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Improvement of nutritional and nutraceutical value of nixtamalized maize tortillas by addition of extruded chia flour

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Mejora del valor nutricional y nutracéutico de tortillas de maíz nixtamalizado mediante la adición de harina de chía extrudida

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ABSTRACT

In this work the objectives were: (1) To optimize the extrusion conditions of defatted chia seeds maximizing antioxidant activity (*AoxA*), total phenolic content (*TPC*) and *in vitro* protein digestibility (*IVPD*), and (2) To evaluate the nutritional, nutraceutical, and sensory (acceptability) properties of tortillas formulated with commercial nixtamalized maize flour (MASECA^MR) and optimized extruded defatted chia flour. Response surface methodology was applied as an optimization tool. A central composite rotatable design with two factors [Extrusion temperature (*ET*=50-160°C), screw speed (*SS*=50-240 rpm)] in five levels were used. The extruded from each treatment (13) were dried and ground to obtain extruded defatted chia flours (*EDCF*). The desirability function was applied as optimization technique. The prediction models for each response variable were adequate and reproducible. The optimal extrusion conditions to obtain optimized extruded defatted chia flour (*OEDCF*) were *ET*=147°C/SS=237 rpm; a high global desirability (*D*=0.815) was associated with these optimal conditions. The tortillas elaborated with MASECA^TM flour + *OEDCF* had higher *IVPD*, *C-PER*, *TPC* and *AoxA*, and better antihypertensive potential than elaborated with only MASECA**™** flour. The addition of *OEDCF* to MASECA^TM flour increased the nutritional and nutraceutical value of the tortillas.
Keywords: *Tortillas, chia, extrusion, optimization, antioxidant/ antihypertensive activity*.

RESUMEN

En este trabajo los objetivos fueron: (1) Optimizar condiciones de extrusión para semillas de chía desgrasada maximizando actividad antioxidante (*AAox*), compuestos fenólicos totales (*CFT*) y digestibilidad proteínica *in vitro* (*DPIV*), y (2) Evaluar propiedades nutricionales, nutracéuticas y sensoriales (aceptabilidad) de tortillas formuladas con harinas de maíz nixtamalizado comercial (MASECA^MR) y chía desgrasada extrudida optimizada. Se aplicó metodología de superficie de respuesta como herramienta de optimización. Se utilizó diseño central compuesto rotable con dos factores [Temperatura de extrusión (*TE*=50-160°C) / Velocidad del tornillo (*VT*=50-240 rpm)] y cinco niveles. Los extrudidos de cada tratamiento (13) se secaron y molturaron para obtener harinas de chía desgrasada extrudida (*HCDE*). Como técnica de optimización se aplicó la función de deseabilidad. Los modelos de predicción para cada variable de respuesta fueron adecuados y reproducibles. Las condiciones óptimas de extrusión para obtener harina de chía desgrasada extrudida optimizada (*HCDEO*) fueron: *TE*=147°C/*VT*=237 rpm; la deseabilidad global (*D*=0.815) asociada con estas condiciones óptimas fue alta. Las tortillas elaboradas con harina MASECA^MR+*HCDEO* tuvieron mayor *DPIV*, *C-PER*, *CFT*, *AAox* y mejor potencial antihipertensivo que elaboradas solo con harina MASECA^MR. La adición de *HCDEO* a la harina MASECA^MR incrementó el valor nutricional/nutracéutico de las tortillas.
Palabras claves: *Tortillas, chía, extrusión, optimización, actividad antioxidante/antihipertensiva*.

INTRODUCTION

In México, especially among low socioeconomic groups, maize tortillas, wheat flour tortillas, and table bread are the basic cereal foods of the diet. Maize-based tortillas and wheat-based bakery items lack the EAA lysine as well as optimum levels of relevant micronutrients such as iron, zinc, and vitamins A, D, E, and B$_{12}$ (Amaya-Guerra *et al*., 2004; Chuck-Hernández *et al*., 2015). Some researchers have reported improvements in tortilla quality by increasing nutritional, nutraceutical, and sensorial properties throughout the addition of several kind of flours: common bean-amaranth (Vázquez-Rodríguez *et al*., 2013), oat (Cortes-Soriano *et al*., 2016), soybean (Acevedo-Pacheco and Serna-Saldívar, 2016), common bean (Grajales-García *et al*., 2012; Treviño-Mejía *et al*., 2016).The fortification of maize tortillas with chia flour also represents an alternative of nutritional and nutraceutical improvement for this product. Rendón-Villalobos et al. (2012), are the only researchers who have reported the elaboration of tortillas from nixtamalized maize flour added with chia flour. In that research, they evaluated the effect of only raw chia flour supplementation on the physicochemical and sensorial properties, as well as starch digestibility of tortillas. They mentioned that these tortillas can be considered as a nutraceutical food, due to the increase in the total dietary fiber and the decrease in the predicted glycemic index. However, in that research work, the improvement in the nutraceutical quality, related to the phenolic compounds content and the antioxidant activity, as well as the nutritional quality, were not studied.

Mayas and Aztec used Chia (*Salvia hispanica* L) seeds, as medical food 3,500 years B.C. These seeds have good protein (15-25%), lipids (29-34% of which 35-64% α-Linolenic acid and 17-35% Linoleic acid), carbohydrates (26-41%), and dietary fiber (18-40%) content (Ullah *et al*., 2015; Bochicchio *et al*., 2015). Chia seeds are also rich in phenolic compounds and have a high antioxidant capacity. The most commonly polyphenols responsible for antioxidant activity are flavonoids and phenolic acids (phenolic acids: rosmarinic, chlorogenic, protocatechuic, gallic, ferulic and caffeic acids; flavonols: myricetin, quercetin and kaempferol; isoflavones: genistin, genistein, daidzin, daidzein, glycitin and glycitein) (Martínez-Cruz and Paredes-López, 2014; Bochicchio *et al*., 2015; Valdivia-López and Tecante, 2015). In addition to antioxidant activity, the dietary polyphenols also have several beneficial biological activities as such anti-allergic, anti-inflammatory, anti-hypertensive, anti-mutagenic, anti-diabetic, anti-carcinogenic, among others (Ullah *et al*., 2015; Orona-Tamayo *et al*., 2016). Numerous methods are used to evaluate antioxidant activities of natural compounds in foods or biological systems with varying results. Due to its good repeatability and simplicity to perform, the three most frequently methods used to assess *in vitro* antioxidant activity are ABTS, DPPH, and ORAC. The advantage of the ORAC method over ABTS and DPPH is that it is automated and largely standardized; hence, values can be easily compared among laboratories. In addition, the ORAC method is reported to mimic phenols antioxidant activity in biological systems, better than other methods, since it uses biologically relevant free radicals and integrates both time and degree of activity of antioxidants. However, the method often requires the use of expensive equipment (Awika *et al*., 2003). These assays have been employed to evaluate antioxidant activity in maize and chia (Mora-Rochín *et al*., 2010; Grajales-García *et al*., 2012; Martínez-Cruz and Paredes-López, 2014; Valdivia-López and Tecante, 2015; Espinoza-Moreno *et al*., 2016; Orona-Tamayo *et al*., 2016; Gómez-Favela *et al*., 2017; Reyes-Moreno *et al*., 2018; Argüelles-López *et al*., 2018).

Reyes-Moreno *et al*. (2018) reported that, the extrusion process is an adequate technology to generate functional foods. This process increases the digestibility and biological value of proteins, and maintains or increases the content of bioactive compounds and nutraceutical properties of foods, due to the use of high-temperature, high-pressure, and high shear conditions in a short period. These accelerate process conditions, used during the extrusion, cause important molecular transformation and chemical reactions (modification in starch, proteins, dietary fiber and lipids, inactivation of enzymes and microorganisms, and formation of color polymers and volatile flavor components) on the food materials. In addition, this process is a highly efficient alternative technology that minimizes energy and water consumption, and does not produce polluting effluents (Reyes-Moreno *et al*., 2018).

Due to the above mentioned, it could be said that the addition of flours elaborated from promising materials as chia, processed with efficient alternative technologies as extrusion, are a viable option to improve the nutritional and nutraceutical quality of tortillas made from nixtamalized maize flours. For this reason, the objectives in this research were to find the optimum extrusion conditions of defatted chia seeds that would maximize antioxidant activity (*AoxA*), total phenolic content (*TPC*), and *in vitro* protein digestibility (*IVPD*), and evaluate the nutritional, nutraceutical and sensory (acceptability) properties of tortillas formulated with commercial nixtamalized maize flour (MASECA™) and optimized extruded defatted chia flour.

MATERIALS AND METHODS
Materials
Whole chia seeds obtained in a market of the locality (Mercado Rafael Buelna, Culiacán, Sinaloa, México) were used as materials of the study.

Methods
Production of extruded defatted chia flours (*EDCF*)
Chia seeds lots (500 g) were defatted using an oil extractor press (model DL-ZYJ05). Pellets of defatted chia were placed in a domestic blender at high velocity to obtain meals that passed through an 40-US mesh (0.425 mm) screen, which were mixed with lime (0.21 g/100 g meal) and water to achieve a water content of 28 g/100 g, and then packed in polyethylene bags and stored for 12 h at 4°C. Before extrusion, samples were tempered at 25°C for one hour. The extrusion experiments were carried out on Model 20 DN single-screw laboratory extruder (CW Brabender Instruments, Inc, NJ, USA) with a 19 mm screw-diameter, length-to-diameter 20:1; nominal compression ratio 1:1, and die to open of 5 mm. A screw-operated feed hopper fed the extruder at a feed screw of 30 rpm (fed rate was 70 g/ min). Extrudates were cooled and dried at environmental conditions (25°C, RH=65%) and milled (UD Cyclone Sample Mill, UD Corp, Boulder, CO, USA) to pass through an 80-US mesh (0.180 mm) screen, packed in plastic bags, and stored at 4°C.

Extraction of free and bound phenolic compounds
A flour sample (0.5 g) was mixed with 10 mL of ethanol (80 %, v/v), agitated (50 rpm/10 min), and centrifuged (3000×g, 10 min). This extraction step was performed two more times. The recovered supernatant was vacuum evaporated to obtain the free phenolic compound. The pellet was hydrolyzed with 10 mL of sodium hydroxide (2 M) first at 95 °C for 30 min, and then at 25 °C by 60 min, using a water bath with agitation (60 rpm). Then, it was neutralized with chloride acid, followed by lipid removal with hexane. The bound phenolic compounds were extracted with 10 mL of ethyl acetate, repeating the extraction step four more times. Finally, extracts were vacuum evaporated and stored at -20 °C (Salas-López *et al*., 2018).

Antioxidant activity (AoxA) and total phenolic content (TPC)
The *ABTS* assay was performed diluting free and bound

extracts with ethanol. Aliquots of 20 µL of each dilution were taken and mixed with 2.0 mL of diluted radical cation ABTS•+, and 6 minutes later the absorbance was determined at 734 nm in a UV-visible spectrophotometer (GENESYS 10UV, Thermo electron, Inc, Madison, WI, USA) (Re *et al.*, 1999). To carry out the *ORAC* assay, free and bound phenolic extracts were diluted in 75 mM phosphate buffer (pH 7.4). Aliquots of 25 µL of diluted extracts were mixed with 150 mL of fluorescein (0.1 mM) and 25 µL of the peroxyl radical AAPH (200 mM). After 30 minutes, fluorescence (485 nm for excitation and 538 nm for emission) was measured (37 °C) at 2 min intervals for 60 min, using a Synergy Microplate Reader (Synergy™ HT Multi-Detection, BioTek, Inc., Winooski, VT) (Mora-Rochín *et al.*, 2010). The results of *ABTS* and *ORAC* assays are expressed as micromol of Trolox equivalent (TE) per 100 g of dry weight sample. The *TPC* of free and bound extracts was determined using 20 mL of appropriate dilutions of extracts, oxidized with 180 mL of Folin-Ciocalteu reagent. After 20 min, absorbance of the resulting blue color was measured at 750 nm using the Synergy Microplate Reader. *TPC* is expressed as mg of gallic acid equivalent (GAE) / 100 g of dry weight sample (Espinoza-Moreno *et al.*, 2016). All measurements were carried out in triplicate.

In vitro protein digestibility (*IVPD*)

The *IVPD* was evaluated in *EDCF* according to Salas-López *et a*l. (2018). Five milliliters of a multi-enzyme solution [pancreatic trypsin, bovine pancreatic chymotrypsin, porcine intestinal peptidase and distilled water, pH=8.0] were mixed with 50 mL of the protein suspension (6.25 g of protein/L,

pH=8.0) employing constant stirring in a water bath at 37 °C. The pH was measured after 10 min, and the following equation used to evaluate *IVPD*:

$$IVPD = 210.46 - 18.10\ X$$

where X = pH after 10 min. The pH assay was carried out in triplicate.

Optimization using Response surface methodology (*RSM*)

To optimization of extrusion condition, we used a central composite experimental design. The employed factors were extrusion temperature (X_1=*ET*= 50-160°C) and screw speed (X_2=*SS*= 50-240 rpm), and the analyzed responses were antioxidant activity (*AoxA*), total phenolic content (*TPC*) and *in vitro* protein digestibility (*IVPD*) (Table 1). We applied the stepwise regression procedure to obtain reproducible prediction models (second-order polynomial) to each response variable, and the desirability numeric method of the *RSM* to determine the best combination of extrusion process variables (*ET*, *SS*). The fitted models for dependent variables were evaluated at any point X = (X1, X2) of the experimental zone, where each model predicted response variable values, Ŷ1 (X), Ŷ2 (X) and Ŷ3 (X). Each Ŷi (X) was transformed into a value *di(X)*, which fells in the range (0, 1) and measured the desirability degree of the response in reference to the optimum value intended to be reached. In this case, the highest possible of *AoxA, TPC*, and *IVPD* values was searched. The multi-response global desirability (*D*) was determined from the individual desirabilities with the mathematical function $D=(d_1 d_2 d_3)^{1/3}$, where the ideal optimum value is *D*=1; an ac-

Table 1. Combination of extrusion process variable used to produce extruded defatted chia flours, and experimental result of response variable of each one treatment flours.
Tabla 1. Combinación de variables del proceso de extrusión utilizadas para producir harinas de chía desgrasada extrudida y resultados de las variables de respuesta de cada uno de los tratamientos.

Treat	Process variables		Response variable		
	Extrusion temperature (°C)	Screw speed (rpm)	AoxA[2]	TPC[3]	IVDP[4]
1	66.11	77.82	18,131	526.68	81.83
2	143.89	77.82	22,613	561.58	84.56
3	66.11	212.18	21,572	551.78	81.48
4	143.89	212.18	24,968	575.06	83.56
5	50.00	145.00	18,629	541.99	80.92
6	160.00	145.00	23,483	597.54	84.43
7	105.00	50.00	24,443	541.42	84.94
8	105.00	240.00	28,949	558.60	84.06
9	105.00	145.00	26,910	573.33	82.23
10	105.00	145.00	26,196	577.93	81.22
11	105.00	145.00	25,883	568.19	82.84
12	105.00	145.00	24,318	564.10	81.99
13	105.00	145.00	25,107	581.56	81.66

[1] Rotable central composite experimental design with two factors and five levels (13 assays), [2] *AoxA*= Antioxidant activity [µmol Trolox equivalents (TE) / 100 g sample, DW], [3] *TPC*= Total phenolic compounds [mg Gallic acid equivalents (GAE) / 100 g sample, DW], [4] *IVPD* = *In vitro* protein digestibility (%)

ceptable value for *D* can be between 0.6 and 0.8. The *RSM* analyzed was realized using the software Design Expert version 7.0.0 (Stat-Ease, Minneapolis, MN, USA).

Chemical composition, soluble and insoluble dietary fiber (*SDF/IDF*) of the optimized extruded defatted chia flour (*OEDCF*)

Protein (Nx6.25), lipids, and moisture contents were determinate according to official AOAC (1999) methods 960.52, 920.39C, 925.09B, respectively. To determine SDF and IDF, the official AOAC (1999) 985.29 method and the TDF assay kit from Sigma-Aldrich (TDF 100 A) were used.

Nutritional properties: Essential amino acid (*EAA*), *in vitro* protein digestibility (*IVPD*), chemical score (*CS*) and calculated protein efficiency ratio (*C-PER*) of the *OEDCF*

The nutritional properties (*EAA*, *IVPD*, *CS* and *C-PER*) of the *OEDCF* were determined according to Salas-López *et al.* (2018). *EAA* composition was determined using an analytical scale (4.6mm×250mm) hypersil ODS C18 column (SGE, Dandenong, Australia) kept at 38 °C and connected to an HPLC system (GBC, Dandenong, Australia) equipped with a fluorescence detector >LC 5100 set at 270 and 316 nm for excitation and emission, respectively. Using an ultraviolet detector at 280 nm the tryptophan was detected. The *IVPD* was evaluated using a multi-enzyme system, above described. The chemical score (*CS*) was calculated as follows: CS = (Content of the most limiting *EAA/REAAR*) x 100; where *EAA* is the essential amino acid and *REAAR* is the recommended amino acid requirements for three years old children and older, adolescents, and adults (FAO, 2013). *C-PER* was calculated based on the *IVPD* and the *EAA* composition of the sample. All determinations carried out in triplicate.

Antihypertensive potential (*IC_{50}*) of the *OEDCF*

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 is based on the colorimetric detection of an indicator after a redox reaction, where the absorbance (Abs) at 450 nm was measured using a Microplate Reader (Synergy**™** HT Multi-Detection, BioTek, Inc., Winooski, VT, USA). 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).

Quality evaluation of tortillas elaborated from *OEDCF*

Tortillas were elaborated from the flour mixture of 75 % MASECA™ flour + 25 % *OEDCF*; tortillas made with MASECA™ were used as control. The tortillas were prepared with water at 30 °C until obtaining an adequate consistency (Corrales-Bañuelos *et al*., 2016). Tortillas' puffing was evaluated throughout their cooking using a 1 to 3 scale, where: 1= tortillas with no puffing, 2= tortillas with an intermediate

puffing, and 3= tortillas with a complete puffing (Milán-Carrillo *et al.*, 2006). The tortilla rollability was performed 30 min after elaboration; these were rolled in a glass stick of 2.54 cm of diameter, and the degree of breakage of tortilla surface (0-100%) indicated the rollability (1-5) as follows: 0%=1, 25%=2, 50%=3, 75%=4 and 100%=5 (Bedolla and Rooney, 1984). For sensory evaluation of tortillas, squared pieces pre-heated at 45°C were presented in one plate to taste. The testing panel was composed of 120 consumers (aged between 18 and 35 years of age) who were habitual tortilla consumers. Plain water was used as a palate cleanser between samples. Consumers assessed the flavor, odor, color, texture and general acceptance. The consumers were asked to indicate his/her degree of liking/disliking using a 9-category hedonic scale (1 = dislike extremely to 9 = like extremely). To determine chemical composition, as well as nutritional and nutraceutical properties of tortillas, samples were dried and milled to pass through an 80-US mesh (0.180 mm) sieve and packed in plastic bags.

Statistical analyses of *OEDCF and tortillas properties*

One-way ANOVA and Duncan's multiple range test for means comparisons were applied to the result of the evaluated properties in *OEDCF*, unprocessed defatted chia flour (*UDCF*), and tortillas using a 5 % of significance level.

RESULTS AND DISCUSSION
Optimal conditions for defatted chia flour extrusion
Prediction models for response variables

The experimental values for *AoxA*, *TPC*, and *IVPD* varied from 18,131 to 28,949 µmol TE / 100g sample (DW), from 526.68 to 581.56 mg GAE/ 100 g sample (DW), and from 80.92 to 84.94 % (DW), respectively (Table 1).

Antioxidant activity (AoxA)

ANOVA showed that the *AoxA* of the *EDCF* depended significantly (p < 0.05) on linear terms of extrusion temperature [*$ET(X_1)$*] and screw speed [*$SS (X_2)$*], as well as the extrusion temperature quadratic term [(*ET*)**²**] (p < 0.0001). The prediction models for *AoxA* of the *EDCF* were:

Coded variables:

$$Y_{AoxA} = 25,676.63 + 1,842.86\,(X_1) + 1,522.86\,(X_2) - 2,825.46\,(X_1)^2$$

Uncoded variables:

$$AoxA = -3,181.49 + 439.68\,(ET) + 22.67\,(SS) - 1.86\,(ET)^2$$

This prediction model explained 88.8% of the total variability (p = 0.0001) of the values of *AoxA* of the *EDCF*. The lack of fit test was non-significant (p =0.2987). It was found that the relative dispersion of the experimental points from the models (CV) predictions was < 10%. The process variables (*ET*, *SS*) had a significant effect on the behavior of the *AoxA* in its linear terms for both, as well as the quadratic term of *ET* (Fig 1A). The lowest value of *AoxA* (15,286 µmol TE/100 g) was located at *ET* = 50 °C/*SS* = 50 rpm. In addition, we observed that increasing *SS* increased *AoxA*, while this response also increased with *ET* to reach a maximum about 117 °C and then there was a decrease in *AoxA*. The desirable highest value of
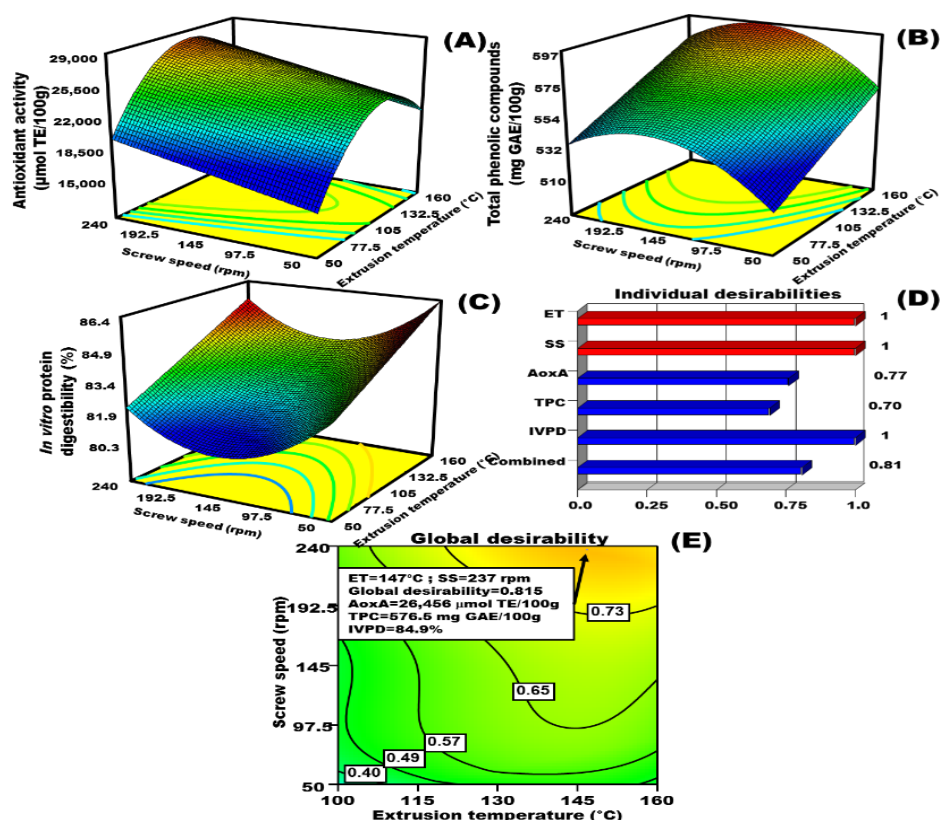
Fig 1. Response Surface plots showing the effect of extrusion temperature and screw speed on: A) Antioxidant activity (AoxA), (B) Total phenolic compounds (TPC); and (C) *In vitro* protein digestibility (IVPD). (D) Individual desirabilities for AoxA, TPC and IVPD. (E) Global desirability plot showing the region with the best combination of extrusion process variable for producing optimized extruded defatted chia flour (OEDCF).

Fig 1. Gráficas de superficie de respuesta mostrando el efecto de la temperatura de extrusión y velocidad de tornillo sobre (A) Actividad antioxidante (AAox), Compuestos fenólicos totales (CFT) y (C) Digestibilidad proteínica *in vitro* (DPIV). (D) Deseabilidades individuales para AAox, CFT y DPIV, (E) Gráfico de deseabilidad global que muestra la región con la mejor combinación de variables de proceso de extrusión para producir harina de chía desgrasada extrudida optimizada (HCDEO).

AoxA (28,114 µmol TE/100 g) was located at *ET* = 117 °C/*SS* = 239 rpm.

In general, heat treatments are thought to be the primary cause of a decrease in natural antioxidants content (Kaur and Kapoor, 2001). However, different researchers have reported increases in *AoxA* in extruded products, due to the release and generation of antioxidant compounds, as well as the inactivation of enzymes responsible for the oxidation of this type of compounds (Milán-Carrillo *et al*., 2012; Espinoza-Moreno *et al*., 2016). Korus *et al*. (2007) reported a decrease in antioxidant activity and polyphenols content during the extrusion of common bean and a bean/maize mixture, respectively, mainly attributed to the process conditions. They did not perform the optimization of the extrusion process. The potential of chia seeds as functional food has been related to their antioxidant activity and other nutraceutical properties (hypocholesterolemic, anti-hypertensive) (Salazar-Vega *et al*., 2012; Orona-Tamayo *et al*., 2016).

Total phenolic content (TPC)

ANOVA showed a significant quadratic model (p < 0.0001) for *TPC*. This analysis also showed that the *TPC* con-tent of the *EDCF* was significantly depended both on the linear terms of the extrusion temperature (*ET*) (p < 0.0001) and the screw speed (*SS*) (p = 0.0122), as well as the quadratic screw speed term (*SS*)^2. The prediction models for the *TPC* of the *EDCF* were:

Coded variables:

$$Y_{TPC} = 570.83 + 17.09\,(X_1) + 7.86\,(X_2) - 12.62(X_2)^2$$

Uncoded variables:

$$TPC = 448.91 + 0.4394\,(ET) + 0.9281(SS) - 2.7969E^{-3}\,(SS)^2$$

This prediction model explained 89.69% of the total variability (p < 0.0001) of the *TPC* values of the *EDCF*. The adjusted determination coefficient R^2 of the model was 0.8625, and the lack of adjustment was non-significant (p = 0.5059). Furthermore, the relative dispersion of the experimental points concerning the predicted values with the model (CV) was 1.26%. These values indicate that the experimental model for the *TPC* of the *EDCF* was adequate and reproducible.

The lowest *TPC* value (510 mg GAE/100 g, DW) reached, was at *ET*= 50 °C/*SS*= 50 rpm. The desirable highest value (596 mg GAE/100 g, DW) of *TPC* was observed at top

ET and intermediate *SS* (*ET*=160 °C/*SS*=165 rpm). According to some researchers, as Hirth *et al*. (2015), increasing the *SS* during the extrusion process, the residence time can decrease by up to 50%; less processing time may result in higher retention of thermolabile compounds, such as some phenolic compounds. However, an increase in *SS* also produces an increase in the specific extruder mechanical energy, with a consequent increment in temperature inside the extruder barrel. This temperature increase could affect the retention of thermolabile compounds in the processed material, although the processing time is shorter. These behaviors are shown in Fig 1B**.** As the *SS* increases the *TPC* content also increases, however, by exceeding 145 rpm, a decrease in the *TPC* levels is evident, which is attributed to the increase in temperature associated to an increased cutting effort produced by a higher *SS*.

Sarawong *et al*. (2014) suggest that the disruption of phenolic compounds attached to the matrix of the cell wall by the high-temperature effect, during the extrusion process, translates into the increased release of bound phenolic compounds; in Fig 1B it is observed that by increasing the *ET* the content of *TPC* also increases.

In vitro protein digestibility (IVPD)

ANOVA showed a significant quadratic model (p = 0.0002) for *IVPD*. This analysis also showed that the *IVPD* of the *EDCF* depended significantly both on the linear terms of the extrusion temperature (*ET*) and the screw speed (*SS*), as the quadratic term of screw speed (*SS*)². The prediction model for *IVPD* of the *EDCF* were:

Coded variables:

$Y_{IVPD}=82.10+1.22(X_1)-0.32(X_2)+1.05(X_2)^2$

Uncoded variables:

$IVPD=84.40+0.0314(ET)-0.0725(SS)+2.333 E^{-4}(SS)^2$

This prediction model explained 87.88% of the total variability (p = 0.0002) of the *IVPD* values of the *EDCF*. The adjusted determination coefficient R^2 of the model was 0.8385, and the lack of fit test was non-significant (p = 0.6355). In addition, the relative dispersion of the experimental points concerning the predicted values with the model (CV) was 0.68%. These values indicate that the experimental model for the *IVPD* of the *EDCF* was adequate and reproducible. Fig 1C shows that both process variables (*ET*, *SS*) had a significant effect on the behavior of *IVPD*. The lowest value (80.35%) of *IVPD* was located at *ET*=50 °C/*SS*=156 rpm. Two desirable highest values (85.5 and 86.4%) of *IVPD* can be observed; they correspond to the highest *ET* (160 °C) and to the highest and lowest values of *SS* (240 and 50 rpm, respectively) (Fig 1C).

Optimization of the defatted chia flour extrusion process

Figure 1E shows the global desirability (*D*) as a function of the extrusion process variables *ET* and *SS*. *D* was obtained as the geometric average of the individual desirabilities of each one of the response variables. It was utilized to determine the best combination of process variables (optimum con-

ditions) for the production of optimized extruded defatted chia flour (*OEDCF*) with high values of *AoxA*, *TPC*, and *IVPD*. The optimum extrusion conditions were *ET*= 147 °C and *SS*= 237 rpm showing a global desirability of *D*=0.815 (Fig 1E); the individual desirabilities of the response variables associated with "*D*" were d_{AoxA}= 0.77, d_{TPC}=0.70, and d_{IVPD}=1.00. Using the *ET/SS* extrusion optimized condition the software predicted values of 26,456 µmol Trolox equivalents (TE) / 100g sample (DW), 576.5 mg gallic acid equivalents (GAE) / 100g sample (DW), and 84.9 % for *AoxA*, *TPC* and *IVPD*, respectively. *OEDCF* was produced applying the best combination of extrusion process variables. The experimental values of *AoxA*, *TPC* (Table 3), and *IVPD* (Table 2) of *OEDCF* were similar to the predicted values, above mentioned, indicating that the optimal conditions of extrusion process were appropriated and reproducible.

Table 2. Chemical composition and essential amino acid content in unprocessed and extruded defatted chia flours.
Tabla 2. Composición química y contenido de aminoácidos esenciales en harinas de chía desgrasadas sin procesar y extruida.

Property	Unprocessed defatted chia flour (UDCF)	Optimized extruded defatted chia flour (OEDCF)	FAO[1] requirements
Chemical composition (%, DW)			
Proteins	31.09±0.37[A]	30.66±0.29[A]	
Lipids	5.27±0.06[A]	5.12±0.07[B]	
Minerals	6.88±0.07[A]	6.91±0.08[A]	
Dietary fiber			
Soluble	6.71±0.09[B]	7.36±0.09[A]	
Insoluble	41.21±0.13[B]	43.01±0.13[A]	
Total	47.92±0.27[B]	50.37±0.27[A]	
Carbohydrates	8.84±0.03[A]	6.94±0.03[B]	
Nutritional			
EAA[2] (g/100 g protein)			
His	1.99±0.04[A]	2.01±0.03[A]	1.60
Ile	3.37±0.03[A]	3.29±0.04[B]	3.00
Leu	6.03±0.03[A]	6.00±0.04[A]	6.10
Lys	4.63±0.02[A]	4.60±0.02[B]	4.80
Met+Cys	2.69±0.03[A]	2.48±0.02[B]	2.30
Phe+Tyr	5.18±0.02[A]	5.02±0.03[B]	4.10
Thr	2.58±0.04[A]	2.51±0.04[B]	2.50
Trp	1.63±0.02[A]	1.54±0.05[B]	0.66
Val	4.23±0.02[A]	4.25±0.04[A]	4.00
Total	32.33	31.70	29.06
EAA Chemical score	96.45	95.83	
Limitant *AAE*	Lys	Lys	
IVPD[3] **(%)**	77.9[B]	84.05[A]	
C-PER[4]	1.70[B]	2.15[A]	

[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.

Table 3. Antioxidant activity, total phenolic content, and antihypertensive potential of unprocessed and extruded defatted chia flours.
Tabla 3. Actividad antioxidante, compuestos fenólicos totales y potencial antihipertensivo de harinas de chía desgrasadas sin procesar y extruida.

Property	Unprocessed defatted chia flour (UDCF)	Optimized extruded defatted chia flour (OEDCF)
Antioxidant activity[1]		
ABTS		
Free phenolics	2,965±133[B]	4,833±150[A]
Bound phenolics	11,587±543[B]	12,698±589[A]
Total	14,552±717[B]	17,531±698[A]
ORAC		
Free phenolics	8,456±230[B]	10,781±198[A]
Bound phenolics	14,268±392[B]	15,986±375[A]
Total	22,724±612[B]	26,767±504[A]
Phenolic compounds [2]		
Free phenolics	248.94±2.97[B]	302.44±4.81[A]
Bound phenolics	252.32±4.19[B]	339.40±3.61[A]
Total	501.26±12.70[B]	641.84±14.2[A]
Antihypertensive potential (IC_{50})[3]	0.51±0.02[A]	0.34±0.01[B]

[A-B] Means with different superscripts in the same row are significantly different (Duncan, $p \leq 0.05$).[1] µmol Trolox equivalents (TE) / 100 g sample, DW.
[2] mg gallic acid equivalents (GAE) / 100 g sample, DW.
[3] mg extract/ ml.

Effect of optimized extrusion process on chemical composition and nutritional properties of defatted chia flour

Table 2 shows protein, lipid and dietary fiber content of unprocessed and optimized extruded defatted chia flours (*UDCF*, *OEDCF*). The extrusion process caused slight changes in the chemical composition of defatted chia flours during the production of *OEDCF*. The essential amino acid (EAA) content of *UDCF* and *OEDCF* is shown in Table 2. In general, the EAA content of *UDCF* and *OEDCF* were higher than those suggested by FAO (2013) for the requirement of essential amino acids for children (3 years and older), adolescents and adults, except Lys and Leu content, which were slightly below the FAO standard; in both *UDCF* and *OEDCF* Lys and Leu were presented as limiting amino acids. The extrusion process caused a slight decrease ($p < 0.05$) in the EAA content of defatted chia flour. Both flours present a high EAA chemical score (95.83-96.45). On the other hand, the extrusion process increased *IVPD* of defatted chia flour. During food processing by extrusion, both an *IVPD* increase and an *IVPD* decrease may occur. This increase of *IVPD* occurs due to the destruction of labile anti-nutritional factors (lectins, trypsin and chymotrypsin inhibitors) and protein denaturation, as a result of the applied conditions (shearing forces, temperature, and humidity) during processing (Gamel *et al*., 2006; Montoya-Rodriguez *et al*., 2015). The decrease of *IVPD* in extrusion occurs because the components of dietary fiber, phenolic compounds, and the products of the Maillard reaction (reaction carried out between reducing sugars and amino acids of the proteins) can bind to protein, partially unavailing it for its digestion (Reyes-Moreno *et al*., 2018). However, the increase of *IVPD* during food processing by extrusion is the most commonly reported phenomenon in literature, which agrees with the results obtained in the present investigation. Results in Table 2 also indicated that *C-PER* increased during the *OEDCF* generation. This nutritional parameter was calculated using the *IVPD* and the *EAA* composition of the sample, so its increase was mainly due to the increase in *IVPD*, since the *EAA* content only slightly decreased.

Gutiérrez-Dorado *et al*. (2008a) carried out a study to improve the nutritional quality of tortillas, employing as a strategy the use of flours produced by extrusion from high quality protein maize (QPM). In this study, we evaluated nutritionally the protein quality of tortillas obtained by *in vitro* and *in vivo* assays. Tortillas produced with extruded QPM flour had an improved amino acid profile and higher *IVPD*, *C-PER*, *PER*, *NPR* and *PDCAAS* values than tortillas made from a commercial nixtamalized corn flour (MASECA™). These researchers also reported that tortillas made with extruded QPM flour had higher dietary fiber content than tortillas made with MASECA™, since, during the flour production by extrusion, all components of the grain were retained, including the pericarp, which is a rich source of fiber. However, the dietary fiber content of the extruded QPM tortillas was slightly lower than that of the tortillas obtained in this research work with the mixture of MASECA™ and *OEDCF* (15.22 vs. 17.11%, respectively). Comparing our results on protein nutritional evaluation and the extruded QPM tortillas from the aforementioned study, both kinds of tortillas had a similar chemical score of the protein (71 vs 70, respectively), and the limiting amino acid in the tortillas with *OEDCF* was Lys, while in the tortillas with extruded QPM was Trp. However, although the content of Lys in the tortillas with *OEDCF* was lower than that of the extruded QPM tortillas, the chemical score of the protein was higher than that of tortillas produced from MASECA™ flour. Regarding the *IVPD* and *C-PER*, tortillas with *OEDCF* had higher values than the extruded QPM tortillas (*IVPD*: 83.22 vs 77.40, *C-PER*: 1.98 vs 1.80, respectively). Another study conducted by Gutierrez-Dorado *et al*. (2008b), concluded that the *PER* obtained using *IVPD* and amino acids underestimated the values obtained in rats, however the absolute differences between the two treatments were similar, indicating that the *C-PER* technique predicted the same difference observed in the bioassay in rats. They recommend using *in vitro* techniques as a rapid and accurate indicator of *IVPD* and *PERs* in foods.

Effect of extrusion on antioxidant activity (*AoxA*) and total phenolic compounds (*TPC*) of defatted chia seeds

Unprocessed defatted chia seeds (*UDCF*) had ABTS antioxidant values of 2,965, 11,587, and 14,552 µmol Trolox equivalent (TE) / 100 g sample, and ORAC antioxidant values of 8,456, 14,268, and 22,724 µmol TE / 100 g sample, to free, bound, and total phenolic compounds, respectively (Table 3). Gómez-Favela *et al*. (2017) reported similar values of *AoxA* to free, bound, and total phenolic compounds in chia

seeds (ABTS: 2,412, 9,843, and 12,255 µmol TE / 100 g sample, respectively; ORAC: 6961, 12,169, and 19,130 µmol TE / 100 g sample, respectively). Marineli *et al*. (2014) reported an ORAC antioxidant value (51,730 µmol TE/100 g free fraction of defatted sample) in Chilean chia seeds higher than that of *UDCF*. This difference could be due to several agronomic factors such as soil type, harvesting time, weather conditions, and others, which influence on secondary metabolites in plants, leading to the increase or decrease of phenolic compounds with antioxidant activity and, in this way, to the increase or decrease of the seeds antioxidant activity. The high antioxidant activities in chia seeds could be due to a high content of polyphenolic compounds (Gómez-Favela *et al*., 2017). The application of the extrusion process to produce *OEDCF* increased ($p < 0.05$) the *AoxA*, evaluated by ABTS, and ORAC procedures, by 20.47 % and 17.79 %, respectively, when compared to *UDCF* (Table 3**)**. The *AoxA* of bound and free phenolic compounds, quantified by ABTS method in defatted chia seeds, increased ($p < 0.05$) by 9.58% and 63.0%, respectively, after extrusion process (Table 3). The *AoxA* of *OEDCF*, evaluated in bound and free phenolics using the ORAC assay, increased by 12.04% and 27.49%, respectively, when compared to *UDCF*. This *AoxA* increase could relate to the release of antioxidant phenolic compounds during the extrusion process, prevention of oxidation of phenolic compounds in the extruded product (by enzymatic inactivation during the processing), and the presence of Maillard reaction products with antioxidant activity (generated during extrusion of raw material that contain amino acids and reducing sugars) (Milán-Carrillo *et al*., 2012; Espinoza-Moreno *et al*., 2016). The *AoxA* mechanism of the melanoidins is based on their ability to trap positively charged electrophilic species, eliminate oxygen radicals or carry out the chelation of metals to form inactive complexes (Delgado-Andrade and Morales, 2005). In addition, *TPC* increased significantly (21.86%) during the preparation of *OEDCF* by extrusion; being bound phenolic compounds the main fraction responsible for the improvement on *TPC* (Table 3). This increase in *TPC* may be accountable for the improvement in *AoxA* of *OEDCF*, explained above. The extrusion process increases phenolic compounds content due to the destruction of cell walls, release of phenolic compounds, and formation of Maillard reaction products, quantified as phenolic compounds (Espinoza-Moreno *et al*., 2016). In the literature, it has been reported that melanoidins, like phenolic compounds, react with the Folin-Ciocalteu reagent; in some studies, this method has even been used to indirectly quantify melanoidins (Pérez-Hernández *et al*., 2013).

Effect of optimized extrusion process on antihypertensive potential [inhibition of angiotensin-converting enzyme (*ACE*)] of phenolic compounds extracted from chia flours

The anti-hypertensive potential was defined as IC_{50} [concentration (mg of extract/mL) required to produce an inhibition of 50 % of the *ACE* activity]. Phenolic compounds extracted from *OEDCF* had better anti-hypertensive potential (lower IC_{50} value) than the phenolic compounds extracted from *UDCF* (0.34 vs. 0.51 mg extract/mL, respectively) (Table 3). The benefits of IC_{50} obtained in this research are in concordance with reported results for phenolic extracts of soybean (0.143-0.160 mg/mL) and grape seeds (0.1-3.5 mg/L) (Ademiluyi and Oboh, 2013). The IC_{50} value improvement during the extrusion process of defatted chia seeds could have occurred by release and formation of bioactive compounds (phenolic compounds and Maillard reaction products) with anti-hypertensive potential. As mentioned above, in this research work an increase in the phenolic compounds content occurred during processing of chia by extrusion (Table 3). It has been reported that 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 action mode is closely related with the class (subclass) and the structure of the phenolic compound that is employed (Massaretto *et al*., 2011; Al-Shukor *et al*., 2013). According to our results, phenolic compounds present in extruded chia flour, obtained by extrusion process at optimal conditions, could be used as functional food supplements or natural medicines for the treatment of hypertension.

Effect of optimized extruded defatted chia flour (*OEDCF*) addition on quality of nixtamalized maize tortillas

The chemical composition, nutritional and nutraceutical properties, and sensory acceptability of tortillas from commercial nixtamalized maize flour (MASECA™) and maize tortillas enriched with optimized extruded defatted chia flour (*OEDCF*) is shown in Table 4. Tortillas, elaborated with 75% MASECA™+25% *OEDCF*, had higher protein, lipids, *IVPD*, C-PER, dietary fiber, and *TPC* than tortillas elaborated only with MASECA™ flour. The addition of *OEDCF* to MASECA™ flour increased the tortillas nutritional properties. Rendón-Villalobos *et al*. (2012) evaluated the effects of chia powders supplementation on the physicochemical and sensorial characteristics as well as starch digestibility of tortillas. They used composite flours containing 5%, 10%, 15%, and 20% of chia seed flour and corn. In agreement with the present research, they reported that nutritionally, all chia tortillas had significantly higher levels of protein, lipids, and total dietary fiber than the control. Additionally, they observed that the reduced enzymatic starch hydrolysis rate and predicted glycemic index recorded for the chia seed flour-added tortilla indicated slow digestion features. According to these researchers, owing to the increase in the total dietary fiber, lower digestion, and predicted glycemic index values, chia seed flour-added tortilla can be considered as a nutraceutical food. Cortes-Soriano *et al*. (2016) studied the effect of the addition of nixtamalized oatmeal on the quality of commercial maize tortillas. They made tortillas with mixtures of nixtamalized maize and oat flours (NMF, NOF); the NOF was obtained from the variety Obsidiana, and NMF was the brand MINSA. The mixtures NOF: NMF evaluated were: 10:90,

Table 4. Chemical composition, nutritional and nutraceutical properties, and sensory acceptability of tortillas from commercial nixtamalized maize flour (MASECA™) and tortillas added with optimized extruded defatted chia flour (MASECA™+ OEDCF).
Tabla 4. Composición química, propiedades nutricionales y nutracéuticas, y aceptabilidad sensorial de tortillas de harina de maíz nixtamalizado comercial (MASECA™) y tortillas añadidas con harina de chía desgrasada extrudida optimizada (MASECA™+ HCDEO).

Property	Tortillas from MASECA	Tortillas from MASECA + OEDCF [1]	FAO [2] requirements
Chemical composition (%, DW)			
Proteins	8.88±0.05[B]	14.33±0.13[A]	
Lipids	5.30±0.02[A]	5.23±0.05[B]	
Dietary fiber			
Soluble	1.37±0.06[B]	2.85±0.09[A]	
Insoluble	4.70±0.10[B]	14.26±0.13[A]	
Total	6.07±0.12[B]	17.11±0.18[A]	
Nutritional			
EAA[3] (g/100 g protein)			
His	2.49±0.02[A]	2.39±0.03[B]	1.60
Ile	2.83±0.03[B]	3.01±0.04[A]	3.00
Leu	11.94±0.06[A]	11.58±0.05[B]	6.10
Lys	2.44±0.04[B]	3.42±0.05[A]	4.80
Met+Cys	3.39±0.03[A]	3.20±0.02[B]	2.30
Phe+Tyr	7.58±0.05[A]	6.94±0.03[B]	4.10
Thr	2.70±0.02[A]	2.67±0.04[A]	2.50
Trp	0.56±0.04[B]	0.79±0.05[A]	0.66
Val	4.23±0.05[A]	4.19±0.04[A]	4.00
Total	38.16	38.19	29.06
Chemical score	50.83	71.25	
Limitant EAA	Lys	Lys	
In vitro protein digestibility (%)	75.90±0.21[B]	83.22±0.20[A]	
C-PER[4]	1.12±0.06 [B]	1.98±0.05[A]	
Nutraceutical			
Antioxidant activity[5]	5,985±246[B]	12,499±438[A]	
Antihypertensive potential (IC$_{50}$)[6]	Non detected	0.67	
Sensory			
Acceptability[7]	8.5[A]	8.3[A]	
Puffing	3[A]	3[A]	
Rollability	5[A]	5[A]	

[A-B] Means with different superscript letters in the same row are significantly different (Duncan, p<0.05).
[1] Tortillas elaborated with 75 % MASECA™ flour + 25 % Optimized extruded defatted chia flour (**OEDCF**).
[2] **EAA** requirements for children (3 years and older), adolescents and adults according to FAO (2013).
[3] **EAA** = Essential amino acid.
[4] **C-PER** = Calculated protein efficiency ratio.
[5] **μmol Trolox equivalents (TE) / 100 g sample, DW**.
[6] mg extract/ mL.[7] Degree of liking/disliking using a 9-category hedonic scale (1 = dislike extremely to 9 = like extremely).

20:80, 30:70, and 40:60, respectively. The quality of the tortilla was measured based on the diameter (cm), thickness (mm), the weight of the tortilla hot and cold (g), the rollability, the performance of the hot and cold tortilla and colorimetry. Additionally, compositional analysis and sensory evaluation of tortillas showed that the 40:60 mixture had a high protein and fiber content, but less acceptability; while those made with 10:90 and 20:80 had the highest protein content, and better acceptability, taste, and texture compared to tortillas from NMF.

Tortillas, elaborated with a mixture of MASECA™ flour and *OEDCF*, had higher antioxidant activity (ORAC: 12,499 vs 5,985 µmol TE/100 g, DW), and better anti-hypertensive potential (0.67 mg extract/mL vs ND) than tortillas elaborated only with MASECA™ flour (Table 4). The extruded chia flour, obtained by extrusion process under optimal conditions, could be used as functional food supplements or natural medicine for hypertension treatment. The improvement of *AoxA* and anti-hypertensive potential during the extrusion process of defatted chia flour could have occurred by release and formation of bioactive compounds (phenolic compounds and Maillard reaction products) with antioxidant and anti-hypertensive potential. Phenolic compounds (Phenolic acids, flavonoids, tannins, stilbenes) inhibit the *in vitro ACE* activity (Massaretto *et al*., 2011; Al-Shukor *et al*., 2013).

The newly developed tortilla, elaborated with 75% MASECA™ flour+25% *OEDCF* could represent a valuable staple in improving the nutritional value of the original food product.

CONCLUSIONS

The best combination of process variables to produce optimized extruded defatted chia flour with maximum antioxidant activity, total phenolic content, and *in vitro* protein digestibility was ET=147 °C/SS= 237 rpm. The addition of optimized extruded defatted chia flour to the nixtamalized maize tortillas caused an increase in the quantity and nutritional quality of the protein, as well as an increase in its dietary fiber content. In addition, tortillas added with chia had a higher content of total phenolic compounds, and antioxidant and antihypertensive activity than tortillas produced only from nixtamalized maize flour. The use of extruded chia flour in the preparation of nixtamalized maize tortillas may have a positive effect on the healthy status of people from countries where these products are widely consumed.

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