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"El saber de mis hijos hará mi grandeza"

Effect of the extruded amaranth flour addition on the nutritional, nutraceutical and sensory quality of tortillas produced from extruded creole blue maize flour

Efecto de la adición de harina de amaranto extruido sobre la calidad nutricional, nutracéutica y sensorial de tortillas producidas a partir de harina de maíz azul criollo extrudido

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ABSTRACT

Mexico suffers from serious malnutrition, overweight and obesity problems, affecting a large part of the population, as well as, chronic degenerative diseases (CDD), among which cardiovascular diseases (CVD) and diabetes, are the main causes of mortality in the country. The tortilla represents a viable vehicle to improve the Mexicans nutritional status. In the present work, the effect of the addition of 30 % extruded amaranth flour (EAF) on the nutritional [essential amino acid profile (EAA), *in vitro* protein digestibility (IVPD), calculated protein efficiency ratio (C-PER)], nutraceutical [antioxidant activity (AoxA), and antihypertensive and hypoglycemic potentials] and sensory properties of extruded creole blue maize flour (ECBMF) tortillas were evaluated. The functional tortillas (ECBMF tortillas added with 30 % of EAF) presented more protein, dietary fiber, IVPD and C-PER than tortillas from ECBMF alone; however, they showed lower AoxA (13,187 vs. 15,298 µmol TE / 100 g, DW) and better antihypertensive and hypoglycemic potentials than 100 % ECBMF tortillas. The addition of EAF, resulted in sensorially acceptable functional tortillas with better nutritional and nutraceutical properties. Functional tortillas could reduce malnutrition and chronic degenerative diseases in Mexico.

Keywords: Creole blue maize, amaranth, extrusion, functional tortillas, sensory properties

RESUMEN

México sufre graves problemas de desnutrición, sobrepeso y obesidad, afectando gran parte de la población. Asimismo, las enfermedades crónicas degenerativas (ECD), como enfermedades cardiovasculares (ECV) y diabetes son la principal causa de mortalidad en el país. La tortilla representa un vehículo viable para mejorar el estado nutricional de los mexicanos. En el presente trabajo se evaluó el efecto de la adición de 30 % de harina de amaranto extrudido (HAE) sobre las propiedades nutricionales [perfil de aminoácidos esenciales (AAE), digestibilidad de proteínas *in vitro* (DPIV), relación de eficiencia proteica calculada (C-PER)], nutracéuticas [actividad antioxidante (AAox) y potenciales antihipertensivo e hipoglucémico] y sensoriales de tortillas de harina de maíz azul criollo extrudido (HMACE). Las tortillas funcionales (tortillas de HMACE adicionadas con 30 % de HAE) presentaron más proteínas, fibra dietética, DPIV y C-PER que las tortillas de solo HMACE; sin embargo, presentaron AAox **más baj**a (13,187 vs. 15,298 µmol ET / 100 g, BS) y mejores potenciales antihipertensivo e hipoglucémico que las tortillas 100 % HMACE. La adición de HAE, permitió obtener tortillas funcionales sensorialmente aceptables con mejores propiedades nutricionales y nutracéuticas. Las tortillas funcionales podrían reducir la desnutrición y las enfermedades crónicas degenerativas en México.

Palabras clave: Maíz criollo azul, amaranto, extrusión, tortillas funcionales, propiedades sensoriales.

INTRODUCTION

Mexico suffers from issues with malnutrition, anemia, overweight, and obesity. Malnutrition is a problem that affects development, human growth, and general health, particularly in children under five years of age and pregnant women. The prevalence of low height is a severe public health problem (13.6 % on average), especially in rural and marginal urban areas (Ramírez-Jaspeado *et al.*, 2018).

Chronic degenerative diseases (CDD) affect all age groups and progress over a long time. CDD were the leading cause of mortality in 2018; with cardiovascular diseases (CVD) and diabetes being the top two causes of death in Mexico with 149,368 and 101,257, respectively (INEGI, 2019). It is necessary to prevent CDD and combat modifiable risk factors: Eliminate tobacco consumption, limit excessive intake of alcohol and salt/sodium, perform regular physical activity, and follow a healthy diet (WHO, 2019).

In Mexico, maize's main food product is the tortilla, with an average daily consumption of 1,400 million tortillas. Mexico's annual consumption amounts to more than 120 kg per capita; in adulthood, 8 to 10 tortillas are consumed daily. One of its main tortillas limitations is the lack of lysine. The nixtamalization process to prepare tortillas presents problems of high liquid waste discharges. Extrusion, an

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alternative to nixtamalization, does not generate polluting effluents, and all the components of the grain, including the pericarp, are retained, producing a tortilla with higher fiber content (Milán-Carrillo *et al.*, 2006; Escalante-Aburto *et al.*, 2013; León-López *et al.*, 2019).

Tortillas represent an excellent vehicle to enhance the nutritional status of tortillas consumers (Mexicans). Several researchers have reported improvements in maize tortilla quality with regard to nutritional and nutraceutical properties throughout the addition of several types of flours: Common bean (Treviño-Mejía *et al.*, 2016), soybean (Chuck-Hernández and Serna-Saldívar, 2019), and sprouted soybean (Inyang *et al.*, 2019). The fortification of creole blue maize tortillas with pseudocereals flours represents an alternative to improve their nutritional and nutraceutical properties.

Mexico has the most considerable genetic diversity of maize (*Zea mays* L.) in the world. Approximately 59 different maize races or landraces have been classified based on morphological characters and isozyme frequencies. In these Mexican maize landraces, pigmented genotypes such as blue, red, and yellow are the most common (Sánchez *et al.*, 2000). Creole genotypes of pigmented maize **are** cultivated in different regions of Mexico. Chalqueño, Bolita, and Elotes Conico landraces predominate in the central mesa (Pineda Hidalgo *et al*., 2013). Currently, interest in pigmented maize has received increased attention owing to consumer awareness of their potential health benefits and color properties. Blue genotypes have multiple functional properties (protection against oxidative stress, hypoglycemic activity, lowers blood pressure, serum cholesterol, and triglyceride levels). These nutraceutical properties are associated with phytochemicals' presence (phenolic acids, anthocyanins) (Urias-Lugo *et al.*, 2015; Gaxiola-Cuevas *et al.*, 2017; Guzmán Gerónimo *et al.*, 2017).

Amaranth seeds possess a protein content near to 15 % (w/w); the essential amino acid composition is close to the optimum amino acid balance required in the human diet (Ogrodowska *et al.*, 2014; Orona-Tamayo and Paredes-López, 2016). They are an excellent source of healthy lipids, like such as unsaturated fatty acids and bioactive compounds like polyphenols, flavonoids, tannins, tocopherols, squalene, and biopeptides, among others (Tovar-Perez *et al.*, 2019; Velarde-Salcedo *et al.*, 2019). Several researchers have observed that amaranth biopeptides not only present cholesterol-lowering activity, but also present antioxidant, antihypertensive, and antithrombotic activities, which is why they have a potential application in foods as a functional ingredient (Orsini-Delgado *et al.*, 2016; Sabbione *et al.*, 2016; Quiroga *et al.*, 2017).

Extrusion cooking uses a combination of high-temperature, pressure, and shear conditions in a short period. This results in molecular transformation and chemical reactions within the extruded products. Furthermore, it improves the starch and protein digestibility and increases bioactive compounds' retention and soluble dietary fiber. Extrusion causes lipid modifications, enzymes and microorganisms inactivation, and the formation of volatile flavor components. It is also a highly efficient alternative technology, which minimizes energy consumption and water pollution (Ramos-Enríquez *et al*., 2018; Ortiz-Cruz *et al.*, 2020).

The work's objective was to evaluate the effect of extruded amaranth flour addition on the quality (nutritional, nutraceutical, sensory) of creole blue maize tortillas.

MATERIALS AND METHODS

Materials

Whole creole blue maize (*Zea mays* L) and amaranth (*Amaranthus hypochondriacus* L) seeds **were** obtained in markets of the localities of Tepeaca, Puebla, México, and Temoac, Morelos, Mexico, respectively. The grains were cleaned and packed in 1 kg lots, and stored in refrigeration (5-10 °C) until their use.

Production of extruded creole blue maize (ECBMF) and amaranth flours (EAF)

Production of ECBMF and EAF was according to the methodology proposed by Milán-Carrillo *et al.* (2006) and Milán-Carrillo *et al.* (2012), respectively. Five hundred gram lots of seeds (creole blue maize, amaranth) were placed in a domestic blender to obtain grits that passed through a 40-US mesh (0.425 mm) screen but were retained over a 200-US mesh (0.074 mm) screen, and fine powder. The seeds grits were mixed with lime (0.21 g/100 g fragmented grain) and water to achieve a water level of 28 g/100 g and 20 g/100 g, respectively. All lots were packed in polyethylene bags and stored (4 °C/12 h). Before extrusion, the grits were tempered (25 °C). The extrusion treatments were carried out in a single screw laboratory extruder Model 20 DN (CW Brabender Instruments, Inc, NJ, USA). A screw-operated hopper-fed the feedstock into the extruder at 30 rpm. The feed rate was set at 70 g/min. The extrusion temperature is defined as the temperature at the die end barrel. The extruder operation conditions were as follows [Extrusion temperature (ET), and screw speed (SS)]: Creole blue maize: ET=85 °C/SS=240 rpm; amaranth: ET=130 °C/SS=124 rpm. Extrudates cooled and equilibrated at environmental conditions (25 ºC, 65 % RH), were milled to pass through an 80-US mesh (0.180 mm) screen, and packed in plastic bags. Extruded creole blue maize and amaranth flours (ECBMF, EAF) were then stored at 4 ºC until use.

Quality evaluation of functional tortillas elaborated from ECBMF and EAF

Functional tortillas elaboration consisted in the flour mixture of 70 % ECBMF+30 % EAF, to compare to tortillas made with 100 % ECBMF, and as control, blue MASECA™. The tortillas were prepared with water at 30 °C until obtaining an adequate consistency. Tortillas' puffing was evaluated throughout their cooking using a 1 to 3 scale, where: 1= No puffing, 2= Intermediate puffing, and 3= Complete puffing (Milán-Carrillo *et al.*, 2006). The tortilla rollability was evaluated 30 min after elaboration. They were rolled using a glass

stick, 2.54 cm in diameter, and the degree of breakage of the tortilla surface (0-100 %) indicated the rollability (1-5) as follows: 100 %=5, 75 %=4, 50 %=3, 25 %=2, and 0 %=1 (Bedolla and Rooney, 1984). For sensory evaluation, squared tortilla pieces pre-heated at 45°C were presented to one hundred twenty consumers (ages18-35), who were habitual tortilla consumers. The panelists used plain water as a palate cleanser between samples. Consumers assessed flavor, odor, color, texture, and general acceptance. Each consumer was asked to indicate his/her degree of liking/disliking using a 9-category hedonic scale (1=dislike extremely to 9=like extremely). Samples were dried and milled to evaluate the chemical composition and the nutritional and nutraceutical properties of tortillas.

Chemical composition of the flours and tortillas

The official AOAC (2012) methods 960.52, 920.39C, and 925.09B were used to determine protein (Nx6.25), lipids, and moisture contents. Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) evaluation, was according to the enzymatic-gravimetric method for total dietary fiber (TDF) (method 985.29), using the TDF assay kit from Sigma-Aldrich (TDF 100 A) (AOAC, 2012).

EAA (Essential amino acid*), IVPD (in vitro* protein digestibility), CS (Chemical score), and C-PER (Calculated protein efficiency ratio) of the flours and tortillas.

Nutritional properties of flours and their tortillas (from blue MASECA™, 100 % ECBMF, and 70 % ECBMF+30 % EAF) were determined according to Salas-López *et al.* (2018). EAA composition was evaluated using an analytical scale (4.6mm×250mm) hypersil ODS C18 column kept at 38 °C, which was connected to an HPLC system (GBC, Dandenong, Australia) equipped with a fluorescence detector >LC 5100 set at 270 nm (excitation) and 316 nm (emission). The tryptophan was determined using an ultraviolet detector at 280 nm. IVPD evaluation was as reported by Rathod *et al.* (2016) with slight modifications. One gram of defatted sample dissolved in a pepsin solution (20 mL 0.1N HCl + 15 mg pepsin) in a 250 mL Erlenmeyer flask, was incubated in a water bath for 3 hours at 37 ° C. After incubation, it was neutralized with 10 mL of 0.2N NaOH, and a pancreatin solution (7.5 mL of phosphate buffer +40 mg pancreatin) was added, adjusting to pH 8, and incubated in a water bath (37 ° C x 24h). At the end of the incubation, 700 µL of trichloroacetic acid were added, centrifuged at 5,000 rpm for 10 minutes; the pellet recovered and dissolved in 30 mL of distilled water to later be dried at 40 ° C for 12 hours. The protein content of the dry sample was determined using the micro-Kjeldahl method and the percentage of *in vitro* protein digestibility was determined using the following equation:

$$IVPD\ (\%) = \frac{Initial\ protein\ content - Residual\ protein\ content}{Initial\ protein\ content}\ x\ 100$$

The CS calculation was as follows:

$$CS = \left(\frac{Content\ of\ the\ most\ limiting\ EAA}{REAAR}\right) \times 100$$

where EAA=Essential amino acid and REAAR=Recommended EAA requirements for children (three years and older), adolescents, and adults (FAO, 2013). The IVPD and EAA composition of the sample were used for C-PER calculation. All evaluations were made in triplicate.

Extraction of free and bound phenolic

The extraction of free phenolic was according to the method described by Adom and Liu (2002). For free phenolic extraction, 0.5 g of sample, mixed with 10 mL of ethanol at 80 % (v/v), was stirred in a rotator (OVAN Noria R, USA 2010) at a speed of 25 rpm for 10 min. Then, centrifuged at 3,000xg, 10 °C, 10 min. This extraction step was performed two more times. The recovered supernatant was placed in a conical tube, and then concentrated to dryness at 45 °C under low pressure (Apud Vac Concentrator, Thermo Elector Corporation). The precipitate was stored to obtain the bound phenolic extracts. The extraction of bound phenolic was according to the procedure of Adom and Liu (2002), with slight modifications (Mora-Rochín *et al.*, 2010). The precipitate was digested with 10 mL of 2 M NaOH, the oxygen eliminated with the presence of N2 gas and the sample was subjected to heat treatment, in a water bath at 95 °C / 30 min, and stirred for 1 h at room temperature (25 ° C). The mixture was neutralized with 2 mL of concentrated HCl, vortexed for 2 min and centrifuged at 3,000xg, 10 °C, 10 min. Subsequently, an extraction with hexane was carried out to remove lipids and extractions of the bound phenolic with ethyl acetate was carried out, which was collected and stored in conical tubes; this extraction procedure was done 4 more times. The ethyl acetate fraction was evaporated to dryness (Apuc Vac Concentrator, Thermo Electror Corporation). The concentrate of extracted free and bound phenolic compounds were stored at -20 °C until its later use in the determination of nutraceutical properties and total phenolic compounds. The concentrates were reconstituted with methanol until reaching a final volume of 2 mL prior to use.

Antioxidant activity (AoxA)

ABTS assay

The ABTS assay for AoxA was performed diluting free and bound extracts with ethanol. Twenty microliters' aliquots of each dilution were taken and mixed with 2.0 mL of diluted radical cation ABTS•+, and six min later, the absorbance read at 734 nm in a UV-visible spectrophotometer (GENESYS 10UV, Thermo electron, Inc, Madison, WI, USA) (Re *et al.*, 1999). The results of assays are expressed as µmol of Trolox equivalents (TE)/100 g of dry weight (DW). This assay was realized by triplicate.

ORAC assay

The ORAC assay was carried out by diluting free and bound phenolic extracts in 75 mM phosphate buffer (pH 7.4). Aliquots (25 µL) of diluted extracts were mixed with 0.1 mM fluorescein (150 mL) and peroxyl radical AAPH (200 mM) (25 **µL**). After 30 min, fluorescence [485 nm (excitation), 538 nm

(emission)] was measured (37 °C) over a 60 min period, at 2 min intervals, using a Synergy Microplate Reader (SynergyTM HT Multi-Detection, BioTek, Inc., Winooski, VT) (Mora-Rochín *et al*., 2010). The results of ORAC assays are expressed as micromole of Trolox equivalent (TE)/100 g of dry weight (DW). The ORAC evaluation was performed in triplicate.

Total phenolic compounds (TPC)

The TPC of free and bound extracts were determined using 20 µL of appropriate extracts dilutions, oxidized with 180 µL of Folin-Ciocalteu reagent (Singleton *et al*., 1999). After 20 min, the resulting blue color's absorbance was measured at 750 nm using the Synergy Microplate Reader. TPC is reported as mg Gallic acid equivalent (GAE)/100 g of dry weight (DW). The TPC measurements were carried out in triplicate.

Antihypertensive potential (IC_{50}) of the flours and their tortillas

The ACE (angiotensin-converting enzyme) inhibitory activity in free and bound phenolic extracts was determined using the Dojindo ACE Kit-WST test kit (Dojindo Laboratories, Kumamoto, Japan). This method relies on an indicator's colorimetric detection after a redox reaction, at an absorbance (Abs) of 450 nm measured using a Microplate Reader (Synergy™ HT Multi-Detection, BioTek, Inc., Winooski, VT, USA). The ACE inhibitory activity (inhibition of the color formation) of the phenolic extracts was calculated using the following equation:

$$\text{ACE inhibitory activity (\%)} = \left[\frac{(Abs_{450}\ control - Abs_{450}\ extract)}{Abs_{450}\ control - Abs_{450}\ blank}\right] \times 100$$

Where: Abs_{450} extract= Absorbance at 450 nm of the reaction solution containing phenolic extract; Abs_{450} control = Absorbance at 450 nm of the reaction solution without phenolic extract; Abs_{450} blank = Absorbance at 450 nm of the reaction solution with enzyme and without both substrate and phenolic extract. The IC_{50} (concentration of phenolic extract that caused an inhibition of 50 % in the ACE activity) values were calculated from different concentrations of the phenolic extracts and ACE inhibitory activity values using the Prism v5 software (GraphPad Prism) (Argüelles-López *et al*., 2018).

Hypoglycemic potential (IC_{50}) [α-amylase and α-glucosidase inhibition activities] of the flours and their tortillas

The inhibitory activity of bound and free phenolic extracts against α-amylase was determined by measuring the color (absorbance at 540 nm) inhibition of the maltose released after stopping the reaction between starch and α-amylase with 3,5-dinitrosalicylic acid (Astawan *et al*., 2020). The inhibitory activity of these extracts against α-glucosidase was determined by measuring the color (absorbance at 405 nm) inhibition of the p-nitrophenol formed by α-glucosidase after reacting with p-nitrophenyl-α-D-glucopyranoside (PNP) (Astawan *et al*., 2020). α-amylase and α-glucosidase inhibition activities of the phenolic extracts were reported as % inhibition of enzyme activity and calculated using the following equation:

$$\text{\% inhibition} = \left[\frac{(Abs\ control - Abs\ extract)}{Abs\ control}\right] \times 100$$

Where: Abs extract= Absorbance of the reaction solution containing phenolic extract; Abs control = Absorbance of the reaction solution without phenolic extract. The IC_{50} (concentration of phenolic extract that caused an inhibition of 50 % in the α-amylase and α-glucosidase activities) values were calculated from the plots of % inhibition vs log of the phenolic extract concentration using the Prism v5 software (GraphPad Prism).

Statistical analyses

The experimental results of chemical composition, nutritional and nutraceutical properties of flours and tortillas, were subjected to one-way analysis of variance (ANOVA) followed by Tukey test (5 % of the significance level).

RESULTS AND DISCUSSION

Nutrimental and nutritional properties of extruded grain flours

Table 1 shows the proteins, lipids, and dietary fiber content of extruded creole blue maize and amaranth flours (ECBMF, EAF). The protein content of ECBMF and EAF was 10.08 and 15.52 % (DW), respectively. Extruded grain flours' lipid content ranged from 6.11 to 6.98 % (DW); the ECBMF had the lowest value. The dietary fiber contained in the extruded grain flours varied from 12.43 % (DW) (ECBMF) to 13.68 % (DW) (EAF); in both flours, the highest dietary fiber content corresponded to the insoluble dietary fiber fraction whose content ranged from 9.37 % (DW) (EAF) to 10.61 % (DW) (ECBMF).

The essential amino acid (EAA) content of ECBMF and EAF is also shown in Table 1. In general, the EAA content of extruded grain flours was higher than those suggested by FAO (2013) for the requirement of EAA for children (3 years and older), adolescents, and adults; however in ECBMF and EAF Lys and Leu, respectively, resulted limiting amino acids. The *in vitro* protein digestibility (IVPD) of extruded grain flours ranged from 78.26 % (ECBMF) to 81.05 % (EAF). The highest C-PER (1.72-2.22) value corresponded to extruded amaranth flour (EAF) (Table 1). The IVPD of seeds increased due to the destruction of antinutritional factors (trypsin, and chymotrypsin inhibitors) and protein denaturation, because of the applied conditions (cutting forces, temperature, and humidity) during the extrusion – cooking process (Gamel *et al*., 2006; Montoya-Rodriguez *et al*., 2015).

Antioxidant activity (AoxA) and total phenolic content (TPC) of extruded grain flours

Table 2 shows the antioxidant activity, total phenolic content, and antihypertensive and hypoglycemic potential

Tabla 1. Composición química y contenido de aminoácidos esenciales en harina comercial de maíz azul nixtamalizado (MASECA™ azul) y harinas de maíz azul criollo y amaranto extrudidos (HMACE, HAE)**.**
Table 1. Chemical composition and essential amino acid content in commercial nixtamalized blue maize flour (blue MASECA™), and extruded creole blue maize and amaranth flours (ECBMF, EAF).

Property	Blue MASECA™	ECBMF	EAF	FAO[1]
Chemical composition (%, DW)				
Proteins	7.83±0.08^{C}	10.08±0.11^{B}	15.52±0.07^{A}	
Lipids	2.40±0.10^{C}	6.11±0.08^{B}	6.98±0.06^{A}	
Minerals	1.52±0.06^{B}	1.56±0.05^{B}	3.47±0.06^{A}	
Dietary fibre				
Soluble	0.72±0.04^{C}	1.82±0.10^{B}	4.31±0.07^{A}	
Insoluble	8.75±0.12^{C}	10.61±0.08^{A}	9.37±0.07^{B}	
Total	9.47±0.10^{C}	12.43±0.12^{B}	13.68±0.09^{A}	
Carbohydrates	78.80±0.15^{A}	69.82±0.17^{B}	60.35±0.14^{C}	
Nutritional				
EAA[2] (g/100 g protein)				
His	2.57±0.04^{B}	3.18±0.02^{A}	2.46±0.03^{C}	1.60
Ile	2.88±0.02^{B}	2.62±0.05^{C}	4.02±0.02^{A}	3.00
Leu	13.88±0.08^{A}	7.90±0.04^{B}	5.88±0.04^{C}	6.10
Lys	2.48±0.03^{C}	3.85±0.05^{B}	5.94±0.03^{A}	4.80
Met+Cys	3.73±0.06^{C}	5.49±0.04^{A}	3.88±0.03^{B}	2.30
Phe+Tyr	7.82±0.04^{A}	6.65±0.06^{C}	7.77±0.05^{B}	4.10
Thr	2.98±0.05^{C}	3.18±0.03^{B}	4.18±0.04^{A}	2.50
Trp	0.56±0.03^{C}	0.77±0.02^{B}	1.29±0.02^{A}	0.66
Val	4.48±0.04^{B}	5.88±0.05^{A}	4.17±0.03^{C}	4.00
Total	41.38	39.52	39.59	29.06
EAA Chemical score	0.52	0.80	0.96	
Limitant AAE	Lys	Lys	Leu	
IVPD [3] (%)	74.05±0.10^{C}	78.26±0.12^{B}	81.05±0.14^{A}	
C-PER[4]	1.00±0.02^{C}	1.72±0.04^{B}	2.22±0.09^{A}	

A-C Means with different superscripts in the same row are significantly different (Tukey, $p \leq 0.05$); **1** EAA requirements for children (3 years and older), adolescents, and adults according to FAO (2013); **2** EAA = Essential amino acid(s); **3** IVPD = *In vitro* protein digestibility (%); **4** C-PER = Calculated protein efficiency ratio.

of extruded creole maize and amaranth flours (ECBMF, EAF). The AoxA, evaluated by ORAC methodology, of ECBMF and EAF, was 15,587 and 5,387 µmol Trolox equivalents (TE)/100 g sample DW, respectively. The commercial nixtamalized blue maize flour (blue MASECATM) showed an AoxA=12,403 µmol TE/100 g sample DW. The AoxA of the extruded grains, evaluated by ABTS methodology, showed a similar tendency. Extruded grain flours' total phenolic content ranged from 64.65 to 282.61 mg Gallic acid equivalents (GAE)/100 g sample DW, showing the extruded amaranth flour (EAF) the lowest value.

Tabla 2. Actividad antioxidante, contenido total de fenólicos y potencial antihipertensivo e hipoglucémico de harina comercial de maíz azul nixtamalizado (MASECA™ azul) y harinas de maíz azul criollo y amaranto extrudidos (HMACE, HAE).
Table 2. Antioxidant activity, total phenolic content, and antihypertensive and hypoglycemic potentials of commercial nixtamalized blue maize flour (blue MASECA™), and extruded creole blue maize and amaranth flours (ECBMF, EAF).

Property	Blue MASECA™	ECBMF	EAF
Antioxidant activity[1]			
ORAC			
Free phenolics	2,295±79^{C}	3,021±98^{A}	2,510±130^{B}
Bound phenolics	10,108±42^{B}	12,566±597^{A}	2,977±141^{C}
Total	12,403±581^{B}	15,587±605^{A}	5,387±136^{C}
ABTS			
Free phenolics	847±58^{B}	1,218±85^{A}	1,267±72^{A}
Bound phenolics	2,708±167^{B}	3,879±156^{A}	2,389±59^{C}
Total	3,555±120^{B}	5,097±160^{A}	3,656±87^{B}
Phenolic compounds[2]			
Free phenolics	61.85±0.58^{B}	64.18±0.37^{A}	20.14±0.23^{C}
Bound phenolics	134.59±0.83^{B}	218.43±2.83^{A}	44.51±0.19^{C}
Total	196.44±0.92^{B}	282.61±3.70^{A}	64.65±0.37^{C}
Antihypertensive potential (IC_{50})[3]			
ACE inhibition	2.51±0.07^{A}	0.49±0.03^{B}	0.40±0.03^{C}
Hypoglycemic potential (IC_{50})[3]			
α-amylase inhibition	27.22±1.37^{A}	28.67±1.11^{A}	10.85±0.76^{B}
α-glucosidase inhibition	18.22±1.01^{B}	20.98±0.89^{A}	14.35±0.83^{C}

A-C Means with different superscripts in the same row are significantly different (Tukey, $p \leq 0.05$); **1** µmol Trolox equivalents (TE) / 100 g sample, DW; **2** mg Gallic acid equivalents (GAE) / 100 g sample, DW; **3** mg extract/ mL

Extrusion-cooking technology has the potential for the development of functional foods since it allows the retention or even increase, phenolic compounds contents, related to antioxidant activity, in a higher proportion than traditional nixtamalization (Mora-Rochín *et al.*, 2010; Escalante-Aburto *et al.*, 2013). Applying the extrusion process at optimal conditions, when the process is optimized to obtain maximum TPC values, specific bioactive compounds, and AoxA, allows us to produce extruded grain flours with high AoxA and TPC. The highest proportion of AoxA (either by retention or increase) could result from the release of phenolic compounds during the extrusion process, preventing oxidation of phenolic compounds in the extruded product by enzymatic inactivation during the processing, and the presence of Maillard reaction products (MRP). The generation of MRP occur during the extrusion of raw materials that contain amino acids and reducing sugars (Escalante-Aburto *et al.*, 2013; Espinoza-Moreno *et al.*, 2016). During the preparation of extruded grain flours, both in creole blue maize and amaranth, bound phenolic compounds were the main contributors (up to 80 %) to the

values of total phenolic compounds antioxidant activity (Table 2). The content of total phenolic compounds in extruded grain flours relates to the destruction of the cell walls, the release of phenolic compounds, and the formation of MRP quantified as phenolic compounds (Espinoza-Moreno *et al.*, 2016).

Antihypertensive and hypoglycemic potentials of phenolic compounds extracted from extruded grain flours

The antihypertensive potential is defined as IC_{50} [concentration (mg of extract/mL) required to produce inhibition of 50 % of the activity of ACE]. The phenolic compounds extracted from ECBMF and EAF had potential antihypertensive activity with IC_{50} of 0.49 and 0.40 mg extract/mL, respectively, while the phenolic extracts from blue MASECA™ flour had an IC_{50}=2.51 mg extract/mL (Table 2). The benefits of the IC_{50} obtained in this research are in concordance with reported results for phenolic extracts from soybean (0.143-0.160 mg/mL), and unprocessed and extruded defatted chia seeds (0.35-0.51 mg/mL) (Ademiluyi and Oboh, 2013; León-López *et al*., 2019). The improvement of IC_{50} values during the extrusion process of creole blue maize and amaranth seeds could have occurred by the release and formation of bioactive compounds (phenolic compounds and MRP) with antihypertensive potential. Phenolic compounds (phenolic acids, flavonoids, tannins, stilbenes) inhibit the *in vitro* ACE activity. The degree of inhibition of the ACE activity depends on the absorption and metabolism of these compounds, and its mode of action related to the class (subclass) and the structure of the phenolic compound that is employed (Massaretto *et al.*, 2011; Al-Shukor *et al.*, 2013). According to this research, the phenolic compounds present in extruded grains flours are suitable for use as functional food supplements or natural medicines to treat hypertension.

The hypoglycemic potential is defined as IC_{50} [concentration (mg of extract/mL) required to produce inhibition of 50 % of the activity of α-amylase or α-glucosidase enzymes]. Extruded amaranth flour (EAF) showed better hypoglycemic potential [EAF: α-amylase, IC_{50}=10.85 mg/mL), α-glucosidase, IC_{50}=14.35 mg/mL / ECBMF: α-amylase, IC_{50}= 28.67 mg/mL, α-glucosidase, IC_{50}=20.98 mg / mL /) than ECBMF (Table 2). The commercial nixtamalized maize flour had potential hypoglycemic values (blue MASECA™: α-amylase, IC_{50}=27.22, α-glucosidase, IC_{50}=18.22 mg/mL) very similar to ECBMF. These results suggest that extruded amaranth flour is a potential source of antioxidant phenolics and great sources of strong natural inhibitors for ACE, α-amylase, and α-glucosidase activities (Ademiluyi and Oboh, 2012; 2013). This information may help with the effective utilization of EAF as a functional food ingredient for promoting health.

Effect of EAF addition on the quality of maize tortillas

The chemical composition, nutritional, nutraceutical, and sensory properties of tortillas are shown in Table 3. Functional tortillas had higher protein and lipid values, as well as IVPD, C-PER, dietary fiber, and antihypertensive and hypoglycemic potentials than tortillas from 100 % ECBMF.

Tabla 3. Composición química, propiedades nutricionales y nutracéuticas, y características sensoriales/tecnológicas de tortillas de harina comercial de maíz azul nixtamalizado (MASECA™ azul), harina de maíz azul criollo extrudido (HMACE) y la mezcla de 70 % HMACE + 30 % HAE (tortillas funcionales).
Table 3. Chemical composition, nutritional and nutraceutical properties, and sensory/technological characteristics of tortillas from commercial nixtamalized blue maize flour (blue MASECA™), extruded creole blue maize flour (ECBMF), and the 70% ECBMF+30% EAF mixture (functional tortillas).

Property	Blue MASECA™ tortillas	ECBMF tortillas	Functional tortillas	FAO[1]
Chemical composition (%, DW)				
Proteins	8.07±0.05^{C}	10.05±0.08^{B}	11.69±0.07^{A}	
Lipids	2.41±0.02^{C}	5.92±0.05^{B}	6.21±0.04^{A}	
Minerals	1.57±0.04^{B}	1.63±0.06^{B}	2.15± 0.5^{A}	
Dietary fiber				
Soluble	0.76±0.03^{C}	1.81±0.03^{B}	2.43±0.04^{A}	
Insoluble	8.66±0.07^{B}	9.38±0.08^{A}	9.49±0.07^{A}	
Total	9.43±0.06^{C}	11.19±0.06^{B}	11.92±0.05^{A}	
Carbohydrates	78.52±1.08^{A}	71.21±1.03^{B}	69.03±1.11^{C}	
Nutritional				
EAA[2](g/100g protein)				
His	2.49±0.02^{C}	3.13±0.04^{A}	2.88±0.02^{B}	1.60
Ile	2.83±0.03^{B}	2.50±0.03^{C}	3.03±0.03^{A}	3.00
Leu	12.94±0.06^{A}	7.39±0.03^{B}	7.19±0.05^{C}	6.10
Lys	2.44±0.04^{C}	3.01±0.02^{B}	4.51±0.04^{A}	4.80
Met+Cys	3.39±0.03^{B}	3.42±0.03^{B}	5.00±0.02^{A}	2.30
Phe+Tyr	7.58±0.05^{B}	9.8±0.02^{A}	6.92±0.05^{C}	4.10
Thr	2.70±0.02^{B}	2.56±0.04^{C}	3.41±0.03^{A}	2.50
Trp	0.56±0.04^{C}	0.64±0.02^{B}	0.91±0.02^{A}	0.66
Val	4.23±0.05^{C}	5.50±0.03^{A}	5.35±0.03^{B}	4.00
Total	39.16	37.95	39.20	29.06
Chemical score	0.51	0.62	0.94	
Limitant EAA	Lys	Lys	Lys	
IVPD (%)[3]	76.86±0.21^{C}	77.21±0.20^{B}	79.39±0.19^{A}	
C-PER[4]	1.52±0.06^{B}	1.54±0.05^{B}	1.95±0.07^{A}	
Nutraceutical				
AoxA[5]	12,031±561^{C}	15,298±505^{A}	13,187±545^{B}	
Antihypertensive potential (IC_{50})[6]				
ACE inhibition	2.43±0.11^{A}	0.45±0.04^{B}	0.37±0.05^{C}	
Hypoglycemic potential (IC_{50})[6]				
α-amylase inhibition	24.63±0.86^{A}	25.17±1.05^{A}	18.33±0.59^{B}	
α-glucosidase inhibition	17.55±0.61^{B}	18.37±0.55^{A}	15.04±0.46^{C}	
Sensory/ Technological				
General acceptability[7]	8.40 ± 0.21^{A}	8.10 ± 0.35^{B}	8.01 ± 0.21^{B}	
Color[7]	8.40 ± 0.30^{A}	8.15 ± 0.25^{B}	8.00 ± 0.29^{B}	
Flavor[7]	8.50 ± 0.25^{A}	8.22 ± 0.44^{B}	7.88 ± 0.34^{C}	
Texture[7]	8.41 ± 0.35^{A}	8.21 ± 0.22^{B}	7.97 ± 0.25^{C}	
Puffing[8]	2.92 ± 0.28^{A}	2.92 ± 0.28^{A}	2.13 ± 0.34^{B}	
Rollability[9]	2.00 ± 0.00^{A}	2.08 ± 0.28^{A}	1.96 ± 0.20^{A}	

$^{A-C}$ Means with different superscript letters in the same row are significantly different (Tukey, $p \leq 0.05$); [1] Essential amino acids requirements for children (3 years and older), adolescents and adults according to FAO (2013); [2]EAA = Essential amino acid; [3] IVPD = *In vitro* protein digestibility (%); [4] C-PER = Calculated protein efficiency ratio; [5]µmol Trolox equivalents (TE) / 100 g, DW; [6] mg extract/ mL; [7]Degree of liking/disliking using a 9-category hedonic scale (1 = dislike extremely to 9 = like extremely); [8] 1= No puffing, 2= Intermediate puffing, and 3= Complete puffing; [9]degree of breakage of the tortilla surface (0-100 %) indicated the rollability (1-5) as follows: 100%=5, 75%=4, 50%=3, 25%=2, and 0%=1.

The addition of EAF to ECBMF increased the nutritional and nutraceutical properties of tortillas.

Pacheco de Delahaye and Portillo (1990) studied the effect of the enrichment of white maize flour (*Zea mays*) with amaranth seed flour (*Amaranthus* sp). They prepared three mixes of precooked white maize flour with processed graniferous amaranth flour at the 10, 20, and 30 % substitution levels. All three mixtures showed gradual increases in fiber, fat, and ash content relative to precooked white maize flour. Arepas were elaborated from these mixtures and acceptability tests were carried out, finding that the mixtures with the levels of 10 % and 20 % of substitution were acceptable from the sensory point of view. Protein quality of arepas produced from the 80 % mixture precooked white maize flour + 20 % amaranth flour, was analyzed in terms of protein efficiency (PER) and was superior to the arepas of the precooked white corn flour; the arepas added with amaranth flour also presented high apparent digestibility (92 %). The lysine content in the arepas elaborated from the mixtures with substitution of 10 % (1.6 g lysine / 100 g of protein) and 20 % (2.0 g lysine / 100 g of protein) was also higher than those prepared with white maize flour (0.7 g lysine / 100 g protein).

Vázquez-Rodríguez *et al.* (2013) evaluated the bean and amaranth flours' effects on nixtamalized maize tortillas nutritional characteristics. They fortified nixtamalized maize flour with common bean (*Phaseolus vulgaris*) and amaranth (*Amaranthus* spp.) flours in three different bean:amaranth proportions (3:7, 5:5, 7:3) concerning commercial maize flour. They recommended this fortified product as an alternative to resolving low protein quantity/quality of maize-based food products with amaranth and common bean, particularly with 3 % bean and 7 % amaranth. In agreement with the present research, they reported that nutritionally these tortillas had significantly higher levels of protein, lysine, and tryptophan than the control.

Functional tortillas had lower antioxidant activity as evaluated by ORAC (Functional tortillas: AoxA=13,187 µmol TE/100 g, DW, 100 % ECBMF tortillas: AoxA=15,298 µmol TE/100 g, DW). This is due to a dilution effect by the addition of EAF (flour with lower content of phenolic compounds) to the EBCM for the elaboration of the functional tortilla, as well as, an antagonistic effect by the interaction of phenolic compounds of both grains. Hajimehdipoor *et al.* (2014), found an antagonistic effect and antioxidant activity decrease by a ternary combination of rutin and the caffeic, rosmarinic, chlorogenic, and gallic acids. They concluded that the possibility of interact could neutralized the effects of antioxidant activity.

On the other hand, functional tortillas showed better antihypertensive (0.37 vs 0.45 mg/mL) and hypoglycemic [100 % ECBMF tortillas: α-amylase (IC_{50}=25.17 mg/mL), α-glucosidase (IC_{50}=18.37 mg/mL) / Functional tortillas: α-amylase (IC_{50}=18.33 mg/mL), α-glucosidase (IC_{50}=15.04 mg/mL)] potentials than tortillas elaborated with 100 % ECBMF (Table 3), since lower IC_{50} values represent higher antihypertensive and hypoglycemic potentials. The improvement in the functional tortilla antihypertensive potential could relate to the presence of bioactive compounds [mainly phenolic acids (gallic, rosmarinic, caffeic, chlorogenic, and vanillic acids) and flavonoids (quercetin, rutin, isoquercitrin, nicotiflorin)] with high ACE-inhibitory potential in the EAF added to ECBMF (Peyrat-Maillard *et al.*, 2003; Barba de la Rosa *et al.*, 2009; López-Mejía *et al.*, 2014). Different hypotheses exist regarding the inhibition mechanisms of phenolic compounds on ACE: i) competitive inhibition, by the structure of phenols that altering their function by agglutination; ii) non-competitive inhibition, both substrate and inhibitor bind to the enzyme simultaneously and reversibly; iii) metal sequestration, ACE is a Zn^{2+}-dependent metalloproteinase, and phenolic compounds can chelate non-specific metals exhibiting an ACE inhibitory effect; iv) the interactions between phenols and the disulfide bridges (oxidized cysteines) that reside on the surface of the ACE, causing slight modifications in the structure of the ACE (Arenas-Carvajal *et al.*, 2009; Ademiluyi and Oboh, 2013; Al-Shukor *et al.*, 2013).

Regarding the hypoglycemic potential, this may relate to the type of phenolic compounds and the non-covalent interactions between polyphenols and enzymes. This is due to hydroxyl and galloyl groups present in the molecular structure of polyphenols. Phenolics can form hydrogen bonds with the enzyme's polar groups. In contrast, there are many hydrophobic amino acids found in enzymes (proteins). Galloyl groups in polyphenols show hydrophobicity and therefore polyphenols can bind to enzymes through hydrophobic association. The galloyl group can play an important role in interacting with α-amylase and α-glucosidase and their positions mainly affect the efficiency of these enzymes (Ali-Asgar, 2012).

Alu'datt *et al.* (2017) found that the inhibition of ACE, α-amylase, and α-glucosidase activity depended on the method of extraction, and the concentration of phenolic compounds. They reported that the most predominant soluble phenolic acids in *Rosmarinus officinalis* were rosmarinic, vanillic, chlorogenic and caffeic acids. Khan *et al.* (2017) found high concentration of gallic acid and rutin in moringa extract, which showed α- amylase and α-glucosidase IC_{50} inhibition values of 52.5 and 33.4 µg/mL, respectively. Therefore, the presence of these phenolic compounds improves both potentials by the inhibition of the activity of ACE, α-amylase, and α-glucosidase enzymes (Ademiluyi and Oboh, 2012; 2013).

The sensory evaluation of the tortillas was carried out by means of a test of general acceptability and attributes, using a hedonic scale of 9 points, where 1 corresponds to "Dislike extremely", 5 to "Neither like or dislike", and 9 to "Like extremely". The functional tortillas showed a general acceptability value of 8.01, which corresponded to the descriptor "Like very much". This value was similar ($p > 0.05$) to that of the tortillas made from ECBMF (general acceptability = 8.10). The general acceptability is correlated with the color, flavor, and texture attributes of the tortillas, which indicates that these characteristics are decisive in the sensory quality of

this product. As was the case for the general acceptability attribute, there was no significant difference (p> 0.05) for the property of color (8.00 vs 8.10) between the functional tortilla and the ECBMF tortillas. Although the functional tortillas presented slightly lower values of flavor (7.88 vs 8.15) and texture (7.97 vs 8.20) than the ECBMF tortillas, the general acceptability was no different between these two types of tortillas, which indicates that the incorporation of 30 % EAF in the functional tortillas did not affect in an important way the sensory characteristics evaluated by the panelists in this product. Likewise, the texture sensory property as a quality parameter associated with the degree of puffing and rollability of the tortillas, showed no significant differences in rollability between the functional tortillas and the ECBMF tortillas, while the functional tortillas presented a lower puffing than the ECBMF tortillas.

Since the newly functional tortillas elaborated with a mixture of ECBMF and EAF could represent a valuable staple to improve the original food product's nutritional and nutraceutical values, the results of sensory properties of the functional tortillas are promising, since the tortilla is an ideal vehicle to improve the general health status of consumers, especially in countries where this product is massively consumed by the population, such as the case of México.

CONCLUSIONS

Functional tortillas had higher proteins, dietary fiber, *in vitro* protein digestibility, calculated protein efficiency ratio, and better nutraceutical properties than tortillas elaborated with 100 % creole blue maize flour**.** The addition of extruded amaranth flour **to** extruded creole blue maize flour allows us to obtain functional tortillas with enhanced nutritional, antihypertensive, and hypoglycemic properties, and sensorially acceptable. As part of public policy, functional tortillas could reduce malnutrition and chronic degenerative diseases in Mexico. The results suggest that the addition of amaranth flour increased the nutritional and nutraceutical value in tortillas. The nutraceutical properties found *in vitro* should be confirmed further in animal or human models.

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