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Additional Information

Content and bioaccessibility of phenolic compounds in blue corn products and tortillas using traditional and ecological nixtamalization Lilia L. Méndez Lagunas¹, Daniel Alberto García Rojas¹, Ana M. Andrés Grau², Luis Gerardo Barriada Bernal, Juan Rodriguez Ramirez

¹Instituto Politécnico Nacional, CIIDIR Oaxaca, Hornos 1003 Sta. Cruz Xoxocotlán Oaxaca, México, 71230

²Universitat Politècnica de València, Dpto. de Tecnología de Alimentos, Camino de Vera, s/n Valencia, España, 46022

Abstract

Food transformation processes effect the composition and bioaccessibility of bioactive compounds. Tortillas are part of the basic diet in Mexico and are made through both industrial and traditional processes. The objective of this work was to evaluate the effect of tortilla processing conditions, using traditional nixtamalization with $\text{Ca}(\text{OH})_2$ and ecological nixtamalization (EN), on the content and bioaccessibility of total phenolic compounds (TFC). The calcium salts: CaCl_2 , CaCO_3 and CaSO_4 were used for the EN process. The color, content of phenolic compounds (FC) in the products (corn grain, nixtamalized corn and tortillas) and bioaccessibility in the oral, gastric and intestinal phases were evaluated using the CIELab, Folin-Ciocalteu and simulated digestion methods, respectively. Blue and purple colors predominated in all products. The nixtamalization released up to 64% more FC than corn grain. TFC retention of up to 87–96% was found in tortillas. A TFC retention >92% was observed in tortillas prepared by the EN method with CaCl_2 , whereas traditional nixtamalization with $\text{Ca}(\text{OH})_2$ resulted in the retention of 96% relative to that in the grain. The bioaccessibility of tortilla FCs was greater than 390%, indicating a bioavailability far superior to that found in other foods.

Resumen

Los procesos de transformación de alimentos afectan la composición y la bioaccesibilidad de los compuestos bioactivos. Las tortillas son parte de la dieta básica en México y se hacen a través de procesos industriales y tradicionales. El objetivo de este trabajo fue evaluar el efecto de las condiciones de procesamiento de tortillas, utilizando la nixtamalización tradicional con $\text{Ca}(\text{OH})_2$ y la nixtamalización ecológica (EN), sobre el contenido y la bioaccesibilidad de los compuestos fenólicos totales (TFC). Las sales de calcio: CaCl_2 , CaCO_3 and CaSO_4 se usaron para el proceso de EN. El color, el contenido de compuestos fenólicos (FC) en los productos (grano de maíz, maíz

nixtamalizado y tortillas) y la bioaccesibilidad en las fases oral, gástrica e intestinal fueron evaluadas utilizando los métodos CIELab, Folin-Ciocalteu y digestión simulada respectivamente. Los colores azul y morado predominaban en todos los productos. La nixtamalización liberó hasta un 64% de FC más que el grano de maíz. En tortillas se encontró una retención de TFC de hasta 87-96%. Se observó una retención de TFC > 92% en tortillas preparadas por el método EN con CaCl_2 , mientras que la nixtamalización tradicional con Ca(OH)_2 resultó en la retención del 96% con respecto al grano. La bioaccesibilidad de las tortillas FC fue superior al 390%, lo que indica una biodisponibilidad muy superior a la encontrada en otros alimentos.

Keywords

Tlayuda, tortilla, phenolic compounds, ecological nixtamalization, bioaccessibility

Palabras clave

Tlayuda, tortilla, compuestos fenólicos, nixtamalización ecológica, bioaccesibilidad

1. Introduction

Tortillas are a staple of the daily diet of the Mexican population and are consumed by 94% of the population. In the southeast of Mexico, tortillas are made by hand, in a traditional way. The amount

of tortillas that are produced using this method is lower than those made industrially but the process is still widely used. Two types of tortillas can be obtained in this way: soft tortillas, which are small and thin, and tlayuda tortillas, which are less flexible and chewier. Traditional tortillas are prepared from fresh dough (masa) obtained from nixtamalized corn, in contrast to tortillas made industrially by using rehydrated nixtamalized flour and industrial machines.

Tlayudas have a diameter of 30 to 40 cm, a thickness of 1.5 mm, an approximate weight of 75 to 80 g, average humidity of 19% and a shelf life of approximately 7 days, whereas industrial tortillas have a diameter of 13 cm, a thickness of 2 mm and shelf life of 2 days (Vázquez-Bocanegra *et al.* 2003).

The process for making tortillas includes the following steps: grain nixtamalization, washing, grinding and cooking. The traditional nixtamalization (TN) process consists of cooking corn with water and $\text{Ca}(\text{OH})_2$, during which the pericarp is removed, which makes the corn more digestible and increases its nutritional value (Ocheme *et al.* 2010). A new nixtamalization process using calcium salts (CaCl_2 , CaCO_3 and CaSO_4) was proposed by Figueroa *et al.* (2006), which is called an ecological nixtamalization (EN) process; wastewater with a pH of 4.3–7.3 and less pollutants are generated through the EN process compared with the traditional method.

Soft and tlayudas tortillas are made with fresh masa and the discs are made manually. The discs are cooked directly on a clay hotplate (comal) for soft tortillas, whereas the tlayudas tortillas are cooked using a metal mesh placed on a ceramic hotplate; the cooking time is approximately 6 min. These processes influence the composition and concentration of compounds in corn, mainly that of the phenolic compounds (FC). The polyphenol family comprises compounds such as flavonoids, which have important biological, antioxidant, antimutagenic and microbicidal activity (Salinas-Moreno *et al.* 2017). Anthocyanins are blue-colored flavonoids found in the aleurone layer of blue corn grain; cyanidine 3-glucoside is the most common anthocyanin in corn (Nakatami *et al.* 1979, Aoki *et al.* 2002, Zhao *et al.* 2008, Escalante-Aburto *et al.* 2016). Anthocyanins play an important role in the prevention of diverse diseases such as cancer; cardiovascular diseases involving mechanisms of antioxidant activity, detoxification activity, anti-proliferation, induction of apoptosis, and anti-angiogenic activity; anti-inflammatory activity and improvement of the immune system (Ames *et al.* 1993, Jing, 2006; Nikkhah *et al.* 2008).

Bordignon-Luiz *et al.* (2007) reported that the stability of anthocyanins depends on the presence of light, oxygen, pH, metal ions and temperature. The pH has an effect on the structure of anthocyanins, which effects the change in color. Anthocyanins are more stable in an acidic medium

than in a neutral or alkaline medium. On the other hand, the high temperature destroys the anthocyanins. Two mechanisms lead to the degradation of anthocyanins: the hydrolysis of the glycosidic bond and hydrolytic breakdown, which cause the formation of aglycone and chalcones, respectively (Timberlake, 1980).

During traditional nixtamalization, the compounds are subjected to conditions of high moisture content, heat (80 to 105 °C) and pH (11 to 12). TN significantly reduces the phenolic compound content, particularly anthocyanin found in pigmented corn. During the nixtamalization process, a large amount of these compounds is lost, solubilized in the cooking water with a high pH and extreme temperature. In addition, other chemical structures derived from the polyphenols are affected by the breakage of ester bonds, and as a consequence the phenols are released into the cooking solution. Most of these compounds are found in the pericarp of the grain and are removed during the washing of the nixtamal (De la Parra *et al.* 2007).

Even if 8000 phenolic structures are currently known, and among them over 4000 flavonoids have been identified, there is little information on the effect of food processing on the bioaccessibility and bioavailability of phenolic compounds (Ribas-Agustí *et al.* 2017, Harborne *et al.* 1992, Bravo, 1998, Cheynier, 2005).

The content of phenolic compounds in corn grain and tortillas prepared through the traditional nixtamalization process with $\text{Ca}(\text{OH})_2$ and through the ecological method with calcium salts such as CaCO_3 , CaCl_2 , CaSO_4 has been investigated (Adom and Liu, 2002, Abdel-Aal *et al.* 2006, De la Parra *et al.* 2007, Rodríguez-Méndez *et al.* 2013, Hernández-Martínez *et al.* 2016). However, the effect of these processes on the bioaccessibility and bioavailability of phenolic compounds present in corn has not been previously reported. Also, the effect of the non-industrial process for making tortillas (soft and tlayudas) on the content of phenolic compounds and their bioaccessibility is not known.

Therefore, the objective of this work was to quantify the phenolic compounds in different corn products (nixtamalized corn and tortillas) made using the TN and EN processes as well as the bioaccessibility of FC in soft and tlayudas tortillas.

2. Methodology

2.1. Raw material

The VC-42 blue variety Bolita corn breed donated by the INIFAP Campus Oaxaca was used to make tortillas. $\text{Ca}(\text{OH})_2$, CaCO_3 , CaCl_2 , CaSO_4 salts were acquired from Sigma–Aldrich (USA).

2.2. Tortilla making process

The traditional nixtamalization process, with calcium hydroxide and ecological nixtamalization, with calcium carbonate, calcium chloride and calcium sulfate, were performed according to the methods reported by Rodríguez-Méndez *et al.* (2013). A nixtamalization time of 30 min was determined according to the corn flotation index according to the Mexican Norm NMX-FF-034. The flotation index of the blue Bolita corn was 75, which has the characteristic of a soft corn according to the hardness classification. To obtain the masa, the washed nixtamal was ground in a stone mill (Model M100, Fumasa, Querétaro, Mexico).

The tlayudas tortillas were prepared using a manual press; the dough was molded into a circle with a diameter of 30 ± 2 cm and a thickness of 2 ± 0.5 mm and then placed on a metal mesh with a diameter of 40 cm. The mesh was then placed on a preheated ceramic grid at 250 ± 10 °C, leaving a space of 1 cm between the plate and the tortilla. Each tortilla was cooked for 17 s on one side then cooked on the other side for 30 s, forming a thick crust; the tortilla is then flipped to the first side and cooked for 10 s to allow the steam formed inside the dough during the cooking process to inflate the tortilla; then, it is deposited on the same face on the metal mesh on a preheated ceramic at 250 ± 10 °C until it is fully cooked.

The soft tortillas, with a diameter of 12 cm and thickness of 1.5 mm, were molded in the same way as described for the tlayudas tortillas. The tortillas were then cooked directly on a ceramic plate for 17 s on one side, on the opposite side for 50 s, forming a crust, then the first side was finally repositioned on the preheated ceramic plate for 17 s to allow inflation and to be fully cooked.

2.3. Color

A Mini Scan spectrometer (EZ 4500S, US) and the CIELab scale (L^* , a^* , b^*) were used to determine the color. Chroma (C^*) and Hue (h^*) were determined according to equation 1 and 2.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h^* = \arctan \left[\frac{b^*}{a^*} \right] \quad (2)$$

L^* is the brightness color parameter; a^* is a color parameter, negative values the color is in the range of green and positive values in the range of red; b^* is the color parameter, negative values is

blue, positive values represent the color yellow; C^* is the Chroma parameter and h^* is the Hue parameter.

The color of the grain, masa and tortilla was determined according to the procedure of Salinas Moreno *et al.* (2013). A sample of 5 g of masa and tortilla were crushed and the powder obtained was passed through 100 mesh and placed in a circular container 1 cm high and 3 cm in diameter. The color of the corn was determined directly on the surface of the grain.

2.4. Quantification of bioactive compounds

2.4.1. Preparation of extracts

Samples of 100 grains of corn or nixtamalized corn and three tortillas were taken. The samples were ground, homogenized and 1 g of sample was taken for the determination of phenolic compounds.

2.4.2. Total phenolic content

The total phenolic content was analyzed using the modified Folin-Ciocalteu method according to Ancillotti *et al.* (2016). Gallic acid (0–36 mg GAE /100 g_{ds}) was used to prepare the calibration curve. The absorbance was measured at 759 nm. The concentration of total phenolic compounds was expressed in mg GAE/100 mL.

2.4.3. Conversion of concentration units

To compare the effect of treatments on the total phenolic compounds (TFC), the units of measure were expressed on a dry basis (g_{ds}) using equation 3:

$$TFC \left[\frac{mg}{g_{ds}} \right] = \frac{C_e \cdot V_s}{100 \cdot DS} \quad (3)$$

in which TFC is the total phenolic content; C_e is the concentration of phenolic compounds (mg/100 mL); V_s is the volume of the solution used for extraction (mL) and DS (g) are the dry solids in the sample used to measure the concentration according to Equation 4.

$$DS = X - 100 \quad (4)$$

in which X is the dry base moisture content (g_{ds}) determined with the A.O.A.C. method 14003 (1980).

2.5. Determination of bioaccessibility

After the consumption of tortillas, gastric digestion releases food compounds into the gastrointestinal tract. To assess the bioaccessibility of these compounds, the simulated

gastrointestinal digestion method was used, which measures the amount of a compound available to be absorbed by the intestine mucosa (Versantvoort et al., 2005). To determine the amount the bioactive compounds that are potentially available for intestinal absorption, the original protocol by Miller *et al.* (1981) and subsequently modified by Luten *et al.* (1996) was used. The bioaccessibility (B) of TFC was calculated with equation 5.

$$B (\%) = \frac{C_f}{C_i} \cdot 100 \quad (5)$$

in which C_f is the final concentration of TFC after 24 h of rest (mg/kg); C_i is the initial content of TFC (mg/kg).

2.6. Design and data analysis

A fully randomized $2 \times 4 \times 2$ factorial design with factors: type of tortilla and type of salt used in the nixtamalization. The type of tortilla at two levels, soft tortilla and tlayuda tortilla, while the type of salt at four levels, $\text{Ca}(\text{OH})_2$, CaCl_2 , CaCO_3 and CaSO_4 . Each treatment was performed in triplicate. An analysis of variance was applied to the data with a level of significance $\alpha < 0.05$ and the Tukey test for the comparison of treatments for each phase ($\alpha < 0.05$).

3. Results

The color of the grains of fresh corn, nixtamalized corn and tortillas was determined (Table 1). The color parameters were similar between soft and tlayudas tortillas, predominantly blue-gray-purple colors. The parameters a^* and b^* indicated shades of blue in the corn grain, nixtamalized corn and tortillas, for all treatments. The Chroma indicates opaque tones and the Hue shows low tone intensity. The nixtamalization with $\text{Ca}(\text{OH})_2$ and CaCl_2 favored a blue hue in the nixtamalized grain. The masa showed the greatest luminosity, which was owing to the higher moisture content (59% wb). On the other hand, the masa was a mixture of the pericarp with the endosperm, which decreases the blue tone and was most notable after the treatment with $\text{Ca}(\text{OH})_2$.

The brightness of the tlayudas tortillas was slightly greater than that of the soft tortillas. The tlayudas tortillas showed a higher Hue value; in particular, those prepared with $\text{Ca}(\text{OH})_2$ and CaCO_3 showed a more intense blue or gray tones compared with those prepared with the other salts. All treatments with acid-neutral pH showed degradation or transformation of the TFC responsible for the blue color in tortillas, probably owing to the process temperature effect.

The content of TFC in corn grain was 213 ± 12 mg GAE/100 g_{ds}, which was in the 140–266.2 mg GAE/100 g_{ds} range reported for other varieties of blue corn (De la Parra *et al.* 2007, Mora-Rochin *et al.* 2010).

The TFC at each stage of the process is shown in Figure 1. The highest TFC content was found in the nixtamalized corn subjected to all treatments, and the lowest in the soft tortilla subjected to a CaCO₃ treatment.

In nixtamalized corn, significant differences were found between treatments, except for Ca(OH)₂ and CaCl₂, which showed similar results. On the other hand, the highest TFC content in nixtamalized corn was found by treatment with CaSO₄.

De la Parra *et al.* (2007) reported TFC for masa of 158.5 mg GAE/100 g_{ds}, which was much lower than that found in nixtamalized corn (Fig. 1), in which the nixtamalized grain has not yet been ground. This suggested that phenolic compounds are degraded during the milling process.

Significant differences were found between all the treatments applied to the tortilla (Fig. 1). The tlayudas and soft tortillas prepared by treatment with Ca(OH)₂ and CaCl₂, respectively, showed the highest retention of TFC.

The TFC in tortillas was lower than in nixtamalized corn; losses of 40.7–49% between nixtamalized corn and tlayudas tortillas and 46.3–57.5% between nixtamalized corn and soft tortillas were found. However, an increase of 49–71.2% was found between corn grain (213 ± 12 mg GAE/100 g_{ds}) and nixtamalized corn (319–364 mg GAE/100 g_{ds}).

De la Parra *et al.* (2007) reported a TFC of 161.8 mg GAE/100 g_{ss} in tortillas made industrially with blue corn, which represents a retention of 60.7% in relation to the initial content found in the grain. In our work we found TF concentrations of 186.0–204.6 mg GAE/100 g_{ss} in tlayudas tortillas and 135.5–184.2 mg GAE/100 g_{ss} in soft tortillas, which represented 87.1–95.7% retention in tlayudas tortillas and 63.4–86.3% of CFT in soft tortillas.

Mostly free phenols were released from all the products at each process stage (nixtamal, dough and tortillas). These results contrast with those reported by De la Parra *et al.* (2007), who found the highest presence of TFC in the bound fraction.

The process of nixtamalization with Ca(OH)₂ and industrial cooking causes losses of phenolic compounds in tortillas of 36.2% for white and yellow genotypes, 44–48% for red genotype and 39–44.4% for blue genotype (De la Parra *et al.* 2007, Mora-Rochin *et al.* 2010). The ecological process

presented losses of phenolic compounds in tortillas of 0–37% in white genotypes, 26–58% in yellow genotypes, 17–38% in red genotypes and 36–40% in black genotypes (Rodríguez-Méndez *et al.* 2013). The differences compared with our results may be owing to the grain genotype, process and proportion of the anatomical fractions of the grain.

The processes of nixtamalization with calcium salts, the thermal cooking treatment of the tortillas and the thickness of the tortilla change the proportion of bound and free phenolic compounds. Previous studies indicate that >80% of CFT were found in bound form (De la Parra *et al.* 2007) in raw grain, masa and tortillas; however, our results showed a higher proportion of TFC in its free form (> 62%) than in the bound form (< 37%) in nixtamalized corn, whereas in tortillas the majority of TFC were found in the free fraction (> 56%), except for the tlayudas tortillas prepared with CaCO₃ and CaSO₄ salts, in which the highest proportion was found in the bound fraction (> 59%).

According to De la Parra *et al.* (2007), the majority of phenolic compound in corn (ferulic acid) is found in the pericarp and represents 85% of the total phenolic compounds, both in its free form and esterified into heteroxylanes, which assemble into hemicelluloses in the plant wall cells. The anatomical fractions of the VC-42 blue variety Bolita maize breed (pericarp = 0.038; endosperm 0.827; germ 0.118) indicate that the pericarp has the lowest proportion in the grain, which could explain the FC in the free fraction.

The total phenolic content at different digestion stages is shown in Figure 2. In general, similar levels of TFC were present in the oral and gastric phase, mostly in the free fraction, whereas after the intestinal phase, the concentration of TCF significantly increased relative to the previous digestion stages, particularly in soft tortillas. The bioaccessibility of TFC in tlayudas and soft tortillas was 390–520% and 487–666%, respectively.

The results in this work suggest that bioactive compounds are released from the grain matrix during traditional processing even if calcium salts other than Ca(OH)₂ are used, increasing the bioaccessibility of TCFs. The nixtamalization process with CaCl₂ resulted in the highest bioaccessibility of TCF at all stages of digestion (Fig. 2). The proportion of pericarp and the acid-neutral pH of the nejayote could explain this result.

There are several factors that interfere with the bioaccessibility of these compounds, such as the food matrix and chemical interactions with other phytochemicals and/or biomolecules (Parada and Aguilera, 2007). The nature of phytochemicals, their stability and their antioxidant activity depend

on many factors, such as the food matrix, pH, temperature and other related factors (McDougall *et al.* 2005, Saura-Calixto *et al.* 2007; Tagliacruz *et al.* 2010).

Our results indicate that the processes of nixtamalization, grinding and cooking releases or dissociates CF, facilitating solubility and availability for absorption (Pohl *et al.* 2012, Pohl, 2009). Food processing can induce chemical and physical modifications in foods, which can influence bioaccessibility. Those changes include: 1) chemical modifications; 2) excision of covalent bonds, hydrogen bonds or hydrophobic forces that bind phenolic compounds to the matrix of the macromolecules; 3) damage of microstructural barriers such as cell walls that impede the release of the matrix and 4) development of microstructures that protect the phenolic compounds until they are absorbed. All these changes can occur during the tortilla making processes. The nixtamalization causes physicochemical changes such as starch gelatinization, partial lipid saponification, solubilization of some proteins around the starch granules and the conversion of the components of the hemicellulose wall cells to soluble gums (Arámbula *et al.* 1999). Thermal and mechanical energy are variables that dictate the content, bioavailability and activity of bioactive compounds (Oghbaei and Prakash, 2015). The microstructural barriers are strongly damaged during the milling process, whereas the cooking temperatures of the tortillas favor the cleavage of the bonds between the phenolic compounds of the cells of the plant wall.

4. Conclusions

We investigated the effect of various calcium salts in traditional nixtamalization processing on the TFC content of nixtamalized corn and tortillas. The color of the products (nixtamalized corn and tortillas) ranged from opaque shades of blue to purple, consistent with the presence of TFC. Both methods of nixtamalization, traditional and ecological, release CFs into nixtamalized grain, mainly in the free form. However, the concentration of CF is significantly reduced during the tortilla cooking process.

In soft tortillas, FCs were better retained using ecological nixtamalization than with $\text{Ca}(\text{OH})_2$. More than 64% of TFC was retained in traditionally made tortillas. The use of the metal mesh during cooking, which separates the tortilla from direct contact with the ceramic plate, seems to reduce the degradative effect of temperature on TCF in tlayuda tortillas.

On the other hand, the highest bioaccessibility of FC was found in tortillas made using ecological nixtamalization with CaCl_2 .

In general, traditional tortilla making processes release phenolic compounds found in the corn kernel and increase their bioaccessibility by more than 390%, a higher value than that found in other foods.

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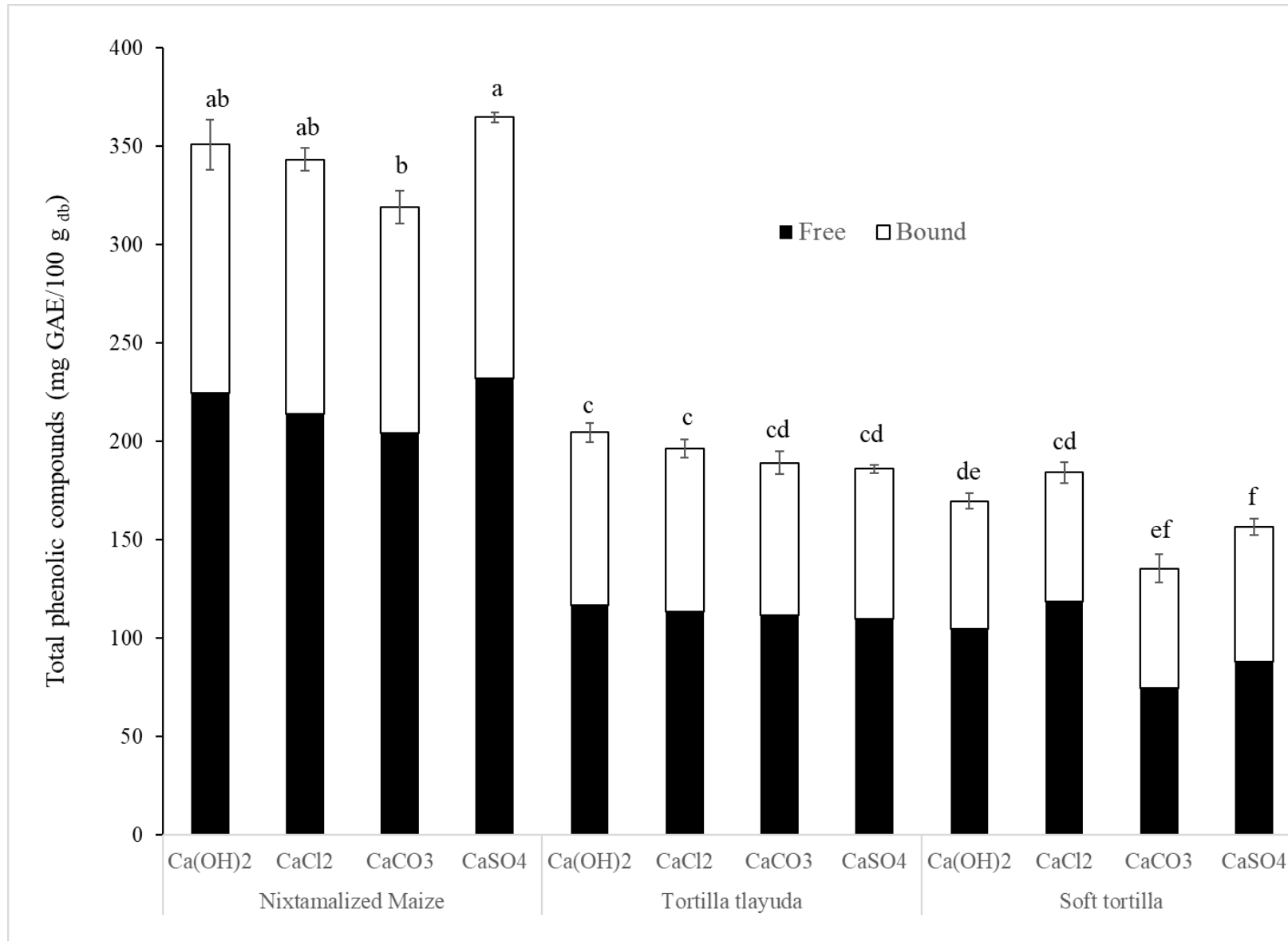
1 Table 1. Color parameters in maize corn and their products

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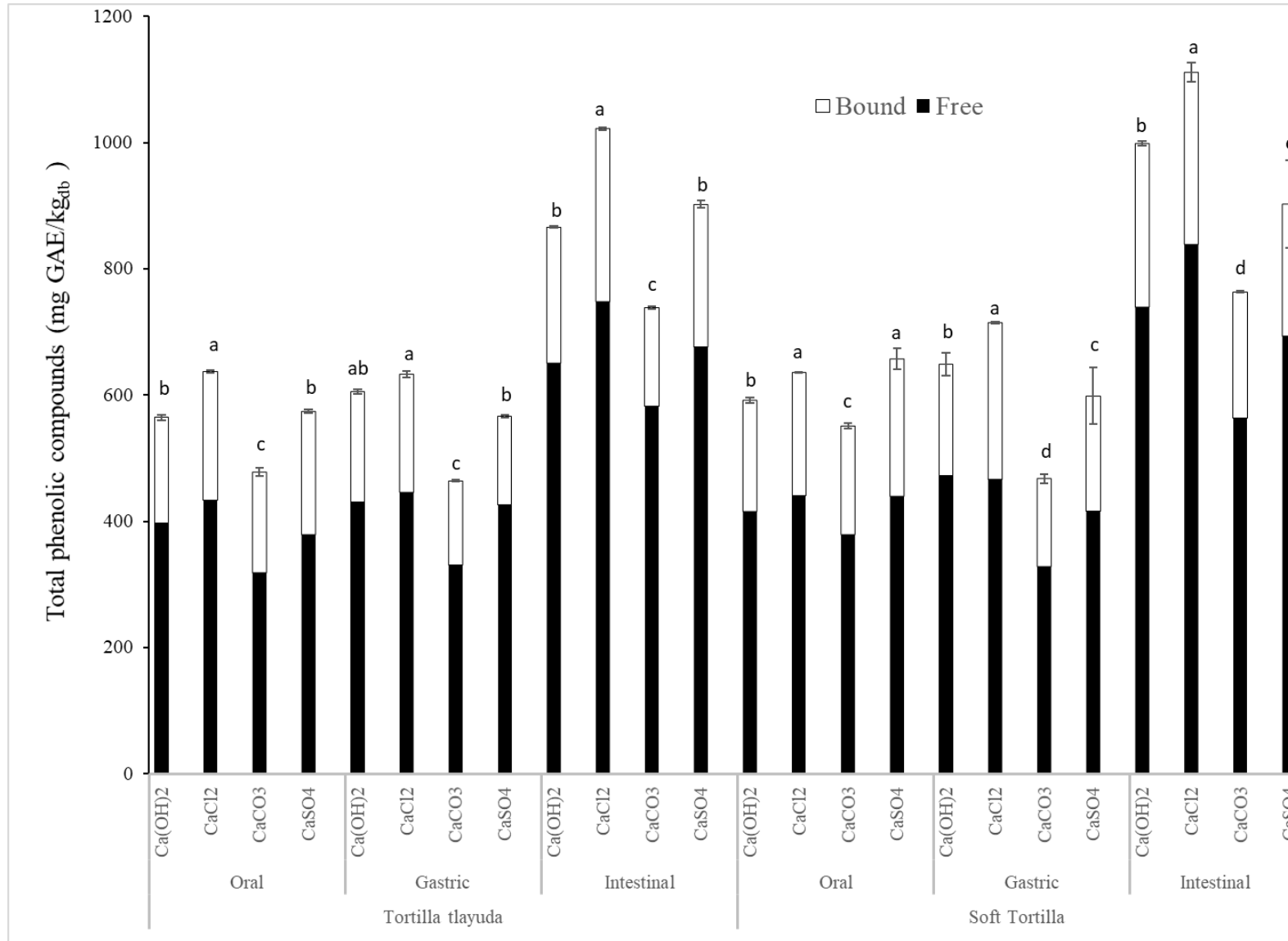
| | L* | a* | b* | c | h |
|---------------------|--------------------------|--------------------------|--------------------------|-----------|------------|
| Maíz | 19.05±0.59 ^a | 1.41±0.16 ^b | 0.04±0.01 ^b | 1.41±0.16 | 1.63±0.5 |
| Nixtamalized corn | | | | | |
| Ca(OH) ₂ | 14.59 ±0.23 ^c | 2.24 ±0.23 ^a | -0.36 ±0.02 ^c | 2.27±0.22 | -9.31±1.0 |
| CaCl ₂ | 14.37 ±0.34 ^c | 2.16 ±0.18 ^a | -0.32 ±0.02 ^c | 2.18±0.18 | -8.43±1.1 |
| CaCO ₃ | 15.89 ±0.41 ^b | 1.42 ±0.26 ^{*b} | 1.67 ±0.12 ^b | 2.20±0.14 | 49.8±6.5 |
| CaSO ₄ | 18.26 ±0.36 ^a | 1.10 ±0.09 ^b | 2.27 ±0.10 ^a | 2.53±0.13 | 64.2±1.0 |
| Masa | | | | | |
| Ca(OH) ₂ | 56.17 ±0.14 ^d | 0.22 ±0.01 ^d | -0.18 ±0.01 ^c | 0.29±0.00 | -39.8±3.6 |
| CaCl ₂ | 58.03 ±0.22 ^a | 9.22 ±0.11 ^a | 0.36 ±0.04 ^a | 9.22±0.11 | 2.26±0.2 |
| CaCO ₃ | 58.20 ±0.09 ^c | 7.78 ±0.04 ^c | 0.31 ±0.01 ^a | 7.78±0.04 | 2.33±0.1 |
| CaSO ₄ | 57.3 ±0.04 ^b | 8.95 ±0.03 ^b | 0.10 ±0.01 ^b | 8.95±0.03 | 0.64±0.1 |
| Tlayuda tortilla | | | | | |
| Ca(OH) ₂ | 34.73 ±0.38 ^c | -2.37 ±0.09 ^c | 4.49 ±0.20 ^c | 5.08±0.21 | -62.13±0.4 |
| CaCl ₂ | 41.23 ±0.18 ^b | 4.54 ±0.02 ^b | 4.03 ±0.02 ^d | 6.07±0.03 | 41.58±0.0 |
| CaCO ₃ | 43.28 ±0.15 ^a | 4.63 ±0.07 ^b | 6.01 ±0.17 ^a | 7.59±0.17 | 52.4±0.4 |
| CaSO ₄ | 42.90 ±0.15 ^a | 6.83 ±0.04 ^a | 5.11 ±0.04 ^b | 8.53±0.03 | 36.8±0.1 |
| Soft tortilla | | | | | |
| Ca(OH) ₂ | 33.70 ±0.24 ^d | -2.56 ±0.35 ^c | 3.81 ±0.12 ^b | 4.59±0.30 | -56.11±2.8 |
| CaCl ₂ | 39.97 ±0.21 ^a | 4.24 ±0.04 ^b | 4.25 ±0.10 ^b | 6.01±0.10 | 45.09±0.4 |
| CaCO ₃ | 39.18 ±0.21 ^b | 4.58 ±0.03 ^b | 5.03 ±0.24 ^a | 6.80±0.16 | 47.72±1.5 |
| CaSO ₄ | 37.75 ±0.08 ^c | 6.52 ±0.16 ^a | 5.27 ±0.44 ^a | 8.38±0.40 | 38.97±1.7 |

Values with different superscripts in the same column and section (a, b, etc.) differ significantly (P <0.05).

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21 Figure 1. Total phenolic content in nixtamalized maize, tortilla tlayuda (TTL) y soft tortilla (TTR)



22 Figure 2. Total phenolic content at different digestion stages