







## Effect of the lentil flour addition on the nutritional content and polyphenols of tortillas from blue maize

## Efecto de la adición de harina de lenteja en el contenido nutricional y polifenoles en tortillas de maíz azul

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### ABSTRACT

Tortilla consumption has been disseminated throughout the world. Blue maize has gained popularity due to its higher protein content and distinctive color provided by anthocyanins. The transformation of maize into tortillas reduces nutritional components and polyphenols. The objective of this research was to elaborate a functional tortilla based on blue maize and lentils (FT), which would increase protein and polyphenols compared to traditional tortilla (CT). The FT was made with a 70:30 ratio (nixtamalized blue maize flour: lentil flour). Sensory quality, chemical composition, color, anthocyanins, polyphenols, and antioxidant activity were evaluated. Protein and ashes increased by 48 % and 7 %, in FT compared to CT, respectively. The color was lighter in FT, confirmed with increases in L\* (67.8 and 59.7) and decreases in ΔE (29.08 and 38.74) ( $p < 0.05$ ). Likewise, the total polyphenols and antioxidant activity were higher ( $p < 0.05$ ) in FT (168.1 mg GAE/100 g, db and 6,223.7 μmol TE/100 g, db), with increases of 21.4 and 82 %,-compared to CT. FT will serve as a nutritional vehicle with acceptable sensorial qualities, allowing a more excellent supply of proteins and bioactive compounds with antioxidant properties.

**KEY WORDS:** Tortilla, blue maize, lentil, protein, polyphenols, antioxidant activity.

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## RESUMEN

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El consumo de tortilla se ha expandido a través del mundo. El maíz azul ha ganado popularidad debido a su mayor aporte proteico y color característico proporcionado por antocianinas. La transformación de maíz a tortilla disminuye componentes nutricionales y polifenoles. El objetivo de esta investigación fue elaborar una tortilla funcional (FT) a base de maíz azul y lenteja, para incrementar proteínas y polifenoles respecto a la tortilla tradicional (CT). La FT fue elaborada en una relación 70:30 (harina de maíz azul nixtamalizado: harina de lenteja). Se evaluó calidad sensorial, composición química, color, antocianinas, polifenoles y actividad antioxidante. El contenido de proteínas y cenizas incrementó 48 % y 7 %, respectivamente, en FT con respecto a CT. El color fue más claro en FT, con incrementos en  $L^*$  (67.8 y 59.7) y disminuciones en  $\Delta E$  (29.08 y 38.74) ( $p < 0.05$ ). Asimismo, polifenoles totales y actividad antioxidante fue mayor ( $p < 0.05$ ) en FT (168.1 mg GAE/100 g, db y 6,223.7  $\mu\text{mol TE}/100\text{ g db}$ ), con incrementos del 21.4 y 82 %, respectivamente, con relación a CT. La FT servirá como un vehículo nutricional con características sensoriales aceptables para un mayor aporte de proteínas y compuestos bioactivos con propiedades antioxidantes.

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**PALABRAS CLAVE:** Tortilla, maíz azul, lenteja, proteína, polifenoles, actividad antioxidante.

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## Introduction

Maize tortillas (*Zea mays* L.) are popular worldwide, but their main intake is in Mexico and Central America, where they are considered a staple food. They are a source of protein, fiber, and calcium, as well as polyphenols with antioxidant properties (Mora-Rochin *et al.*, 2019; Domínguez-Hernández *et al.*, 2022). In Mexico, there is an approximate daily intake of 155 to 217 g per person in urban and rural areas (Salinas-Moreno *et al.*, 2024). Likewise, other types of products made from maize (totopos, tostadas, tamales, and nixtamal) are also widely consumed (Mora-Rochin *et al.*, 2019).

Currently, some research lines consider pigmented maize a source of genotypic and phenotypic biodiversity in exploration (Hidalgo-Ramos *et al.*, 2024; Salinas-Moreno *et al.*, 2024). However, blue maize has gained popularity among consumers because it has a different flavor from white maize and more attractive color, which is attributed to the anthocyanins presence, secondary metabolites with antioxidant (chemical and cellular), anti-inflammatory, and anticarcinogenic

properties, among others (Gaxiola-Cuevas *et al.*, 2017; Herrera-Sotero *et al.*, 2017; Domínguez-Hernández *et al.*, 2022; Gutiérrez-Llanos *et al.*, 2023; Hidalgo-Ramos *et al.*, 2024).

The traditional nixtamalization process is used mainly to produce masa and tortillas. During this process, the maize kernel is subjected to cooking in alkali ( $\text{Ca(OH)}_2$ ) and high temperature, followed by long resting times. These cooking conditions cause a decrease in labile compounds at high temperatures and alkaline pH. Likewise, amino acids, vitamins, minerals, and polyphenols with antioxidant properties leach into the cooking liquor during nixtamalization. In addition, the pericarp fraction, which is rich in polyphenols, vitamins, and fiber, is hydrolyzed, resulting in a nixtamalized flour that will give rise to corn-based products with a nutritional deficit and bioactive compounds (Mora-Rochin *et al.*, 2010; Astorga-Gaxiola *et al.*, 2023).

The aforementioned has led to the search for improvements to tortillas by fortification or enrichment with other grains or ingredients to increase nutritional quality, such as the addition of common bean (Treviño-Mejía *et al.*, 2016), jatropha (Argüello-García *et al.*, 2017), soybean (Chuck-Hernández & Serna-Saldívar, 2019), germinated soybean (Inyang *et al.*, 2019), avocado and nopal flour (Rodiles-López *et al.*, 2019), extruded amaranth (Gámez-Valdez *et al.*, 2021), and extruded chickpea (Bon-Padilla *et al.*, 2022). Fortification of tortillas with these ingredients has shown to be effective in increasing their nutritional value by improving protein, essential amino acids, and dietary fiber content, as well as increasing polyphenol content and antioxidant activity. Likewise, these authors have shown that it improves antihypertensive and hypoglycemic potential, as well as the ability to lower LDL cholesterol levels in *in vivo* studies. This demonstrates that the nutritional attributes and functional properties of foods can be improved through the combination of food matrices.

In Mexico, other grains of high intake are legumes, which are an important source of proteins, vitamins, and bioactive compounds. Lentil is a legume with high fiber content, even higher than beans and chickpeas, and possesses a low lipid content, and a low glycemic index (Brummer *et al.*, 2015; Joshi *et al.*, 2017; Shrestha *et al.*, 2023). Lentil protein is an important source of essential amino acids, particularly leucine, lysine, threonine, and phenylalanine, but is deficient in the sulfur amino acids methionine and cysteine. Incidentally, cereal proteins are a source of methionine but are low in lysine. Therefore, a combination of lentils and maize provides a complete protein profile of all essential amino acids (Samaranayaka & Khazaei, 2024). In addition to their nutritional contribution, lentils are high in phenols, flavonoids, and condensed tannins, compounds related to oxidative damage reduction, which is associated with different cardiovascular diseases, diabetes, and cancer, among others (Yeo & Shahidi, 2017; Pathiraja *et al.*, 2023).

Therefore, lentils are a promising alternative as an ingredient for the fortification of maize tortillas, to produce functional tortillas, whose intake could benefit populations that have nutritionally deficient diets and reduce the development of malnutrition diseases, such as anemia, obesity, and/or overweight (Gámez-Valdez *et al.*, 2021), in addition to being a vehicle to increase the intake of compounds with antioxidant properties. The objective of this research was to elaborate a functional tortilla with a high protein value and increased polyphenolic content from nixtamalized blue maize flour and lentil flour.

## Materials and Methods

Blue maize and lentil kernels harvested in 2022 in Sinaloa, Mexico, were used. The grains were stripped of extraneous organic and inorganic matter and stored in 500 g batches at refrigerated temperatures (5 to 10 °C) until use.

### Flour processing

The nixtamalized blue maize flour was obtained, as indicated by Mora-Rochin *et al.* (2010). The blue maize kernels were subjected to cooking (85 °C for 30 min) in alkaline medium [5.4 g  $\text{Ca}(\text{OH})_2/\text{L}$ ] maintaining a grain/cooking water ratio of 1:3. The grains, together with the cooking liquor, were left to stand for 8 h. Subsequently, the grains were washed with sufficient water for lime removal. The nixtamal obtained was dehydrated for 12 h at 55 °C and ground until a fine flour was obtained that passed through a mesh No. 80 (0.180 mm). The nixtamalized blue maize flour was stored under refrigeration until further use.

To obtain flour from lentil grains, they were crushed in a hammer mill until fine flour was obtained, which was then sieved through a mesh No. 80 (0.180 mm). The flour was stored in polyethylene bags and refrigerated until use.

### Preparation of tortillas

Functional tortillas (FT) were prepared using 500 g of flour in a 70:30 ratio (nixtamalized blue maize flour: lentil flour). To this, sufficient water was added to reach a consistency suitable for tortilla production. The dough obtained was divided into 30 g portions to be molded in a circle using a manual press. The discs formed were placed on a griddle pan to cook at  $290 \pm 10$  °C for 27 s on one side and 30 s on the back side. The tortillas were turned over again until they expanded or puffed (Mora-Rochin *et al.*, 2010). Also, for comparative purposes, control tortillas (CT), made with 100 % nixtamalized blue maize flour, were prepared using the same methodology used to elaborate the FT.

### Tortilla quality and sensory evaluation

The degree of puffing of the tortillas was evaluated subjectively, using a scale from 1 to 3, where 1 (30 %) = little or no puffing, 2 (30-70 %) = medium puffing, and 3 (70-100 %) = full puffing. The rollability test was evaluated 30 min after cooking, where the degree of fracture on the tortilla surface was observed (0 to 100 %) according to a scale of 1 to 5, where 1 = 0 %, 2 = 25 %, 3 = 50 %, 4 = 75 %, and 5 = 100 %. These tests were performed on 30 tortillas. For the sensory test, samples were used in squares at 45 °C, and provided to 100 consumers with an average age ranging from 18 to 35 years old. Consumers evaluated color, flavor, texture, and overall acceptability characteristics using a category-9 hedonic scale, where 1 = extremely dislike and 9 = extremely like (Gómez-Valdez *et al.*, 2021). Finally, the tortillas were cooled to room temperature, dried at 55 °C for 12 h, processed in a mill to obtain fine flour, and stored at -20 °C until use.

## Proximate chemical composition

The chemical composition of blue maize kernels and tortillas fortified with lentil flour (FT) and control tortillas (CT) was determined following the AOAC (Association of Official Analytical Chemists, 2005) protocol. The moisture of the samples under study was determined by the drying method at 105 °C for 24 h. Protein content was determined by the micro-Kjeldahl method (N x 6.25), while a Soxhlet apparatus and petroleum ether as solvent were used for lipid determination. For ash determination, the samples were incinerated at 550 °C. The carbohydrate content was calculated as the difference between 100 and the sum of the other components. All determinations were performed in triplicate.

## Color

The methodology indicated by Gutiérrez-Llanos *et al.* (2023), with minor adjustments, was used to evaluate the color of the grains and tortillas under study. A Minolta Chroma-meter mod CR-210 (Minolta LTD, Japan) with the Ciel\*a\*b\* system was used. For this determination, 100 g of nixtamalized blue maize flour, lentil, and/or tortilla (FT and CT) were taken and placed in a Petri dish of 15 cm diameter. The values of L\*, a\*, and b\* parameters were recorded. The determination was performed in triplicate.

## Total anthocyanins

Total anthocyanin content was determined according to the method by Abdel-Aal & Hucl (1999) and Mora-Rochin *et al.* (2016). Samples (maize, lentils, and/or tortillas) were extracted with acidified cold methanol (95 % methanol and 1 N HCl, 85:15, v/v). The mixture was centrifuged at 3000 x g for 10 min, and the supernatant was collected. The absorbance of the extracts was recorded at 535 and 700 nm (turbidity correction) on a microplate reader (Synergy HT, Bio-Tek Instrument, Inc., Winooski VT, USA). The concentration (C) of anthocyanins was expressed as mg cyanidin 3-glucoside equivalents (CGE)/100 g dry basis (db). The molar extinction coefficient ( $\epsilon = 25965 \text{ Abs/M} \times \text{cm}$ ) and molecular mass (MW = 449.2 g/mol) of CGE were used to perform calculations in the following equation:

$$C = \left[ \left( \frac{A_{535 \text{ nm}} - A_{700 \text{ nm}}}{\epsilon} \right) \times (\text{total volume of extract}) \times \text{MW} \right] / (\text{sample weight})$$

## Soluble and insoluble polyphenol extraction

For the extraction of these fractions, the method indicated by Mora-Rochin *et al.* (2010), was used. One g sample of flour (maize, lentils, and/or tortillas) was homogenized with 10 mL of cold ethanol (80 %) and stirred for 10 min. The mixture was centrifuged at 2500 g for 10 min, and the supernatant was recovered and placed to dryness. Subsequently, it was resuspended in a volume of 2 mL. The extract was stored at -20 °C until use.

The residue or pellet was digested using NaOH 2 mol/L at 90 °C for 30 min and placed in stirring at room temperature for 1 h. The mixture was acidified with concentrated HCl and stirred again for 30 min. Sample lipids were removed using hexane, and polyphenols were recovered from the reaction mixture by five washes with ethyl acetate. The final acetate fraction was evaporated at 45 °C and low pressures using a concentrator (Speed Vac Concentrator, Thermo Electron Corporation). Finally, the residue was reconstituted with 2 mL of 50 % methanol. Extracts were obtained in triplicate and stored at -20 °C until use.

### **Determination of total polyphenols**

The content of soluble and insoluble polyphenols was determined according to the Folin-Ciocalteu colorimetric method reported by Singleton *et al.* (1999). In a 96-well plate, 20 µL of the appropriate extract was added and oxidized with 180 µL of Folin-Ciocalteu's reagent. The reaction was neutralized with 50 µL of 7 % Na<sub>2</sub>CO<sub>3</sub>. The reaction mixture was incubated in the dark for 90 min. The absorbance was recorded at 750 nm on a microplate reader (Synergy HT, Biotek Instrument). Polyphenol content was expressed as milligram gallic acid equivalents (mg GAE)/100 g, dry basis (db). Total polyphenol content was calculated as the sum of the soluble and insoluble fractions.

### **Determination of antioxidant activity**

The determination of antioxidant activity using the oxygen radical absorbance capacity (ORAC) method was performed by appropriate dilution with 75 mmol/L phosphate buffer solution of the soluble and insoluble polyphenol extracts. 25 µL of each dilution were placed in a black 96-well plate and homogenized with 150 µL of 0.1 mmol/L fluorescein. 25 µL of the free radical generator AAPH 200 mmol/L was used for fluorescence loss. After 30 min of incubation at 37 °C, the fluorescence loss was recorded every 2 min for 1 h at 485 nm excitation and 538 nm emission on a microplate reader (Synergy HT Multi-Detection Microplate Reader; Bio Tek Instruments, Inc., Winooski, VT, USA). The results obtained were expressed as micromole (µmol) Trolox equivalents (TE)/100 g, dry basis (db) (Mora-Rochin *et al.*, 2010).

### **Statistical analysis**

Data were expressed as the mean ± standard deviation. Statistical analysis and comparison of means were performed with the MINITAB version 19 statistical package. Statistical analysis was performed with Student's t-test for paired two-sample means with an  $\alpha$  of 0.05.

## **Results and Discussion**

### **Grain characterization**

#### **Proximate chemical composition**



The proximate chemical composition of the grains used was expressed as a percentage on a dry basis (% db) (Table 1). The chemical composition found in this study is similar to that obtained by other authors for lentil grain, where a 25.8 % protein content, 2.2 % ash, 1.1 % lipids, and 60.1 % carbohydrates are reported (Urbano *et al.*, 2007; Aslani *et al.*, 2015; Gasinski & Kawa-Rygielska, 2022; Shrestha *et al.*, 2023). While, in maize grain, Ramírez-Jiménez *et al.* (2023) reported values of 8.68, 4.43, and 1.20 % for protein, lipids, and ash, respectively, values that are slightly different from those found in this study. These differences could be attributed to the variety of grain used and the climatic conditions where the grains were grown (Domínguez-Hernández *et al.*, 2022).

It is noteworthy that lentil grain has higher protein ( $25.3 \pm 0.68$  %) and ash ( $2.4 \pm 0.07$  %) content ( $p < 0.05$ ) than blue maize grain. In contrast, lipid and carbohydrate content is lower ( $p < 0.05$ ) in lentils than in maize (Table 1). Lentil grain is an important source of protein (22-26 %, db) and contains vitamins, minerals, and dietary fiber (Pathiraja *et al.*, 2023). Incidentally, it has been reported that the lipid and trans-fatty acid contents are low compared to other legumes, such as chickpeas, peas, lupin, and beans (Halima *et al.*, 2022). Because of all these nutritional attributes of lentil grain, it is necessary to increase its intake and use it as an alternative ingredient to fortify essential products in the Mexican diet, such as maize tortillas.

**Table 1. Chemical characteristics, color, and phytochemical content of maize and lentil grains**

Parameter / Feature	Blue maize	Lentil
<b>Nutritional composition<sup>1</sup></b>		
Proteins	$9.08 \pm 0.094^b$	$25.3 \pm 0.68^a$
Lipids	$3.90 \pm 0.08^a$	$0.8 \pm 0.05^b$
Ash	$1.62 \pm 0.001^b$	$2.4 \pm 0.07^a$
Carbohydrates	$85.32 \pm 0.16^a$	$71.4 \pm 0.67^b$
<b>Color</b>		
L*	$57.74 \pm 0.26^b$	$68.4 \pm 1.2^a$
a*	$5.31 \pm 0.01^a$	$-1.3 \pm 0.0^b$
b*	$1.82 \pm 0.15^b$	$21.6 \pm 0.4^a$
$\Delta E$	$39.02 \pm 0.3^a$	$36.0 \pm 0.8^a$
<b>Phytochemicals</b>		
Total anthocyanins <sup>2</sup>	$20.74 \pm 1.43$	ND
Total polyphenols <sup>3</sup>	$230.2 \pm 11.7^a$	$189.3 \pm 2.9^b$
Soluble	$61.4 \pm 5.5^a$	$61.2 \pm 4.5^a$
Insoluble	$168.8 \pm 12.6^a$	$128.1 \pm 1.9^b$

Data are expressed as the mean  $\pm$  standard deviation. Different letters within the same row are different ( $p < 0.05$ ). <sup>1</sup> Values expressed as a percentage on a dry basis (db). <sup>2</sup> mg cyanidin-3-glucoside equivalents (CGE)/100 g, db. <sup>3</sup> mg gallic acid equivalents (GAE)/100 g, db. ND= not detected.

## Color

In the grains used, it was observed that the  $L^*$  parameter (achromatic coordinate of luminosity) was higher ( $p < 0.05$ ) in the lentil grain ( $68.4 \pm 1.2$ ) compared to the blue maize grain ( $57.7 \pm 0.26$ ). Likewise,  $\Delta E$  was lower in lentils ( $36 \pm 0.8$ ), indicating greater clarity. In maize grain, the variables  $a^*$  and  $b^*$  showed positive values ( $5.31 \pm 0.01$  and  $1.82 \pm 0.15$ ) indicating red and yellow tones, while, in lentils, negative  $a^*$  ( $-1.31 \pm 0.0$ ) is indicative of green tones and positive  $b^*$  ( $21.6 \pm 0.4$ ) of yellow tones. Values in lentil grain agree with those reported by Erdogan (2015) in the variables  $L^*$ ,  $a^*$ , and  $b^*$  (71, 0.72, and 17). While in blue maize grain, Cetin-Babaoğlu *et al.* (2021) reported values of 47.12, 10.64, and 6.81, respectively, for the same variables. The differences found in regard to kernel tone could be due to the different genotypes used.

## Phytochemicals

Anthocyanins are secondary metabolites in blue maize that provide the characteristic color of their kernels and products. Their content was expressed as milligram equivalents of cyanidin-3-glucoside on a dry basis (mg CGE/100 g, db). Table 1 shows that blue maize kernels have a concentration of  $20.74 \pm 1.43$  mg CGE/100 g, db, while, in lentil kernels, they were not detected. The anthocyanin content may vary depending on the maize genotype used, as well as the harvest season, among other factors that may affect the concentration of these secondary metabolites (Hidalgo-Ramos *et al.*, 2024). In a study by Hidalgo-Ramos *et al.* (2024) with 300 genotypes of pigmented maize, the authors found variations in anthocyanin content ranging from 24.8 to 31.7 mg CGE/100 g, db. Other studies conducted by Herrera-Sotero *et al.* (2017) in blue maize grain reported values of 70.50 mg CGE/100 g, db.

Total polyphenol content was higher ( $p < 0.05$ ) in blue maize grain ( $230 \pm 11.7$  GAE/100 g, db) compared to lentil grain ( $189.3 \pm 2.9$  mg GAE/100 g, db) ( $p < 0.05$ ). Likewise, the fraction of insoluble polyphenols was higher in maize ( $168.8 \pm 12.6$  GAE/100 g, db) compared to lentil grain ( $128.1 \pm 1.9$  mg GAE/100 g, db) ( $p < 0.05$ ) (Table 1). The insoluble fraction represents 73 and 67 %, respectively, of the total polyphenols in maize and lentil grain, and it is important to note that insoluble polyphenols are the ones that provide the highest total antioxidant activity (Adom & Liu, 2002).

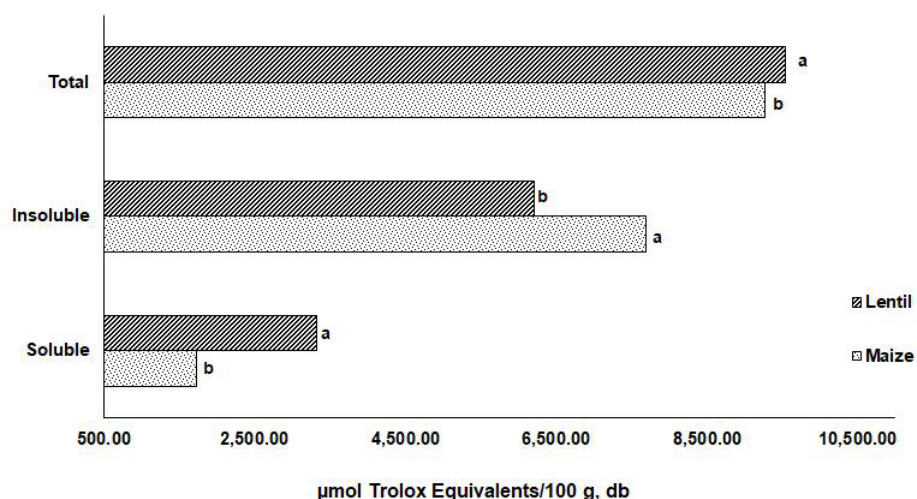
Herrera-Sotero *et al.* (2017) reported a total polyphenol content of 287.3 mg GAE/100 g, db, in blue maize, a value close to that found in the present study. Meanwhile, Gaxiola-Cuevas *et al.* (2017) reported 41.2 mg GAE/100 g, db, in the soluble fraction in blue maize grain. Incidentally, Manco *et al.* (2023) determined the concentration of polyphenols in 5 lentils varieties. These authors found values ranging from 416 to 220 mg GAE/100 g, db, values higher than those indicated in this study. However, polyphenol content can be affected by genotypic factors and environmental growing conditions.



## Antioxidant activity

Total antioxidant activity (sum of soluble and insoluble polyphenol fractions) was higher ( $p < 0.05$ ) for lentil grain ( $9,536.7 \pm 614.4 \mu\text{mol TE}/100 \text{ g}$ , bs) compared to maize grain ( $9,272.3 \pm 554.6 \mu\text{mol TE}/100 \text{ g}$ , bs) (Figure 1). Manco *et al.* (2023) evaluated this property by the ORAC method in 5 lentil varieties, indicating a range from 500 to 15,000  $\mu\text{mol TE}/100 \text{ g}$ , db. Herrera-Sotero *et al.* (2017) found 4,900  $\mu\text{mol TE}/100 \text{ g}$  of antioxidant activity in blue maize grain.

Of the fractions evaluated for the determination of total antioxidant activity in maize and lentil, the insoluble fraction showed the highest activity in maize ( $7,698.03 \pm 278.7 \mu\text{mol TE}/100 \text{ g}$ , db) compared to  $6,208.4 \pm 391.1 \mu\text{mol TE}/100 \text{ g}$ , db, in lentil ( $p < 0.05$ ) (Figure 1). In a study, Adom & Liu (2002) evaluated the content of polyphenols in the soluble and insoluble fractions in different cereal grains. These authors mentioned that maize kernels contain more than 80 % of polyphenols in the insoluble or bound fraction (from the pericarp and/or aleurone layer) and concluded that, of the total antioxidant activity in maize, the insoluble fraction represents the greatest contribution.



**Figure 1. Antioxidant activity of maize and lentil grains.**

Means with the same letter are not significantly different ( $p < 0.05$ ).

## Tortilla characterization

### Quality and sensory characteristics

The quality of the tortilla evaluated with the characteristics of swelling and rollability can be observed in Table 2. During tortilla cooking, the water contained in the dough evaporates, causing

inflation or blistering (Salinas-Moreno *et al.*, 2011). The control tortilla (CT), made with 100 % nixtamalized blue maize flour, and the functional tortilla (FT), made with 30 % lentil flour and 70 % nixtamalized blue maize flour, showed no differences ( $p > 0.05$ ) in puffiness ( $2.8 \pm 0.40$  and  $2.63 \pm 0.49$ ). According to the scale used, both tortillas showed a puffing greater than 70 %. Bon-Padilla *et al.* (2022) elaborated a tortilla with a mixture of nixtamalized maize flour and extruded chickpea. These authors found no significant differences in puffing compared to the control tortilla.

Incidentally, significant differences ( $p < 0.05$ ) were observed in the degree of rollability of the tortillas under study, where the CT ( $1.83 \pm 0.37$ ) showed the lowest average value (lowest fracture) compared to the FT ( $2.03 \pm 0.18$ ). According to the results obtained, the lentil flour addition causes a 25 % increase in fracture compared to the CT. This characteristic in tortillas is related to their flexibility, and the ease of forming a taco without fracture (Salinas-Moreno *et al.*, 2011).

The sensory characteristics evaluated showed that the FT presented an overall acceptability of  $8.52 \pm 1.9$  compared to  $8.6 \pm 1.75$  for the CT ( $p > 0.05$ ). The average obtained for both tortillas corresponds to a description that the tortillas “like very much.” Consumer acceptability of a food product is related to color, flavor, and texture attributes. During the evaluation of color ( $8.84 \pm 1.12$  and  $8.76 \pm 1.37$ ), flavor ( $8.52 \pm 1.90$  and  $8.04 \pm 2.6$ ), and texture ( $8.6 \pm 1.75$  and  $8.12 \pm 2.5$ ), no significant differences ( $p > 0.05$ ) were observed between the CT and FT by consumers (Table 2). According to the quality and sensory attributes evaluated, it is possible to incorporate 30 % lentil flour to produce a fortified or functional nixtamalized blue maize tortilla. Tortilla consumers accept this proportion of added legume.

**Table 2. Quality and sensory characteristics of control and functional tortillas**

Parameter / Feature	Control tortilla	Functional tortilla
Quality		
Puffing <sup>1</sup>	$2.8 \pm 0.40^a$	$2.63 \pm 0.49^a$
Rollability <sup>2</sup>	$1.83 \pm 0.37^b$	$2.03 \pm 0.18^a$
Sensory		
General acceptability <sup>3</sup>	$8.6 \pm 1.75^a$	$8.52 \pm 1.9^a$
Color <sup>3</sup>	$8.84 \pm 1.12^a$	$8.76 \pm 1.37^a$
Flavor <sup>3</sup>	$8.52 \pm 1.90^a$	$8.04 \pm 2.6^a$
Texture <sup>3</sup>	$8.6 \pm 1.75^a$	$8.12 \pm 2.5^a$

Data are expressed as the mean  $\pm$  standard deviation. Different letters within the same row are different ( $p < 0.05$ ). <sup>1</sup> Subjective scale where 1 = 30 %, 2 = 30-70 %, and 3 = 70-100 %. <sup>2</sup> Subjective fracture scale where 1 = 0 %, 2 = 25 %, 3 = 50 %, 4 = 75 % and 5 = 100 %. <sup>3</sup> Degree of liking/dislike, according to a category-9 hedonic scale (1 = extremely dislike, and 9 = extremely like).

Other research has focused on the elaboration of fortified tortillas, such as tortillas fortified with oat flour (Cortes-Soriano *et al.*, 2016), common bean (Treviño-Mejía *et al.*, 2016), jatropha (Argüello-García *et al.*, 2017), germinated soybean (Inyang *et al.*, 2019), avocado and nopal (Rodiles-López *et al.*, 2019), amaranth (Gámez-Valdez *et al.*, 2021), and chickpea (Bon-Padilla *et al.*, 2022), which presents this research as another alternative of maize tortilla fortified with lentil flour with higher nutrient contribution.

Studies related to maize tortilla fortification using cereal, pseudocereal, legume, and/or oilseed flours presently report that these flours and/or ingredients are obtained by different processes and/or technologies, such as extrusion cooking, germination, drying, and defatted flours, among others (Inyang *et al.*, 2019; Rodiles-López *et al.*, 2019; Gámez-Valdez *et al.*, 2021; Bon-Padilla *et al.*, 2022). These ingredients may have a higher cost due to the equipment used or the time invested to obtain them. However, to date, there are no reports of the fortification of a blue maize tortilla with lentil flour obtained only by milling. This technique can be performed traditionally, which would not generate an excessive cost; even broken grains of low commercial value could be used.

### Proximate chemical composition

Table 3 shows the proximate chemical composition expressed as a percentage on dry basis (% db) of the control tortilla (CT) and functional tortilla (FT) or tortilla added with lentil flour. The CT showed a protein content of  $11.53 \pm 0.04$  %, while in the FT a significant increase of 48 % in this parameter ( $17.07 \pm 0.99$  %) was found due to the addition of lentil flour. The protein content of the FT in this study was higher than that reported by Bon-Padilla *et al.* (2022) in tortillas fortified with extruded chickpea (13.27 %) and by Treviño-Mejía *et al.* (2016) in corn tortillas added with common bean (10.89 %). The main objective of this study was to increase the protein content compared to the traditional tortilla, which was achieved by adding lentil flour for the preparation of the FT.

In other studies, on blue maize tortillas, authors such as Colín-Chávez *et al.* (2020) and Astorga-Gaxiola *et al.* (2023) reported lower protein values (9.45 and 9.20 %, respectively), while in the present investigation, the protein content in the CT was higher ( $11.53 \pm 0.04$  %) than reported by these authors. It is important to remark that the differences found could be attributed to the different maize genotypes used, as well as to the different conditions of the nixtamalization process.

**Table 3. Chemical characteristics, color and phytochemicals content of control and functional tortillas**

Parameter / Feature	Control tortilla (CT)	Functional tortilla (FT)
<b>Nutritional composition<sup>1</sup></b>		
Proteins	11.53 ± 0.04 <sup>b</sup>	17.07 ± 0.99 <sup>a</sup>
Lipids	4.44 ± 0.11 <sup>a</sup>	4.38 ± 0.03 <sup>a</sup>
Ash	1.68 ± 0.02 <sup>b</sup>	1.80 ± 0.13 <sup>a</sup>
Carbohydrates	82.4 ± 0.07 <sup>a</sup>	76.2 ± 0.31 <sup>b</sup>
Color		
L*	59.7 ± 0.6 <sup>b</sup>	67.8 ± 0.8 <sup>a</sup>
a*	3.4 ± 0.08 <sup>a</sup>	1.23 ± 0.04 <sup>b</sup>
b*	1.7 ± 0.05 <sup>b</sup>	6.9 ± 0.01 <sup>a</sup>
ΔE	38.74 ± 1.0 <sup>a</sup>	29.08 ± 0.7 <sup>b</sup>
Phytochemicals		
Total anthocyanins <sup>2</sup>	12.37 ± 0.34 <sup>a</sup>	9.99 ± 0.69 <sup>b</sup>
Total polyphenols <sup>3</sup>	138.5 ± 7.8 <sup>b</sup>	168.1 ± 6.0 <sup>a</sup>
Soluble	63.8 ± 4.5 <sup>a</sup>	63.7 ± 2.8 <sup>a</sup>
Insoluble	74.7 ± 3.0 <sup>b</sup>	104.4 ± 4.2 <sup>a</sup>

Data are expressed as the mean ± standard deviation. Different letters within the same row are different ( $p < 0.05$ ). <sup>1</sup> Values expressed as a percentage on a dry basis (db). <sup>2</sup> mg cyanidin-3-glucoside equivalents (CGE)/100 g, db. <sup>3</sup> mg gallic acid equivalents (GAE)/100 g, db.

Ash content in the FT ( $1.80 \pm 0.13$  %) increased ( $p < 0.05$ ) 7 % compared to the CT ( $1.68 \pm 0.02$  %) (Table 3). This significant increase is attributed to the higher ash content of lentil kernels compared to blue maize kernels (Table 1). However, it was observed that carbohydrate content decreased ( $p < 0.05$ ) by 7.5 % in the FT. This significant decrease in carbohydrates in the FT can be attributed to the addition of lentil flour, which increased protein and ash. However, in lipid content, no significant differences were observed in the tortillas under study (Table 3).

As in the present study, tortillas have been made with other legumes. Treviño-Mejía *et al.* (2016) elaborated a maize tortilla fortified with common bean. The authors reported significant increases in protein (10.89 compared to 9.43 %) and ash (1.81 compared to 1.26 %) and a decrease in lipids (6.45 compared to 4.13 %), compared to the unfortified tortilla. Likewise, Bon-

Padilla *et al.* (2022) observed that the addition of extruded chickpea to make a fortified tortilla increased the protein (13.27 compared to 8.88 %), lipid (3.18 compared to 2.50 %), and ash (2.37 compared to 2.10 %) contents, compared to the regular or unfortified tortilla. Incidentally, the addition of pseudocereals, such as amaranth flour and chia in maize flour to make fortified tortillas, favors the increase in protein and dietary fiber (León-López *et al.*, 2019; Gámez-Váldez *et al.*, 2021).

The results obtained in the present study suggest that the FT (tortilla with lentil flour) could be an alternative to cover the nutritional deficiencies of the traditional tortilla. Therefore, this research constitutes an important advance for the vulnerable Mexican population, where the intake of foods with high protein content is scarce. Adding a low-cost legume, such as lentils, to fortify a staple food such as tortillas represents an alternative to reduce diseases related to poor nutrition. However, it is important to conduct future studies to determine the content of essential amino acids after tortilla fortification with lentil flour.

## Color

The consumption of blue maize tortillas is becoming more frequent; however, in central Mexico, it is more rooted because consumers relate the blue maize grain to a native or ancestral grain and also prefer the blue tortilla for the health benefits provided by anthocyanins, compounds that give the maize grain its color (Gutiérrez-Llanos *et al.*, 2023; Hidalgo-Ramos *et al.*, 2024).

The transformation of maize grain to tortilla resulted in an increase in the “L\*” parameter ( $57.74 \pm 0.26$  compared to  $59.7 \pm 0.6$ ) (Tables 1 and 3). This increase in luminosity caused the tortilla to be lighter in color compared to the maize grain. During the traditional nixtamalization process, the grain is subjected to produce nixtamalized flours, and losses of more than 50 % of anthocyanins occur (Mora-Rochin *et al.*, 2016). These losses are related to the conditions used during grain cooking, such as alkaline pH (>10) and high temperatures (85-90 °C), which affect the stability and structure of anthocyanins and, therefore, the color of the final product, in this case, the tortilla (Herrera-Sotero *et al.*, 2016; Mora-Rochin *et al.*, 2016).

The addition of lentil flour to make the FT favored an increase ( $p < 0.05$ ) in the luminosity parameter ( $L^*=67.8 \pm 0.8$ ), indicative of a brighter tortilla, and a decrease ( $p < 0.05$ ) in the total color difference, represented as  $\Delta E$  ( $29.08 \pm 0.7$ ) compared to the CT ( $L=59.7 \pm 0.6$  and  $\Delta E=38.74 \pm 1.0$ ) (Table 3). These significant changes in the parameters  $L^*$  and  $\Delta E$  in the FT compared to the traditional tortilla or the CT could be attributed to the greater brightness that the lentil grain has compared to the blue maize grain, where it can be corroborated that greater brightness is represented by a high value in the luminosity ( $L^*$ ) and a lower value in the total color difference ( $\Delta E$ ) ( $L^*=68.4 \pm 1.2$  and  $57.74 \pm 0.26$ ,  $\Delta E = 39.02 \pm 0.3$  and  $36.0 \pm 0.8$ ) (Table 1).

## Phytochemicals

The transformation of maize grain to the CT decreased the anthocyanin content by 40 %. These secondary metabolites are sensitive to factors such as alkaline pH and high temperatures

(conditions used during maize grain cooking and tortilla cooking), generating colorless structures (Mora-Rochin *et al.*, 2016; Gutiérrez-Llanos *et al.*, 2023). In a study by Mora-Rochin *et al.* (2016) with 15 genotypes of blue maize, it was shown that the nixtamalization process generated significant decreases greater than 70 % in anthocyanin content.

In the FT, the anthocyanin content (9.99 mg CGE/100 g, db) represents the lowest ( $p < 0.05$ ) concentration compared to the CT (12.37 mg CGE/100 g, db) (Table 3). This significant decrease compared to the CT could be attributed to the fact that lentil grain is not a source of these secondary metabolites (Table 1); therefore, the addition of lentil flour to nixtamalized blue maize flour caused the decrease of these compounds in the final product.

Regarding the traditional tortilla, other authors reported lower anthocyanin content compared to the CT in this study. Astorga-Gaxiola *et al.* (2023), in a study with a commercial blue tortilla, found a content of 6.61 mg CGE/100 g, db. While Herrera-Sotero *et al.* (2017), when performing nixtamalization of blue maize grain to produce tortillas, reported higher concentrations in traditional tortillas (21.8 and 27.8 mg CGE/100 g, db). The differences found in the anthocyanin content could be attributed to the different maize genotypes used, and the different temperatures, cooking and resting times, and alkali concentrations used during the cooking of the maize kernels. Gutiérrez-Llanos *et al.* (2023) used different alkali concentrations during the cooking of maize kernels, reporting that, as the alkali concentration increases (0.5 to 1.5 %), the anthocyanin content decreases (52.2 to 10.8 mg CGE/100 g, db).

The anthocyanins concentration in a product derived from blue maize will depend, in part, on whether the anatomical fractions, where these secondary metabolites are located, are preserved during kernel processing. Anthocyanins can be found in different anatomical parts: pericarp and/or aleurone cells. They should be located in the aleurone if the grain will be destined for the production of nixtamalized flour (Salinas-Moreno *et al.*, 2013). The anthocyanins loss during nixtamalization is due, on the one hand, to high temperatures and alkaline pH. In this sense, the anthocyanins located in the aleurone cells, which are the innermost part compared to the pericarp, will be affected to a lesser extent. However, those present in the pericarp will be lost during traditional nixtamalization since this fraction is partially or detached during the process, thus causing a greater decrease of these compounds in the final product (Salinas-Moreno *et al.*, 2013; Mora-Rochin *et al.*, 2016; Domínguez-Hernández *et al.*, 2022).

The total polyphenol content (sum of the soluble and/or insoluble fractions) decreased due to the effect of the transformation of maize grain to CT, where the maize cooking and tortilla preparation caused decreases of 40 % ( $138.5 \pm 7.8$  mg GAE/100 g, db) (Table 3). This decrease can be attributed to the characteristics of the nixtamalization process, where the cooking of the maize kernel uses high temperature and pH greater than 10, causing the partial or total detachment of the pericarp fraction, the anatomical part where more than 80 % of the polyphenols in the maize kernel are concentrated (Adom & Liu, 2002; Aguayo-Rojas *et al.*, 2012; Olšaníková *et al.*, 2022). Therefore, the products obtained from the nixtamalization process in tortillas, contain a lower concentration of polyphenols than unprocessed grain (Mora-Rochin *et al.*, 2010). Colín-Chávez *et al.* (2020), in a study of blue maize tortilla, reported 120.8 mg GAE/100 g, db, values similar



to those found in the CT in this study, while Herrera-Sotero *et al.* (2017) reported lower values (70.3 mg GAE/100 g, db). Due to the aforementioned, it is important to consider the addition of a legume, in this case, lentils, as a source of polyphenols that could compensate for the loss of these compounds that occur during the nixtamalization and tortilla manufacturing process.

The addition of lentil flour to make the FT managed to favor an increase of 21.4 % ( $p < 0.05$ ) of total polyphenols compared to the CT ( $168.1 \pm 6.0$  compared to  $138.5 \pm 7.8$  mg GAE/100 g, db) (Table 3). This significant increase is because the lentil grain possesses polyphenols that could resist the tortilla cooking process. In a similar study, Treviño-Mejía *et al.* (2016) observed increases of 8 % of total polyphenols in tortillas added with common bean compared to the control tortilla made only with maize.

Of the fractions used for polyphenol quantification, the insoluble fraction represents more than 50 % in both tortillas. The addition of lentil flour to make the FT shows a 40 % ( $p < 0.05$ ) increase in insoluble polyphenols with respect to the CT ( $104.4 \pm 4.2$  compared to  $74.7 \pm 3.0$  mg GAE/100 g, db). Although lentil grain has a lower content of insoluble polyphenols than maize grain (Table 1), the addition of lentil flour to make the FT increases the polyphenols that were reduced after the nixtamalization process of the maize grain, where partial or total loss of the pericarp occurs, the fraction where the highest content of polyphenols in corn is concentrated (Adom & Liu, 2002; Mora-Rochin *et al.*, 2010).

Astorga-Gaxiola *et al.* (2023) conducted an *in vitro* colonic fermentation study with commercial tortillas (blue and white). These authors mention that the colonic microbiota favors the release of polyphenols found in the insoluble fraction, promoting an antioxidant environment in the colon. They also indicated that the polyphenols released could reduce cells that promote the development of colon cancer. The aforementioned demonstrates the health impact of the intake of a blue maize tortilla fortified with lentil flour.

Incidentally, the fraction of soluble polyphenols in both tortillas showed no significant differences (Table 3). The results found in this study are higher than those indicated by other authors in blue maize tortilla, who reported values of 41.2 and 30.64 mg GAE/100 g, db (Gaxiola-Cuevas *et al.*, 2017; Astorga-Gaxiola *et al.*, 2023).

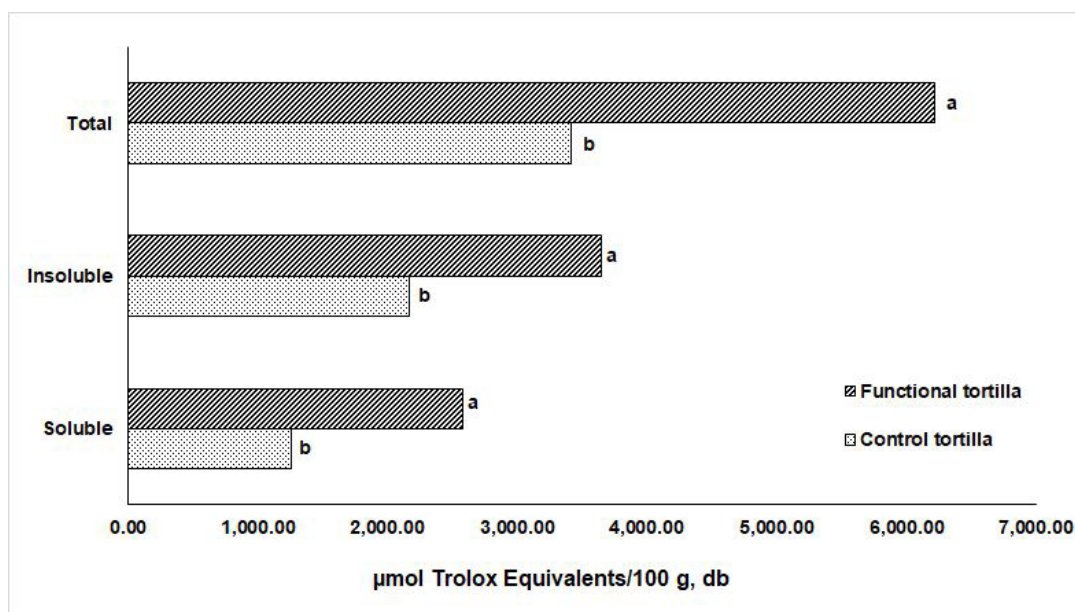
## Antioxidant activity

Antioxidant activity is attributed to the different secondary metabolites present in foods, and this property, or bioactivity, is related to the prevention of various types of diseases; however, it can be affected by the processing conditions applied to the food matrix (Tsoupras *et al.*, 2024). Of the tortillas under study, the lowest ( $p < 0.05$ ) antioxidant activity evaluated by the ORAC method was observed in the CT ( $3,419.3 \pm 131.6$   $\mu\text{mol TE}/100$  g, db) (Figure 2). The process of nixtamalization of maize grain for the production of nixtamalized flour and tortillas causes a decrease of 63 %. The important decrease in this property is caused by the processing conditions to which the maize kernel is subjected, where pericarp detachment and leaching to the cooking liquor of polyphenols with antioxidant properties occurs, which is discarded, leaving the tortilla

with a lower content of bioactive compounds that provide antioxidant effects (Gaxiola-Cuevas *et al.*, 2017). Those losses confirm the need to produce tortillas with other grains or ingredients that, in addition to increasing the protein content, increase the content of polyphenols with antioxidant properties.

The FT presented higher ( $p < 0.05$ ) antioxidant activity with respect to CT ( $6,223.7 \pm 411.2$  compared to  $3,419.3 \pm 131.6 \mu\text{mol TE}/100 \text{ g, db}$ ) (Figure 2). Figure 1 shows that both grains have similar antioxidant activities; however, as mentioned before, the conditions to which maize is subjected for transforming into flour generate important losses of polyphenols that provide this property to the tortilla. This decrease in nixtamalized flour was recovered by adding lentil flour to make the FT.

In similar studies, Gámez-Valdez *et al.* (2021) and León-Murillo *et al.* (2021) made tortillas with extruded blue maize flour fortified with extruded amaranth flour and extruded defatted chia flour. These authors found higher antioxidant activity values ( $13,187$  and  $15,531 \mu\text{mol TE}/100 \text{ g, db}$ ) than reported in this study. These differences in antioxidant activity are attributed to the characteristics of the process used to obtain extruded maize flour, which is considered whole wheat flour. In this process, there is no loss of anatomical parts and/or leaching of polyphenols; moreover, the high temperatures used are for a short time (Aguayo-Rojas *et al.*, 2012; Olšaníková *et al.*, 2022). In another study, Bon-Padilla *et al.* (2022) found an antioxidant activity of  $8,031 \mu\text{mol TE}/100 \text{ g, db}$ , in nixtamalized blue maize tortilla fortified with extruded chickpea, where it is observed that these values are similar to those found in this study. The difference in this parameter, as evaluated by the different authors, is that the maize flours were processed using different technologies.



## Figure 2. Antioxidant activity of control and functional tortillas.

Means with the same letter are not significantly different ( $p < 0.05$ )

Regarding the fraction of polyphenols that provide the highest antioxidant activity, insoluble polyphenols represent more than 55 % and soluble polyphenols more than 35 % in both tortillas. In Figure 2, it can be observed that the FT has the highest ( $p < 0.05$ ) insoluble and soluble antioxidant activity ( $3,647.7 \pm 400.1$  and  $2,575.9 \pm 90.1 \mu\text{mol TE}/100 \text{ g, db}$ ) compared to the CT ( $2,164.2 \pm 251.1$  and  $1,255.2 \pm 120 \mu\text{mol TE}/100 \text{ g, db}$ ). As previously mentioned, the higher polyphenol content and antioxidant activity in the FT are due to the addition of lentil flour, which compensates for the polyphenols that were depleted during the alkaline cooking of the maize kernel to produce nixtamalized flour. However, future studies are needed to determine the polyphenol profile in the soluble and insoluble fractions and to be able to attribute the effect on the antioxidant property to one or several compounds.

## Conclusions

The functional tortilla shows better nutritional and antioxidant characteristics than the traditional one made with 100 % nixtamalized blue maize flour. In the same manner, the overall acceptability of the functional tortilla is similar or equal to that of the control tortilla. The incorporation of lentil flour to make the functional tortilla showed significant increases in protein content, ash, total polyphenols, and antioxidant activity. Those increases demonstrate the importance of incorporating ingredients that improve nutritional aspects, mainly protein content, and increase bioactive compounds that are related to antioxidant properties. The intake of fortified tortillas through this strategy could positively impact the nutritional status of the population in underprivileged sectors. Likewise, the synergy of bioactive compounds from combining both grains could reduce the development of some chronic degenerative diseases in consumers. However, further studies are needed regarding the amino acid and polyphenol profile, and *in vitro* and *in vivo* studies to confirm that the functional tortilla made from blue maize and lentils has nutritional and nutraceutical properties superior to those of the traditional tortilla.

## Authors' contribution

"Conceptualization of the work, Sánchez-Magaña, L.M.; Mora-Rochin, S.; development of the methodology, Rodríguez-Heráldez, D.A.; León-López, L.; Domínguez-Arispuro, D.M.; Cuevas-Rodríguez E.O.; software management, Rodríguez-Heráldez, D.A.; Sánchez-Magaña, L.M.; Mora-Rochin, S.; experimental validation, Reyes-Moreno, C.; Mora-Rochin, S.; Sánchez-Magaña, L.; analysis of results, Rodríguez-Heráldez, D.A.; Mora-Rochin, S.; Sánchez-Magaña, L.; analysis of results, Rodríguez-Heráldez, D.A.; Mora-Rochin, S.; Sánchez-Magaña, L.; León-López, L.; data management, Mora-Rochin, S.; Sánchez-Magaña, L.M.; writing and preparation of the manuscript, Mora-Rochin, S.; Sánchez-Magaña, L.M.; León-López, L.; drafting, revising, and editing, Mora-Rochin, S.; Sánchez-Magaña, L.; Liliana-León, Domínguez-Arispuro, D.M.; Reyes-

Moreno, C.; project manager, Mora-Rochin, S.; Sánchez-Magaña, L.M.; acquisition of funds, Mora-Rochin, S.

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## Conflict of interest

“The authors declare that they have no conflict of interest”.

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