rotation of the PC. Additionally, a linear discriminant analysis was performed to estimate the similarity of the three groups of tortillas obtained from maize landraces and to predict the likelihood that a maize tortilla sample will belong to a particular landrace based on the metric independent variables: that is color, phenolic content and AA (Hair, Anderson, Tatham, & Black, 1998). Statgraphics Ver. 6.0 software (Manugistics Corp., Rockville, MD, USA) was used to provide both the PCA and the discriminant analysis. All analyses were performed with at least three repetitions.

Results and discussion

Color of tortillas

Color is an attribute of foodstuffs that can strongly influence selection decisions of consumers. Color can be expressed in terms of lightness (L*), hue angle (h°) and color saturation index (chroma). In some cases, the values of Hunter parameters a* and b* are also reported, which permits appreciation of the color tones that predominate in the samples. According to the parameters we evaluated, the color of the tortillas prepared from grain from the three races was statistically different (p ≤ 0.05). Bol tortillas showed the highest value of L*, while Ec tortillas had the lowest value (Figure 1). This means that Bol tortillas were lighter colored than Ec and Chal tortillas. All tortillas had a greenish tint, indicated by the negative values of a*, and that was more pronounced in Chal tortillas. Both Chal and Bol tortillas presented positive values of b* that are related to yellow tint, which was more intense in Bol tortillas. However, Ec tortillas showed a negative value of b*, indicative of a bluish tint. In their assessment of the color of extruded blue maize flours, Sánchez-Madrigal et al. (2015) reported negative values in the b* parameter, which is related to a bluish tone. In nixtamalized blue maize flours, they obtained positive values, which are related to a yellowish tint. Ec tortillas presented a hue value of 266.3 ± 48.1° that is related with a blue tone. Chal and Bol tortillas had hue values associated with greenish (176.8 ± 9.9°) and yellowish tones (127.7 ± 9.5°). Maize tortillas from all 18 landraces had very low chroma values (2.8–5.5), indicating a high presence of gray tones and low color purity.

In the central region of Mexico, blue tortillas are commonly consumed, and consumer’s preferences are toward deep blue tortillas, which are more expensive than white tortillas (Keleman & Hellin, 2009). Of the three maize races, tortillas from Ec landraces meet consumer expectations on blue tortillas. Figure 2 presents graphics of lightness (L*) in function of hue, chroma, and aa* and bb* color parameters of the tortillas obtained from the 18 landraces. L*/Hue allowed the blue tortillas from the three races to be differentiated. Only tortillas from one landrace within the Ec race was grouped with tortillas from the Chal race (Figure 2a); the L*/chroma could separate only two groups: one consisting of tortillas from Bol...
races, and the other with tortillas from Chal and Ec races (Figure 2b). The greenish tint of the blue tortillas is shown in Figure 2c (L*/a*); Figure 2d shows the yellowish tone in tortillas of the 18 landraces. Tortillas from Bol showed the highest values. The yellowish tone in maize tortillas is related to the amount of solubilized pericarp that remains attached to maize grain after nixtamalization. We did not determine this variable, but it is possible that Bol grains retained higher amounts of solubilized pericarp after nixtamalization than did Ec and Chal grains, increasing the yellowish tone in their tortillas.
Tortilla color depends on the color of maize grains and on the interactions of its chemical components with the alkaline pH present during nixtamalization. In the 18 blue/purple-grained landraces used in this study, anthocyanins are located in the aleurone layer (Salinas-Moreno et al., 2012), which is laid below the pericarp. During nixtamalization, the pericarp is solubilized by the alkali, and anthocyanins are directly exposed to the highly alkaline pH. The color observed in tortillas from blue/purple maize grain is consistent with that reported by Torskangerpoll and Andersen (2005) who pointed out that the degradation of CG in solution at pH 10.5 shows a greenish blue hue, with a considerably decreased chroma value. The pH of the dough (masa) and tortilla from the different maize landraces was 7–8 (data not shown), and at this pH the color of an extract of anthocyanins from purple maize grain was dark purple-red (Cevallos-Casals & Cisneros-Zevallos, 2004). Collison et al. (2015), who examined the relationship between maize grain color and tortilla chips, have reported similar results. They worked with four colors of maize grain (red, red/blue, blue and purple) and found that the chroma value of products decreased with increased alkali concentrations during nixtamalization. Chroma was the color parameter that best separated the tortilla chips by color. However, in our present work, we found that chroma was not useful to separate the blue tortillas of the 18 landraces by color; L* and hue were.

Phenolic composition of blue tortillas

Findings on total content of anthocyanins (TAC) and TSP in blue tortillas are presented in Table 1. Statistical differences (p ≤ 0.05) were observed among tortillas for these two variables. The highest TAC values were measured for tortillas from Ec race, and the lowest in tortillas from Bol race. White tortilla showed a marginal TAC value. The TAC values found in this study are comparable to those reported for blue tortillas by Aguayo-Rojas et al. (2012) (12.68 mg of CGE/100 g DW) and Mendoza-Diaz et al. (2012) (34.5 mg of CGE/100 g DW). Considering tortilla TAC by race, Ec and Chal tortillas were statistically equal and had a higher TAC than Bol (data not shown). In maize grain landraces, TAC was in the order Ec > Chal > Bol (Salinas-Moreno et al., 2012). After transforming grain into tortillas, the order was Ec = Chal > Bol, the percentage of anthocyanin losses were similar among samples from the three races (data not shown). Anthocyanin losses are caused by the alkaline pH and high temperatures that characterize the nixtamalization process, conditions under which these flavonoids are unstable (Brouillard, 1982).

The content of TSP was higher than the content of TAC in blue tortillas, result that agreed with that from Mora-Rochin et al. (2010). Several tortillas from Bol race had similar TSP than tortillas from Chal race; but Bol tortillas had lower TAC than Chal tortillas. These results could be related to the differences in maize grain hardness between the two races. Bol maize grains are harder than Chal maize grains (Salinas-Moreno et al., 2012), and hard maize grains have higher ferulic acid contents than soft grains (Chiremba, Taylor, Rooney, & Beta, 2012). Most of the total ferulic acid in maize grain is attached to cell wall components by ester bonds (Saumier & Thibault, 1999). During nixtamalization, alkaline pH hydrolyzes the ester bond and releases ferulic acid (De La Parra et al., 2007). It is possible that more ferulic acid was released in Bol grains than in Chal grains, and this extra ferulic acid remained in the tortilla to be quantified as part of TSP, together with anthocyanins.

The ratio TAC/TSP showed higher values in tortillas from Ec and Chal than in tortillas belonging to Bol race. This is related with the higher contents of anthocyanins observed in Ec and Chal tortillas.

Antioxidant activity

With the DPPH method, tortillas from Chal landraces 11, 16 and 19 had the highest AA (p ≤ 0.05), while the lowest AA corresponded to tortillas from the Bol population 107 and from white maize grain (H-40) Figure 3. A lower variability of AA was observed among tortillas from Ec landraces (coefficient of variation [CV] = 12.1%), while high variability was observed among tortillas from landraces of Bol race (CV = 18.6%). The Trolox equivalents antioxidant capacity (TEAC) method showed similar AA results to those obtained with the DPPH method. With this method (TEAC), the tortillas from Ec landraces 16, 27 and 54 demonstrated outstanding AA. Despite the low TAC in tortillas from the Bol race, the AA of tortillas from some of its landraces (16, 89, 120 and 151) were statistically not different than those of certain Ec and Chal landraces with almost double TAC. These results could be due to differences in the phenolic composition of the non-anthocyanin fraction of TSP between landraces of races with floury (Ec and Chal) and vitreous (Bol) grains. So far, the most studied phenolics in pigmented maize grain have been anthocyanins (Lopez-Martinez et al., 2009; 2012).
Moreno, Sanchez, Hernandez, & Lobato, 2005) and phenolic acids (De La Parra et al., 2007; Mora-Rochin et al., 2010). However, recent studies indicate a significant presence of flavonols such as kaempferol and morin in a larger proportion than phenolic acids in the TSP fraction of purple maize grain (Ramos-Escudero, Muñoz, Alvarado-Ortíz, Alvarado, & Yáñez, 2012). It is possible that these compounds (kaempferol and morin) were present in the blue/purple grains of Bol race and they contributed to the high AA observed in these tortillas, despite their low TAC. The reported high presence of kaempferol and morin in purple maize grains (Ramos-Escudero et al., 2012) besides the high content of phenolic amides in blue and purple maize grains (Collison et al., 2015) suggest that soluble phenolic composition in maize grains with these types of colors is much more complex than anthocyanins and phenolic acids.

The higher AA in tortillas from blue/purple grain compared with tortillas from white grain is attributed to anthocyanins, which are more powerful antioxidants than the phenolic acids (Rice-Evans et al., 1996) that are common in both tortilla colors.

Using more than one method to evaluate AA in foodstuffs is highly recommended because the sensitivity of each of the phenolic compounds present in the extracts of the food matrix is different to free radicals of the various models (Alam, Bristi, & Rafiquzzaman, 2013). Of the color parameters determined in blue tortillas, lightness (L*) and hue (H) showed highly significant correlations with TSP ($r = -0.550^*$ and $r = 0.629^{**}$, respectively); both color
parameters displayed highly significant correlations with TAC \((r = -0.816^{**} \text{ and } r = 0.836^{**})\) for \(L^*\) and \(H\), in this order). In blue tortillas, a deep blue color (high \(H\)) was associated with high TAC and low \(L^*\) value. \(L\) showed a significant highly negative correlation with DPPH \((r = -0.754^{**})\), but the correlation was not significant with TEAC. In contrast, \(H\) was not significantly correlated with DPPH, but was with TEAC \((r = 0.583^{*})\). Croma \((Cr)\) was highly correlated with TAC \((r = -0.70^{*})\) and with TEAC \((r = -0.647^{**})\). Interestingly, TSP was not correlated with TAC, nor with DPPH and TEAC. A likely explanation is that most anthocyanins, which are mainly responsible for the AA of raw grain, are degraded during the alkaline cooking of maize grain. However, other phenolics such as ferulic acid increase their proportion in the TSP extract (De La Parra et al., 2007), but their AA is lower than anthocyanins (Rice-Evans et al., 1996). In raw pigmented maize grains, Lopez-Martinez et al. (2009) reported a close correlation between free phenolics (TSP) and the AA evaluated by both DPPH and ABTS\(^+\) methods. TAC showed highly significant correlations with DPPH \((r = 0.615^{**})\) and TEAC \((r = 0.706^{**})\) which means that the higher the TAC in blue tortilla, the higher its antioxidant content.

**Grouping of blue maize tortillas by color and antioxidant activity variables using multivariate statistical tools**

The PCA model with two PC already explained 79.8% of the total variance in the data (PC1 captured 63% and PC2 16.8% of the variance). As shown in Figure 4a, the first PC is represented in the positive quadrant by Hue, TSP and TEAC.
Table 2. Standardized coefficients and significance of the functions derived from the linear discriminant analysis of blue tortillas from 18 landraces with blue/purple grain from Mexican maize races (Elotes Cónicos, Chalqueño and Bolita).

| Variable  | Function 1 | Function 2 |
|-----------|------------|------------|
| H         | -1.158     | 0.891      |
| TEAC      | -0.338     | 0.732      |
| DPPH      | 0.154      | 0.006      |
| TAC       | 0.960      | -0.049     |
| Cr        | 0.989      | 0.203      |
| L         | 1.507      | 0.996      |
| TSP       | 1.535      | 0.293      |
| p-Value   | 0.000      | 0.003      |

Table 2. Coeficientes estandarizados y significancia de las funciones derivadas del análisis discriminante lineal de tortillas azules de 18 variedades locales con grano azul/morado de tres razas Mexicanas de maíz (Elotes Cónicos, Chalqueño y Bolita).

Conclusions

There were significant differences for tortilla color, its phenolic composition and AA among the maize landraces studied. The tortillas from Elotes Cónicos were the only blue tortillas, according to their color parameters, and they had high TAC and the highest AA, which was around 0.7–1.0 times higher than that of white tortillas. Color parameters, phenolic composition and AA can be used to group blue tortillas made from different maize landraces that have blue/purple grain, what could be useful to maize landraces selection for tortilla preparation.

Acknowledgments

The help of Gilberto Esquivel Esquivel and Flavio Aragón Cuevas in maize landraces classification within races is appreciated. We also appreciate the valuable help of Dr. Stephen Waddington for correction of the English language of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Viridiana Hernández-Martínez and Yolanda Salinas-Moreno thank the Instituto de Ciencia y Tecnología del Distrito Federal (ICYTDF) through Project PICS010-53 for the financial support used to conduct this study.

References

Abdel-Aal, E.-S.M., & Hucl, P.A. (1999). A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. Cereal Chemistry, 76, 350–354. doi:10.1094/CCHM.1999.76.3.350

Aguayo-Rojas, J., Mora-Rochin, S., Cuevas-Rodríguez, E.O., Serna-Saldivar, S.O., Gutierrez-UrIBE, J.A., Reyes-Moreno, C., & MILán-Carrillo, J. (2012). Phytochemicals and antioxidant capacity of tortillas obtained after lime-cooking extrusion process of whole pigmented Mexican maize. Plant Foods for Human Nutrition, 67, 178–185. doi:10.1007/s11130-012-0288-y

Alam, M.N., Bristi, N.J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal, 21, 143–152. doi:10.1016/j.jspsj.2012.05.002

Aragón, C.F., Tabá, S., Hernández, C.J.M., Figueroa, C.J., Serrano, A.V., & Castro, G.F.H. (2006). Catálogo de Maíces Criollos de Oaxaca. INIFAP-SAGARPA. Libro Técnico Núm. 6. Oaxaca, Oaxaca, México. 344 p.

Brouillard, R. (1982). Chemical structure of anthocyanins. In P. Markakis (Ed.), Anthocyanins as food color (pp. 1–40). New York, NY: Academic Press.

Cevallos-Casals, B.A., & Cisneros-Zevallos, L. (2004). Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. Food Chemistry, 86, 69–77. doi:10.1016/j.foodchem.2003.08.011

Chiremba, C., Taylor, J.R.N., Rooney, L.W., & Beta, T. (2012). Phenolic acid content of sorghum and maize cultivars varying in hardness. Food Chemistry, 134, 81–88. doi:10.1016/j.foodchem.2012.02.067

Collison, A., Yang, L., Dykes, L., Murray, S., & Awika, J.M. (2015). Influence of genetic background on anthocyanin and copigment composition and behavior during thermoalkaline processing of maize. Journal of Agricultural and Food Chemistry, 63, 5528–5538. doi:10.1021/acs.jafc.5b00798

De la Parra, C., Serna-Saldivar, S.O., & Liu, R.H. (2007). Effect of processing on the phytochemical profiles and antioxidant activity of corn for production of masa, tortillas, and tortilla chips. Journal of Agricultural and Food Chemistry, 55, 4171–4183. doi:10.1021/jf063487p

Hair, J.F., Anderson, R.E., Tatham, R.L., & Black, W.C. (1998). Multivariate data analysis (5th ed.). Upper Saddle River, NJ: Prentice Hall.
Keleman, A., & Hellin, J. (2009). Specialty maize varieties in Mexico: A case study in market-driven agro-biodiversity conservation. Journal of Latin American Geography, 8, 147–174. doi:10.1353/lag.00061

Lopez-Martinez, L.X., Oliart-Ros, R.M., Valerio-Alfaro, G., Lee, C.-H., Parkin, K.L., & Garcia, H.S. (2009). Antioxidant activity, phenolic compounds and anthocyanins content of eighteen strains of Mexican maize. LWT-Food Science and Technology, 42, 1187–1192. doi:10.1016/j.lwt.2008.10.010

Mendoza-Díaz, S.M., Ortiz-Valero, C., Castaño-Tostado, E., Figueroa-Cárdenas, J.D., Reynoso-Camacho, R., Ramos-Gómez, M., & Loarca-Piña, G.F. (2012). Antioxidant capacity and antimutagenic activity of anthocyanin and carotenoid extracts from nixtamalized pigmented creole maize races (Zea mays L.). Plant Foods for Human Nutrition, 67, 442–449. doi:10.1007/s11130-012-0326-9

Mora-Rochin, S., Gutiérrez-Unibe, J.A., Serna-Saldivar, S.O., Sánchez-Peña, P., Reyes-Moreno, C., & Milán-Carrillo, J. (2010). Phenolic content and antioxidant activity of tortillas produced from pigmented maize processed by conventional nixtamalization or extrusion cooking. Journal of Cereal Science, 52, 502–508. doi:10.1016/j.jcs.2010.08.010

Moreno, Y.S., Sanchez, G.S., Hernandez, D.R., & Lobato, N.R. (2005). Characterization of anthocyanin extracts from maize kernels. Journal of Chromatographic Science, 43, 483–487. doi:10.1093/chromsci/43.9.483

Ramos-Escudero, F., Muñoz, A.M., Alvarado-Ortiz, C., Alvarado, Á., & Yáñez, J.A. (2012). Purple corn (Zea mays L.) phenolic compounds profile and its assessment as an agent against oxidative stress in isolated mouse organs. Journal of Medicinal Foods, 15, 206–215. doi:10.1089/jmf.2010.0342

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine, 26, 1231–1237. doi:10.1016/S0891-5849(98)00315-3

Rice-Evans, C.A., Miller, N.J., & Paganga, G. (1996). Structure antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology & Medicine, 20, 933–956. doi:10.1016/0891-5849(95)00227-9

Salinas-Moreno, Y., Martínez-Bustos, F., Soto-Hernández, A.R., Ortega-Pacza, R., & Arellano-Vázquez, J.L. (2003). Effect of alkaline cooking process on anthocyanins in pigmented maize grain. Agrociencia, 37, 617–628.

Salinas-Moreno, Y., Pérez-Alonso, J.J., Vázquez-Carrillo, G., Aragón-Cuevas, F., & Velázquez-Cardelías, G.A. (2012). Anthocyanin content and antioxidant activity of maize grain (Zea mays L.) from the races Chalqueño, Elotes Cônicos, and Bolita. Agrociencia, 47, 815–825.

Sánchez, J.J., Goodman, M.M., & Stuber, C.W. (2000). Isozymatic and morphological diversity in the races of maize of Mexico. Economical Botany, 54, 43–59. doi:10.1007/BF02866599

Sánchez-Madrigal, M.A., Quintero-Ramos, A., Martínez-Bustos, F., Meléndez-Pizarro, C.O., Ruiz-Gutiérrez, M.G., Camacho-Dávila, A., … Ramirez-Wong, B. (2015). Effect of different calcium sources on the bioactive compounds stability of extruded and nixtamalized blue maize flours. Journal of Food Science and Technology, 52, 2701–2710. doi:10.1007/s13197-014-1307-9

Saulnier, L., & Thibault, J.-F. (1999). Ferulic acid and diferulic acids as components of sugar-beet pectins and maize bran heteroxylans. Journal of the Science of Food and Agriculture, 79, 396–402. doi:10.1002/(ISSN)1097-0010

Secretaria de Economía. (2002). NMX-FF-034/1-SCFI-2002. Non industrialized food products for human consumption-cereals - Part I: White corn for alkaline process of corn tortillas and nixtamalized corn products-Specifications and Test methods. México.

Singleton, V.L., & Rossi, J.A. (1995). Colorimetric of total phenols with phosphomolybdic, phosphotungstic acid reagent. American Journal of Enology and Viticulture, 16, 144–158.

Torskangerpoll, K., & Andersen, Ø.M. (2005). Colour stability of anthocyanins in aqueous solutions at various pH values. Food Chemistry, 89, 427–440. doi:10.1016/j.foodchem.2004.03.002

Vázquez-Carrillo, M.G., García-Lara, S., Salinas-Moreno, Y., Bergvinson, D. J., & Palacios-Rojas, N. (2011). Grain and tortilla quality in landraces and improved maize grown in the Highlands of Mexico. Plant Foods for Human Nutrition, 66, 203–208. doi:10.1007/s11130-011-0231-7

Wootten-Beard, P.C., & Ryan, L. (2011). Improving public health?: The role of antioxidant-rich fruit and vegetable beverages. Food Research International, 44, 3135–3148. doi:10.1016/j.foodres.2011.09.015

Wu, L.-C., Hsu, H., Chen, Y.-C., Chiu, C.C., Lin, Y.-I., & Ho, J.-A.A. (2006). Antioxidant and antiproliferative activities of red pitaya. Food Chemistry, 95, 319–327. doi:10.1016/j.foodchem.2005.01.002

Zazueta-Morales, J.J., Martínez-Bustos, F., Jacobo-Valenzuela, N., Ordorica-Falomir, C., & Paredes-López, O. (2001). Effect of the addition of calcium hydroxide on some characteristics of extruded products from blue maize (Zea mays L) using response surface methodology. Journal of the Science of Food and Agriculture, 81, 1379–1386. doi:10.1002/(ISSN)1097-0010