

RESEARCH ARTICLE

Tortillas made from nixtamalized maize and extruded chickpea flours: A product with improved in vitro nutritional and antihypertensive properties

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Abstract

Background and Objectives: In this study, tortillas with improved in vitro nutritional and antihypertensive properties were developed using flours produced from nixtamalized maize (NixM) and extruded chickpea (ExtCP). The aims were (1) to find the optimal extrusion conditions to produce optimized ExtCP flour with high values of antioxidant activity (AoxA), total phenolic compounds (TPC), and in vitro protein digestibility (IVPD), and (2) to evaluate the addition effect of 30% of optimized ExtCP flour over the nutritional, technological, sensory y nutraceutical properties of tortillas prepared from commercial NixM flour. A rotatable composite central experimental design with two factors (extrusion temperature [ET] = 50°C–170°C; screw speed (SS) = 50–240 rpm) and five levels was used to optimize the extrusion process.

Findings: The best combination of variables of the extrusion process to produce ExtCP flour was: ET = 143°C/SS = 138 rpm. The tortillas added with ExtCP flour had: proteins = 13.27%, total dietary fiber = 13.40%, protein chemical score = 85.42%, IVPD = 82.01%, calculated protein efficiency ratio (C-PER) = 1.84, AoxA = 8031 μmol TE/100 g, and sensory acceptability = 8.51 (value located between I like it very much and I like it extremely).

Conclusions: The tortillas made from nixtamalized maize and ExtCP flours have better in vitro nutritional and antihypertensive properties than tortillas prepared from only nixtamalized maize flour.

Significance and Novelty: The addition of optimized ExtCP flour to commercial NixM flour allows obtaining functional tortillas with improved and sensory acceptable in vitro nutritional and antihypertensive properties.

KEYWORDS

antioxidant compounds, *Cicer arietinum* L, healthy foods, protein quality, *Zea mays* L

1 | INTRODUCTION

In Mexico, in 2019, according to the National Demographic Dynamics Survey (ENADID by its acronym in Spanish), the population of older adults (OA) (60 years and older) was 16,179,000. Several of the health problems suffered by OA are attributable to age. However, many of them are also due to bad habits during the previous stages of their lives, enhanced by age. Taking preventive measures to modify these habits and guarantee access to health services are individual and institutional actions (INEGI, 2019).

The rapid increase in the OA population has led to the accumulation of chronic degenerative diseases (cardiovascular diseases, type 2 diabetes, hypertension, and some types of cancer) (Prince et al., 2015). Furthermore, significant physiological changes in OA such as difficulty chewing, dry mouth, loss of taste, and appetite loss may also be present. Consequently, there is a reduction in oral processing capacity. Therefore, researchers and the food industry have shown interest in supplying technological solutions for foods with nutritional properties and attractive sensory attributes (Wang & Cheng, 2017).

In Mexico, maize tortillas are a staple of the diet. Unfortunately, maize-based tortillas lack lysine (essential amino acid [EAA]), besides optimal levels of important micronutrients such as minerals (Fe, Zn) and vitamins (A, D, E, B12) (Chuck-Hernández & Serna-Saldívar, 2019). Fortification of tortillas can act as an excellent means to improve the nutritional condition of tortilla consumers (such as Mexicans). Its annual intake sums to more than 90 kg per capita; in adulthood, 8–10 tortillas are ingested per day. The fortification of maize tortillas with pseudo-cereal flours (amaranth, chia, and quinoa) or legumes (chickpea, common bean, tepary bean) is an alternative to enhance the nutritional and nutraceutical quality of this product.

Maize (*Zea mays* L) originated in Mexico more than 6000 years ago (González-Ortega et al., 2017), and is the most widely produced cereal globally (FAO, 2020). In Mexico, a great number of food products and dishes (more than 600) are made from maize, and almost half of them use nixtamalized maize (NixM), meaning, cooked with lime.

Maize kernels provide macronutrients, including protein, starch, fat, and micronutrients, including minerals and vitamins (Prasanna et al., 2019). The main compounds found in the grain are insoluble and soluble dietary fibers (SDFs), including lignin and cellulose, and secondly, arabinoxylans, hemicelluloses, and β -glucans. Insoluble dietary fiber (IDF) improves intestinal transit, decreases constipation, and lowers the glycemic index; NixM tortillas are classified as low-glycemic food (less than 55). SDF

ameliorates intestinal function; as a fermentation product, it produces volatile fatty acids, which are hypocholesterolemic (Palacios-Rojas et al., 2020).

Also, maize contains phytochemicals that supposedly aid in health improvement and disease prevention, such as phenolic acids, carotenoids (yellow and orange maize), and anthocyanins (blue, purple, and black maize) (Palacios-Rojas et al., 2020; Prasanna et al., 2019). The concentrations are dependent on the variety of the grain, the agronomic management, the interaction between the variety and the environment, and the postharvest handling.

Chickpea beans contain a protein percentage similar to other legumes, such as pea and lentils (Bon-Padilla et al., 2019). Besides this, chickpea proteins have excellent bioavailability and great digestibility (48%–89.01%) (Kou et al., 2013). Likewise, chickpea proteins have an appropriate balance of amino acids, highlighting Glu, Asp, Arg, Leu, Phe, Lys, Ser, and to a lesser extent His, Gly, Tre, Ala, Tyr, Val, Ile; however, they are deficient in sulfur amino acids (Met + Cys) (Cortés-Giraldo et al., 2016). Carbohydrates are the major component of chickpea (62%–70%) and are mainly made up of polysaccharides, such as starch (of which 35% is resistant and 65% is available) and dietary fiber (18%–22%), of which 4%–8% is soluble, and 10%–18% is insoluble. Chickpea has 4%–10% of lipids; however, there are mostly unsaturated fatty acids, mainly linoleic (54.7%–56.2%), oleic (21.6%–22.2%), and palmitic (18.9%–20.4%). Also, chickpea is an important source of B vitamins and microelements (Fe, Zn, Ca, Mg, K, Cu, and P) (Bon-Padilla et al., 2019). Finally, chickpea contains phytochemicals such as phenols (phenolic acids, flavonoids), lectins, saponins, phytic acid, trypsin, chymotrypsin, and α -amylase inhibitors, among others (Domínguez-Arispuro et al., 2018; Bon-Padilla et al., 2019; Domínguez-Arispuro et al., 2021). The bioactive compounds present in whole grains of chickpea present a wide range of biological activities, including antioxidant, antihypertensive, anti-inflammatory, anticancer, hypocholesterolemic, and hypoglycemic, among others (Bon-Padilla et al., 2019).

The nutritional and nutraceutical qualities of maize and chickpea make these crops very attractive to the food industry. Many processing methods (germination, solid-state fermentation, extrusion-cooking) are applied to maize and chickpea kernels to retain and enhance their nutritional and nutraceutical properties. Extrusion-cooking applies high temperature, pressure, and shear force (shear stress) for a short period. This technology increases the proteins and starches digestibility and improves the retention of bioactive compounds and SDF. Besides this, it produces lipid changes, inactivation of enzymes and microorganisms, and production of volatile aromatic components. Extrusion-cooking is an alternative

technology that is extremely efficient by lessening energy consumption and water pollution. This technology can also develop functional foods and beverages (Ortiz-Cruz et al., 2020; Ramos-Enríquez et al., 2018).

This study had the following aims: (1) To find the optimal extrusion conditions to produce optimized extruded chickpea (ExtCP) flour with high antioxidant activity (AoxA), total phenolic compounds (TPC), and in vitro protein digestibility (IVPD), and (2) prepare a mixture (70% commercial NixM flour + 30% optimized ExtCP flour) suitable for making a functional tortilla for OA with high values of protein quality, AoxA, and sensory acceptability (A).

2 | MATERIALS AND METHODS

Whole chickpea (*Cicer arietinum* L) seeds variety Blanco Sinaloa 92 were provided by INIFAP Valle de Culiacán, Sinaloa, Mexico. The grains were cleaned, placed in hermetic containers, and stored (5°C–10°C) until used. The commercial NixM flour (MASECA^{MR}) was acquired in a market in the locality of Culiacán, Sinaloa, Mexico.

2.1 | Production of ExtCP flour

The ExtCP flour production was carried out according to Gutiérrez-Dorado et al. (2008). Lots of 500 g of whole chickpea seeds were set in a domestic blender at low speed; broken grains were obtained that were triturated in the same blender to get grain fragments (grits) that passed through a 40-US (0.425 mm) screening mesh but were retained over a 200-US (0.074 mm) screening mesh, and fine powder. The grits were blended with water to achieve a water content of 28/100 g. The conditionate samples were placed in polyethylene bags and stored (4°C/12 h). Before extrusion, the wetted and balanced grits were tempered to 25°C. Extrusion was carried out in a Model 20 DN single-screw laboratory extruder (CW Brabender Instruments, Inc.) with 19 mm screw diameter, 20:1 length to diameter; 1:1 nominal compression ratio, and 3 mm die opening. The feed rate was 70 g/min. The extruder operating conditions were selected from intervals of axial values to the process variables: ET (50°C–170°C) and screw speed (SS, 50–240 rpm) (Supporting Information: Table 1). The extrudates were cooled, equilibrated under ambient conditions (25°C/65% relative humidity (RH)], ground to pass through 80 mesh (0.180 mm), and wrapped in plastic bags. The ExtCP flours were stored (5°C–8°C) until their use. The ExtCP flours were evaluated for AoxA, TPC, and IVPD to carry out the optimization of the process and determine the

optimal extrusion conditions to obtain optimized ExtCP flour with high values of AoxA, TPC, and IVPD.

2.2 | Extraction of phenolic compounds (free and bound fractions)

The extraction of free and bound phenolic compounds was carried out in compliance with the methodology reported by Gámez-Valdez et al. (2021). The solvents used for the extraction of free and bound phenolic compounds were 80% chilled ethanol and ethyl acetate, respectively. All extractions were performed by triplicate.

2.3 | AoxA and TPC

The oxygen radical absorbance capacity (ORAC) assay was used to evaluate AoxA of free and bound phenolic extracts; this assay was carried out by diluting phenolic extracts in 75 mM phosphate buffer (pH 7.4). Aliquots of 25 μ l of diluted extracts were combined with 150 μ l of fluorescein (0.1 mM) and 25 μ l of the peroxy radical 2,2-azobis(2-amidopropane dihydrochloride, AAPH (200 mM)). After 30 min, the fluorescence was determined (485 nm for excitation and 538 nm for emission) (37°C) each 2 min by a 60 min period, using a Synergy microplate reader (SynergyTM HT Multi-Detection, BioTek, Inc.) (León-Murillo et al., 2021). The ABTS (2,2'-azino-bis (3-ethylbenzothiazollone-6-sulfonic acid)) assay to evaluate AoxA was executed by diluting extracts of free and bound phenolic compounds with ethanol. Aliquots of 20 μ l were taken from each dilution and blended with 2.0 ml of diluted radical cation ABTS^{•+}, and 6 min later, the absorbance at 734 nm was determined in a UV-visible spectrophotometer (GENESYS 10UV; Thermo Electron, Inc.) (León-Murillo et al., 2021). The ORAC and ABTS results were stated as μ mol of Trolox equivalents (TE)/100 g sample of dry weight (DW).

The content of phenolic compounds of the free and bound extracts was evaluated using 20 μ l of proper dilutions of extracts, oxidized with 180 μ l of Folin-Ciocalteu reagent (Gámez-Valdez et al., 2021). After 20 min, the resultant blue color's absorbance was calculated at 750 nm using the Synergy microplate reader. The content of phenolic compounds (free, bound, and total) was stated as mg of gallic acid equivalents (GAE)/100 g sample (DW). All measurements were made in triplicate.

2.4 | IVPD

For the determination of IVPD, the methodology of Queiroz-Mendes et al. (2016) was followed. Fifty milliliters

of a protein-water suspension (6.25 mg of protein/ml) were prepared to which the pH was adjusted to 8.0 and was placed in a double-walled recirculation beaker, keeping the temperature at 37°C with stirring. subsequently, 5 ml of a multi-enzyme solution (trypsin 2.5 mg/ml and pancreatin 1.6 mg/ml) were added. The pH was determined with a potentiometer after 10 min of reaction and was used to calculate the IVPD using the following equation:

$$\text{IVPD}(\%) = 93.1359 * \left[1 - e^{-3.1438 * (8 - \text{pH})} \right].$$

2.5 | Optimization procedure using response surface methodology (RSM)

The optimization of extrusion conditions to obtain optimized ExtCP flour was realized using a central composite experimental design. The used factors were ET (=50°C–170°C) and SS (=50–240 rpm), and the analyzed responses were AoxA, total phenolic content (TPC), and IVPD (Supporting Information: Table 1). The prediction models (second-order polynomial) for each response variable were obtained using the stepwise regression procedure, and to determine the best combination of extrusion process variables (ET, SS) the graphical method (conventional method) of the RSM was used, to maximize AoxA, TPC, and IVPD. This was realized using contour graphs constructed with the prediction experimental models obtained for each response variable, that were superimposed to get a contour plot including the optimal conditions (optimal ET and SS) for producing optimized ExtCP flour. The Design-Expert software version 7.0.0 (Stat-Ease) of the RSM was used to realize this analysis.

2.6 | Chemical composition, SDF, and IDF of flours

The official AOAC (2012) methods 960.52, 920.39C, and 925.09B were used to determine the content of proteins (Nx6.25), lipids, and moisture. The SDF and IDF were evaluated according to the enzymatic-gravimetric method for total dietary fiber (TDF) (method 985.29), using the Sigma-Aldrich TDF test kit (TDF 100 A) (AOAC, 2012).

2.7 | Nutritional properties of flours: EAAs, IVPD, protein chemical score (PCS), calculated protein efficiency ratio (C-PER)

The nutritional properties (EAA, IVPD, PCS, C-PER) of the commercial NixM and optimized ExtCP flours, and their tortillas (control tortilla: made with 100% commercial NixM

flour; and functional tortilla: made with 70% commercial NixM flour + 30% optimized ExtCP flour) were evaluated in agreement to Salas-López et al. (2018). The composition of EAA was determined using an analytical scale hypersil ODS C18 column (4.6 mm × 250 mm) (SGE) maintained at 38°C and connected to an high performance liquid chromatography system (GBC) equipped with a fluorescence > LC 5100 detector set at 270 and 316 nm for excitation and emission, respectively. Tryptophan was detected using an ultraviolet detector at 280 nm. IVPD was evaluated using the multi-enzyme system above described. The PCS was calculated as follows: PCS = (limiting EAA content/most limiting REAAR) × 100, where EAA = essential amino acid, and REAAR = recommended essential amino acid requirement for children 3 years and older, adolescents, and adults (FAO, 2013). C-PER was calculated based on the IVPD and the EAA content of the sample. All evaluations were carried out in triplicate.

2.8 | Antihypertensive potential (IC₅₀) of flours

The antihypertensive potential was defined as IC₅₀ (concentration [mg of extract/ml] of phenolic extract required to produce 50% inhibition of angiotensin-converting enzyme (ACE) activity). ACE inhibitory activity in free and bound phenolic extracts was determined using the Dojindo ACE Kit-WST test kit (Dojindo Laboratories). The colorimetric detection of an indicator supports the methodology after a redox reaction. The absorbance (Abs) at 450 nm is determined using a microplate reader (Synergy™ HT Multi-Detection, BioTek, Inc.). The IC₅₀ values were calculated from different phenolic extract concentrations and the ACE inhibitory activity values using the Prism v5 software (GraphPad Prism) (Argüelles-López et al., 2018).

2.9 | Antihyperglycemic potential (inhibition of α-amylase and α-glucosidase activities) of flours

The antihyperglycemic potential was defined as IC₅₀ (concentration [mg of extract/ml] required to produce 50% inhibition of the α-amylase or α-glucosidase enzymes). The total phenolic extract (sum of free and bound phenolic extracts) against α-amylase was determined by colorimetric measurement of the maltose released by α-amylase from starch after stopping enzymatic reaction with 3,5-dinitrosalicylic acid (Astawan et al., 2020). The inhibitory activity of these extracts against α-glucosidase was determined by measuring the

formation of p-nitrophenol by α -glucosidase after reacting with p-nitrophenyl- α -D-glucopyranoside (PNP) (Astawan et al., 2020). The percentages of inhibition of α -amylase and α -glucosidase were calculated using the following equations:

$$\begin{aligned} & \% \text{Inhibition of } \alpha\text{-amylase} \\ &= [(A_{540 \text{ control}} - A_{540 \text{ extract}}) / (A_{540 \text{ control}})] \\ & \quad \times 100\% \text{ inhibition of } \alpha\text{-glucosidase} \\ &= [(A_{405 \text{ control}} - A_{405 \text{ extract}}) / (A_{405 \text{ control}})] \\ & \quad \times 100. \end{aligned}$$

IC₅₀ values were calculated from the graphs of % inhibition versus phenolic extract concentration using Prism v5 software (GraphPad Prism).

2.10 | Tortilla preparation from commercial NixM and optimized ExtCP flours

Two types of tortillas were produced: (1) Tortilla made with 100% commercial NixM flour, and (2) functional tortilla made from the mixture of 70% commercial NixM flour + 30% optimized ExtCP flour. This proportion of flours was chosen based on laboratory preliminary studies. Tortillas were prepared by mixing 6.0 kg of the flour samples in a mixer (Model TA-50; Tortimaq y Diseño S. de R.L.), with the amount of purified water (60°C) to achieve masa (dough) with a suitable consistency (suitable sheeting and forming properties). Masa samples were processed in a commercial tortillería (Tía Violeta). A roller machine (Model TR-60; Tortimaq y Diseño S. de R.L.) was used to mold the tortillas (masa flattened, laminated and cut to take the classic disc shape). A 15-cm mold was used to mold unbaked tortillas (30 g weight). The tortillas were baked in an oven with three tiers at temperatures of 210 ± 15°C; 270 ± 25°C; and 250 ± 20°C, respectively, and a residence time of 50 s. Baked tortillas were cooled (room temperature = 25°C), separated one by one, and packed in polythene bags to avoid moisture loss. The tortillas were transported to the laboratory and stored at room temperature (25°C) for evaluation of sensorial and technological characteristics.

2.11 | Quality evaluation of functional tortillas made from commercial NixM and optimized ExtCP flours

The "swelling" of the tortillas was evaluated during their cooking using a scale of 1–3, where 1 = tortillas "without

swelling," 2 = tortillas with an "intermediate swelling," and 3 = tortillas "fully swollen" (Milán-Carrillo et al., 2006). The "rollability" (rolling capacity, or ability to form a taco) of the tortilla was evaluated 30 min after preparation. The tortillas were rolled on a 2.54 cm diameter glass rod, and the degree of rupture of the tortilla surface (0%–100%) indicated the "rollability" (1–5) as follows: 0% = 1; 25% = 2; 50% = 3; 75% = 4; and 100% = 5 (Bedolla & Rooney, 1984). For the sensory evaluation of the tortillas, square pieces of tortillas, preheated to 45°C, were presented on a plate to the panelists. The test panel comprises 120 panelists (aged between 18 and 65 years old) who are habitual tortilla consumers. The panelists rinsed their mouths with purified water, as a palate cleanser, between samples. Consumers evaluated taste, smell, color, texture, and acceptability (general acceptance). The panelists were asked to indicate their degree of like/dislike using a nine-level hedonic scale (1 = *extremely dislike* to 9 = *extremely liked*).

The remaining tortillas were dried and ground to pass through an 80-US mesh to evaluate their chemical composition, and nutritional and nutraceutical properties. These tortilla properties were evaluated according to the methodologies above described to flours. All evaluations were done by triplicate.

2.12 | Statistical analysis

The experimental results of chemical composition, nutritional, and nutraceutical properties of flours and tortillas were subjected to a *t*-student test at a 5% significance level using the MINITAB 17.0 software.

3 | RESULTS AND DISCUSSION

3.1 | Optimization of the extrusion process to obtain optimized extcp flour

3.1.1 | Prediction models of response variables (AoxA, TPC, and IVPD)

The AoxA, TPC, and IVPD values of ExtCP flours ranged from 1685 to 4453 $\mu\text{mol TE}/100 \text{ g (DW)}$, 57.3–150.1 mg GAE/100 g (DW), and 67.2%–86.3%, respectively (Supporting Information: Table 1). Regression analyzes were carried out on the experimental results, and prediction models were obtained for each response variable (Supporting Information: Table 2). From these prediction models, contour and surface graphs were obtained in which the effect of the process variables on the response

variables is shown (Supporting Information: Figures 1–3). The analysis of variance (ANOVA) (Supporting Information: Table 2) showed that AoxA, TPC, and IVPD depended significantly ($p < .0001$) on the linear term of ET. Likewise, the quadratic term of extrusion temperature (ET)² significantly affected ($p \leq .0001$) the response AoxA and IVPD, while the response of TPC was significantly affected ($p = .0002$) by the quadratic term of speed screw (SS)². The prediction models using the variables coded for the response variables (AoxA, TPC, and IVPD) were:

$$Y_{\text{AoxA}} = 1900.22 + 755.92 (ET) + 629.02 (ET)^2,$$

$$Y_{\text{TPC}} = 100.28 + 25.89 (ET) - 19.50 (SS)^2,$$

$$Y_{\text{IVPD}} = 82.82 + 5.94 (ET) - 3.88(ET)^2.$$

The regression models explained 94.68, 91.22, and 93.12% of the total variation of AoxA, TPC, and IVPD, respectively. The models did not show a lack of fit ($p > .05$), and the coefficient of variation (CV), which refers to the relative dispersion of the experimental points of the prediction models, was less than 10% (Supporting Information: Table 2). According to these values, the prediction models were adequate and reproducible.

3.1.2 | Optimization of the variables of the extrusion process to produce an Extcp Flour

The superposition of the contour graphs of the response variables (Supporting Information: Figures 1–3) generated the superposition graph (Figure 1), which shows in yellow the area where the best combination of the process variables (ET/SS) to which an optimized ExtCP flour can be obtained with high AoxA, TPC, and IVPD values. The best combination of variables was ET = 143°C/SS = 138 rpm, which predicts obtaining values of 2880 $\mu\text{mol TE}/100\text{ g}$ (DW), 120.90 mg GAE/100 g (DW), and 85.13% for AoxA, TPC, and IVPD, respectively. The experimental values of AoxA, TPC, and IVPD of optimized ExtCP flour (Tables 1 and 2) were similar to the predicted values, indicating that the optimal extrusion conditions were appropriate and reproducible.

3.2 | Chemical composition/nutritional properties of flours

Table 1 shows the proteins, lipids, and dietary fiber content of commercial NixM flour and optimized

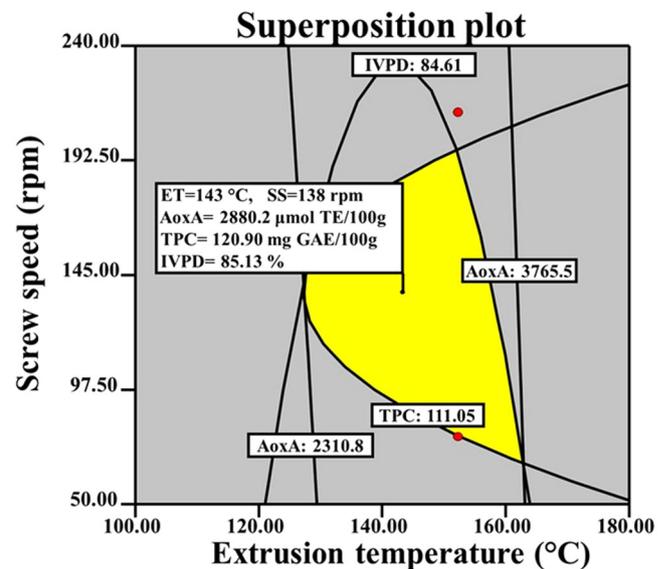


FIGURE 1 Superposition plot that shows the region of the best combination of extrusion temperature/screw speed for producing optimized extruded chickpea (ExtCP) flour with high antioxidant activity, total phenolic compounds, and in vitro protein digestibility. [Color figure can be viewed at wileyonlinelibrary.com]

ExtCP flour. The protein content [% , DW] of commercial NixM flour and optimized ExtCP flour was 9.02% and 23.08%, respectively. The lipid content of the flours ranged from 2.63% to 4.54%, DW. The commercial NixM flour had the littlest value. The dietary fiber content in the flours varied from 9.42%, DW (commercial NixM flour) to 22.61%, DW (optimized ExtCP flour); in both flours, the highest dietary fiber value corresponded to the IDF fraction which content varied from 8.20%, DW (commercial NixM flour) to 18.01%, DW (optimized ExtCP flour).

The EAA total content of the flours, commercial NixM flour, and optimized ExtCP flour, was superior to the pattern suggested by FAO (2013) for the recommendation of EAA for children (3 years and older), adolescents, and adults. In commercial NixM flour, Lys was the limiting EAA; optimized ExtCP flour showed a deficiency in sulfur EAA (Met + Cys). The IVPD of the flours ranged between 74.01% (commercial NixM flour) and 84.70% (optimized ExtCP flour). The highest value of C-PER (1.81 vs. 1.08) corresponded to the optimized ExtCP flour (Table 1). The high IVPD of optimized ExtCP flour is as a result of the inactivation of antinutritional factors (tannins, saponins, phytates, protease [trypsin and chymotrypsin] inhibitors) and dietary protein denaturation. It is the consequence of the conditions applied (cutting forces, temperature, and humidity) during the extrusion-cooking process (Félix-Medina et al., 2021).

TABLE 1 Chemical composition and nutritional properties of commercial NixM flour and optimized ExtCP flour.

Property	Commercial NixM flour	Optimized ExtCP flour	FAO ^a
<i>Chemical composition</i> (% DW)			
Protein	9.02 ± 0.12b	23.08 ± 0.10a	
Fat	2.63 ± 0.06b	4.54 ± 0.05a	
Ashes	2.14 ± 0.02b	3.02 ± 0.02a	
Dietary fiber			
Soluble	1.28 ± 0.05b	4.60 ± 0.07a	
Insoluble	8.20 ± 0.15b	18.01 ± 0.13a	
Total	9.42 ± 0.17b	22.61 ± 0.14a	
Carbohydrates	76.79 ± 1.06a	46.75 ± 1.21b	
<i>Nutritional</i>			
EAA (g/100 g protein)			
His	2.66 ± 0.04a	2.50 ± 0.04b	1.60
Ile	3.02 ± 0.04a	3.08 ± 0.03a	3.00
Leu	12.55 ± 0.08a	7.15 ± 0.06b	6.10
Lys	2.93 ± 0.03b	6.68 ± 0.07a	4.80
Met + Cys	3.65 ± 0.06a	2.08 ± 0.03b	2.30
Phe + Tyr	7.66 ± 0.04b	8.14 ± 0.04a	4.10
Thr	2.87 ± 0.03b	3.90 ± 0.02a	2.50
Trp	0.53 ± 0.03b	1.20 ± 0.03a	0.66
Val	4.60 ± 0.04a	4.12 ± 0.03b	4.00
Total	40.47	38.85	29.06
Chemical score	61.04	90.43	
Limiting EAA	Lys	Met + Cys	
IVPD (%)	74.01 ± 0.10b	84.7 ± 0.15a	
C-PER	1.08 ± 0.02b	1.81 ± 0.03a	

Note: a, b: Means with different letters, in the same row, are different (*t*-student test, $p \leq .05$).

Abbreviations: C-PER, calculated protein efficiency ratio; DW, dry weight; EAA, essential amino acid(s); ExtCP, extruded chickpea; IVPD, in vitro protein digestibility (%); NixM, nixtamalized maize.

^aEAA requirements for children (3 years and older), adolescents, and adults according to FAO (2013).

3.3 | AoxA and phenolic compounds in extruded grain flours

Table 2 shows the AoxA, content of phenolic compounds, and the antihypertensive and antihyperglycemic potentials of commercial NixM flour and optimized ExtCP flour. The AoxA, assessed by ORAC methodology, of commercial NixM flour and optimized ExtCP flour was 10,276 and 2773 $\mu\text{mol TE}/100\text{ g}$ of sample,

TABLE 2 Antioxidant activity, phenolic compounds, and antihypertensive and hypoglycemic potentials of commercial NixM flour and optimized ExtCP flour.

Property	Commercial NixM flour	Optimized ExtCP flour
Antioxidant activity ^a		
ORAC		
Free phenolic	3186 ± 72a	1659 ± 101b
Bound phenolic	7090 ± 142a	1114 ± 110b
Total phenolic	10,276 ± 389a	2773 ± 97b
ABTS		
Free phenolic	2153 ± 44a	1020 ± 65b
Bound phenolic	4279 ± 167a	815 ± 108b
Total phenolic	6432 ± 132a	1835 ± 147b
Phenolic compounds ^b		
Free phenolic	63.04 ± 1.10a	41.76 ± 2.36b
Bound phenolic	139.12 ± 2.49a	81.96 ± 4.01b
Total phenolic	202.16 ± 3.14a	123.72 ± 4.69b
Antihypertensive potential (IC ₅₀) ^c		
ACE inhibition hypoglycemic potential (IC ₅₀) ^c	2.43 ± 0.06a	1.32 ± 0.03b
α -amylase inhibition	34.02 ± 1.42b	64.06 ± 1.03a
α -glucosidase inhibition	23.03 ± 1.31b	81.02 ± 1.01a

Note: a, b: Means with different letter, in the same row, are different (*t*-student test, $p \leq .05$).

Abbreviations: ABTS, 2,2'-azino-bis (3-ethylbenzothiazollone-6-sulfonic acid); ACE, angiotensin-converting enzyme; DW, dry weight; ExtCP, extruded chickpea; NixM, nixtamalized maize; ORAC, oxygen radical absorbance capacity.

^a $\mu\text{mol Trolox equivalents (TE)}/100\text{ g}$ sample, DW.

^bmg gallic acid equivalents (GAE)/100 g sample, DW.

^cmg extract/ml.

DW, respectively. The AoxA of the flours, evaluated by ABTS methodology, showed a similar trend. The TPC of the flours ranged between 202.16 and 123.72 mg of GAE/100 g of sample, DW; the optimized ExtCP flour had the smallest value, however, this TPC value, as well as the AoxA value of optimized ExtCP flour were higher than raw chickpea flour (data not showed). Extrusion-cooking technology could be used to develop new functional foods and beverages since it allows the retention, or causes an increase, of phenolic compounds related to the AoxA when optimized extrusion conditions are applied (Félix-Medina et al., 2020, 2021). The application of the extrusion process under optimal conditions, when the process has been optimized to

get maximum values of TPC, some specific bioactive compounds, and AoxA, allows producing extruded grain flours with high AoxA and TPC values. The retention of the greater proportion of AoxA or the increase of it could be the result of the release of phenolic compounds through the extrusion process, the prevention of the oxidation of phenolic compounds in the extruded products by enzymatic inactivation during the process, and the presence of the Maillard reaction products (MRP). The MRP is generated through the extrusion of raw material which contains amino acids and reducing sugars (Espinoza-Moreno et al., 2016; Félix-Medina et al., 2020). The TPC content in extruded grain flours is related to destroying cell walls and phenolic compounds' release. Also, TPC is associated with the development of MRP, which are quantified as phenolic compounds (Espinoza-Moreno et al., 2016; Félix-Medina et al., 2020).

3.4 | Antihypertensive (inhibition of the ACE) and antihyperglycemic (inhibition of α -amylase and α -glucosidase enzymes) potentials of phenolic compounds extracted from flours

Antihypertensive potential was defined as IC_{50} (concentration [mg of extract/ml] required to produce 50% inhibition of ACE activity). The phenolic compounds obtained from commercial NixM flour and optimized ExtCP flour had a potential antihypertensive $IC_{50} = 2.43$ and 1.32 mg of extract/ml, respectively (Table 2).

The IC_{50} values found for phenolic extracts of optimized ExtCP flour in this study agree with the results reported for extruded defatted chia seeds (León-López et al., 2019). An improvement in the IC_{50} value by extrusion process application to chickpea was observed in this study (IC_{50} value to raw chickpea was not reported); the improvement could be attributed to the release and forming of bioactive compounds (phenolic compounds and MRP) with antihypertensive potential. Phenolic compounds (phenolic acids, flavonoids, tannins, stilbenes) inhibit the in vitro activity of ACE. The grade of Inhibition of ACE activity is influenced by the absorption and metabolism of these compounds, their means of action related to the class (subclass), and the phenolic compound structure (Al-Shukor et al., 2013; Massaretto et al., 2011). The phenolic compounds present in optimized ExtCP flour could be used as functional food supplements or natural remedies to treat hypertension.

The antihyperglycemic potential was described as IC_{50} (concentration [mg of extract/ml] required to

produce 50% inhibition of the α -amylase or α -glucosidase enzymes). Optimized ExtCP flour showed lower antihyperglycemic potential than commercial NixM flour [optimized ExtCP flour: α -amylase, $IC_{50} = 64.06$ mg/ml), α -glucosidase, $IC_{50} = 81.02$ mg/ml. Commercial NixM flour: α -amylase, $IC_{50} = 34.02$ mg/ml, α -glucosidase, $IC_{50} = 23.03$ mg/ml) (Table 2). Commercial NixM flour and optimized ExtCP flour are potential sources of nutraceutical compounds such as phenolic antioxidants, and natural inhibitors for ACE, α -amylase, and α -glucosidase activities. This data can support the effectual use of these flours as functional food ingredients to promote health. The potential physiological effects of α -amylase and α -glucosidase enzymes in vitro inhibition on the glycemic response are relatively easy to foresee, given their occurrence at small intestinal digestion levels. But, the relevance of in vitro ACE inhibition seems more difficult to extrapolate to the in vivo situation. However, the physiological effects of ACE inhibition on hypertension can be inferred from the high values of bioaccessibility and bioavailability of phenolic compounds as well as from in vivo ACE inhibition results of them that have been reported in the literature (Häckl et al., 2002; Kasprzak-Drozd et al., 2021; Salem et al., 2022). However, more research is needed in the future to obtain information regarding the components of ingested functional foods or functional food ingredients that will reach ACE in serum, lungs, and kidneys to inhibit it and thus reduce blood pressure.

3.5 | Effect of the addition of optimized ExtCP flour on the quality of the tortillas

The chemical composition and nutritional properties of tortillas are shown in Table 3. Tortillas added with 30% of optimized ExtCP flour had higher protein content (10.89 vs. 9.47%, DW), dietary fiber (13.40 vs. 9.78%, DW), IVPD (82.01 vs. 76.82%), and C-PER (1.84 vs. 1.17) than 100% commercial NixM flour tortillas. The addition of optimized ExtCP flour to commercial NixM flour improved the tortillas' chemical composition and nutritional properties. Treviño-Mejía et al. (2016) used common bean flour to fortify nixtamalized corn tortillas. The optimum composition was 80% NixM flour and 20% bean flour. According to the investigation, nutritionally, corn and common bean tortillas had higher protein content (10.89% vs. 9.47%) and dietary fiber (12.76% vs. 5.78%) than those made with 100% NixM flour (control).

Inyang et al. (2019) reported that germinated soy flour could improve the nutrient content and amino acid profile of NixM flour. Limiting EAA in both NixM flour and germinated soybean meal improved due to soybean

TABLE 3 Chemical composition and nutritional properties of tortillas from 100% commercial NixM flour and functional tortillas from 70% commercial NixM flour+ 30% optimized ExtCP flour.

Property	Tortillas		FAO ^a
	Control	Functional	
<i>Chemical composition</i> (% DW)			
Proteins	8.88 ± 0.12b	13.27 ± 0.10a	
Lipids	2.50 ± 0.03b	3.18 ± 0.04a	
Ashes	2.10 ± 0.02b	2.37 ± 0.03a	
<i>Dietary fiber</i>			
Soluble	1.37 ± 0.04b	2.28 ± 0.03a	
Insoluble	8.41 ± 0.13b	11.13 ± 0.14a	
Total	9.78 ± 0.13b	13.40 ± 0.12a	
Carbohydrates	76.74 ± 1.06a	67.78 ± 1.06b	
<i>Nutritional</i>			
EAA (g/100 g protein)			
His	2.59 ± 0.03a	2.55 ± 0.03a	1.60
Ile	2.86 ± 0.04b	3.14 ± 0.03a	3.00
Leu	11.94 ± 0.13a	10.71 ± 0.15b	6.10
Lys	2.88 ± 0.02b	4.10 ± 0.03a	4.80
Met + Cys	3.39 ± 0.03a	3.11 ± 0.03b	2.30
Phe + Tyr	7.33 ± 0.07b	7.68 ± 0.06a	4.10
Thr	2.70 ± 0.03b	3.10 ± 0.03a	2.50
Trp	0.56 ± 0.02b	0.70 ± 0.02a	0.66
Val	4.23 ± 0.05b	4.37 ± 0.06a	4.00
Total	38.48	39.46	29.06
Chemical score	60.00	85.42	
Limiting EAA	Lys	Lys	
In vitro protein digestibility (%)	76.82 ± 1.30b	82.01 ± 0.04a	
C-PER3	1.17 ± 0.06b	1.84 ± 0.04a	

Note: a, b: Means with a different letter in the same row are different (*t*-student test, $p \leq .05$).

Abbreviations: C-PER, calculated protein efficiency ratio; DW, dry weight; EAA, essential amino acid(s); ExtCP, extruded chickpea; NixM, nixtamalized maize.

^aEAA requirements for children (3 years and older), adolescents, and adults according (FAO, 2013).

meal supplementation. They reported that products made with mixtures supplemented with soy flour have a higher nutritional value than products made with 100% nixtamalized corn flour.

The nutraceutical and sensory properties of the tortillas are shown in Table 4. The tortillas added with 30% of optimized ExtCP flour had a lower AoxA (ORAC:

TABLE 4 Nutraceutical and sensory properties of control tortillas from 100% commercial NixM flour and functional tortillas from 70% commercial NixM flour + 30% optimized ExtCP flour.

Property	Tortillas	
	Control	Functional
<i>Nutraceutical</i>		
Antioxidant activity ^a (μmol TE/100 g, DB)		
ORAC	9918 ± 402a	8031 ± 387b
ABTS	6285 ± 320a	5008 ± 259b
Antihypertensive potential (IC ₅₀) ^b	2.29 ± 0.06a	1.93 ± 0.06b
Hypoglycemic potential (IC ₅₀) ^b		
α-amylase	36.05 ± 1.02b	41.12 ± 0.85a
α-glucosidase	22.14 ± 1.02a	37.01 ± 0.89b
<i>Sensory/technological</i>		
Global acceptability ^c	8.63 ± 0.25a	8.51 ± 0.32a
Color ^c	8.51 ± 0.39a	8.37 ± 0.32a
Flavor ^c	8.72 ± 0.23a	8.23 ± 0.42a
Texture ^c	8.68 ± 0.29a	8.59 ± 0.31a
Puffing	2.98 ± 0.02a	2.87 ± 0.12a
Rollability	1.20 ± 0.22a	1.40 ± 0.29a

Note: a, b: Means with a different letter, in the same row, are different (*t*-student test, $p \leq .05$).

Abbreviations: ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); C-PER, calculated protein efficiency ratio; DW, dry weight; EAA, essential amino acid(s); ExtCP, extruded chickpea; NixM, nixtamalized maize; ORAC, oxygen radical absorbance capacity.

^aμmol Trolox equivalents (TE)/100g, DW.

^bMg extract/ml

^cDegree of liking/disliking using a nine-category hedonic scale (1 = *dislike extremely* to 9 = *like extremely*).

8031 vs. 9918 μmol TE/100 g, DW; ABTS: 5008 vs. 6285 μmol ET/100 g, DW) and antihyperglycemic potential (IC₅₀ [α-amylase]: 41.12 vs. 36.05 mg extract/ml, IC₅₀ [α-glucosidase]: 37.01 vs. 22.14 mg extract/ml) potential but better antihypertensive potential (IC₅₀: 1.93 vs. 2.29 mg extract/ml) than 100% commercial NixM flour tortillas.

Treviño-Mejía et al. (2016) used common beans for the fortification of maize tortillas. They observed that bean flour addition to maize tortillas caused an increase in AoxA (evaluated in ethanol extracts [free phenolics] by two methods: ABTS and DPPH). AoxA is an essential characteristic of food products. Antioxidants decrease or delay the formation of reactive oxygen species (ROS). The disproportion in ROS concentration in the human body has been linked to oxidative stress, which induces noncommunicable diseases (Gülçin, 2012).

The in vitro results could suggest that the ExtCP flour, acquired by the extrusion process under optimal conditions, has the potential to be used as a functional food or natural medication to prevent or treat hypertension, however, this nutraceutical property evaluated in vitro needs to be further confirmed in animal or human models. The improvement in nutraceutical potential during the extrusion of whole chickpea "grits" was produced by releasing and forming bioactive compounds (phenolic compounds and MRP) with antihypertensive potential. As reported by some researchers (Al-Shukor et al., 2013; Massaretto et al., 2011), phenolic compounds (phenolic acids, flavonoids, stilbenes, tannins) inhibit ACE activity in vitro.

According to Vinayagam et al. (2016), some phenolic acids and their derivatives exhibit meaningful clinical or in vivo antidiabetic activity. Currently, the treatment of metabolic syndrome and the prevention of diabetes implicates lifestyle modifications, for instance, enhancing physical activity and weight control by reducing caloric intake. Ever more, dietary advice for people at risk of diabetes highlight the consumption of plant food products, like whole grains, known as excellent sources of dietary fiber and phenolic compounds. The role of phenolic compounds in glucose metabolism has been tested both in vitro and in vivo, as indicated by the review made by Vinayagam et al. (2016); they reported that one of the most proved mechanisms of dietary phenols involved in glucose control include the inhibition of carbohydrate digestion and glucose absorption from the intestine.

Tortillas from commercial NixM flour added with optimized ExtCP flour (70% commercial NixM flour + 30% optimized ExtCP flour) could be a potential alternative to the improvement of the original food's nutritional/antihypertensive value product (100% maize tortilla), however, as was above mentioned, this nutritional/nutraceutical potential evaluated in vitro need to be further confirmed in animal or human models. If these properties evaluated in vitro are in vivo confirmed, it could be said that these tortillas could be used, through appropriate public policies, to reduce malnutrition and the incidence of chronic degenerative diseases (e.g., hypertension, diabetes) in Mexico.

4 | CONCLUSIONS

The addition of 30% of optimized ExtCP flour to commercial NixM flour to produce functional tortillas yielded a functional food with higher protein and dietary fiber contents, better IVPD, C-PER, and antihypertensive potential than the tortillas made with 100% commercial

NixM flour. The addition of optimized ExtCP flour to commercial NixM flour allows obtaining functional tortillas with improved and sensory-acceptable in vitro nutritional and antihypertensive properties. The functional tortillas fortified with 30% optimized ExtCP flour have the potential to be used, as part of public policy, to reduce malnutrition and the incidence of chronic degenerative diseases (e.g., hypertension, diabetes) in Mexico. However, the nutritional and nutraceutical properties found in vitro need to be further confirmed in animal or human models.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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