Impact of supplementing blue and white corn tortillas with huauzontle flour on the nutritional, rheological, structural properties and *in vitro* digestibility

Impacto de la suplementación de tortillas de maíz azul y blanco con harina de huauzontle sobre las propiedades nutricionales, reológicas, estructurales y digestibilidad *in vitro*

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Abstract

The objective of this work was to evaluate the effect on the nutritional, antioxidant, rheological, and structural properties of blue and white corn tortillas added with huauzontle flour. Supplementation of corn tortillas with huauzontle flour (HF) significantly improved their nutritional and functional properties. Blue corn tortillas (TA) had a higher initial protein content (10.10 g/100 g) than white corn tortillas (TM, 7.00 g/100 g). Supplementation with HF increased the protein content in both. Fat content was higher in TM (4.50 g/100 g) than in TA (3.58 g/100 g) and also increased with HF. Moisture content was higher in white corn tortillas ($42.60 \pm 0.36\%$) and increased with the addition of HF ($45.73 \pm 0.66\%$ moisture in TM_{15}), attributed to differences in nixtamalization. Fortification with HF significantly improved functional properties: it increased antioxidant capacity, polyphenol content, and resistant starch (TA_{15}: $45.91 \pm 2.0\%$ RS), thus reducing starch digestibility. Blue corn tortillas exhibited superior viscoelastic properties (higher G' values). Texturally, hardness decreased in the HF-fortified blue tortillas but increased in the white corn tortillas. HF also shifted the color toward green tones (a* from 0.08 ± 0.15 to -6.51 ± 0.25 and b* from 3.52 ± 0.26 to 21.98 ± 1.24). Fourier transform infrared spectroscopy analysis revealed that HF reduced the ordered structure of the starch, promoted stronger starch-water interactions, and increased β-sheet structures in the amide I region. This indicates the formation of a three-dimensional interpolymer network, which explains the improved rheological properties. In conclusion, huauzontle flour effectively improves the nutritional profile, functional benefits, and dough quality of corn tortillas, offering a viable strategy to increase the nutritional value of this staple food.

Keywords: Tortillas, huauzontle, *Zea mays*, digestibility, rheological properties.

Resumen

El objetivo fue evaluar el efecto de la adición de harina de huauzontle sobre las propiedades nutricionales, antioxidantes, reológicas y estructurales de tortillas de maíz azul y blanco. La fortificación de tortillas de maíz con harina de huauzontle (HF) mejoró significativamente sus propiedades nutricionales y funcionales. Las tortillas de maíz azul (TA) presentaron un mayor contenido proteico inicial (10.10 g/100g) que las de maíz blanco (TM, 7.00 g/100g). La suplementación con HF incrementó el contenido proteico en ambas. El contenido de grasa fue mayor en TM (4.50 g/100g) que en TA (3.58 g/100g) y también aumentó con HF. La humedad fue mayor en las tortillas de harina blanca (42.60 ± 0.36 %) y aumentó con la adición de HF (45.73 ± 0.66 % de humedad en TM_{15}), atribuido a diferencias en la nixtamalización. La fortificación con HF mejoró significativamente las propiedades funcionales: incrementó la capacidad antioxidante, el contenido de polifenoles y el almidón resistente (TA_{15}: 45.91 ± 2.0 % de RS), reduciendo así la digestibilidad del almidón. Las tortillas de maíz azul exhibieron propiedades viscoelásticas superiores (valores G' más altos). Texturalmente, la dureza disminuyó en las tortillas azules con HF, pero aumentó en las tortillas de maíz blanco. HF también desplazó el color hacia tonos verdes (a* de 0.08 ± 0.15 a -6.51 ± 0.25 y b* de 3.52 ± 0.26 a 21.98 ± 1.24). El análisis de espectroscopia infrarroja por transformada de Fourier reveló que el HF redujo la estructura ordenada del almidón, promovió interacciones almidón-agua más fuertes y aumentó las estructuras de lámina β en la región amida I. Esto indica la formación de una red interpolimérica tridimensional, lo que explica la mejora de las propiedades reológicas. En conclusión, la harina de huauzontle mejora eficazmente el perfil nutricional, los beneficios funcionales y la calidad de la masa de las tortillas de maíz, ofreciendo una estrategia viable para aumentar el valor nutricional de este alimento básico.

Palabras clave: Tortillas, huauzontle, *Zea mays,* digestibilidad, propiedades reológicas.

1   Introduction

The high consumption of products with low nutritional value is one of the main catalysts of the global obesity pandemic, which is associated with a higher risk of chronic diseases such as type 2 diabetes, hypertension, and certain types of cancer. Public health interventions generally focus on treating existing obesity, with limited effect on its prevention (Vidaña-Pérez *et al.*, 2022). Limited access to nutritious food exacerbates this problem, increasing the risk of malnutrition and chronic diseases. A promising strategy lies in the phenolic compounds of plants, which also protect plants from environmental stress (Sahu et al., 2023) possess a potent antioxidant activity that can surpass that of vitamins such as C and E, and numerous studies have reported powerful antimicrobial, anti-inflammatory and anticancer effects (Domínguez-Hernández *et al.*, 2022).

Currently, pseudocereals have gained significant importance due to their high content of bioactive compounds and their exceptional nutritional profile (Hernández-Aguilar *et al.*, 2025). In this sense, huauzontle is a pseudocereal native to Mexico, which has been consumed since pre-Hispanic times, having a high content of quality protein, dietary fiber, essential amino acids, phenolic compounds and vitamins content. Current scientific literature explores the potential of huauzontle as a functional food ingredient. García *et al.* (2022) developed a fermented huauzontle beverage that exhibited high antioxidant capacity, as measured by ORAC (856.21 μM Trolox equivalents) and ABTS (351.80 μM Trolox equivalents) assays. This activity was attributed to polyphenols, flavonoids, and other phytochemicals present in huauzontle. Hernández-Aguilar *et al.* (2025) demonstrated that incorporating fermented huauzontle (2% w/w) significantly increased phenolic compound content (1.66-fold higher) and enhanced inhibition of lipoxygenase and trypsin by 3.98% and 9.34%, respectively. Furthermore, native bacteria isolated from huauzontle not only meet fundamental probiotic requirements but also produce bioactive metabolites with antioxidant, anti-inflammatory, and immunomodulatory functions (Santiago-López et al., 2024). However, research on the effects of supplementing foods with huauzontle flour remains limited.

On the other hand, corn and its by-products form an important part of the caloric and nutrient supply of 4.5 billion people around the world, mainly in rural areas of Latin America and Africa (Domínguez-Hernández *et al.*, 2022). In Mexico, the consumption of corn-based foods, e.g. tortillas, is part of the daily diet of Mexicans, reaching an annual consumption of tortillas greater than one hundred kilograms per capita (Salinas-Moreno *et al.*, 2024). Due to the high consumption of this type of products, the formulation of the corn dough is essential to improve the health of Mexicans. Added to this, currently there is a growing interest in healthy foods, such as those rich in antioxidants. In this sense, pigmented corn varieties are rich in antioxidants (Álvarez-Poblano *et al.*, 2020), such as polyphenols, e.g. blue corn varieties have been reported to contain between 918.9 to 1165.5 mg/kg (gallic acid equivalents (GAE); dry weight) (Damián-Medina *et al.*, 2020). Mendez-Lagunas *et al.* (2022) found that blue and purple corn tortillas prepared with an ecological nixtamalization method had a total phenols retention greater than 92%. Phenolic compounds are bioactive compounds responsible for the control of postprandial serum glucose levels and the incidence of type 2 diabetes.

The high resilience, nutritional, and nutraceutical profile of ancestral grains has led to them being the subject of numerous studies, where the goal is not only to characterize their physicochemical properties but also to evaluate ancestral grains as functional ingredients in processed foods with a sustainable approach (Silva-Fernández *et al.*, 2025; Beltran-Medina *et al.*, 2025). Therefore, it was hypothesized that incorporating huauzontle flour (HF) into blue and white corn tortillas, one of the most widely consumed foods nationwide, would improve their nutritional profile and technological properties while providing bioactive compounds with antioxidant potential. For this reason, this study evaluated the nutritional properties, total phenolic content, antioxidant activity, *in vitro* digestibility, as well as the rheological properties of blue corn and commercial white corn tortillas supplemented with huauzontle flour at different concentrations. Furthermore, the structural features induced in the tortillas starch, water and protein organization were evaluated using Fourier Transform Infrared Spectroscopy (FTIR).

2   Materials and methods

2.1   Materials

Blue corn (*Zea mays* L.) was sourced from a local producer in the community of Zacatlan, Puebla, Mexico (19°56'00" N, 97°57'38" W; 2039 m above sea level). Huauzontle (*Chenopodium nuttalliae* Saff.) was obtained from a local grower in Tepoztlan, Morelos, Mexico (18°59'07" N, 99°05'59" W; 1706 m above sea level). Hydrochloric acid (HCl), sodium hydroxide (NaOH), and ethanol of analytical grade were supplied by J.T. Baker (Xalostoc, State of Mexico, Mexico). Commercial nixtamalized corn flour (Maseca®, GRUMA S.A.B. de C.V., Monterrey, Nuevo Leon, Mexico) was purchased

from a local Walmart supermarket in Mexico City. Amyloglucosidase (A7095, 260 U/mL), porcine pancreatic α-amylase (A3176, Type VI-B, $\geq$5 U/mg solid), 3,5-dinitrosalicylic acid (DNS), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Huauzontle flour preparation

For obtaining huauzontle flour (HF) the huauzontle inflorescence was used, and placed in metal trays in a convection oven (HCF-62, Riossa Digital, Puebla, State of Puebla, Mexico) at 40 °C for 12 h. It was later ground in a food processor (NutribulletTM, NB-101B, Hong King Ltd., Guandong, China) until it could be sieved through an 80 mesh (Manufacturers of Industrial and Commercial Supplies, St. Louis, MI, USA) to ensure a fine flour and subsequently a homogeneous dough. The HF was stored in hermetic bags (Ziploc, S. C. Johnson & Son, Inc., Mexico City, Mexico) at room temperature. The composition of huauzontle flour was 23.07 ± 0.10% protein, 12.19 ± 0.08% crude fiber, 2.29 ± 0.03% ether extract, 9.32 ± 0.05% ash, and 53.13 ± 0.12% carbohydrates (% dry weight basis).

2.3 Nixtamalization process

While the commercial corn flour was produced by a traditional nixtamalization process involving cooking and steeping dried maize kernels in an alkaline solution, the blue corn grains were subjected to the ecological nixtamalization process described by Mariscal-Moreno et al. (2022). Maize was processed by cooking at 94 °C for 30 min in water (1:2, w/v) containing 1% calcium carbonate, followed by steeping at room temperature for 16 h. The nixtamal was milled using a conventional disc grinder (Molino del Rey®, Mexico City, Mexico) to obtain the masa (dough). The masa was dehydrated in a convection oven (HCF-62, Riossa Digital, State of Puebla, Puebla, Mexico) at 40 °C for 24 h.

2.4 Nutrient content determination

Moisture, total protein, and total lipid contents were determined according to AOAC methods (2000). Protein content was quantified using the Kjeldahl method, applying a nitrogen conversion factor of 6.25.

2.5 Tortilla variations

The nixtamalized flour of blue corn and commercial corn was supplemented with huauzontle flour, for obtaining the different tortilla variations studied, as shown in **Table 1**. The dough for the tortillas was made by mixing 40 g of flour with 60 g of water.

Approximately 15 ± 0.2 g of masa were shaped into spherical portions and placed between two plastic sheets in a manual wooden tortilla press to obtain circular masa sheets. The formed discs were then transferred to a preheated hotplate for cooking (CIMAREC, Thermo Fisher Scientific Inc., Madrid, Spain) at 350 ± °C for 17 s, then turned over and placed for further 50 s and flipped again for 17 s. Tortillas were left to cool at room temperature and were individually packed into polyethylene bags. The tortillas with different supplementation levels were coded as follows: TA_5, TA_{10}, and TA_{15} for blue corn tortillas, where the A stands for "azul" (blue in Spanish) and TM_5, TM_{10}, and TM_{15}, where M stands for "Maseca" (the commercial corn flour brand) for tortillas made with commercial corn flour. The subindexes refer to the amount of huauzontle flour with which the tortillas were supplemented. The treatments without addition of huauzontle flour were used as controls and were coded as TA and TM.

2.6 Moisture content and thickness of the tortillas

Moisture content of fresh tortillas and those stored for 4 days was determined using the AOAC (2005) vacuum oven drying method (930.15). Measurements were taken at different locations on the tortilla and subsequently averaged. The thickness of the tortillas was measured after one day of storage with a digital caliper (Coolant-Proof Micrometer IP 65, Mitutoyo, Apodaca, State of Nuevo Leon, Mexico).

2.7 Tortilla hardness

One of the most important parameters in tortillas is hardness, due to its relationship with the tortilla aging (Vernon-Carter *et al.*, 2020). Whole tortillas were placed in a Brookfield CT3-4500 texturometer (AMETEK Brookfield, Middleborough, MA, USA) with a bottom plate having a round (0.0254 μm diameter) drilled hole. The tortilla sample was compressed with a TA18 spherical probe (1.27 cm in diameter) at a rate of 1 mm s^{-1}, until achieving 50 % penetration in one compression cycle. Hardness values were obtained directly from the instrument software and expressed in Newtons.

2.8 Color of tortillas

Color parameters were measured using a hand-held Chroma Meter colorimeter (CR-400, Konica Minolta, Ramsey, NJ, USA) equipped with Spectra Magic NX software and operated under illuminant D65. Prior to measurements, the instrument was calibrated with a standard white reference tile. Color coordinates L*, a*, and b* were recorded, where L* represents lightness (0 = black, 100 = white), a* indicates

greenness (-) to redness (+), and b* corresponds to blueness (-) to yellowness (+).

2.9 Total phenolic content and antioxidant activity

2.9.1 Extraction of free phenolic compounds

On the day of analysis, 4 g of sample were extracted with 10 mL of acidified methanol (methanol:distilled water:HCl, 80:10:1, v/v/v) at 50 °C for 2 h in a shaking water bath set at 300 rpm. The resulting extracts were then centrifuged at $1008 \times g$ for 10 min and supernatant was collected (Yaver & Bilgiçli, 2021).

2.9.2 Extraction of bound phenolic compounds

Bound phenolic compounds in samples were extracted according to the method reported previously by Mora-Rochin *et al.* (2010). Following the extraction of free phenolic compounds, the remaining solid was treated with 10 mL of 2 M sodium hydroxide in a water bath at 95 °C for 30 minutes. Afterwards, the mixture was stirred at room temperature for an additional hour. To eliminate lipids, the sample was acidified to a pH below 2.0 using 2 mL of hydrochloric acid and then extracted with hexane. Subsequently, phenolic compounds were recovered by performing three successive extractions with 10 mL of ethyl acetate each. The ethyl acetate layers were combined and evaporated under vacuum at 35 °C until dry. Finally, the bound phenolic compounds were dissolved in 2 mL of a methanol:water mixture (50:50, v/v) to enhance solubility and obtain a clear, uniform solution.

2.9.3 Total phenolic content

The phenolic content, both free and bound, is analyzed by mixing a 0.125 mL aliquot of extract with 0.5 mL of Folin-Ciocalteu reagent. The samples were vortexed for 15 s and allowed to react for 6 min at 20 °C. Subsequently, 1.25 mL of a 7% (w/v) Na_2CO_3 solution was added, and the mixture was brought to a final volume of 12 mL with deionized water. The reaction was left to develop for 90 min, after which absorbance was recorded at 760 nm using a Genesys 10S UV–Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Quantification was performed using a gallic acid calibration curve, and results were expressed as milligrams of gallic acid equivalents (mg GAE).

2.9.4 DPPH radical scavenging activity

The antioxidant capacity of the extracts was assessed using the DPPH method. Briefly, 0.1 mL of extract was reacted with 3.9 mL of 0.6 mM DPPH in 80% methanol. After vortexing 15 s and 30 min of incubation, the absorbance was measured at 517 nm (Genesys 10S UV-Vis spectrophotometer, Thermo Fisher Scientific). A solvent blank and a DPPH control were used for the corresponding calculations.

2.9.5 ABTS radical scavenging activity

Following the standard ABTS$^+$ assay methodology, the radical ABTS was generated from (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) by reaction with potassium persulfate. The resulting stock solution (7.00 mM) was kept in the dark for 16 h to allow complete radical formation. Prior to analysis, this solution was adjusted by dilution to an absorbance of 0.70 ± 0.02) at 734 nm, thus obtaining the working solution. In the assay itself, 40 μL of extract were combined with 960 μL of the ABTS$^+$ solution. After a reaction time of exactly 6 minutes, the absorbance was recorded at a wavelength of 734 nm (Genesys 10S UV-Vis spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA). The percentage of inhibition was calculated as:

$$Antioxidant\ activity\ (\%\ inhibition) = \left[\left(Abs_{Blank} - Abs_{Sample} \right) \right] \times 100 \qquad (1)$$

2.10 Fourier Tranform Infrared Spectroscopy (FTIR)

FTIR spectra of tortilla samples were recorded using a PerkinElmer Spectrum 100 spectrophotometer (PerkinElmer, Waltham, MA, USA) equipped with a diamond crystal universal ATR accessory. Four scans were collected for each sample, and the spectra reported correspond to the average of these measurements. Spectral deconvolution was carried out using Gaussian functions to analyze specific absorption regions. The starch fingerprint region (1200–900 cm^{-1}) was evaluated to assess changes in short-range molecular order, while the amide I region (1700–1600 cm^{-1}) was analyzed to investigate variations in protein secondary structure (Vernon-Carter *et al.*, 2020).

2.11 Rheological properties of masa

The dynamic viscoelastic properties of the masa were determined according to Álvarez-Poblano *et al.* (2020) using oscillatory measurements. A strain sweep test was conducted, during which the storage modulus (G') and loss modulus (G") were measured. Measurements were made over a strain range of 0.1–100 % at 1 Hz with a Physica MCR 300 rheometer (Physica Messtechnik GmbH, Stuttgart, Germany). The geometry consisted of plate-plate, with a 50 mm diameter and normal force of 5 N. Samples (~5.0 g) were placed in the equipment measuring and allowed to stabilize for 5 min at 25 °C for structure recovery.

Mineral oil was applied to the sample edges between the geometry plates to prevent dehydration during measurements. Temperature was maintained with a Physica TEK 150P Peltier system.

2.12 In vitro starch digestibility

On the day of the determination, an enzymatic solution was prepared according to Ye *et al*. (2019). Briefly, pancreatic *alpha*-amylase (0.0225 g, 10 U/mg) was suspended in 7.5 mL deionized water with magnetic stirring for 30 min and then centrifuged at 1500 ×g for 5 min. The supernatant was transferred into a beaker and mixed with 0.75 mL amyloglucosidase (260 U/mL). The sample (0.5 g) was dispersed in 25 mL of deionized water and equilibrated at 37 °C for 30 min with shaking in a water bath.

The *in vitro* digestion of samples was performed by the DNS method for the quantitative determination of reducing sugars (Miller, 1959). 0.75 mL of the enzyme solution was added to each sample. The samples were incubated at 37 °C with slight shaking for 120 min. Aliquots were taken at times 0 min, 20 min and 120 min. A standard curve was obtained by measuring the absorbance at 540 nm of known concentrations of glucose solutions. Measurements were made in duplicate for each sample and the data provided is an average of these results. The percentages of RDS (rapidly digestible starch), SDS (slowly digestible starch), and RS (resistant starch) in the samples were calculated using the following equations:

$$RDS = ((G_{20} - FG) \times 0.9)/TS \qquad (2)$$

$$SDS = ((G_{120} - G_{20}) \times 0.9)/TS \qquad (3)$$

$$RS = (TS - RDS - SDS)/TS \qquad (4)$$

where FG is the free glucose content, TS is the total starch content, G_{20} and G_{120} represent the amounts of glucose released after 20 and 120 min of hydrolysis time of the sample.

2.13 Statistical analysis

All measurements were carried out in triplicate using three independent experiments under a randomized design. Results are reported as mean ± standard deviation. Data were analyzed by one-way analysis of variance (ANOVA), and when significant differences were detected, means were compared using Tukey's test. Statistical significance was set at $p < 0.05$. All analyses were performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA).

3 Results and discussion

3.1 Nutrient content

The control tortilla made with nixtamalized blue corn had a protein content of 10.10 ± 0.44 g/ 100 g, similar to that reported by other researchers 9.45 ± 0.24 g/100 g (Colín-Chávez *et al*., 2020). On the other hand, control tortilla made with commercial nixtamalized corn flour presented a protein content, 7.00 ± 0.01 g/100 g. For both types of tortilla, the protein content increased significantly with the addition of huauzontle flour (**Table 1**). The increase in protein content could contribute to the reduction in malnutrition in Mexico, which represents the most consumed product, particularly in nutritionally vulnerable social strata. On the other hand, the crude fat content in TA was 3.58 ± 0.07 g/100 g. TM had a crude fat content of 4.50 ± 0.01 g/100 g, higher than that reported (3.42 ± 0.15 g/100 g) by Colín-Chávez *et al*. (2020) for tortillas made from white maize.

Table 1. Effect of huauzontle flour incorporation on crude protein and crude fat content of blue and white corn tortillas.

Tortilla variations	Corn (g of	Huauzontle flour)	Crude protein (N x 6.25) (g/100 g, dry weight)	Crude fat
TA	100	0	10.10 ± 0.44[c]	3.58 ± 0.07[c]
TA$_5$	95	5	10.27 ± 0.85[c]	4.49 ± 0.25[b]
TA$_{10}$	90	10	10.53 ± 0.15[b]	4.59 ± 0.04[b]
TA$_{15}$	85	15	10.97 ± 0.46[a]	4.79 ± 0.02[ab]
TM	100	0	7.00 ± 0.01[e]	4.50 ± 0.01[b]
TM$_5$	95	5	7.18 ± 0.43[e]	4.68 ± 0.10[b]
TM$_{10}$	90	10	7.64 ± 0.07[d]	5.09 ± 0.01[ab]
TM$_{15}$	85	15	7.96 ± 0.29[d]	5.37 ± 0.02[a]

Note: Values are presented as mean ± standard deviation of triplicates ($p < 0.05$). [abcd]Significant differences in column ($p < 0.05$). TA$_n$ is the sample of blue tortilla and TM$_n$ is the sample of white tortilla, where the subscript "n" denotes the proportion of huauzontle flour added (%).

Table 2. Moisture content, thickness and hardness of the tortillas.

| | Moisture content (%) | | Lost moisture | Thickness | Hardness |
	Day 1	Day 4	(%)	(mm)	(N)
TA	33.41 ± 0.50^{eA}	27.81 ± 0.14^{dB}	16.76 ± 1.02^{ab}	1.32 ± 0.10^{a}	4.47 ± 0.08^{a}
TA$_5$	39.16 ± 0.13^{dA}	33.61 ± 0.36^{cB}	14.16 ± 1.21^{b}	1.43 ± 0.08^{a}	3.01 ± 0.01^{d}
TA$_{10}$	39.56 ± 0.41^{dA}	34.22 ± 0.23^{cB}	13.50 ± 0.35^{b}	1.41 ± 0.17^{a}	2.69 ± 0.24^{d}
TA$_{15}$	39.24 ± 0.30^{dA}	33.61 ± 0.06^{cB}	14.34 ± 0.80^{b}	1.47 ± 0.05^{a}	1.09 ± 0.08^{e}
TM	42.60 ± 0.36^{cA}	35.49 ± 0.48^{bcB}	16.69 ± 0.98^{ab}	1.37 ± 0.21^{a}	3.46 ± 0.42^{c}
TM$_5$	44.19 ± 0.31^{bA}	37.95 ± 0.85^{aB}	14.12 ± 2.19^{b}	1.42 ± 0.10^{a}	3.97 ± 0.05^{b}
TM$_{10}$	45.10 ± 0.24^{bA}	36.51 ± 1.09^{abB}	19.04 ± 2.83^{a}	1.35 ± 0.14^{a}	3.64 ± 0.32^{bc}
TM$_{15}$	45.73 ± 0.66^{aA}	38.35 ± 0.65^{aB}	16.14 ± 0.30^{ab}	1.47 ± 0.13^{a}	3.98 ± 0.01^{b}

Note: Values are presented as mean $\pm$ standard deviation of triplicates ($p < 0.05$). [abcd]Significant differences in column ($p < 0.05$). [ABCD]Significant differences in row (p < 0.05). TA$_n$ is the sample of blue tortilla and TM$_n$ is the sample of white tortilla, where the subscript "n" denotes the proportion of huauzontle flour added (%).

The crude fat content was significantly increased in the blue tortillas supplemented with HF, but the increase remained independent of HF concentration, while the crude fat content of the commercial corn tortillas remained non-significantly different when supplemented with HF levels up to 10%, but was significantly higher at 15% HF (**Table 1**). The fat content of corn tortillas is affected by both the corn variety and the nixtamalization process, since lipids from the grain germ may be solubilized during alkaline cooking or leached out during the maceration stage (Colín-Chávez *et al.*, 2020).

3.2 Moisture content and thickness of the tortillas

In this study, organically nixtamalized blue corn was used to preserve its bioactive compounds, and commercial white corn flour was used to reflect typical consumption. While this approach introduces technological differences between the matrices, it allows for the evaluation of the potential of fresh corn flour in both traditional and industrial settings, highlighting the importance of analyzing its impact within each specific food system. Moisture content of the HF fortified blue and commercial tortillas was significantly higher than that of their TA and TM control counterparts (**Table 2**). TM had significantly higher moisture content than TA. Moisture content remained non-significantly different as HF content increased in TA and TM tortillas, except for TM$_{15}$. The moisture content in blue tortillas varied from 33.3% for TA (control) to 39.2% for TA$_{15}$, while for the white tortillas it varied from 42.6% for TM (control) to 45.9% for TM$_{15}$ on day 1. Salinas-Moreno *et al.* (2024) reported that the moisture content of tortillas made from commercial corn flour was up to five percentage points than those made from native corn landrances. The difference in moisture content between the tortillas made with blue and commercial white corn flour could be due to the

different method of nixtamalization of the grain. Ecological nixtamalization causes less pericarp loss in the grain, which decreases the water absorption capacity (Mariscal-Moreno *et al.*, 2022). In addition, the presence of carboxymethyl cellulose and other gums in commercial nixtamalized flour may have promoted water absorption and retention (Salinas-Moreno *et al.*, 2024). According to Colín-Chávez *et al.* (2020), the humidity of tortillas ranges from 35 to 50 %, depending on the variety of corn and the nixtamalization conditions used. A moisture range between 42-46% is considered acceptable for quality tortillas (Mariscal-Moreno *et al.*, 2022).

After 4 days of storage at 4 °C, the different tortilla variations suffered a moisture loss of about 13.50 % to 19.04 %, respect the moisture content they possessed at day 1. The blue corn tortillas variations, nixtamalized using the ecological method, presented lower moisture losses than the commercial corn tortillas, which were obtained by traditional nixtamalization. Mariscal-Moreno *et al.* (2022) found similar results for tortillas made with ecological and traditional nixtamalized corn flour. Lower moisture loss resulted in tortillas with lower starch retrogradation during storage.

Tortilla thickness is a parameter that influences the moisture content of tortillas. The thickness of the tortillas ranged from 1.32 ± 0.10 to 1.47 ± 0.13 mm (**Table 2**). The tortilla thickness of all the tortilla variations kept within the range reported for commercially produced tortillas (1.3 to 1.5 mm) (Salinas-Moreno *et al.*, 2024).

3.3 Hardness

The hardness of the tortillas ranged from 1.09 N to 4.47 N (**Table 2**), with TA (control) exhibiting the highest hardness, and the lowest hardness was displayed by TA$_{15}$. Argüello-García *et al.* (2017) observed that tortillas enriched with *Jatropha curcasha* L. flour, suffered a decrease in hardness

from 3.13 ± 0.08 N in the control group to 2.30 ± 0.01 N in tortillas supplemented with 15 % *Jathropha* flour. The decrease was attributed to the increased protein content resulting from the incorporation of the *Jatropha* flour, as proteins have the ability to hold water, contributing to the softness of tortillas. In this sense, huauzontle protein has shown a high-water holding capacity of 3.3 ± 0.2 g/g (López-Monterrubio *et al.*, 2020), which may be linked to decrease in hardness in blue corn tortillas. However, the hardness of the tortillas made with commercial nixtamalized flour remained almost at the same level when HF was added. This suggests that the interactions between huauzontle flour components and corn flour constituents were not strong enough to significantly modify the hardness of the enriched tortillas (Álvarez-Poblano *et al.*, 2020). This behavior can be attributed to differences in the structural matrix of commercial corn flour, which includes additives such as gums that already confer a high-water retention capacity and a more rigid protein network (Salinas-Moreno *et al.*, 2024).

3.4 Color

The color parameters measured in the tortillas can be observed in **Table 3**. The results show significant differences (p < 0.05) in color measurements between all the tortillas variations. **Figure 1** is composed of an a*-b* circle, divided in 4 quadrants. All tortilla variations fell within the -a*+b* quadrant, with the intensity of the -a* (green) values predominating over the +b* (yellow) values. Increasing HF addition shifted the color of TA (almost located in the origin), towards a predominantly -a* (greener) value. On the other hand, the TM variation had a greater predominance of +b* (yellower) than of -a* (greener), and addition of HF shifted the color predominance to -a* (greener) than to +b* (yellower) color. The right-hand side photographs provide a measure of the observed overall color hue and luminosity of the tortillas variations. It can be observed that the blue corn tortillas become increasingly greener and more luminous, while the white corn tortillas become less luminous and also increasingly greener. These results agree with what was expected, because the huauzontle flour is green in color and increasing its content intensifies the green hue of the tortillas obtained.
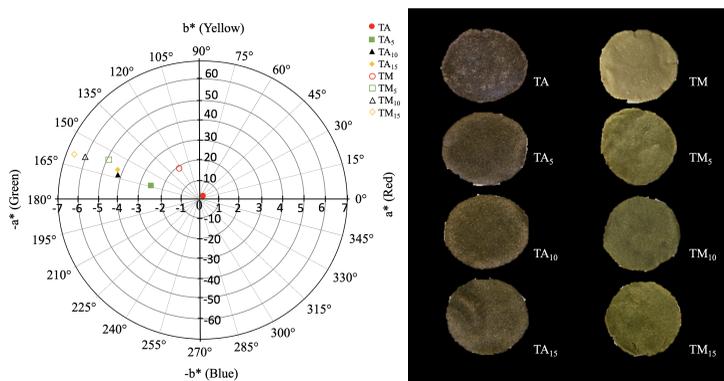

Figure 1.- a) *CIELab color parameters (L, a*, and b*) of tortilla variations. b) Representative images of the tortilla* samples obtained from different formulations.

Table 3. Instrumental color parameters (L, a*, and b*) of blue and white corn tortillas enriched with huauzontle flour.

Sample	L*	a*	b*
TA	38.66 ± 0.76[e]	0.08 ± 0.15[a]	3.52 ± 0.26[e]
TA$_5$	37.71 ± 0.85[e]	-2.48 ± 0.34[c]	9.62 ± 0.66[d]
TA$_{10}$	36.94 ± 0.44[e]	-4.01 ± 0.17[d]	14.38 ± 0.66[c]
TA$_{15}$	37.62 ± 0.40[e]	-4.08 ± 0.15[d]	16.21 ± 1.03[b]
TM	71.33 ± 2.02[a]	-1.16 ± 0.09[b]	17.12 ± 0.38[b]
TM$_5$	62.74 ± 0.47[b]	-4.69 ± 0.18[e]	20.53 ± 0.33[a]
TM$_{10}$	55.81 ± 0.77[c]	-5.82 ± 0.19[f]	21.12 ± 0.59[a]
TM$_{15}$	52.38 ± 1.04 [d]	-6.51 ± 0.25[g]	21.98 ± 1.24[a]

Note: Values are presented as mean ± standard deviation of triplicates (*p* < 0.05). [abcd]Significant differences in column (*p* < 0.05). TA$_n$ is the sample of blue tortilla and TM$_n$ is the sample of white tortilla, where the subscript "n" denotes the proportion of huauzontle flour added (%).
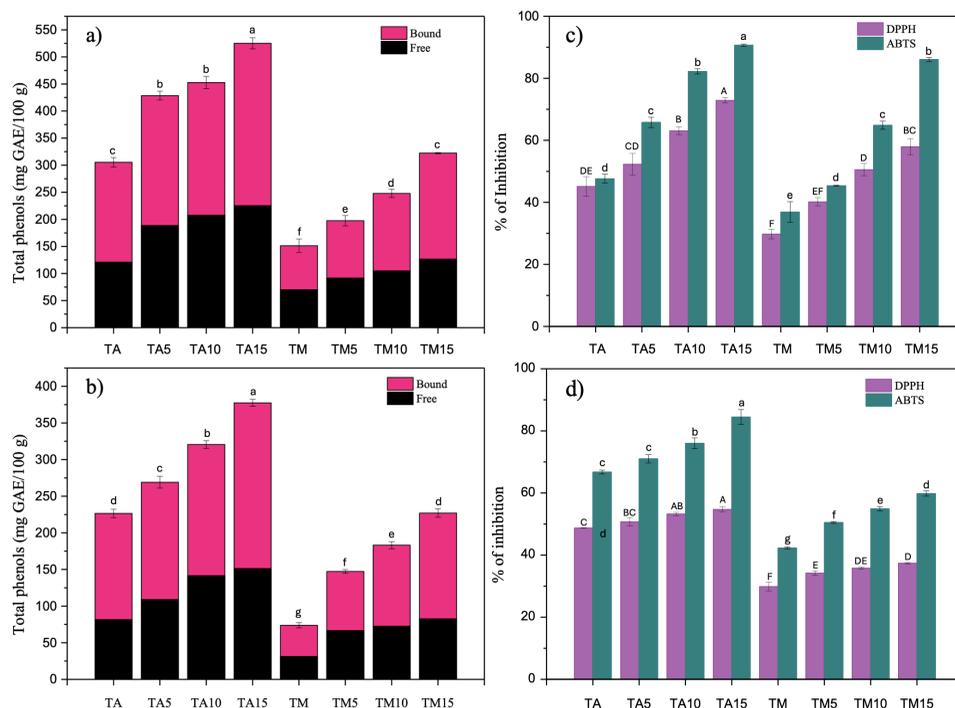
Figure 2. Phenolic content and antioxidant capacity of flour and tortilla samples obtained from different formulations: a) total phenolic compounds in flours, b) total phenolic compounds in tortillas, c) antioxidant capacity of free phenolic fraction, and d) antioxidant capacity of bound phenolic fraction.

3.5 Total phenolic content

The polyphenol content of the blue and white corn tortillas and flour is shown in **Figure 2a** and **2b**. The total phenolic content in all tortilla and flour variations increased significantly with the incorporation of huauzontle flour. The content of free phenolic compounds in huauzontle flour was 314.57 ± 1.10 mg GAE/100 g dry weight. Román-Cortés *et al*. (2018) reported a content of free phenolic compounds in huauzontle of 432.99 mg GAE/100g fresh weight. The differences found could be due to the different method used for the determination of phenolic compounds. On the other hand, the content of total polyphenols in the raw blue corn grain was 305.39 ± 1.36 mg GAE/100g dry weight. Méndez-Lagunas *et al*. (2022) reported a free polyphenol content in blue corn grains of 213 ± 12 mg GAE/100 g dry weight, and Vázquez-Carrillo et al. (2023) found 185.3 ± 8 mg ferulic acid equivalents /100 g dry weight in blue corn grains. The content of bound phenolic compounds was higher than free phenolic compounds in all treatments (**Figures 2a and 2b**). The results showed that, on average, free phenolic compounds in tortillas ranged from 31.37 to 151.26 mg GAE/100 g dry weight, while bound phenolics ranged from 42.52 to 226.09 mg GAE/100 g dry weight. Previous studies have shown that over 80 % of phenolic compounds in corn grains and their tortillas exist in cell wall-bound form, primarily associated with hemicelluloses in the pericarp, aleurone layer,

and germ (Mora-Rochin *et al*., 2010).

The content of phenolic compounds in tortillas was significantly reduced when compared to flour (loss percentage: 25.84 to 55.23 %), with the TM tortilla variations exhibiting the lowest degree of retention of phenolic compounds. Similar results were found by López-Vásquez *et al*. (2020) where, despite the short time of exposure of white corn tortillas to high temperatures when baked, caused a high percentage of degradation of the phenolic compounds (46.7 %). Méndez-Lagunas *et al*. (2022) reported a loss between 46.3-57.5% between nixtamalized corn and tortillas, attributed to the thermal-alkaline conditions of the nixtamalization that caused the leaching of phenolic compounds towards the cooking liquid. The addition of huauzontle flour to both blue and white corn flour, could represent an excellent alternative in the production of products with a high content of bioactive compounds with antioxidant activity.

3.6 DPPH radical scavenging activity

To overcome the inherent limitations of individual antioxidant evaluation methods, two complementary techniques were employed: the ABTS assay, which quantifies both hydrophilic and hydrophobic antioxidants, along with the DPPH assay, more specific for lipophilic compounds. According to the DPPH and ABTS test, the antioxidant activity of tortillas increased significantly ($p < 0.05$) as the

amount of HF in the variations increased (**Figure 2**). The blue corn tortillas supplemented with HF exhibited significantly higher ($p < 0.05$) antioxidant capacity compared to that of the white corn tortillas. This was attributed to the high content of phenolic compounds that are considered potent antioxidants carried by blue corn. In addition, the presence of ascorbic acid, vitamin E and flavonoids in huauzontle flour also contributes to the high antioxidant activity displayed (Garcia *et al.*, 2022). Blue corn tortillas had almost double the antioxidant activity of white corn tortillas. The antioxidant capacity of blue corn is mainly attributed to ferulic acid, its most abundant phenolic acid, as well as to the interaction with other phytochemicals present in this grain (Mora-Rochin *et al.*, 2010).

Moreover, a higher inhibition percentage was observed for the ABTS radical compared to the DPPH radical. These findings are consistent with those of Samardžic *et al.* (2025), who demonstrated that corn ferulic acid was more effective in neutralizing the ABTS radical than DPPH. In addition to this, the DPPH method generally yields lower values than ABTS because the DPPH radical is more stable, which reduces its reactivity. However, the fortification of commercial white corn flour with huauzontle flour produced a significant increase in the antioxidant capacity compared to the control.

3.7 FTIR

The FTIR spectra of the tortilla's variations are shown in **Figure 3a**. All the variations showed similar characteristic bands. The broad band between 3700-3000 cm^{-1} is related to the superimposed combination of stretching OH, due to hydration effects, and NH stretching attributed to the presence of Amide A (Vernon-Carter *et al.*, 2020). The band observed at approximately 2930 cm^{-1} can be attributed to CH stretching, associated with the presence of amylose-fatty acid inclusion complexes during retrogradation (Álvarez-Poblano *et al.*, 2020). The presence of amylose-fatty acid complexes was expected due to the lipid content in both flours (*see section 3.1*).
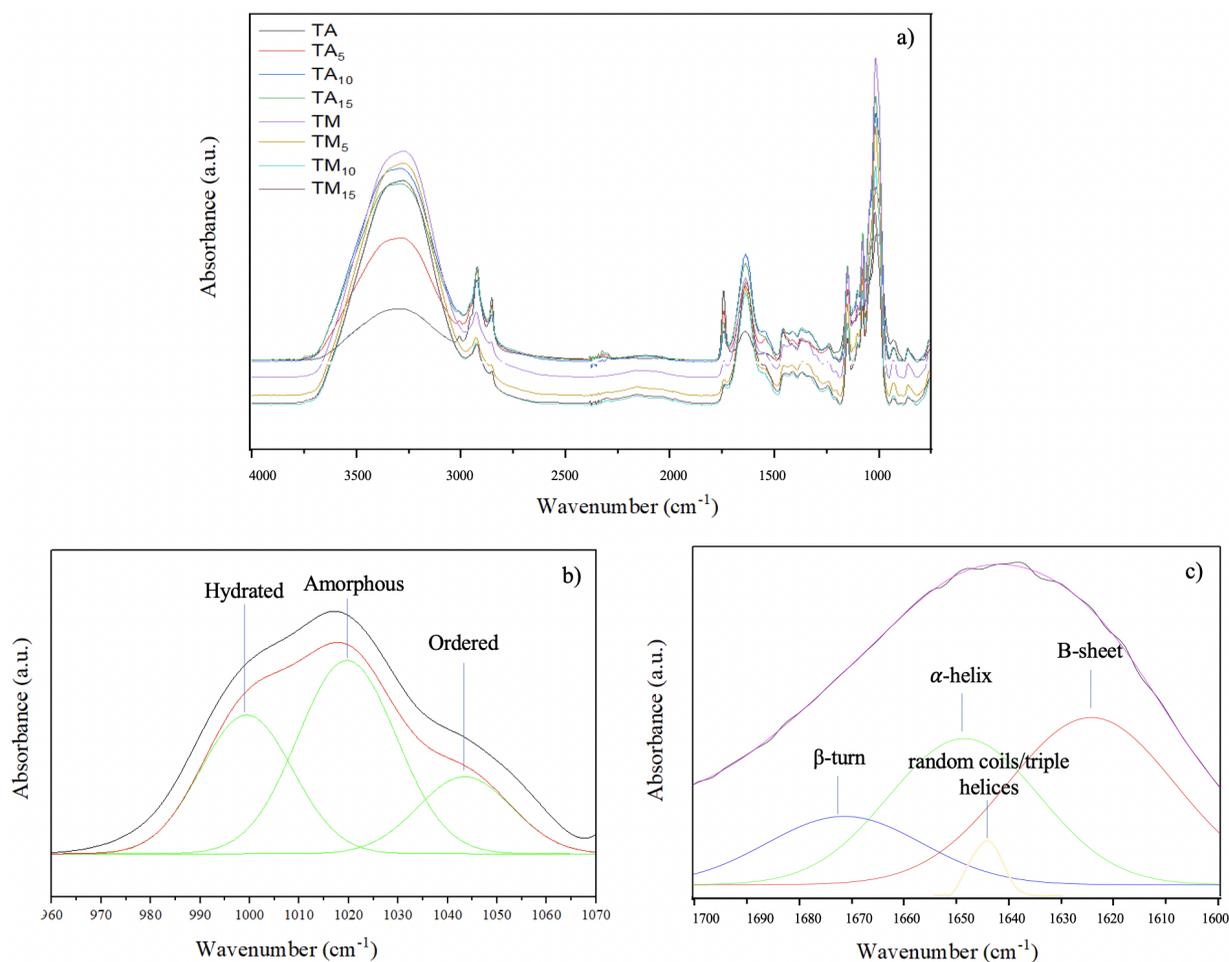

Figure 3. a) FTIR spectra of tortilla variations. b) Illustrative example of the numerical deconvolution applied to the starch fingerprint region. c) Illustrative example of the numerical deconvolution of the amide I region associated with protein structure.

The bands found between 1700-1600 cm^{-1}, 1600-1500 cm^{-1} and 1330-1500 cm^{-1} are attributed to the vibration of Amide I, Amide II and Amide III, respectively. The peak observed between 1420–1370 cm^{-1} has been associated with CH group bending vibrations of starches and lipids (Zhang *et al.*, 2025). The bands between 1080-950 are considered the fingerprint of starch structures and have been associated with stretching vibrations of CO and CH and bending vibrations of CHO (Vernon-Carter *et al.*, 2020). The absorption peak at 995 cm^{-1} could reflect the interaction between water and starch (**Figure 3b**). The intensity ratios of 1047/1022 cm^{-1} and 1022/995 cm^{-1} are commonly used to assess variations in short-range molecular order and double-helix organization, respectively. In particular, the ratio between the 1047 and 1022 cm^{-1} bands serves as an indicator of starch structural order, as it is sensitive to changes associated with crystalline and amorphous regions (Mariscal-Moreno *et al.*, 2022). The incorporation of huauzontle flour decreased the ratio 1047/1022 cm^{-1} in all cases, which may be associated with more disordered structures. TM$_{15}$ presented the highest value of 1022/995 and the lowest value of 1047/1022. This behavior can be associated with the loss of the ordered structure of starch and a strong interaction between water and starch through hydrogen bonds. Vernon-Carter *et al.* (2020) attributed this behavior to the interaction between starch and phenolic compounds through non-covalent interactions, promoting the formation of single amylose helices due to hydrophobic effects or the formation of amylose-polyphenol complexes through hydrogen bonds.

Amide I arises from the stretching vibrations of the C=O groups in peptide bonds and, therefore, is related to the conformation of the main chain and the hydrogen bonding pattern, which is why it is used for the characterization of the secondary structure of proteins. From the deconvolution of Amide I, four main bands were observed: *alpha*-helix, β-sheet, β-turns, and random coils/triple helix, at 1650–1660 cm^{-1}, 1610–1640 cm^{-1}, 1660–1700 cm^{-1}, and 1640–1650 cm^{-1}, respectively (López-Monterrubio *et al.*, 2020). With the addition of huauzontle flour to TA and TM, an increase in β-sheet structures was observed. This increase could be attributed to two factors: *i*) the interaction of huauzontle flour proteins with other components, such as lipids and/or carbohydrates, favoring a more compact and stable structural rearrangement; *ii*) the gelation of proteins during the tortilla cooking process, which often increases the β-sheet content due to the ordering of proteins during the formation of a three-dimensional interpolymetric network (Cortez-Trejo *et al.*, 2022).
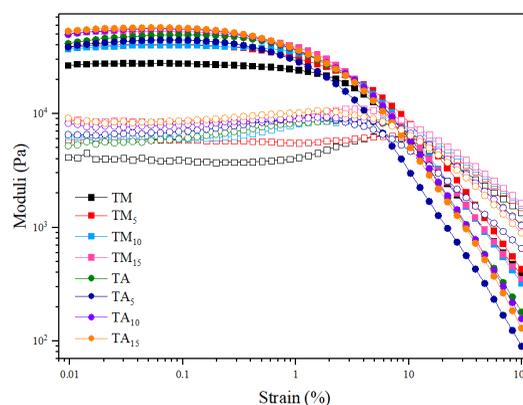

Figure 4. Storage (G') and loss (G") moduli of masa as a function of strain amplitude. Storage (G') (filled symbols) and loss (G") (empty symbols) moduli.

3.8 Rheological behavior of the dough

The rheological properties, particularly viscoelasticity, are crucial for dough malleability and affect processes such as laminating, cutting, and shaping, impacting tortilla production and quality (Vernon-Carter et al., 2020). **Figure 4** shows the variation of the storage (G') and loss (G") moduli as a function of strain amplitude. In all formulations, G' > G" within the linear viscoelastic region (LVR), indicating predominantly elastic behavior. The control tortillas (TM and TA) showed a wider LVR, reflecting a more robust structural network (Vernon-Carter *et al.*, 2020). This behavior suggests that components of huauzontle, such as dietary fiber and protein, may have disrupted the continuity of the gelatinized starch matrix, decreasing the system's ability to withstand deformations without structural damage (Amador-Rodríguez *et al.*, 2025). An increase in the storage modulus was observed with increasing levels of HF supplementation in both tortilla types. The results of this work suggest that the addition of huauzontle flour improved the elastic properties of the dough. This behavior could be related to increased interaction between the components of the huauzontle flour and the starch matrix in the tortilla, resulting in a more rigid structure. This effect can be attributed to two main mechanisms: i) hydrophobic interactions and hydrogen bonds between proteins and amylose/amylopectin chains (Gentile, 2020); ii) the possible formation of starch-polyphenol complexes, including inclusion and non-inclusion interactions, which restrict the molecular mobility of amylose chains and contribute to a more rigid and compact starch matrix (Álvarez-Poblano *et al.*, 2020; Vernon-Carter *et al.*, 2020). Furthermore, the increase in β-sheet structures (*see section 3.7*) with the highest HF supplementation provides direct structural evidence supporting mechanism i). Higher β-sheet content has previously been associated with a more ordered

protein network and strong hydrogen bonds (Wang *et al.*, 2017). These results differ from those reported, where supplementation with 2.5% huauzontle flour did not affect storage modulus values (Sánchez-Villa *et al.*, 2020). The differences found could be due to the different substitution levels used, the nixtamalization method, and/or the variety of corn employed. Additionally, tortillas made with white corn flour showed lower G' values. This behavior may be associated with the higher moisture content of white corn tortillas compared to blue corn tortillas (*see Table 2*). Thakur *et al.* (2017) observed a decrease in G' and G" moduli values with the increase in water content in corn flour. This could be associated with the weakening of protein-starch interactions, leading to a reduction in viscoelastic behavior.

3.9 *In vitro starch digestibility*

Resistant starch (RS) is defined as the portion of starch that is not hydrolyzed in the small intestine within 2 hours of consumption and is instead fermented in the colon (Robles-Arias *et al.*, 2025). The RS fraction in tortillas fortified with huauzontle flour increased compared to their non-fortified counterparts TA and TM (**Table 4**). Similar results have been previously reported. Vernon-Carter *et al.* (2020) observed a decrease in starch digestibility in tortillas fortified with anthocyanins. This was likely due to enzymatic inhibition caused by phenolic compounds, as well as the formation of starch-polyphenol inclusion and non-inclusion complexes (Fan *et al.*, 2025). Additionally, the higher lipid content in fortified tortillas may have contributed to the formation of a greater proportion of RS. According to Reyes-Moreno *et al.* (2013), the hydrophobic core of amylose can trap lipid molecules through the formation of amylose-lipid complexes, which can inhibit starch digestion by amylase.

Table 4. Effect of huauzontle flour incorporation on starch digestibility fractions (RDS, SDS, and RS) of corn tortillas.

	RDS (%)	SDS (%)	RS (%)
TA	53.19 ± 1.10^b	22.28 ± 1.21^a	24.52 ± 0.44^d
TA_5	48.14 ± 1.87^d	22.03 ± 0.59^a	29.82 ± 0.15^c
TA_{10}	45.21 ± 0.86^e	22.15 ± 0.08^a	32.62 ± 0.65^b
TA_{15}	31.67 ± 0.66^f	22.40 ± 1.43^a	45.91 ± 2.04^a
TM	59.83 ± 1.48^a	20.37 ± 0.39^b	19.79 ± 0.08^e
TM_5	51.05 ± 1.07^c	19.97 ± 0.61^b	28.97 ± 1.01^c
TM_{10}	51.19 ± 2.24^c	20.17 ± 1.10^b	28.62 ± 1.54^c
TM_{15}	47.58 ± 0.62^d	20.57 ± 2.62^b	31.83 ± 0.47^b

Note: Values are presented as mean ± SD of triplicates (p < 0.05). [abcd]Significant differences in column (p < 0.05). RDS = Readily digestible starch. SDS = Slowly digestible starch. RS = Resistant starch. TA_n is the sample of blue tortilla and TM_n is the sample of white tortilla, where the subscript "n" denotes the proportion of huauzontle flour added (%).

Likewise, the formation of complexes with other components, such as proteins, could have limited the enzymatic hydrolysis of starch. Similar effects have been reported in cereal-based systems supplemented with plant-derived ingredients, where changes in the molecular organization of starch and proteins were associated with reduced in vitro starch digestibility (Godoy-Ramírez *et al.*, 2024). These results suggest that supplementing corn flour with huauzontle flour may contribute to better digestion and a lower glycemic index, making it suitable for regular consumption.

Conclusions

This study demonstrates that supplementing tortillas with huauzontle flour can significantly improve their nutritional and functional profile. Tortillas made with nixtamalized blue corn showed higher protein content and antioxidant activity compared to commercial tortillas, suggesting potential for reducing malnutrition in Mexico. Additionally, the incorporation of huauzontle flour increased starch resistance to digestion and improved the viscoelastic properties of the dough, which could contribute to a lower glycemic response. Although the effect on tortilla firmness was variable, huauzontle's ability to retain water appears to be linked to reduced hardness in blue corn tortillas. Differences in nixtamalization methods, substitution levels, and corn varieties may explain the variability of results compared to previous studies. On the other hand, fortifying commercial corn tortillas with 15% huauzontle flour could match the phenolic compound content of blue corn tortillas. Therefore, the addition of huauzontle represents a viable strategy to enhance the nutritional and functional quality of tortillas, with positive implications for public health. In addition, the results presented provide a solid framework for future studies aimed at further elucidating the molecular and structural factors that govern the rheological and nutritional behavior of tortilla systems enriched with huauzontle. They also provide a starting point for the scaling up to a semi-industrial level for producing tortillas with huauzontle flour, and for establishing standardized steps for preparing ingredients, handling dough, suggesting potential packaging that might help preserve and minimize textural and sensorial quality of tortillas.

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