



## Research Paper

## Comparison of nutritional properties and bioactive compounds between industrial and artisan fresh tortillas from maize landraces



Citlali Colín-Chávez<sup>a</sup>, Jose J. Virgen-Ortiz<sup>a,\*</sup>, Luis E. Serrano-Rubio<sup>a</sup>,  
Miguel A. Martínez-Téllez<sup>b</sup>, Marta Astier<sup>c,\*\*</sup>

<sup>a</sup> CONACYT - Centro de Investigación en Alimentación y Desarrollo, A.C. - CIDAM, Km. 8 Antigua Carretera a Pátzcuaro s/n, C.P. 58341, Morelia, Michoacán, México

<sup>b</sup> Centro de Investigación en Alimentación y Desarrollo, A. C., Carretera Gustavo Enrique Astiazarán Rosas 46, C.P. 83304, Hermosillo, Sonora, México

<sup>c</sup> Centro de Investigaciones en Geografía Ambiental, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro 8701, C.P. 58190, Morelia, Michoacán, México

## ARTICLE INFO

## Keywords:

Small-scale artisan producer  
Handmade tortillas  
Maize landraces  
Native-traditional foods

## ABSTRACT

Consumers are seeking for native-traditional foods to improve their intake of both nutrients and health-promoting phytochemicals. This study was designed to evaluate the difference in content of nutrients and bioactive compounds from handmade tortillas elaborated by a small-scale artisan producer and tortillas sold by a large food retailer available to consumers. All tortillas were analyzed for chemical composition, dietary fiber, calcium and phytochemical content, antioxidant capacity, and phenolic acids profile. Chemical and nutritional variation in the tortillas was estimated using principal component analysis. Data showed that artisan tortillas made from blue and white maize landraces had significantly ( $p < 0.05$ ) higher content of nutritional and bioactive compounds compared to those of the supermarket. Handmade blue maize tortillas (HBMT) had a high content of free phenolics content and the highest antioxidant capacity (DPPH and ABTS methods), which was around 1.7–2.1 fold higher than that of commercially produced white maize tortillas (CWMT). Total dietary fiber was higher in HBMT ( $15.7 \pm 1.06$  g/100 g) than in CWMT ( $11.6 \pm 0.96$  g/100 g). CWMT had the lowest calcium content ( $42.1 \pm 0.9$  mg/100 g) compared to handmade tortillas ( $155.5 \pm 4.5$  mg/100 g). HPLC results indicated the presence of ferulic, *p*-coumaric, caffeic, syringic and 4-hydroxybenzoic acids. Interestingly, handmade tortillas from blue maize had 4.5-fold ferulic acid content compared with commercially produced white maize tortillas, consequently it can be a good source of phenolic antioxidants, particularly ferulic acid. This study showed that artisan fresh tortillas had superior nutritional-nutraceutical properties compared to CWMT.

## 1. Introduction

Maize originated in Mexico more than 6000 years ago and is a key grain for the world's economy and food security (González-Ortega et al., 2017). In Mexico, the maize agroecosystem in addition to being a fundamental part of local and national food production, it also has considerable social, cultural and historical relevance since pre-Hispanic times (Dominguez-Hernandez et al., 2018). Maize food produced domestically under industrial conditions is primarily distributed in urban areas, where it is consumed principally as tortillas. Mexico is the main tortilla consumer in the world, with an annual consumption about to 11 million tons of maize, which represents a *per capita* consumption of 79.5 kg of tortillas in rural areas, and 56.7 kg in the urban areas

(CEDRSSA, 2014). However, the tortillas also have an important role in the diet of people from Central America, and recently United States due to awareness of their health benefits (González-Ortega et al., 2017). Artisan tortillas are traditionally elaborated using the process named nixtamalization (Bello-Perez et al., 2016). The nixtamalization confers several benefits in the final products compared to unprocessed grains such as improves bioavailability of niacin and protein quality, reduces mycotoxins in raw kernels, increases resistant starch and calcium content and reduces phytate content in the final products (Gutiérrez-Urbe et al., 2014).

Traditional tortilla production contributes to the conservation of native maize and indigenous agriculture, and it promotes local and traditional food systems (Astier et al., 2019). However, commercial

\* Corresponding author.

\*\* Corresponding author.

E-mail addresses: [jose.virgen@ciad.mx](mailto:jose.virgen@ciad.mx) (J.J. Virgen-Ortiz), [mastier@ciga.unam.mx](mailto:mastier@ciga.unam.mx) (M. Astier).

tortillas made from corn flours have gradually replaced the handmade ones made with nixtamalized maize. Even though Mexican consumers prefer the handmade tortillas because they are “natural” and due to its sensory attributes, the commercial ones are mainly consumed because they are cheaper and more convenient to get it (García-Sempere et al., 2019).

The color of maize is important to some consumers in Mexico as well in Central and South America, and white maize is preferred for food consumption (Gwirtz and Garcia-Casal, 2014). However, recently the pigmented maize has received considerable interest from a nutraceutical perspective due to their potential health benefits. Pigmented genotypes as blue, purple, and red are the most common Mexican maize and have been identified as suitable for tortilla production. Phytochemical contents and their antioxidant activity in this type of maize such as phenolic, anthocyanins and carotenoids have been documented (Mendoza-Díaz et al., 2012). The health beneficial effects of these bioactive compounds have been mainly attributed to their antioxidant and antiradical capacity but they also increase the activity of endogenous antioxidant enzymes and exhibit antimutagenic, anticarcinogenic and chemoprotective properties (Jing et al., 2008; Mendoza-Díaz et al., 2012). However, even though maize is an important source of plant bioactive metabolites, it needs to be processed into final food previously to consumption, which may change or degrade their bioactive compounds (Das et al., 2017). In this sense, it has been reported that the nixtamalization process reduces antioxidant capacity and total phenolics when compared to raw kernels and such losses vary according to the type of corn (de la Parra et al., 2007).

Some studies have documented on some nutritional properties and bioactive compounds of different maize tortillas. Hernández-Martínez et al. (2016) studied the phenolic content and antioxidant activity of blue tortillas from some Mexican maize landraces. They found that tortillas made from “Elotes conicos” blue maize landrace had the highest antioxidant activity. Corrales-Bañuelos et al. (2016) evaluated the effect of traditional nixtamalization and lime cooking extrusion processes on total carotenoids content and major carotenoids, as well as lipophilic antioxidant activity of tortillas elaborated from Mexican pigmented maize landrace. They concluded that nixtamalized tortillas had higher content of total carotenoids and oxygen radical absorption capacity. Bello-Pérez et al. (2015) evaluated the effect of nixtamalization with calcium salts on antioxidant capacity and carbohydrate digestibility during the production of blue maize tortillas. These researchers found that the nutraceutical properties (antioxidant capacity and dietary fiber content) of blue tortillas are enhanced when made using the nixtamalization process with calcium salts.

Although the contribution of previous researches to the knowledge of the nutraceutical content of tortillas made from some maize landraces, the nutritional characteristics of tortillas from landraces maize cultivated under traditional agronomic management practices in the Purhépecha Region in the Mexican state of Michoacán remain unexplored. In this region, handmade tortilla making is one of the most important activities supporting local economy and conservation of native maize varieties (Astier et al., 2019). Therefore, the objective of this work was to evaluate the nutritional properties and bioactive compounds of tortillas prepared by a small-scale artisan food manufacturer from blue maize landrace and white maize and to compare them to those sold by a large food retailer. The present study on handmade fresh tortillas from locally-grown maize landraces free from agrochemicals, represents an opportunity to enhance its consumption and take advantage of its nutritional and nutraceutical properties for human health benefits. Additionally, this study will promote the conservation of this genetic resource supporting the livelihood of the peasant and indigenous communities that produce this crop under agroecological practices.

## 2. Materials and methods

This study was performed with fresh tortillas as available to the consumers. Three contrasting tortillas corresponding to handmade blue

maize tortillas (HBMT), handmade white maize tortillas (HWMT), and commercially produced white maize tortillas (CWMT). Both maize used for preparing tortillas by a small-scale artisan tortilla producer were purchased from a traditional farmer in the Purhépecha plateau region in the state of Michoacán, México. Blue and white maize landraces were cultivated under traditional agronomic management practices. Three batches of tortillas were made on different days for each type. Similarly, three batches of tortillas CWMT, on different days, were bought in a large food retailer (supermarket) that produces its own tortillas.

### 2.1. Masa and tortilla production

Handmade tortillas were collected fresh, on the time of production, from a micro-scale tortilla producer in the Pátzcuaro, Michoacán (México) area. Handmade fresh tortillas were elaborated using masa produced from traditional nixtamalized maize. For this purpose, blue maize landrace and white maize was lime-cooked with 1% calcium hydroxide solution in a proportion of 1:2 (maize: solution) at 92–95 °C for 45 min and then allowed to stand overnight. The cleaned and washed lime-cooked maize was stone-ground into masa using a commercial mill. The fresh masa (approximately 40 g per tortilla) was hand-molded into little ball and pressed to form thin flat discs using a manual tortilla pressing machine. The resulting masa discs were baked on a hot griddle called a “comal” using an ecological wood-fired oven (Patsari cookstove) (Maserá et al., 2005). Tortillas were baked at 220 ± 10 °C for 30 ± 5 s on one side, followed by 20 ± 3 s on the other side, and then turned them back on the first side until they expanded. Tortillas were allowed to cool down for 45 min at 25 ± 3 °C and packaged in polyethylene bags, and transported to laboratory for the different studies.

### 2.2. Proximal chemical analysis and calcium content

Proximate composition analysis of the maize tortillas were determined by the AOAC methods (Official Methods of, 2006). The analysis of insoluble and soluble dietary fiber was performed by the enzymatic-gravimetric method (AOAC 991.43), and the results were expressed in g/100 g fresh weight basis (FW). The content of calcium was determined by inductively coupled plasma-atomic emission spectrometry according to United States Environmental Protection Agency 6010 B method (USEPA, 1996).

### 2.3. Extraction and determination of total soluble phenolic content

Phenolic compounds in tortillas samples were extracted as previously documented (Palafox-Carlos et al., 2012) with minor changes. A sample of 2 g was mixed with 5-mL chilled methanol:water (80:20, v/v) for 10 min in a shaker at 100 rpm and then the mixture was sonicated for 30 min at 20 ± 2 °C in an ultrasonic bath. After sonication the mixture was centrifuged (10,000×g, 10 min) and the supernatant recovered. The pellet was re-extracted once time as described above and both supernatants were combined. This extract was utilized to quantify total soluble phenolics, to evaluate antioxidant activity and for the determination of phenolic acids profile by HPLC. The total soluble phenolic content was measured using 0.2 N Folin-Ciocalteu reagent according to the method adapted from Singleton et al. (1999). Results were expressed as mg gallic acid equivalents (GAE) per 100 g DW.

### 2.4. Determination of total anthocyanins and antioxidant capacity

The content of total anthocyanins was evaluated using a colorimetric method previously described (Carreño et al., 1997) and expressed as mg of cyanidin-3-glucoside per 100 g of tortilla DW. The free-radical-scavenging activity was measured by DPPH radical method as previously described in Floegel et al. (2011). Also the antioxidant capacity was evaluated by ABTS radical scavenging activity measured as previously described (Re et al., 1999). The antioxidant capacity in each

case was expressed as percentage of inhibition.

## 2.5. Determination of phenolic composition

*p*-Coumaric, 4-hydroxy-3-methoxycinnamic (ferulic), syringic, 4-hydroxybenzoic, and caffeic acids were quantified using an HPLC (Agilent model 1260, Germany) equipped with a photodiode array detector (Agilent model G4212B, 320 and 280 nm) and a 4.6 × 250 mm XSelect® HSSC18 (5 μm) (Waters, Milford, MA, USA). An isocratic mobile phase (at 1 mL/min) consisting of 30% and 70% aqueous formic acid was used. Standard solutions of phenolic acid in methanol at concentrations between 0.036 and 20 μg/mL were used to produce a calibration curve. The retention times were 6.36 min (*p*-coumaric acid, 280 nm), 12.62 min (4-hydroxy-3-methoxycinnamic acid, 320 nm), 17.88 min (syringic acid, 320 nm), 6.04 min (4-hydroxybenzoic acid, 280 nm), and 6.92 min (caffeic acid, 320 nm). The limits of quantification (LOQ) were 0.068 (*p*-coumaric acid), 0.060 (3-hydroxy-4-methoxycinnamic acid), 0.036 (syringic acid), 0.104 (4-hydroxybenzoic acid) and 0.058 μg/mL (caffeic acid).

## 2.6. Statistical analysis

Results were expressed as mean ± standard deviation of three independent determinations. Data were evaluated using one-way ANOVA, and the significance of their variance was verified by means of the Tukey test ( $p < 0.05$ ) performed using Matlab software (MathWorks, Inc.). Principal component analysis was carried out using the composition parameters (protein, fat, calcium, ash, moisture, dietary fiber, antioxidant capacity, ferulic acid, and anthocyanins) as variables in the derivation of the principal components, and to elucidate the similarity of tortillas samples.

## 3. Results and discussion

### 3.1. Chemical composition of maize tortilla

The chemical composition of HBMT, HWMT, and CWMT are shown in Table 1. The moisture contents of the different tortillas varied between values of 45.9%–48.1% with no significant difference ( $p < 0.05$ ). Moisture content of tortillas has been documented to be between 35 and 50%, values that depend on the maize variety used and the conditions during the nixtamalization process (Rendón-Villalobos et al., 2009), (Hernández-Uribe et al., 2007). The lowest protein content was found in CWMT, and both handmade tortillas showed the highest values. The protein content for handmade tortillas from blue maize is consistent with results reported by (Hernández-Uribe et al., 2007) for tortillas from blue maize. Diverse studies using nixtamalized corn flour and masa for the elaboration of white tortillas have reported protein content between 7.5% and 9.6% (Rendón-Villalobos et al., 2009), (Agama-Acevedo et al., 2004). CWMT exhibited a higher lipid content ( $3.42 \pm 0.15\%$ ) than both handmade tortillas HBMT ( $1.87 \pm 0.09\%$ ) and HWMT ( $1.78 \pm 0.08\%$ ). Argüello-García et al., 2017 reported a lipid content of 3.14% for tortillas prepared from commercial maize flour. Other authors have reported that tortillas made with commercial corn flours had lipid contents

between 2.75 and 4.10% (Agama-Acevedo et al., 2004). Fat content in corn tortillas depends on nixtamalization conditions, because lipids from the germ of the kernel can be solubilized during the alkaline treatment or during the steeping time, and on the maize variety used (Agama-Acevedo et al., 2004). As shown in Table 1 the ash content was significantly higher ( $p < 0.05$ ) in handmade tortillas than in CWMT. Our results are in agreement to the ash values previously reported for tortillas from nixtamalized maize of diverse maize cultivars (Agama-Acevedo et al., 2004). The ash content in both types of handmade tortillas increased due to the lime that was incorporated into the corn kernels during the nixtamalization process.

As regards to total dietary fiber, HBMT presented a high amount of total dietary fiber (15.72 g/100 g FW), similar to results reported by other authors (Bello-Pérez et al., 2015) for tortillas from blue maize subject to non-traditional nixtamalization with calcium salts. In general, total dietary fiber contents in HWMT and CWMT (Table 1) were similar to those ranges value previously reported (Bello-Pérez et al., 2014). All tortillas had higher content of insoluble dietary fiber than soluble one. The insoluble dietary fiber fraction is important because of the more rapid transit through the stomach and intestine and its bulking properties (Bello-Pérez et al., 2014). Daily intake of dietary fiber has been recommended, mainly due to can contribute to gastrointestinal health and the evidence of cardiovascular as well as other health benefits such as their ability to reduce cholesterol levels and improve glucose tolerance and the insulin response. The recommended adequate intake of dietary fiber for adults is 25–38 g/day (King et al., 2012). In this context, the intake of tortillas can provide *per se* a significant portion of the recommended daily intake of dietary fiber, especially the handmade tortillas from blue maize landrace.

### 3.2. Calcium content

As shown in Fig. 1, the calcium content was significant highest ( $p < 0.05$ ) in HWMT ( $155.5 \pm 4.5$  mg/100 g) followed by HBMT ( $136.6 \pm 3.2$  mg/100 g). The lowest calcium content ( $42.1 \pm 0.9$  mg/100 g) was found in CWMT. (Maya-Cortés et al., 2010) reported a calcium content of 162.98 mg/100 g for tortillas prepared by the traditional method of nixtamalization from white corn and of 105.42 mg/100 g for tortillas prepared using an ecological method of nixtamalization from the same maize. The calcium content found in CWMT was lower than the reported by other authors in tortillas prepared with instant commercial corn flour (Maya-Cortés et al., 2010), (Mora-Avilés et al., 2007). It is known that the production of nixtamalized maize flour by the tortilla industry does not use the traditional method for nixtamalization. In the conventional tortilla industry, the maize for nixtamalization is processed through a different procedure, avoiding the use of long periods of cooling and soaking. In this sense, the lower value found for calcium in CWMT could be due to low steeping time that does not permit the entrance of calcium into the maize kernel, and the intentional reduction of most of the pericarp during the industrial process (Mora-Avilés et al., 2007), (Palacios-Fonseca et al., 2009), (Cornejo-Villegas et al., 2010). Previous studies have shown that the main calcium content is located in the pericarp and the calcium in the corn flour has a dependence on the pericarp thickness (Gutierrez et al., 2007). In the present study, the low

**Table 1**  
Chemical composition of different maize tortillas (%).

Sample	Moisture	Protein	Fat	Ash	Insoluble dietary fiber	Soluble dietary fiber
<sup>1</sup> CWMT	45.9 ± 3.5 <sup>a</sup>	8.66 ± 0.20 <sup>a</sup>	3.42 ± 0.15 <sup>a</sup>	1.27 ± 0.01 <sup>a</sup>	11.27 ± 0.96 <sup>a</sup>	0.36 ± 0.11 <sup>a</sup>
<sup>2</sup> HWMT	47.8 ± 3.9 <sup>a</sup>	9.63 ± 0.15 <sup>b</sup>	1.78 ± 0.08 <sup>b</sup>	1.80 ± 0.07 <sup>b</sup>	12.65 ± 0.94 <sup>a</sup>	0.56 ± 0.10 <sup>a</sup>
<sup>3</sup> HBMT	48.1 ± 3.6 <sup>a</sup>	9.45 ± 0.24 <sup>b</sup>	1.87 ± 0.09 <sup>b</sup>	1.85 ± 0.05 <sup>b</sup>	14.84 ± 1.06 <sup>b</sup>	0.88 ± 0.10 <sup>b</sup>

<sup>1</sup>CWMT: commercially produced white maize tortillas (supermarket).

<sup>2</sup>HBMT: handmade blue maize tortillas.

<sup>3</sup>HWMT: handmade white maize tortillas.

Values presented as means of three replicates ± standard error. Values followed by different literals into the same column indicate statistical differences ( $p < 0.05$ ).

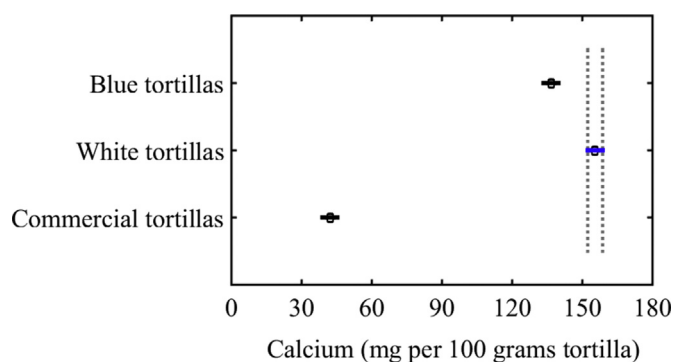


Fig. 1. Multiple comparison test (Tukey) for calcium content in both artisan handmade tortillas from blue maize landrace and white maize, and tortillas from a commercial producer (supermarket).

level of calcium content found in tortillas from commercial producer is cause for concern from a nutritional point of view because tortillas are the main source of calcium available at low cost.

### 3.3. Free phenolic and anthocyanins content

Free phenolic and anthocyanins content in handmade tortillas from blue maize landrace and white maize hybrid, and from commercial tortillas producer are shown in Table 2. HBMT had the highest free phenolic content at  $120.8 \pm 4.2$  mg GAE/100 g DW (based on the ANOVA,  $p < 0.05$ ), followed by HWMT ( $74.4 \pm 3.1$  mg GAE/100 g DW), and lastly, CWMT ( $38.4 \pm 1.6$  mg GAE/100 g DW). These values were higher than those reported by other researchers (Aguayo-Rojas et al., 2012), who studied tortillas from extruded pigmented Mexican maize flours. Hernández-Martínez et al. (2016) reported a total soluble phenolic content of 59.3 mg GAE per 100 g for tortillas from white corn, and a content of  $81.6 \pm 8.9$  mg GAE/100 g DW for tortillas from blue maize “Elotes Cónicos”.

Anthocyanins are the main phytochemicals responsible for the final tortilla blue color. Foods rich in anthocyanin have been proposed as potential agents to decrease the risk of colon cancer by inhibiting proliferation of human colon cancer cells *in vitro* (Jing et al., 2008). In this sense, anthocyanins contained in the tortillas made with blue corn of the Mixteco race showed antiproliferative effects on breast and prostate cancer cells (Herrera-Sotero et al., 2020). Additionally, anthocyanins have been proposed as modulators of gut microbiota that contribute to obesity control and these bioactive metabolites may be considered to have a prebiotic action (Jamar et al., 2017). As shown in Table 2 the anthocyanins content in HBMT was 145 fold higher than in HWMT, whilst in CWMT were at very low levels ( $\leq 0.01$  mg cyanidin 3-glucoside equivalents per 100 g of sample). Our reported anthocyanin values are similar than those found in tortillas produced from traditional nixtamalized white maize reported by Mora-Rochin et al. (2010)

**Table 2**  
Phenolic and anthocyanins content of different maize tortillas.

Sample	<sup>1</sup> Phenolic mg/100 g	<sup>2</sup> Total anthocyanins mg/100 g
Tortillas from commercial producer	$38.4 \pm 1.6^a$	$\leq 0.01$
Handmade tortillas from white maize	$74.4 \pm 3.1^b$	$0.15 \pm 0.04^a$
Handmade tortillas from blue maize	$120.8 \pm 4.2^c$	$21.80 \pm 1.23^b$

Values followed by different literals into the same column indicate statistical differences ( $p < 0.05$ ).

<sup>1</sup> Expressed as mg gallic acid equivalents.

<sup>2</sup> Expressed as mg cyanidin 3-glucoside equivalents.

( $0.19 \pm 0.05$  mg cyanidin-3-glucoside equiv/100 g DW). However, in the present study, the anthocyanins content was higher than those documented by these authors for tortillas elaborated from nixtamalized flours of blue maize ( $13.80 \pm 0.14$  mg cyanidin-3-glucoside equivalents/100 g DW). The anthocyanins content value found in this study for tortillas from blue maize is comparable to those reported by Hernández-Martínez et al. (2016) ( $24.5 \pm 4.8$  mg cyanidin-3-glucoside equivalents/100 g DW) for tortillas prepared from blue maize “Chalqueño”.

### 3.4. Phenolic acid profile

There is currently much interest in the potential health benefits and antioxidant activities associated with individual phenolic acids (Liu et al., 2015), (Gaxiola-Cuevas et al., 2017). The contents of phenolic acids in tortillas are given in Table 3. Five major phenolic acids compounds, including ferulic, *p*-coumaric, caffeic, syringic and 4-hydroxybenzoic acid, were identified and quantified in the extracts of the samples assayed (Table 3). Our findings on all the phenolic acids are in agreement with that of Das and Singh (2016) found in botanical fractions of Indian specialty maize (*Zea mays* L.) genotypes. In the same way, Gaxiola-Cuevas et al. (2017) reported a similar phenolic acids profile in free phenolic fraction of tortillas of Mexican native maize landraces made from nixtamalization or lime cooking extrusion processes. The total content of phenolic acids of both handmade tortillas HBMT ( $6903 \pm 94$   $\mu$ g/100 g DW) and HWMT ( $5949 \pm 198$   $\mu$ g/100 g DW) was significantly ( $p < 0.05$ ) higher compared to that of CWMT ( $4318 \pm 205$   $\mu$ g/100 g DW). Our reported values for total free phenolic acid in tortillas from commercial producer are similar than the total free phenolic acids content reported by (Gaxiola-Cuevas et al., 2017) ( $4.4 \pm 0.3$  mg/100 g DW) for tortillas elaborated from nixtamalized Mexican maize landrace. The main phenolic acid found in handmade tortillas was ferulic acid followed by 4-hydroxybenzoic acid. This last phenolic acid was the most abundant one detected in tortillas from commercial producer. Interestingly, HBMT contained more than 4.5 times higher content of ferulic acid compared to CWMT (Table 3). Ferulic acid exhibits antioxidant, antihyperlipidemic, hypotensive, antimicrobial, anticarcinogenic and radioprotective properties. Recently it has been reported that ferulic acid inhibits the proliferation and promotes apoptosis in an osteosarcoma cell line (Wang et al., 2016). Since the beneficial effects of ferulic acid on human health, HBMT receive additional nutritive value. As depicted in Table 3, *p*-coumaric acid was present almost exclusively in both HWMT and CWMT, whereas HBMT were practically devoid of this compound.

### 3.5. Antioxidant capacity

Table 4 depicts the antioxidant capacity for the three tortillas types. CWMT had the lowest antioxidant capacity compared to both handmade tortillas HWMT and HBMT. HBMT had 2.0- and 2.2-fold stronger free radical scavenging capacity in DPPH and ABTS system, respectively, than CWMT. HBMT had a higher ( $p < 0.05$ ) antioxidant capacity compared to

**Table 3**  
Phenolic acid composition of different maize tortillas.

Phenolic acid ( $\mu$ g phenolic acid/100 g DW)	Tortilla from commercial producer	Handmade tortilla from white maize	Handmade tortilla from blue maize
Ferulic	$1127 \pm 48^a$	$3547 \pm 102^b$	$5151 \pm 21^c$
<i>p</i> -Coumaric	$789 \pm 35^a$	$517 \pm 15^b$	$13 \pm 1^c$
Caffeic	$521 \pm 22^a$	$186 \pm 8^b$	$222 \pm 10^c$
Syringic	$111 \pm 9^a$	$571 \pm 20^b$	$451 \pm 19^c$
4-Hydroxybenzoic	$1770 \pm 91^a$	$1129 \pm 53^b$	$1066 \pm 43^b$
Total phenolic acid	$4318 \pm 205^a$	$5949 \pm 198^b$	$6903 \pm 94^c$

Data correspond to the means  $\pm$  standard deviation. Means in the same line that do not share the same superscript letters (a-c) are significantly different ( $p < 0.05$ , Tukey test).

**Table 4**

Antioxidant capacity values of different maize tortillas as determined by ABTS and DPPH radical scavenging methods.

Sample	% Inhibition	
	<sup>1</sup> ABTS	<sup>2</sup> DPPH
Tortillas from commercial producer	32.0 ± 1.2 <sup>a</sup>	34.6 ± 1.4 <sup>a</sup>
Handmade tortillas from white maize	41.1 ± 1.8 <sup>b</sup>	43.6 ± 2.2 <sup>b</sup>
Handmade tortillas from blue maize	70.8 ± 2.2 <sup>c</sup>	78.6 ± 3.1 <sup>c</sup>

Data correspond to the means ± standard deviation. Means in the same column that do not share the same superscript letters (a-c) are significantly different ( $p < 0.05$ , Tukey test).

<sup>1</sup>ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) method.

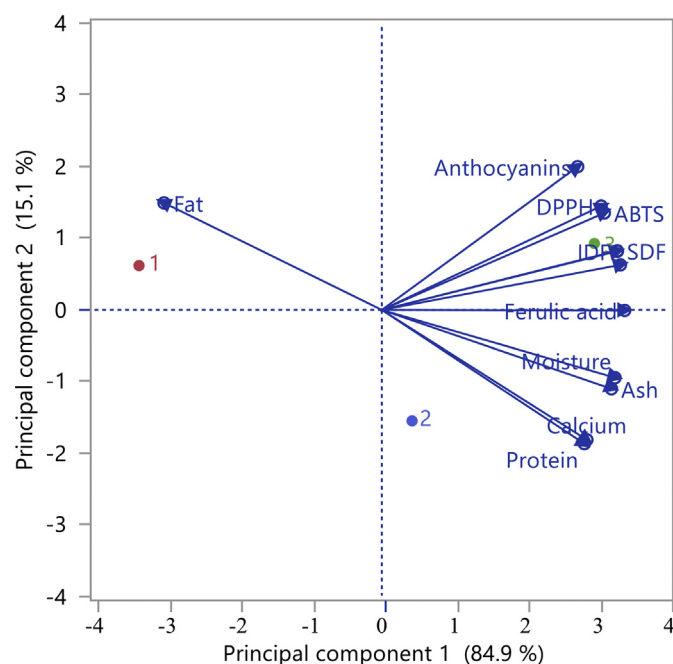
<sup>2</sup>DPPH: 2,2-diphenyl-1-picrylhydrazyl method.

others tortillas evaluated in this work, and this is attributed to their anthocyanins content that are considered potent antioxidants. This is in accordance to [Poza-Insfran et al. \(2007\)](#) who reported that products made from the blue genotypes contained a higher antioxidant activity than their counterparts produced from white maize. Other authors ([de la Parra et al., 2007](#)) did not find significant differences in antioxidant capacity between tortillas from blue and white maize.

Summarizing, the data obtained in this study could motivate some consumers to prefer consumption of artisanal tortillas due to its superior nutritional value and content of health promoting molecules.

### 3.6. Principal component analysis

To determine and verify the variations as well as find similarities among the tortilla samples, the nutritional composition data were submitted to principal component analysis (PCA). The PCA shows how the samples are distributed by different variables, and it can be used to separate samples that are similar or distinct to one another. According to principal component analysis ([Fig. 2](#)), the first two components represented 100% of the total variance in the nutritional analyzed variables. The first principal component (PC1) alone accounted for 84.9% of the total variance and predominantly determine the differentiation among



**Fig. 2.** Biplot graph with two principal components of the chemical and nutritional content found in tortillas. 1: commercially produced white maize tortillas (supermarket); 2: handmade white maize tortillas; 3: handmade blue maize tortillas.

the analyzed samples. PC1 correlated with ferulic acid, phenolics, soluble and insoluble dietary fiber, ABTS and DPPH scavenging capacity, ash, and calcium content. On the other hand, the PCA indicates that the second principal component (PC2) accounted for 15.1% of the total variance and it is determined by the contents of anthocyanins and protein. The biplot graphic of PC1 and PC2 ([Fig. 2](#)) shows quite well the separation among the tortillas samples according to their respective scores. Samples with higher protein, calcium, ash, ferulic acid, dietary fiber, DPPH and ABTS scavenging capacity, and anthocyanin content are located at the right side of the graph (HWMT and HBMT samples), while the CWMT with higher fat content is located at the left side of the graph. Overall, the PCA allowed the analyzed tortilla samples to be clearly discriminated based on their main nutritional and bioactive compounds.

## 4. Conclusions

The results of this study demonstrate that there are considerable differences in the nutritional properties and bioactive compounds content between industrial and artisan fresh tortillas from maize landraces. The handmade blue maize tortillas exhibited noticeably higher total dietary fiber content and *in vitro* antioxidant capacity. The strong antioxidant capacity of the blue maize tortillas can be attributed to the presence of a higher amount of anthocyanins and phenolic acids. Overall, this research indicates that artisanal tortillas from blue maize landrace may represent a better source of nutrients and phytochemicals, and can be an alternative for consumers seeking for healthy and functional foods.

## Funding

This work was supported by the Mexican Commission for the Knowledge of Biodiversity (CONABIO), Mexican Council for Science and Technology (Cátedras CONACyT project 770), and the National Autonomous University of Mexico (DGPA-UNAM).

## Declaration of Competing Interest

No potential conflict of interest was reported by the authors.

## CRediT authorship contribution statement

**Citlali Colín-Chávez:** Methodology, Investigation, Validation, Formal analysis. **Jose J. Virgen-Ortiz:** Conceptualization, Writing - original draft, Formal analysis, Supervision. **Luis E. Serrano-Rubio:** Methodology, Investigation. **Miguel A. Martínez-Téllez:** Writing - review & editing. **Marta Astier:** Conceptualization, Writing - review & editing, Funding acquisition.

## Acknowledgments

The authors wish to thank Emmanuel Aispuro Hernández for his assistance in proofreading the manuscript.

## References

- Agama-Acevedo, E., Rendón-Villalobos, R., Tovar, J., Paredes-López, O., Islas-Hernández, J.J., Bello-Pérez, L.A., 2004. *In vitro* starch digestibility changes during storage of maize flour tortillas. *Food/Nahrung* 48 (1), 38–42. <https://doi.org/10.1002/food.200300352>.
- Aguayo-Rojas, J., Mora-Rochín, S., Cuevas-Rodríguez, E.O., Serna-Saldivar, S.O., Gutierrez-Urbe, 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 Hum. Nutr.* 67 (2), 178–185. <https://doi.org/10.1007/s11130-012-0288-y>.
- AOAC, 2006. Official Methods of Analysis of AOAC International, eighteenth ed. Association of Official Analytical Chemists (AOAC), Gaithersburg, MD.
- Argüello-García, E., Martínez-Herrera, J., Córdova-Téllez, L., Sánchez-Sánchez, O., Corona-Torres, T., 2017. Textural, chemical and sensorial properties of maize tortillas fortified with nontoxic *Jatropha curcas* L. flour. *CyTA - J. Food* 15 (2), 301–306. <https://doi.org/10.1080/19476337.2016.1255915>.

- Astier, M., Odenthal, G., Patricio, C., Orozco-Ramírez, Q., 2019. Handmade tortilla production in the basins of lakes Pátzcuaro and Zirahuén, Mexico. *J. Maps* 15 (1), 52–57. <https://doi.org/10.1080/17445647.2019.1576553>.
- Bello-Pérez, L.A., Flores-Silva, P.C., Agama-Acevedo, E., de Dios Figueroa-Cardenas, J., Lopez-Valenzuela, J.A., Campanella, O.H., 2014. Effect of the nixtamalization with calcium carbonate on the indigestible carbohydrate content and starch digestibility of corn tortilla. *J. Cereal. Sci.* 60 (2), 421–425. <https://doi.org/10.1016/j.jcs.2014.05.001>.
- Bello-Pérez, L.A., Flores-Silva, P.C., Camelo-Méndez, G.A., Paredes-López, O., Figueroa-Cárdenas, J.d.D., 2015. Effect of the nixtamalization process on the dietary fiber content, starch digestibility, and antioxidant capacity of blue maize tortilla. *Cereal Chem.* 92 (3), 265–270. <https://doi.org/10.1094/CCHEM-06-14-0139-R>.
- Bello-Pérez, L.A., Osorio-Díaz, P., Agama-Acevedo, E., Gonzalez-Soto, R.A., 2016. Functional and beneficial properties of corn tortilla. In: Kristbergsson, K., Ötles, S. (Eds.), *Functional Properties of Traditional Foods*. Springer US, Boston, MA, pp. 139–155. [https://doi.org/10.1007/978-1-4899-7662-8\\_11](https://doi.org/10.1007/978-1-4899-7662-8_11).
- Carreño, J., Almela, L., Martínez, A., Fernández-López, J.A., 1997. Chemotaxonomical classification of red table grapes based on anthocyanin profile and external colour. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 30 (3), 259–265. <https://doi.org/10.1006/food.1996.0174>.
- CEDRSSA, Centro de Estudios para el Desarrollo Rural Sustentable y la Soberanía Alimentaria, 2014. Consumo, distribución y producción de alimentos: el caso del complejo maíz-tortilla. [http://www.cedrssa.gob.mx/post\\_reportes\\_del\\_n-cedrssa-n-volumen\\_ii.htm](http://www.cedrssa.gob.mx/post_reportes_del_n-cedrssa-n-volumen_ii.htm). (Accessed 21 June 2019).
- Cornejo-Villegas, M.A., Acosta-Osorio, A.A., Rojas-Molina, I., Gutiérrez-Cortéz, E., Quiroga, M.A., Gaytán, M., Herrera, G., Rodríguez-García, M.E., 2010. Study of the physicochemical and pasting properties of instant corn flour added with calcium and fibers from nopal powder. *J. Food Eng.* 96 (3), 401–409. <https://doi.org/10.1016/j.jfoodeng.2009.08.014>.
- Corrales-Bañuelos, A.B., Cuevas-Rodríguez, E.O., Gutiérrez-Urbe, J.A., Milán-Noris, E.M., Reyes-Moreno, C., Milán-Carrillo, J., Mora-Rochín, S., 2016. Carotenoid composition and antioxidant activity of tortillas elaborated from pigmented maize landrace by traditional nixtamalization or lime cooking extrusion process. *J. Cereal. Sci.* 69, 64–70. <https://doi.org/10.1016/j.jcs.2016.02.009>.
- Das, A.K., Singh, V., 2016. Antioxidative free and bound phenolic constituents in botanical fractions of Indian specialty maize (*Zea mays* L.) genotypes. *Food Chem.* 201, 298–306. <https://doi.org/10.1016/j.foodchem.2016.01.099>.
- Das, A.K., Bhattacharya, S., Singh, V., 2017. Bioactives-retained non-glutinous noodles from nixtamalized Dent and Flint maize. *Food Chem.* 217, 125–132. <https://doi.org/10.1016/j.foodchem.2016.08.061>.
- de la Parra, C., Serna Saldívar, 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. *J. Agric. Food Chem.* 55 (10), 4177–4183. <https://doi.org/10.1021/jf063487p>.
- Dominguez-Hernandez, M.E., Zepeda-Bautista, R., Valderrama-Bravo, M.d.C., Dominguez-Hernandez, E., Hernandez-Aguilar, C., 2018. Sustainability assessment of traditional maize (*Zea mays* L.) agroecosystem in Sierra Norte of Puebla, Mexico. *Agroecol. Sust. Food Syst.* 42 (4), 383–406. <https://doi.org/10.1080/21683565.2017.1382426>.
- Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S.I., Chun, O.K., 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J. Food Compos. Anal.* 24 (7), 1043–1048. <https://doi.org/10.1016/j.jfca.2011.01.008>.
- García-Sempere, A., Morales, H., Hidalgo, M., Ferguson, B.G., Rosset, P., Nazar-Beutelspacher, A., 2019. Food Sovereignty in the city?: a methodological proposal for evaluating food sovereignty in urban settings. *Agroecol. Sust. Food Syst.* 43 (10), 1145–1173. <https://doi.org/10.1080/21683565.2019.1578719>.
- Gaxiola-Cuevas, N., Mora-Rochín, S., Cuevas-Rodríguez, E.O., León-López, L., Reyes-Moreno, C., Montoya-Rodríguez, A., Milán-Carrillo, J., 2017. Phenolic acids profiles and cellular antioxidant activity in tortillas produced from Mexican maize landrace processed by nixtamalization and lime extrusion cooking. *Plant Foods Hum. Nutr.* 72 (3), 314–320. <https://doi.org/10.1007/s11130-017-0624-3>.
- González-Ortega, E., Piñeyro-Nelson, A., Gómez-Hernández, E., Monterrubio-Vázquez, E., Arleo, M., Dávila-Velderrain, J., Martínez-Debat, C., Álvarez-Buylla, E.R., 2017. Pervasive presence of transgenes and glyphosate in maize-derived food in Mexico. *Agroecol. Sust. Food Syst.* 41 (9–10), 1146–1161. <https://doi.org/10.1080/21683565.2017.1372841>.
- Gutiérrez, E., Rojas-Molina, I., Pons-Hernandez, J.L., Guzman, H., Aguas-Angel, B., Arenas, J., Fernandez, P., Palacios-Fonseca, A., Herrera, G., Rodríguez, M.E., 2007. Study of calcium ion diffusion in nixtamalized quality protein maize as a function of cooking temperature. *Cereal Chem.* 84 (2), 186–194. <https://doi.org/10.1094/CCHEM-84-2-0186>.
- Gutiérrez-Urbe, J.A., Rojas-García, C., García-Lara, S., Serna-Saldívar, S.O., 2014. Effects of lime-cooking on carotenoids present in masa and tortillas produced from different types of maize. *Cereal Chem.* 91 (5), 508–512. <https://doi.org/10.1094/CCHEM-07-13-0145-R>.
- Gwirtz, J.A., Garcia-Casal, M.N., 2014. Processing maize flour and corn meal food products. *Ann. N. Y. Acad. Sci.* 1312 (1), 66–75. <https://doi.org/10.1111/nyas.12299>.
- Hernández-Martínez, V., Salinas-Moreno, Y., Ramírez-Díaz, J.L., Vázquez-Carrillo, G., Domínguez-López, A., Ramírez-Romero, A.G., 2016. Color, phenolic composition and antioxidant activity of blue tortillas from Mexican maize races. *CyTA - J. Food* 14 (3), 473–481. <https://doi.org/10.1080/19476337.2015.1136842>.
- Hernández-Urbe, J.P., Agama-Acevedo, E., Islas-Hernández, J.J., Tovar, J., Bello-Pérez, L.A., 2007. Chemical composition and *in vitro* starch digestibility of pigmented corn tortilla. *J. Sci. Food Agric.* 87 (13), 2482–2487. <https://doi.org/10.1002/jsfa.3008>.
- Herrera-Sotero, M.Y., Cruz-Hernández, C.D., Oliart-Ros, R.M., Chávez-Servia, J.L., Guzmán-Gerónimo, R.I., González-Covarrubias, V., Cruz-Burgos, M., Rodríguez-Dorantes, M., 2020. Anthocyanins of blue corn and tortilla arrest cell cycle and induce apoptosis on breast and prostate cancer cells. *Nutr. Canc.* 72 (5), 768–777. <https://doi.org/10.1080/01635581.2019.1654529>.
- Jamar, G., Estadella, D., Pisani, L.P., 2017. Contribution of anthocyanin-rich foods in obesity control through gut microbiota interactions. *Biofactors* 43 (4), 507–516. <https://doi.org/10.1002/biof.1365>.
- Jing, P., Bomser, J.A., Schwartz, S.J., He, J., Magnuson, B.A., Giusti, M.M., 2008. Structure–function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of colon cancer cell growth. *J. Agric. Food Chem.* 56 (20), 9391–9398. <https://doi.org/10.1021/jf8005917>.
- King, D.E., Mainous, A.G., Lambourne, C.A., 2012. Trends in dietary fiber intake in the United States, 1999–2008. *J. Acad. Nutr. Diet.* 112 (5), 642–648. <https://doi.org/10.1016/j.jand.2012.01.019>.
- Liu, L., Guo, J., Zhang, R., Wei, Z., Deng, Y., Guo, J., Zhang, M., 2015. Effect of degree of milling on phenolic profiles and cellular antioxidant activity of whole brown rice. *Food Chem.* 185, 318–325. <https://doi.org/10.1016/j.foodchem.2015.03.151>.
- Masera, O.R., Díaz, R., Berrueta, V., 2005. From cookstoves to cooking systems: the integrated program on sustainable household energy use in Mexico. *Energy Sustain. Dev.* 9 (1), 25–36. [https://doi.org/10.1016/S0973-0826\(08\)60480-9](https://doi.org/10.1016/S0973-0826(08)60480-9).
- Maya-Cortés, D.C., Figueroa Cárdenas, J.d.D., Garnica-Romo, M.G., Cuevas-Villanueva, R.A., Cortés-Martínez, R., Vélez-Medina, J.J., Martínez-Flores, H.E., 2010. Whole-grain corn tortilla prepared using an ecological nixtamalisation process and its impact on the nutritional value. *Int. J. Food Sci. Technol.* 45 (1), 23–28. <https://doi.org/10.1111/j.1365-2621.2009.02095.x>.
- Mendoza-Díaz, S., Ortiz-Valerio, M.d.C., Castaño-Tostado, E., Figueroa-Cárdenas, J.d.D., Reynoso-Camacho, R., Ramos-Gómez, M., Campos-Vega, R., Loarca-Piña, G., 2012. Antioxidant capacity and antimutagenic activity of anthocyanin and carotenoid extracts from nixtamalized pigmented creole maize races (*Zea mays* L.). *Plant Foods Hum. Nutr.* 67 (4), 442–449. <https://doi.org/10.1007/s11130-012-0326-9>.
- Mora-Avilés, A., Lemus-Flores, B., Miranda-López, R., Hernández-López, D., Pons-Hernández, J.L., Acosta-Gallegos, J.A., Guzmán-Maldonado, S.H., 2007. Effects of common bean enrichment on nutritional quality of tortillas produced from nixtamalized regular and quality protein maize flours. *J. Sci. Food Agric.* 87 (5), 880–886. <https://doi.org/10.1002/jsfa.2801>.
- Mora-Rochin, S., Gutiérrez-Urbe, J.A., Serna-Saldívar, 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. *J. Cereal. Sci.* 52 (3), 502–508. <https://doi.org/10.1016/j.jcs.2010.08.010>.
- Palacios-Fonseca, A.J., Vazquez-Ramos, C., Rodríguez-García, M.E., 2009. Physicochemical characterizing of industrial and traditional nixtamalized corn flours. *J. Food Eng.* 93 (1), 45–51. <https://doi.org/10.1016/j.jfoodeng.2008.12.030>.
- Palafios-Carlos, H., Yahia, E.M., González-Aguilar, G.A., 2012. Identification and quantification of major phenolic compounds from mango (*Mangifera indica*, cv. Ataulfo) fruit by HPLC–DAD–MS/MS–ESI and their individual contribution to the antioxidant activity during ripening. *Food Chem.* 135 (1), 105–111. <https://doi.org/10.1016/j.foodchem.2012.04.103>.
- Pozo-Insfran, D.D., Serna Saldívar, S.O., Brenes, C.H., Talcott, S.T., 2007. Polyphenolics and antioxidant capacity of white and blue corns processed into tortillas and chips. *Cereal Chem.* 84 (2), 162–168. <https://doi.org/10.1094/CCHEM-84-2-0162>.
- 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 Biol. Med.* 26 (9), 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3).
- Rendón-Villalobos, R., Agama-Acevedo, E., Osorio-Díaz, P., Tovar, J., Bello-Pérez, L.A., 2009. Proximal composition and *in vitro* starch digestibility in flaxseed-added corn tortilla. *J. Sci. Food Agric.* 89 (3), 537–541. <https://doi.org/10.1002/jsfa.3490>.
- Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*. Academic Press, pp. 152–178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1).
- USEPA, 1996. *Inductively Coupled Plasma Atomic Emission Spectroscopy Method 6010. SW-846 Test Methods for Evaluating Solid Wastes*. United States Environmental Protection Agency, Washington, DC.
- Wang, T., Gong, X., Jiang, R., Li, H., Du, W., Kuang, G., 2016. Ferulic acid inhibits proliferation and promotes apoptosis via blockage of PI3K/Akt pathway in osteosarcoma cell. *Am. J. Transl. Res.* 8 (2), 968–980.