



Biotecnia

E-ISSN: 1665-1456

biotecnia@ciencias.uson.mx

Universidad de Sonora

México

Argüelles-López, Oscar Daniel; Reyes-Moreno, Cuauhtémoc; Gutiérrez-Dorado, Roberto;  
Sánchez-Osuna, María Fernanda; López-Cervantes, Jaime; Cuevas-Rodríguez, Edith  
Oliva; Milán-Carrillo, Jorge; Perales-Sánchez, Janitzio Xiomara Korina

FUNCTIONAL BEVERAGES ELABORATED FROM AMARANTH AND CHIA FLOURS  
PROCESSED BY GERMINATION AND EXTRUSION

Biotecnia, vol. 20, núm. 3, septiembre-diciembre, 2018, pp. 135-145

Universidad de Sonora

Available in: <https://www.redalyc.org/articulo.oa?id=672971088019>

- How to cite
- Complete issue
- More information about this article
- Journal's homepage in redalyc.org

redalyc.org

Scientific Information System

Network of Scientific Journals from Latin America, the Caribbean, Spain and Portugal

Non-profit academic project, developed under the open access initiative

# FUNCTIONAL BEVERAGES ELABORATED FROM AMARANTH AND CHIA FLOURS PROCESSED BY GERMINATION AND EXTRUSION

BEBIDAS FUNCIONALES ELABORADAS A PARTIR DE HARINAS DE AMARANTO Y CHÍA PROCESADOS POR GERMINACIÓN Y EXTRUSIÓN

Oscar Daniel Argüelles-López<sup>1</sup>, Cuauhtémoc Reyes-Moreno<sup>1,2</sup>, Roberto Gutiérrez-Dorado<sup>1,2</sup>, María Fernanda Sánchez-Osuna<sup>1</sup>, Jaime López-Cervantes<sup>3</sup>, Edith Oliva Cuevas-Rodríguez<sup>1,2</sup>, Jorge Milán-Carrillo<sup>1,2</sup>, Janitzio Xiomara Korina Perales-Sánchez<sup>1,2,\*</sup>

<sup>1</sup> Programa de Posgrado en Ciencia y Tecnología de Alimentos, Facultad de Ciencias Químico Biológicas (FCQB), Universidad Autónoma de Sinaloa (UAS), Ciudad Universitaria, AP 1354, CP 80,000 Culiacán, Sinaloa, México.

<sup>2</sup> Programa de Regional de Posgrado en Biotecnología, FCQB-UAS, Ciudad Universitaria, AP 1354, CP 80,000 Culiacán, Sinaloa, México.

<sup>3</sup> Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, 85000 Ciudad Obregón, Sonora, México.

## ABSTRACT

The aims of this research were to develop functional beverages from amaranth and chia flours, processed by germination and extrusion, and evaluate its nutritional, antioxidant and antihypertensive properties. Optimal conditions, previously obtained, to produce extruded (Extrusion temperature= 141 °C/screw speed = 81 rpm) and germinated (Germination temperature= 30 °C/germination time= 78 h.) amaranth flours (EAF, GAF) were applied. Optimal conditions of germination temperature (29 °C) and germination time (197 h) to elaborate germinated chia flour (GCF), with maximum values of antioxidant activity, total phenolic and protein contents, were obtained. A 200 mL portion of the functional beverages, elaborated with 25 g of 70 % EAF+ 30% GCF or 70 % GAF + 30 % GCF mixtures, had 3.90-4.53 g protein, 5.04-6.81 g dietary fiber, 95-96 kcal of energy, calculated protein efficiency ratio = 2.52-2.69, antioxidant activity= 4,009-6,495 µmol TE, antihypertensive potential (IC<sub>50</sub>) = 0.43-0.47 µg extract/mL, and sensorial acceptability between "I like it very much" and "I like it extremely". These functional beverages, due to its high nutritional value, and antioxidant and antihypertensive potential, can be used for health promotion of consumers.

**Keywords:** Amaranth, chia, functional beverages, antioxidant activity, antihypertensive potential.

## RESUMEN

Los objetivos de esta investigación fueron desarrollar bebidas funcionales a partir de harinas de amaranto y chía procesados por germinación y extrusión, y evaluar sus propiedades nutricionales, antioxidante y antihipertensiva. Se aplicaron condiciones óptimas, previamente obtenidas, para producir harinas de amaranto extrudido (Temperatura de extrusión = 141 °C/velocidad de tornillo = 81 rpm) y germinado (Temperatura de germinación= 30 °C/tiempo de germinación= 78 h.) (HAE, HAG). Se obtuvieron condiciones óptimas de temperatura (29 °C) y tiempo de germinación (197 h), para elaborar harina de chía germinada (HCG), con

valores de actividad antioxidante, contenidos de compuestos fenólicos totales y proteína máximos. Una porción de 200 mL de bebidas funcionales, preparadas con 25 g de las mezclas 70 % HAE + 30 % HCG o 70 % HAG + 30 % HCG, tuvieron 3.90-4.53 g de proteína, 5.04-6.81 g de fibra dietaria, 95-96 kcal de energía, relación de eficiencia proteínica calculada = 2.52-2.69, actividad antioxidante= 4,009-6,495 µmol ET y potencial antihipertensivo (IC<sub>50</sub>) = 0.43-0.47 µg extracto/mL y aceptabilidad sensorial entre "me gusta mucho" y "me gusta extremadamente". Estas bebidas funcionales, por su valor nutricional alto y su potencial antioxidante y antihipertensivo, pueden ser empleadas para promover la salud de los consumidores.

**Palabras clave:** Amaranto, chía, Bebida funcional, actividad antioxidante, potencial antihipertensivo

## INTRODUCTION

In last decades, researchers from around the world have participated in the valorization of crops, such as amaranth and chia, ancestral grains of pre-Columbian American cultures (Aztec, Maya) with outstanding agronomic characteristics and nutritional / nutraceutical value. The rational, sustainable and efficient exploitation of renewable natural resources is an indispensable condition for securing global food security in the future (FAO, 2005).

The amaranth grain contains highly digestible proteins with good levels of essential amino acids; its balance being close to the optimum required in the human diet (FAO, 2013). Depending on the species, the total protein content ranges from 12.5-22%, lipid content 1.9-13%, carbohydrates 51.5-75.8% and fiber 7.6-16.4% (Orona-Tamayo and Paredes-López, 2017a). Furthermore, they contain polyphenolic compounds, such as phenolic acids (ferulic, *p*-coumaric, vanillic, gallic acids) and flavonoids (rutin, vitexin and isovitexin) (Paško *et al.*, 2008; Perales-Sánchez *et al.*, 2014). Amaranth grains are considered functional food due to their properties for the prevention and / or treatment of chronic-degenerative disease such as hypocholesterolemia (Mendonça *et al.*, 2009),

\*Autor para correspondencia: Janitzio Xiomara Korina Perales-Sánchez  
Correo electrónico: xiomara\_perales@hotmail.com

Recibido: 10 de enero de 2018

Aceptado: 21 de marzo de 2018

and hypertension (Martirosyan *et al.*, 2007); also, they are gluten free foods, so they can be consumed by celiac people (Alvarez-Jubete *et al.*, 2010).

Chia (*Salvia hispanica* L.) is native to Mesoamerica; 3,500 years BC, Mayas and Aztecs used it for food and medicine. Its grains contain, dry weight (dw), 15-25% proteins, 29-34% lipids (35-64%  $\alpha$ -Linolenic acid, 17-35% Linoleic acid), 26-41% carbohydrates and 18-40% dietary fiber (Ullah *et al.*, 2015; Orona-Tamayo and Paredes-López, 2017b). In addition, they have phenolic compounds such as rosmarinic, protocatechuic, ferulic and caffeic acids, and flavonols like quercetin, kaempferol, myricetin (Orona-Tamayo and Paredes-López, 2017b), which may be able to protect consumers against different chronic degenerative diseases: hypertension, diabetes, cardiovascular diseases, certain types of cancer, among others (Ullah *et al.*, 2015).

When the mixture of cereal and legume is ingested, a substantial improvement in the nutritional quality of the protein is obtained due to a better amino acid balance. Furthermore, the addition of phytochemicals increases the nutraceutical potential (Reyes-Moreno *et al.*, 2012; Milán-Carrillo *et al.*, 2017). This same phenomenon of nutritional and nutraceutical complementarity must occur when mixing amaranth-chia seeds.

It has been reported that, optimal processing conditions of extrusion and germination increases the biological value of proteins, the content of phenolic compounds, antioxidant, antihypertensive, antidiabetic, anticancer activities in cereals, legumes and pseudo-cereals (Mora-Escobedo *et al.*, 2009; Milán-Carrillo *et al.*, 2012; Perales-Sánchez *et al.*, 2014; Espinoza-Moreno *et al.*, 2016; Mamilla and Mishra, 2017). Functional beverages are a functional food category; they provide a health benefit, beyond simple nutritional function. Beverages are the fastest growing (14% annual) segment of the food sector, because they are excellent means to supply nutrients and bioactive compounds (antioxidants, vitamins, minerals, fiber, prebiotics and probiotics). In addition, they are practical for consumers by their size, shape, and ease storage (Corbo *et al.*, 2014). Pandal (2017), estimated that the sale of functional beverages in the world could be around of \$105.5 billion US dollars.

In Mexico, 76.6% adults, 36.3% adolescents and 33.2% children suffer from overweight and obesity, and 25.5% adults suffer from arterial hypertension (INSP, 2013). By 2017, the estimated direct cost for medical care of diseases attributable to overweight and obesity (hypertension, diabetes mellitus type 2, cardiovascular diseases) will be 77,919 million of Mexican pesos (Barrera-Cruz *et al.*, 2013). Oxidative stress is a crucial causal factor for the initiation and progression of hypertension. An important factor in blood pressure regulation is the angiotensin-converting enzyme (ACE) that catalyzes the conversion of angiotensin I into angiotensin II (vasoconstrictor) and inactivates Bradykinin (vasodilator). One of the strategies to combat hypertension is searching for ACE inhibitors from natural products such as peptides, triterpenes, and phenolic compounds. Previous researches

have demonstrated that phenolic compounds derived from pseudo-cereals (quinoa and amaranth) and chia have antioxidant and antihypertensive effects (Arenas-Carvajal *et al.*, 2009; Asao and Watanabe 2010; Orona-Tamayo and Paredes-López, 2017b).

Actually, the growing interest of the food industry to generate functional beverages with potential therapeutic effect on the consumer, therefore the use of ingredients with high nutritional / nutraceutical value, and the application of technologies with potential to favor an increase in the biological value of proteins, bioactive compounds content, and nutraceutical properties of foods, is relevant. For this reason, the aim of this research was to develop functional beverages from amaranth and chia flours, processed by germination and extrusion, and evaluate their nutritional, antioxidant and antihypertensive properties.

## MATERIALS AND METHODS

### Materials

Whole amaranth and chia seeds obtained in a market of the locality (Mercado Rafael Buelna, Culiacán, Sinaloa, Mexico) were used as materials of the study.

### Chemicals

Folin-Ciocalteu reagent, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), ethanol and ethyl acetate were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

### Production of extruded and germinated amaranth flours

Processing conditions previously optimized in our laboratory (Bioprocesses and Functional Foods) were used to produce extruded and germinated amaranth flours. Optimization was carried out to obtain the maximum possible values of antioxidant activity, total phenolic content, and protein content in extruded and germinated amaranth flours. Extruded amaranth flour was produced in a single screw extruder. The grains were mixed with lime (0.24% in relation to grain weight) and water (28% moisture); the operating optimal conditions were Extrusion temperature = 141 °C/screw speed = 81 rpm. The extruded product was cooled (25 °C), ground, packed in plastic bags and stored (4 °C) (Milán-Carrillo *et al.*, 2012). To produce germinated amaranth flour, germinated amaranth was obtained using a stainless-steel germination chamber with controlled temperature and time conditions. Before germination, grains were soaked in water for six h. Germination conditions were: Germination temperature (GT)= 30 °C/Germination time (Gt)= 78 h. The sprouts were dried (50 °C/8 h), cooled (25 °C), ground, packed in plastic bags and stored (4 °C) (Perales-Sánchez *et al.*, 2014).

### Optimization of the process of chia seeds germination

#### Experimental design

A rotatable central composite experimental design [Response Surface Methodology (RSM)] with two factors or process variables [germination temperature ( $X_1$ )=GT= 18-37

°C), germination time ( $X_2$ =Gt= 12-288 h)] and five levels of variation was used to carry out the optimization of the process of chia seeds germination; 13 treatments were generated (Table 1). Antioxidant activity, total phenolic content, and protein content were selected as response variables.

### Production of germinated chia flours (GCF)

A 25 g portion of whole chia seeds was placed inside plastic trays with absorbent paper previously moisturized with 200 mL of a 100 ppm sodium hypochlorite dissolution. Plates were introduced in the germination chamber (manufactured by Centro de Instrumentos, Universidad Autónoma de Sinaloa) with controlled temperature. A relative humidity of 80–90 % within the chamber was maintained using trays with water. The germination was carried out applying combinations of germination temperature/time (GT/Gt) in the intervals of 18–37 °C, and 12–288 h, respectively (Table 1) (13 treatments). In all cases, seeds were germinated under light/darkness periods of 50/50 % of the germination time daily. The resulting sprouted chia seeds were dried (50 °C/8 h), cooled (25 °C), and milled (UD Cyclone Sample Mill, UD Corp, Boulder, CO, USA) to pass through an 80-US mesh (0.180 mm) screen, to produce germinated chia flours. The germinated chia flours were packed and kept at 4 °C in tightly sealed containers until further analysis. The germinated chia flours were evaluated for antioxidant activity, total phenolic content, and protein content.

**Table 1.** Combination of germination process variable used to produce germinated chia flours, and experimental results of response variables of each treatment.

**Tabla 1.** Combinación de variables de proceso de germinación utilizadas para producir harinas de chía germinada, y resultados experimentales de las variables de respuestas de cada uno de los tratamientos

Treat	Process variables		Response variables		
	Germination temperature (°C)	Germination time (h)	AoxA <sup>2</sup>	TPC <sup>3</sup>	PC <sup>4</sup>
1	20.78	52.42	29,135	507.6	22.8
2	34.22	52.42	30,750	525.9	21.8
3	20.78	247.58	33,167	555.7	23.7
4	34.22	247.58	43,118	587.1	32.1
5	18.00	150.00	29,080	504.7	25.0
6	37.00	150.00	39,522	561.5	26.3
7	27.50	12.00	28,700	498.5	22.2
8	27.50	288.00	47,000	644.8	30.5
9	27.50	150.00	44,133	596.2	24.7
10	27.50	150.00	46,029	612.6	26.9
11	27.50	150.00	44,367	605.8	25.3
12	27.50	150.00	46,953	618.8	27.4
13	27.50	150.00	42,604	597.9	26.1

<sup>1</sup> Rotable central composite experimental design with two factors and five levels (13 assays), <sup>2</sup> AoxA= Antioxidant activity [ $\mu$ mol Trolox equivalents (TE) / 100 g sample, dw], <sup>3</sup> TPC= Total phenolic compounds [mg Gallic acid equivalents (GAE) / 100 g sample, dw], <sup>4</sup> PC= Protein content (% dw).

### Regression Analysis

Least square multiple regression methodology of the RSM was applied to investigate the relationship between the independent [GT ( $X_1$ ) and Gt ( $X_2$ )] and dependent (antioxidant activity, total phenolic and protein content) variables. To each response studied, a second-order polynomial equation was fitted using a multiple regression equation and the experimental data of antioxidant activity, total phenolic content, and protein content of the produced GCF (13 different flours). The prediction models obtained were used to represent the system graphically. Response surface plots were obtained for each response variable showing the effect of process variables on the response variables (Yolmeh and Jafari, 2017).

### Optimization of germination process

To obtain the optimum conditions of the germination process [best combination of GT ( $X_1$ ) and Gt ( $X_2$ )] we applied the desirability numerical method of the RSM (Milán-Carrillo *et al.*, 2012). The statistical software Design-Expert version 7.0 (Stat-Ease, Minneapolis, MN, USA) was used for the RSM analyses.

### Extraction of free and bound phenolic compounds

Extraction of free and bound phenolic compounds was realized according to Perales-Sánchez *et al.* (2014), using 80% chilled ethanol and ethyl acetate as solvents, respectively. All extractions were made in triplicate.

### Antioxidant activity and total phenolic content

Antioxidant activity for free and bound phenolic extracts was determined using the ORAC (oxygen radical absorbance capacity) and total phenolic content (Folin-Ciocalteu reagent) assays, according to the methodology reported by Perales-Sánchez *et al.* (2014), using a Microplate Reader (Synergy™ HT Multi-Detection, BioTek, Inc., Winooski, VT, USA). Extracts were assessed against a standard of Trolox and gallic acid, respectively. The results of antioxidant activity were expressed as  $\mu$ mol of Trolox equivalents (TE) / 100 g of sample dry weight (dw), and total phenolic content were expressed as mg of gallic acid equivalents (GAE) / 100g sample (dw). All measurements were made in triplicate.

### Chemical composition, soluble and insoluble dietary fiber (SDF/IDF)

The official AOAC (1999) methods 960.52, 920.39C, 925.09B, were used to determine protein (Nx6.25), lipids, and moisture contents, respectively. SDF and IDF were evaluated 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, 1999).

### Functional beverages preparation and sensory evaluation

Functional beverages 1 and 2 were developed from two flours mixtures: Mixture 1 (70% extruded amaranth flour



+ 30% germinated chia flour) and Mixture 2 (70% germinated amaranth flour + 30% germinated chia flour), respectively. Each flour mix (25 g) was added with 2.5 g of no calorie sweetener Stevia™, vanillin powder (1 g) and water (170 mL); the suspension was stirred with a domestic shaker (medium velocity), refrigerated (8–10 °C) and sensory evaluated for acceptability (A). The sensory evaluation was carried out by 80 non-trained panelists using a LAM (Labeled Affective Magnitude) scale (values from -100 to 100). The values of A were transformed to 0-100 scale (Cardello and Schutz, 2004).

### Nutritional properties: Essential amino acid, *in vitro* protein digestibility, chemical score and calculated protein efficiency ratio

The essential amino acid composition was determined according to López-Cervantes *et al.* (2006) 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. Tryptophan was detected at 280 nm with an ultraviolet detector. The *in vitro* protein digestibility was evaluated according to Hsu *et al.* (1977) using a multi-enzyme system. The chemical score (CS) was calculated as follows:  $CS = (\text{Content of the most limiting EAA}/\text{REAA}) \times 100$ ; where EAA is the essential amino acid and REAA is the recommended amino acid requirements for three-years old children and older, adolescents, and adults (FAO, 2013). Calculated protein efficiency ratio was calculated as described by Satterlee *et al.* (1982) and summarized by the AOAC (1999). This procedure is based on the *in vitro* protein digestibility and the essential amino acid composition of the optimized mixture. All determinations were made in triplicate.

### Antihypertensive potential ( $IC_{50}$ )

The ACE (angiotensin converting enzyme) inhibitory activity of free and bound phenolic extracts was determined by the Dojindo ACE Kit-WST test kit (Dojindo Laboratories, Kumamoto, Japan), which is based on the colorimetric detection of an indicator after a redox reaction with the 3-hydroxybutyric acid (3 HB), previously generated by enzymatic hydrolysis in a mixture containing ACE, aminoacylase enzyme, 3-hydroxybutyrate glycylglycylglycine (3HB-GGG) and the ACE-inhibitor (phenolic extracts). In the enzymatic reaction solution (ERS), the Gly-Gly dipeptide and 3-hydroxybutyl-Gly (3HB-G) are generated by hydrolysis with ACE from 3HB-GGG, and subsequently, the Gly peptide and 3HB were obtained by the action of an aminoacylase. Then, the 3 HB reacted with an indicator solution and the absorbance (Abs) at 450 nm was 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 by the following equation:

$$\text{ACE inhibitory activity (\%)} = \{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / (\text{Abs}_{\text{control}} - \text{Abs}_{\text{blank}})\} \times 100$$

Where:  $\text{Abs}_{\text{sample}}$  = Absorbance of ERS + indicator containing the ACE-inhibitor;  $\text{Abs}_{\text{control}}$  = Absorbance of ERS + indicator without ACE-inhibitor;  $\text{Abs}_{\text{blank}}$  = Absorbance of ERS without both indicator and ACE-inhibitor.

Different concentrations of the phenolic extracts (g/mL) were plotted versus the corresponding ACE inhibitory activity values (%), and the dose response curves were obtained by nonlinear sigmoid regression with Prism v5 (GraphPad Prism). The  $IC_{50}$  value was calculated as the concentration of phenolic extract that caused an inhibition of 50% in the ACE activity.

### Statistical analyses

The experimental results of chemical composition, nutritional properties, antioxidant activity, phenolic compounds content, and antihypertensive potential of extruded and germinated amaranth flours, germinated chia flour, unprocessed amaranth and chia flours, and beverages prepared from extruded and germinated amaranth flours, and germinated chia flour mixtures were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test comparison among means with 5 % of significance level (Statgraphics Plus ver. 6.0).

## RESULTS AND DISCUSSION

### Optimal conditions for chia seeds germination

### Prediction models for antioxidant activity, and total phenolic and protein contents of germinated chia flours

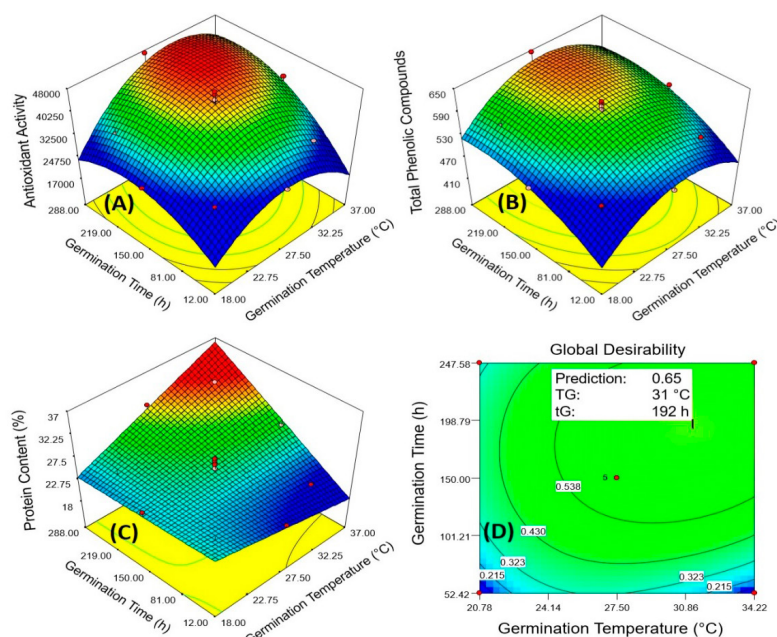
The experimental values for antioxidant activity, total phenolic content, and protein content varied from 28,700 to 47,000  $\mu\text{mol TE} / 100\text{g sample (dw)}$ , 498.5 to 644.8 mg GAE/ 100 g sample (dw), and 21.8 to 32.1 %, dw, respectively (Table 1). ANOVA showed that antioxidant activity and total phenolic content depended significantly ( $p < 0.05$ ) on linear and quadratic terms of GT ( $X_1$ ) and Gt ( $X_2$ ); while, antioxidant activity also depended on the interaction GT-Gt. Protein content depended significantly ( $p < 0.05$ ) on lineal terms of GT and Gt and the interaction between the same. The prediction models, using coded factors (coded process variables), for the response variables were:

$$Y_{\text{AOXA}} = 44,817.20 + 3,291.65(X_1) + 5,285.01(X_2) + 2,084(X_1)(X_2) - 5,766.35(X_1)^2 - 3,991.85(X_2)^2$$

$$Y_{\text{TPC}} = 606.26 + 16.25(X_1) + 39.52(X_2) - 38.66(X_1)^2 - 19.38(X_2)^2$$

$$Y_{\text{PC}} = 25.75 + 1.15(X_1) + 2.7(X_2) + 2.35(X_1)(X_2)$$

The regression models explained 95.13, 93.44 and 89.30 % of the total variability ( $p < 0.05$ ) in antioxidant activity, total phenolic and protein contents, respectively. The lack of fit was not significant ( $p > 0.05$ ); the relative dispersion of the experimental points from the predictions of the models (CV) was found to be  $< 10\%$ . In general, antioxidant activity of germinated chia flours increased with GT and Gt, until maximum reach values around 27–34°C and 160–288 h (Fig 1A); the total phenolic content of GCF showed the same behavior as the antioxidant activity, from GT and Gt to 27–31 °C and 219–288 h, respectively, the total phenolic content increased (Fig 1B). The protein content showed maximum values at highest GT (30–37°C) and Gt (220–288 h) (Fig 1C). However, the factor that most affected these studied variables of response



**Figure 1.** Response surface plots showing the effect of germination temperature and time on: (A) Antioxidant activity, (B) Total phenolic compounds, and (C) Protein content. (D): Global desirability plot showing the region with the best combination of germination process variables for producing germinated chia flour (GCF).

**Figura 1.** Gráficas de superficie de respuesta muestran el efecto de la temperatura y el tiempo de germinación sobre: (A) actividad antioxidante, (B) compuestos fenólicos totales y (C) contenido de proteína. (D): Gráfico de deseabilidad global que muestra la región con la mejor combinación de variables de proceso de germinación para producir harina de chía germinada (HCG).

was the Gt, where the highest values of them were located at the highest times of germination.

Germination is a biological process, where a multitude of chemical changes occurs to mobilize stored carbohydrates and proteins into the growing sprout and simple carbohydrates, free amino acids and essential nutrients in available form can be used readily by the seeds in germination (Fernandez-Orozco *et al.*, 2006). During this process, some of the seeds reserve materials are degraded and used partly for respiration and synthesis of new cell constituents of the developing embryo during germination; therefore, this process causes important changes in the biochemical and nutritional characteristics of seeds. Fats and carbohydrates, which often are at high levels in the seeds, are broken down to supply the energy requirements during the germination, by the increased metabolic activity that occurs during this process (Vidal-Valverde *et al.*, 2002). Perales-Sánchez *et al.* (2014) and Servín de la Mora-López *et al.* (2018) reported that the consumption of these chemical components during germination causes losses in dry weight of sprouting seeds and seedling in development, which results in an increase in protein content; they found that this protein content increase was continuous across all of the germination time, similar to the behavior found in the present research study (Fig 1C). On the other hand, secondary compounds as such

phenolic compounds, which present high antioxidant activity, increase dramatically during germination (Vidal-Valverde *et al.*, 2002). Biosynthesis of phenolic compounds, as a response to oxidative stress, occurs during germination since the metabolic activity of the plant cell is restarted (Servín de la Mora-López *et al.*, 2018). Perales-Sánchez *et al.* (2014) and Servín de la Mora-López *et al.* (2018) found that this increase in phenolic compounds content and antioxidant activity was also continuous as germination time increased, similar to the behavior found in the present research work (Figs 1A and 1B).

### Optimization of the chia seeds germination

Figure 1D shows the global desirability (D) as a function of the germination process variables GT and Gt. D was obtained as the geometric average of the individual desirabilities of each one of the response variables, and it was utilized to determine the best combination of process variables (optimum conditions) for production of germinated chia flour with high values of antioxidant activity, total phenolics, and protein contents. The optimum conditions of germination were GT= 31°C and Gt= 192 h, showing a global desirability of D=0.65 (Fig. 1D); the individual desirabilities of the response variables associated with this optimum global desirability were  $d_{AoxA} = 0.9946$ ,  $d_{TPC} = 0.8089$ , and  $d_{PC} = 0.6177$ . Using the GT/Gt germination optimized condition, the soft-

were predicted values of 46,901  $\mu\text{mol}$  Trolox equivalents (TE) / 100g sample (dw), 616.6 mg gallic acid equivalents (GAE) / 100g sample (dw), and 28.1 % for antioxidant activity, total phenolics, and protein contents, respectively. Germinated chia flour was produced applying the best combination of germination variables. The experimental values of antioxidant activity, total phenolic content (Table 3), and protein content (Table 2) of germinated chia flour were similar to the predicted values, above mentioned, indicating that the optimal conditions of germination were appropriated and reproducible.

### Effect of extrusion and germination processes on chemical composition and nutritional properties of amaranth and chia

#### Chemical composition

Table 2 shows the proteins, lipids and dietary fiber content of unprocessed amaranth and chia flours, as well as extruded and germinated amaranth flours, and germinated

chia flours. The major changes in these chemical components were observed in the flours obtained by the germination process. The protein content had a significant ( $p < 0.05$ ) increase during the production of germinated amaranth and chia flours (28.8 and 28.5 %, respectively), while, the lipid content decreased ( $p < 0.05$ ) between 28.5-57.4 % in germinated flours with respect to raw flours. Several authors have reported that these changes are mainly due to the consumption and degradation of macro-components as carbohydrates that are modified to simple sugars during respiration due to production of carbon dioxide and water, or lipids that are used as a source of energy during germination (Perales-Sánchez *et al.*, 2014; Gómez-Favela *et al.*, 2017). Servín de la Mora *et al.* (2018) reported that the energy requirements during the germination of seeds with high lipid content (oilseeds) could have been provided mainly by lipids instead of carbohydrates, similar to what occurred in the present work with chia seeds. The utilization of lipids as energy sources in this type of seeds is, basically, to initiate the germination process

**Table 2.** Chemical composition and nutritional properties of amaranth and chia flours unprocessed and processed by germination and extrusion.

**Tabla 2.** Composición química y propiedades nutricionales de harinas de amaranto y chía sin procesar y procesadas por germinación y extrusión.

Property	Unprocessed Amaranth	OEAF <sup>1</sup>	OGAF <sup>1</sup>	Unprocessed Chia	OGCF <sup>1</sup>	FAO <sup>2</sup>
Chemical composition(%dw)						
Protein	14.22±0.4 <sup>C</sup>	14.15±0.39 <sup>C</sup>	18.31±0.6 <sup>B</sup>	19.28±0.5 <sup>B</sup>	24.78±0.74 <sup>A</sup>	---
Lipids	7.50±0.05 <sup>C</sup>	6.24±0.09 <sup>D</sup>	5.36±0.10 <sup>E</sup>	31.85±0.3 <sup>A</sup>	13.57±0.59 <sup>B</sup>	---
Dietary fiber						---
Soluble	4.69±0.12 <sup>A</sup>	3.20±0.09 <sup>D</sup>	3.55±0.08 <sup>C</sup>	4.03±0.21 <sup>B</sup>	3.54±0.12 <sup>C</sup>	---
Insoluble	9.74±0.11 <sup>D</sup>	9.40±0.10 <sup>E</sup>	20.31±0.2 <sup>C</sup>	38.12±0.13 <sup>B</sup>	41.67±0.08 <sup>A</sup>	---
Total	14.43±0.11 <sup>D</sup>	12.60±0.09 <sup>E</sup>	23.86±0.1 <sup>C</sup>	42.15±0.15 <sup>B</sup>	45.21±0.22 <sup>A</sup>	---
Nutritional						
EAA <sup>3</sup> (g/100 g protein)						
His	1.96±0.04 <sup>D</sup>	1.85±0.05 <sup>E</sup>	2.62±0.05 <sup>B</sup>	2.06±0.02 <sup>C</sup>	4.07±0.04 <sup>A</sup>	1.60
Ile	3.81±0.05 <sup>BC</sup>	3.52±0.06 <sup>C</sup>	4.40±0.05 <sup>B</sup>	3.31±0.03 <sup>C</sup>	6.39±0.05 <sup>A</sup>	3.00
Leu	6.96±0.04 <sup>B</sup>	6.75±0.05 <sup>C</sup>	6.98±0.06 <sup>B</sup>	5.42±0.04 <sup>D</sup>	7.61±0.05 <sup>A</sup>	6.10
Lys	7.39±0.06 <sup>A</sup>	6.56±0.04 <sup>B</sup>	7.47±0.03 <sup>A</sup>	3.80±0.03 <sup>D</sup>	4.81±0.06 <sup>C</sup>	4.80
Met+Cys	5.28±0.02 <sup>C</sup>	5.08±0.03 <sup>D</sup>	6.54±0.04 <sup>A</sup>	2.95±0.04 <sup>E</sup>	5.73±0.04 <sup>B</sup>	2.30
Phe+Tyr	9.94±0.06 <sup>A</sup>	7.44±0.05 <sup>C</sup>	9.03±0.05 <sup>B</sup>	4.51±0.04 <sup>E</sup>	7.22±0.06 <sup>D</sup>	4.10
Thr	4.55±0.03 <sup>B</sup>	4.37±0.04 <sup>C</sup>	4.91±0.03 <sup>A</sup>	2.39±0.03 <sup>E</sup>	3.12±0.04 <sup>D</sup>	2.50
Trp	0.59±0.02 <sup>D</sup>	0.58±0.03 <sup>D</sup>	1.13±0.03 <sup>C</sup>	1.77±0.01 <sup>B</sup>	1.96±0.03 <sup>A</sup>	0.66
Val	5.49±0.04 <sup>C</sup>	5.22±0.04 <sup>D</sup>	5.95±0.05 <sup>B</sup>	3.83±0.02 <sup>E</sup>	6.51±0.06 <sup>A</sup>	4.00
Total	45.97	41.37	49.03	30.04	47.42	29.06
Chemical score	89	88	100	79	100	---
Limitant EAA <sup>3</sup>	Trp	Trp	None	Lys	None	---
IVPD <sup>4</sup> (%)	76.7±1.7 <sup>C</sup>	81.8±0.20 <sup>B</sup>	85.7±1.6 <sup>A</sup>	79.1±1.5 <sup>C</sup>	82.9±1.2 <sup>B</sup>	---
C-PER <sup>5</sup>	1.72	1.83	2.70	1.54	2.30	---

<sup>A-E</sup> Means with different superscript letters in the same row are significantly different (Duncan,  $p < 0.05$ ).

<sup>1</sup> OEAF = Optimized extruded amaranth flour, OGAF=Optimized germinated amaranth flour, OGCF= Optimized germinated chia flour; <sup>2</sup> Essentials amino acids requirements for child, adolescent, and adults (3 years and older) according to FAO (2013); <sup>3</sup> EAA = Essential amino acid, <sup>4</sup> IVPD = *in vitro* protein digestibility; <sup>5</sup> C-PER = Calculated protein efficiency ratio.

**Table 3.** Antioxidant activity, phenolic compounds and antihypertensive potential of amaranth and chia flours unprocessed and processed by germination and extrusion.**Tabla 3.** Actividad antioxidante, compuestos fenólicos y potencial antihipertensivo de harinas de amaranto y chíá sin procesar y procesadas por germinación y extrusión.

Property	Unprocessed Amaranth	EAF <sup>1</sup>	GAF <sup>1</sup>	Unprocessed Chia	GCF <sup>1</sup>
Antioxidant activity <sup>2</sup>					
Free phytochemicals	2,134±96 <sup>D</sup>	1,675±73 <sup>D</sup>	11,089±187 <sup>B</sup>	10,045±545 <sup>C</sup>	24,767±804 <sup>A</sup>
Bound phytochemicals	2,768±111 <sup>E</sup>	3,765±125 <sup>D</sup>	10,132±21 <sup>C</sup>	17,561±734 <sup>B</sup>	21,938±766 <sup>A</sup>
Total	4,902±10 <sup>D</sup>	5,440±159 <sup>D</sup>	21,221±69 <sup>C</sup>	27,606±811 <sup>B</sup>	46,704±920 <sup>A</sup>
Phenolic compounds <sup>3</sup>					
Free	11.94±0.5 <sup>D</sup>	7.8±1.3 <sup>D</sup>	146.0±5.3 <sup>C</sup>	290.8±10.3 <sup>B</sup>	392±11.7 <sup>A</sup>
Bound	16.11±0.4 <sup>E</sup>	1.5±1.5 <sup>D</sup>	101.6±6.2 <sup>C</sup>	254.4±12.2 <sup>B</sup>	291±10.6 <sup>A</sup>
Total	28.05±0.9 <sup>E</sup>	69.3±3.3 <sup>D</sup>	247.6±4.9 <sup>C</sup>	545.2±9.3 <sup>B</sup>	683±14.3 <sup>A</sup>
Antihypertensive potential (IC <sub>50</sub> ) <sup>4</sup>	ND	0.42	0.49	0.45	0.33

<sup>A-C</sup> Means with different superscript letters in the same row are significantly different (Duncan,  $p < 0.05$ ), <sup>1</sup> EAF = Extruded amaranth flour, GAF= Germinated amaranth flour, GCF= Germinated chia flour; <sup>2</sup> μmol Trolox equivalents (TE) / 100 g sample, dw; <sup>3</sup> mg Gallic acid equivalents (GAE)/ 100 g sample, dw; <sup>4</sup> mg extract/ mL.

or synthesis of certain structural constituents in the young seedling. This metabolic process is initiated by lipases, which catalyze the hydrolysis of triacylglycerols (storage lipids) to release glycerols and free fatty acids, some of which are oxidized into acetyl-CoA, and then transformed into simple carbohydrates that are transferred to the embryo as saccharose. Also, a part of fatty acids and glycerol produced by lipases, are metabolized in glyoxysomes and other organelles. It is well known that germinating seedlings of many oilseeds are a rich source of lipases (triacylglycerol acyl hydrolases) (Dawood *et al.*, 2013). Another important change occur on the total dietary fiber content, which increased ( $p < 0.05$ ) to 65 % during this process by biosynthesis of cell wall components (cellulose, hemicellulose and pectic polysaccharides) (Perales-Sánchez *et al.*, 2014; Gómez-Favela *et al.*, 2017). These results coincide with other researchers who reported a similar behavior during the germination of pseudo-cereals (Perales-Sánchez *et al.*, 2014), and legumes (Salas-López *et al.*, 2018). The extrusion process caused slight changes on chemical composition of amaranth during production of extruded amaranth flours. Milán-Carrillo *et al.* (2012) reported similar results.

### Nutritional properties

Table 2 shows the essential amino acid content of unprocessed and processed flours. In general, the essential amino acid content of unprocessed amaranth and chia flours were higher than those suggested by FAO (2013) for the essential amino acids requirement for children (3 years and older), adolescents and adults; Trp was the only one presented as limiting amino acid in unprocessed amaranth flour, and Leu, Lys, Thr and Val in unprocessed chia flour. This tendency is similar to literature results of essential amino acid content in amaranth (Amare *et al.*, 2015) and chia (Orona-Tamayo and

Paredes-López, 2017b) seeds. Essential amino acids content of amaranth and chia significantly increased ( $p < 0.05$ ) between 6.7–57.9 % after germination bioprocess; germinated amaranth and chia flours did not show limiting amino acid, while the extrusion process caused a slight decrease ( $p < 0.05$ ) in the essential amino acid content of amaranth, and Trp was the limiting amino acid in extruded amaranth flour.

The germination bioprocess increased ( $p < 0.05$ ) 4.8–11.7 % the *in vitro* protein digestibility and 49–57 % the calculated protein efficiency ratio of chia and amaranth, respectively. There are reports indicating that the improvement of *in vitro* protein digestibility during the germination process of seeds could be attributed to the removal/reduction of anti-nutritional factors as such phytic acid, tannins, and enzymatic inhibitors. During germination, there is hydrolysis of compounds (phytates) that contain organic phosphorus for the release of inorganic phosphates, which the plant uses to grow; breakdown of phytate during germination is attributed to the significant increase of the endogenous phytase enzyme activity. The reduction of tannin content in the sprouted seed has been well reported; this may be attributed to the loss of tannins by leaching them into the soaking water during germination process. Also, during germination there is a reduction of the enzymatic inhibitors activity, which causes an increase in the action of proteolytic enzymes as trypsin and chymotrypsin (Elizalde *et al.*, 2009). In the case of the extrusion process, the *in vitro* protein digestibility of amaranth also increased due to the destruction of anti-nutritional factors (tannins, saponins, trypsin and chymotrypsin inhibitors) and protein denaturation, as a result of the applied conditions (cutting forces, temperature, and humidity) during the processing (Elizalde *et al.*, 2009; Montoya-Rodríguez *et al.*, 2015).



## Effect of extrusion and germination processes on antioxidant activity and total phenolic compounds of amaranth and chia

### Antioxidant activity

The application of the extrusion and germination processes to produce extruded and germinated amaranth flours, and germinated chia flour, increased ( $p < 0.05$ ) the total antioxidant activity (sum of antioxidant activity of free and bound phenolic compounds extracts) by 11, 332 and 70 %, respectively, when compared to unprocessed seeds (Table 3). The antioxidant activity in free and bound phenolic compounds extracts of amaranth and chia increased ( $P < 0.05$ ) by 419-146 % and 266-24.92 %, respectively, after germination process. These results are in agreement with those reported by other researchers (Perales-Sánchez *et al.*, 2014; Salas-López *et al.*, 2018) who observed that the germination of amaranth (pseudo-cereal) and tepari bean (legume) seeds increased antioxidant activity when compared to unprocessed grains, which improves its functionality and therapeutic potential. These increases could be due to the release and biosynthesis of phenolic compounds by the action of the endogenous hydrolytic enzymes (Servín de la Mora-López *et al.*, 2018).

The antioxidant activity of extruded amaranth flour, evaluated in bound and total phenolic using the ORAC assay, increased by 36 %, when compared with unprocessed amaranth flour (10.9 %). This antioxidant activity increase could be a result of the release of antioxidant phenolic compounds during extrusion process. Prevention of oxidation by 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, such as amaranth (Milán-Carrillo *et al.*, 2012; Espinoza-Moreno *et al.*, 2016).

### Total phenolic compounds

The germination of amaranth and chia seeds increased ( $p < 0.05$ ) the contents of free, bound and total phenolic compounds by 35-112 %, 14-530 % and 25-782 %, respectively (Table 3). The metabolic activity of the plant cell during seeds germination is restarted, biosynthesizing secondary metabolites as a response to oxidative stress. Reactive oxygen species (ROS) are produced by seeds in germination, and they are neutralized by action of phenolic compounds, which also are synthesized during germination, since ROS need to be maintained below toxic concentration for the plant (Servín de la Mora-López *et al.*, 2018). During this process, cell wall-degrading enzymes (mainly esterases) are activated, and modify the cell wall structure of the seed, releasing phenolic compounds such as hydroxycinnamates (e.g., ferulic and p-coumaric acids) that are linked by bonds of ester and ether to the polysaccharides in the cell walls of the seeds. In addition, during germination of the seeds, the biosynthesis of phenolic compounds is favored by the activation of the phenylalanine ammonia lyase (key enzyme in phenolic biosynthesis) (Duodu, 2014).

Also, total phenolic content increased significantly (147 %) during the preparation of extruded amaranth flour by extrusion; being the bound phenolic compounds the main fraction responsible of the improvement on total phenolic content (Table 3). This increase in phenolic compounds content by extrusion processing has been widely reported in literature, and the mechanism by which occurs this phenomenon was above explained in the section for antioxidant activity (Milán-Carrillo *et al.*, 2012; Espinoza-Moggreno *et al.*, 2016).

### Effect of extrusion of phenolic compounds extract from amaranth and chia flours

The antihypertensive potential was defined as  $IC_{50}$  [concentration (mg of extract/mL) required to produce an inhibition of 50 % of the activity of ACE]. The phenolic compounds extracted from extruded and germinated amaranth flours, and germinated chia flour had better antihypertensive potential (lower  $IC_{50}$  value) than the phenolic compounds extracted from unprocessed amaranth and chia flours [0.33-0.49 vs 0.45-ND (not detected) mg extract/mL, respectively] (Table 3). The values 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 improvement of  $IC_{50}$  value during the extrusion and germination processes of amaranth and chia seeds could have occurred by release and/or formation of bioactive compounds (phenolic compounds and Maillard reaction products) with antihypertensive potential. As above was mentioned, in this research work an increase in the content of phenolic compounds occurred during processing of amaranth and chia by extrusion and germination (Table 3). Recently, natural sources of ACE inhibitors such as sprouts and seedlings obtained from corn, buckwheat, rice, and legumes have been reported (Randhir *et al.*, 2007; Masarretto *et al.*, 2011). It has been reported that phenolic compounds such as phenolic acids, flavonoids, tannins and stilbenes, inhibit the *in vitro* ACE activity. The degree of inhibition of the ACE activity depend on the absorption and metabolism of these compounds, and its action mode are closely related with the class (subclass) and the structure of the phenolic compound that is employed (Masarretto *et al.*, 2011; Al-Shukor *et al.*, 2013). According to the results of this research, it could be said that the phenolic compounds present in the amaranth and chia flours, obtained by extrusion and germination processes, could be used as functional food supplements or natural medicines for the treatment of hypertension.

### Formulation, nutrimental composition, energy content, and sensory properties of functional beverages

#### Formulation

Functional beverages 1 and 2 were developed from two flours mixtures: Mixture 1 (70% extruded amaranth flour + 30% germinated chia flour) and Mixture 2 (70% germinated amaranth flour + 30% germinated chia flour), respectively. The development of the functional beverages was based on

the preparation of traditional drinks consumed in Mexico that are made from grains (e.g. maize, rice, barley). Sensory evaluations were performed to define the quantities of the ingredients. The standard Mexican Norm NMX-F-439-1983 for food and non-alcoholic beverages define a beverage as nutritional when it contains at least 1.5 % protein or protein hydrolysates with a quality equivalent to that of casein; drinks must contain between 10 and 25 % main ingredient. These may contain about 2% ethanol, sweeteners, flavoring agents, carbon dioxide, juices, fruit pulp, vegetables or legumes and other additives authorized by the Ministry of Health (Secretaría de Salud, México). In both cases, the formulation developed originated beverages containing 12.5 % of the primary ingredient (Mixture 1 or Mixture 2) and a protein content > 1.5 % with a high quality, due to the essential amino acids content of them were higher than those the pattern suggested by FAO (2013), which is based in the amino acids content of casein. To sweeten beverages 1-2, non-caloric vegetable sweetener (Stevia™) was used to comply with the recommendation of the Ministry of Health: "A portion of 200 mL of the beverage (food) should not contain more than 140 kcal".

### Nutritional composition and energy content

A 200 mL portion of functional beverages (Beverages 1-2) prepared with 25 g of Mixture 1 or Mixture 2, contains 3.90 y 4.53 g of good quality protein, 1.90 y 1.76 g lipids, 15.86 y 15.25 g carbohydrates, 5.04 y 6.81 g total dietary fiber, and 96 y 95 kcal, respectively. The presence of dietary fiber in food has significant repercussions on the health of consumers because it is considered a functional ingredient for combating obesity and reducing the incidence of colon cancer (Prado-Silva *et al.*, 2014).

### Sensory properties

Panelists assessed with an average value of 83-85 the acceptability of the functional beverages (corresponding to a level of satisfaction between "I like it very much" and "I like it extremely"). It is to be expected that this high acceptability of the functional beverages will allow an adequate consumption of them, and thus they can provide benefits to the health of consumers.

### Nutritional properties of functional beverages

The essential amino acid content in the functional beverage was higher than those of the suggested pattern of essential amino acid requirements for three years old children and older, adolescents and adults (FAO, 2013). This portion of beverage covers between 34.8–30.0 % and 20.5–23.8 % of the daily proteins requirements for children of 1-3 and 4-8 years old, respectively. Its chemical score was 100 and it did not present limiting essential amino acid. The functional beverages had *in vitro* protein digestibility of 80.9–83.9 % and calculated protein efficiency ratio of 2.52-2.69 (Table 4). The excellent nutritional properties of the functional beverages 1 and 2 are essentially due to the improvement in protein quality of the amaranth and chia grains during the processing

**Table 4.** Nutritional and nutraceutical properties and sensory acceptability functional beverages of mixtures of amaranth and chia flours processed by germination and extrusion.

**Tabla 4.** Propiedades nutricionales y nutracéuticas y aceptabilidad sensorial de bebidas funcionales de mezclas de harinas de amaranto y chía procesadas por germinación y extrusión.

Property	Functional beverage 1 <sup>1</sup>	Functional beverage 2 <sup>2</sup>	EAA Requirements (3 years and older)
Nutritional			
EAA <sup>3</sup> (g/100 g protein)			
His	2.52 ± 0.05 <sup>B</sup>	3.06 ± 0.05 <sup>A</sup>	1.60
Ile	4.38 ± 0.06 <sup>B</sup>	4.76 ± 0.04 <sup>A</sup>	3.00
Leu	7.01 ± 0.05 <sup>B</sup>	7.17 ± 0.06 <sup>A</sup>	6.10
Lys	6.04 ± 0.05 <sup>B</sup>	6.67 ± 0.04 <sup>A</sup>	4.80
Met+Cys	5.28 ± 0.03 <sup>B</sup>	6.30 ± 0.04 <sup>A</sup>	2.30
Phe+Tyr	7.37 ± 0.05 <sup>B</sup>	8.49 ± 0.05 <sup>A</sup>	4.10
Thr	4.00 ± 0.04 <sup>B</sup>	4.37 ± 0.03 <sup>A</sup>	2.50
Trp	0.99 ± 0.03 <sup>B</sup>	1.38 ± 0.03 <sup>A</sup>	0.66
Val	5.61 ± 0.05 <sup>B</sup>	6.12 ± 0.05 <sup>A</sup>	4.00
Total	43.2	48.31	29.06
Chemical score	100	100	
Limitant EAA	none	none	
<i>In vitro</i> protein digestibility (%)	80.9 <sup>B</sup>	83.9 <sup>A</sup>	
C-PER <sup>4</sup>	2.52	2.69	---
Nutraceutical			
Antioxidant activity <sup>5</sup>	4,009 ± 87.0 <sup>B</sup>	6,495 ± 73.0 <sup>A</sup>	---
Antihypertensive potential (IC <sub>50</sub> ) <sup>6</sup>	0.43	0.47	
Sensory acceptability <sup>7</sup>	85	83	---

<sup>A-B</sup> Means with different superscript letters in the same row are significantly different (Duncan,  $p < 0.05$ )

<sup>1</sup> Functional beverage 1 = Elaborated with 70% extruded amaranth flour (EAF) + 30% germinated chia flour (GCF); <sup>2</sup> Functional beverage 2 = Elaborated with Mixture 70% germinated amaranth flour (GAF) + 30% germinated chia flour (GCF); <sup>3</sup> EAA = Essential amino acid; <sup>4</sup> C-PER = Calculated protein efficiency ratio; <sup>5</sup> μmol Trolox equivalents (TE)/ 200 mL of beverage; <sup>6</sup> mg extract/ mL; <sup>7</sup> Values between "I like it very much" and "I like it extremely" in 11 points hedonic scale.

(Table 2), as well as to the fact that the main ingredient with which they were prepared corresponded to mixtures of flours obtained from processed grains (Mixture 1=70 % extruded amaranth flour + 30 % germinated chia flour or Mixture 2= 70 % germinated amaranth flour + 30 % germinated chia flour) instead of the individual flours produced (extruded and germinated amaranth flours, and germinated chia flour).

There are few beverage options on the market that offer a high content of both good quality proteins and dietary fiber, and an energy content of less than 100 kcal per portion.

### Antioxidant activity and antihypertensive potential of functional beverages

The 200 mL portion of functional beverages 1 and 2 had a total hydrophilic antioxidant activity of 4,009 and 6,495  $\mu\text{mol TE}$ , respectively, which contributes between 80–217 % of the recommended daily consumption of antioxidants (3,000 to 5,000  $\mu\text{mol TE}$ ) (USDA, 2010). These functional beverages also had an antihypertensive potential, expressed as  $\text{IC}_{50}$  of 0.43-0.47 mg extract/mL (Table 4). This antihypertensive value ( $\text{IC}_{50}$ ) of the functional beverages is basically referred to extracts of phenolic compounds of the Mixture 1 and Mixture 2. There aren't reports in the literature of the ACE inhibitory activity ( $\text{IC}_{50}$ ) for other functional flours prepared from mixtures of grains processed by extrusion and germination.

### CONCLUSIONS

The best combination of process variables to produce optimized germinated chia flour was  $\text{GT}=31^{\circ}\text{C}/\text{Gt}=192$  h. The extrusion and germination processes are excellent technological strategies that increased the nutritional value, the antioxidant activity, the content of total phenolic compounds, and the antihypertensive potential of amaranth and chia seeds. The functional beverages prepared with mixtures of flours from amaranth and chia processed by extrusion and germination presented a high content of both good quality proteins and dietary fiber, with content lower than 100 kcal of energy and a high sensory acceptability. Also, the functional beverages presented a high antioxidant and antihypertensive potential. Therefore, these functional beverages with high nutritional value and nutraceutical potential can be used for health promotion and as an alternative to beverages with high caloric content and low nutritional value, which predominate in a market of consumers with significant trends or incidences of overweight/obesity and chronic degenerative diseases as hypertension.

### ACKNOWLEDGEMENT

This work was financially supported by Consejo Nacional de Ciencia y Tecnología (CONACYT), Ciencia Básica Convocatoria 2014 (No. 241090).

### REFERENCES

- Ademiluyi, A.O. and Oboh, G. 2013. Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) and hypertension (angiotensin I converting enzyme) in vitro. *Experimental and Toxicologic Pathology*, 65: 305-309.
- Al-Shukor, N., Van-Camp, J., Gonzales, G.B., Staljanssens, D., Struijs, K., Zotti, M.J., Raes, K. and Smagghe, G. 2013. Angiotensin-converting enzyme inhibitory effects by plant phenolic compounds: a study of structure activity relationships. *Journal of Agricultural and Food Chemistry*, 61: 11832–11839.
- Alvarez-Jubete, L., Arendt, E.K. and Gallagher, E. 2010. Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. *Trends in Food Science and Technology*, 21: 106-113.
- Amare, E., Mouquet-Rivier, Adrien Servent, A., Morel, G., Adish, A. and Haki, G.D. 2015. Protein Quality of Amaranth Grains Cultivated in Ethiopia as Affected by Popping and Fermentation. *Food and Nutrition Sciences*, 6: 38-48.
- AOAC. 1999. *Official Methods of Analysis* 16<sup>th</sup> ed. Harla, Association of Official Analytical Chemists, St. Paul, USA.
- Arenas-Carvajal, R., Pachón-Gómez, E., Méndez-Callejas, G. and Guzmán-Avendaño, A. 2009. Estudio del efecto inhibitorio de extractos de *Salvia scutellarioides* sobre la actividad de la enzima convertidora de angiotensina. *Universitas SCIENTIARUM*, 14: 141-150.
- Asao, M. and Watanabe, K. 2010. Functional and bioactive properties of quinoa and amaranth. *Food Science and Technology Research*, 16: 163-168.
- Barrera-Cruz, A., Rodríguez-González, A. and Molina-Ayalab, M.A. 2013. Escenario actual de la obesidad en México. *Revista Médica del Instituto Mexicano del Seguro Social*, 51:292-299.
- Cardello, A.V. and Schutz, H.G. 2004. Research note numerical scale-point locations for constructing the LAM (labeled affective magnitude) scale. *Journal of Sensory Studies*, 19: 341-346.
- Corbo, M.R., Bevilacqua, A., Petrucci, L., Casanova, F.P. and Sinigaglia, M. 2014. Functional beverages: The emerging side of functional foods. Commercial trends, research, and health implications. *Comprehensive Reviews in Food Science and Food Safety*, 13:1192-1206.
- Dawood, M., Sadak, M., Rayad, B.Y., El-Sayed, A.S.M. and El-Gayar, S.H. 2013. Changes in chemical composition during germination of some canola varieties Changes in oil content and fatty acid composition. *Scientia Agriculturae* 2:77-82.
- Duodu, K.G. 2014. Effects of processing on phenolic phytochemicals in cereals and legumes. *Cereal Foods World*, 59: 64-70.
- Elizalde, A.D., Porrilla, Y.P. and Chaparro, D.C.C. 2009. Antinutritional factors in eatable seeds. *Facultad de Ciencias Agropecuarias*, 7: 45-54.
- Espinoza-Moreno, R.J., Reyes-Moreno, C., Milán-Carrillo, J., López-Valenzuela J.A., Paredes-López, O. and Gutiérrez-Dorado, R. 2016. Healthy ready-to-eat expanded snack with high nutritional and antioxidant value produced from whole amarantin transgenic maize and black common bean. *Plant Foods for Human Nutrition*, 71: 218–224.
- FAO. 2005. *The State of Food Insecurity in the World 2004*. Rome: FAO.
- FAO. 2013. Findings and Recommendations of the 2011 FAO Expert Consultation on Protein Quality Evaluation in Human Nutrition. In: "Dietary Protein Quality Evaluation in Human Nutrition: Report of a FAO Expert Consultation." FAO Food and Nutrition Paper 92. Food and Agriculture Organization of the United Nations, Rome, Italy, chapter 4, pp. 29.
- Fernandez-Orozco, F.J., Zielinski, H., Muñoz, M., Piskula, M.K., Kozłowska, H. and Vidal-Valverde, C. 2009. Evaluation of bioprocess to improve the antioxidant properties of chickpeas. *Lwt-Food Science and Technology* 42:885-892.
- Gómez-Favela, M.A., Gutiérrez-Dorado, R., Cuevas-Rodríguez, E.O., Canizalez-Román, V.A., León-Sicairos, C.R., Milán-Carrillo, J. and Reyes-Moreno, C. 2017. Improvement of chia seeds with antioxidant activity, GABA, essential amino acids,

- and dietary fiber by controlled germination bioprocess. *Plant Foods for Human Nutrition*, 72:1-8.
- Hsu, H.W., Vavak, D.I., Saterlee, I.D. and Miller, G.A. 1977. A multienzyme technique for estimating protein digestibility. *Journal of Food Science*, 42: 1269-1273.
- INSP. 2013. Encuesta nacional de salud y nutrición 2012. Cuernavaca, México: Instituto Nacional de Salud Pública. Available in: encuestas.insp.mx.
- López-Cervantes, J., Sánchez-Machado, D.I. and Rosas-Rodríguez, J.A. 2006. Analysis of free amino acids in fermented shrimp waste by high-performance liquid chromatography. *Journal of Chromatography*, 1105:106-110.
- Maquilla, R.K. and Mishra, V.K. 2017. Effect of germination on antioxidant and ACE inhibitory activities of legumes. *LWT – Food Science and Technology*, 75:51-58.
- Martirosyan, D.M., Miroshnichenko, L.D., Kulakova, S.N., Pogojeva, A.V. and Zolodov, V.I. 2007. Amaranth oil application for cary heart disease and hypertension. *Lipids in Health and Disease*, 6:1-12.
- Massaretto, I.L., Márcio Fernando Madureira-Alves, M.F., Mussi de Mira, N.V., Carmona, A.K. and Lanfer-Marquez, U.M. 2011. Phenolic compounds in raw and cooked rice (*Oryza sativa* L.) and their inhibitory effect on the activity of angiotensin I-converting enzyme. *Journal of Cereal Science*, 54: 263-240.
- Mendonça, S., Saldiva, P.H., Cruz, R.J. and Arêas J.A.G. 2009. Amaranth protein presents cholesterol-lowering effect. *Food Chemistry*, 116: 738-742.
- Milán-Carrillo, J., Montoya-Rodríguez, A., Gutiérrez-Dorado, R., Perales-Sánchez, J.X.K. and Reyes-Moreno, C. 2012. Optimization of extrusion process for producing high antioxidant instant amaranth (*Amaranthus hypochondriacus* L.) flour using response surface methodology. *Applied Mathematics*, 3: 1516-1525.
- Milán-Carrillo, J., Gutiérrez-Dorado, R., Cuevas-Rodríguez, E.O., Sánchez-Magaña, L.M., Rochín-Medina, J.J. and Reyes-Moreno, C. 2017. Functional Beverage Produced with Bioprocessed Blue Corn and Black Beans with Antihypertensive and Antidiabetic Potential. *Revista Fitotecnica Mexicana*, 40: 451-459.
- Montoya-Rodríguez, A., Milán-Carrillo, J., Reyes-Moreno, C. and González de Mejía, E. 2015. Characterization of peptides found in unprocessed and extruded amaranth (*Amaranthus hypochondriacus*) pepsin/pancreatin hydrolysates. *International Journal of Molecular Sciences*, 16: 8536-8554.
- Mora-Escobedo, R., Robles-Ramírez, M.C., Ramón-Gallegos, E. and Reza-Alemán, R. 2009. Effect of Protein Hydrolysates from Germinated Soybean on Cancerous Cells of the Human Cervix: An In Vitro Study. *Plant Foods for Human Nutrition*, 64: 271-278.
- Orona-Tamayo, D. and Paredes-López, O. 2017a. Amaranth-Sustainable crop for the 21st century: food properties, and nutraceuticals for improving human health. In: *Sustainable Protein Sources* (Sudarshan Nadathur, Eds.), Pp 265-281. Elsevier.
- Orona-Tamayo, D. and Paredes-López, O. 2017b. Chia—The New Golden Seed for the 21st Century: Nutraceutical Properties and Technological Uses. In: *Sustainable Protein Sources* (Sudarshan Nadathur, Eds.), Pp 239-256. Elsevier.
- Pandal N. (2017). Nutraceuticals: global markets, Market Research Reports, bcc Research.
- Paško P., Sajewicz M., Gorinstein S. and Zachwieja Z. 2008. Analysis of selected phenolic acids and flavonoids in *Amaranthus cruentus* and *Chenopodium quinoa* seeds and sprouts by HPLC. *Acta Chromatographica*, 20: 661-672.
- Perales-Sánchez, J.X.K., Reyes-Moreno, C., Gómez-Favela, M., Milán-Carrillo, J., Cuevas-Rodríguez, E.O., Valdez-Ortiz, A. and Gutiérrez-Dorado, R. 2014. Increasing the antioxidant activity, and total phenolic and flavonoid contents by optimizing the germination conditions of amaranth seeds. *Plant Foods for Human Nutrition*, 69: 196-202.
- Prado-Silva, L., Azevedo, L., Oliveira, J.A.C., Moreira, A.P.M., Schmieles, M., Chang, Y.K., Paula, F.B.A. and Clerisi, M.T.P.S. 2014. Sesame and resistant starch reduce the colon carcinogenesis and oxidative stress in 1,2-dimethylhydrazine-induced cancer in Wistar rats. *Food Research International*, 6: 609-617.
- Randhir, R., Kwon, Y. and Shetty, K. 2007. Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. *Innovative Food Science and Emerging Technologies*, 9: 355-364.
- Salas-López, F., Gutiérrez-Dorado, R., Milán-Carrillo, J., Cuevas-Rodríguez, E.O., Canizalez-Román, V.A., León-Sicaños, C.R. and Reyes-Moreno, C. 2018. Nutritional and antioxidant potential of a desert underutilized legume – tepary bean (*Phaseolus acutifolius*). Optimization of germination bioprocess. *Food Science and Technology* (Campinas). In press
- Salazar-Vega, I.M., Segura-Campos, M.R., Chel-Guerrero, L.A. and Betancur-Ancona, D.A. 2012. Antihypertensive and antioxidant effects of functional foods containing chia (*Salvia hispanica*) protein hydrolysates. In: "Scientific, Health and Social Aspects of the Food Industry, (B.Valdez Ed.).
- Satterlee, L.D., Kendrick, J.G., Marshall, H.F., Jewell, D.K., Ali, R.A., Heckman, M.M., Steinke, H.F., Larson, P., Phillips, R.D., Sarwar, G. and Slump, P. 1982. In vitro assay for predicting protein efficiency ratio as measured by rat bioassay: Collaborative study. *Journal of the Association of Official Analytical Chemists*, 65: 798-809.
- Servín de la Mora-López, G., López-Cervantes, J., Gutiérrez-Dorado, R., Cuevas-Rodríguez, E.O., Milán-Carrillo, J., Sánchez-Machado, D.I. and Reyes-Moreno C. 2018. Effect of Optimal Germination Conditions on Antioxidant Activity, Phenolic Content and Fatty Acids and Amino Acids Profiles of Moringa Oleifera Seeds. *Revista Mexicana de Ingeniería Química*, 1-13.
- Ullah, R., Nadeem, M., Khalique, A., Imran, M., Mehmood, S., Javid, A. and Hussain, J. 2015. Nutritional and therapeutic perspectives of Chia (*Salvia hispanica* L.): a review. *Journal of Food Science and Technology*, 53: 1750-1758.
- USDA. 2010. Antioxidants and Health. ACES publications, pp. 4
- Vidal-Valverde, C., Frias, J., Sierra, I. Blazquez, I., Lambein, F. and Kuo, Y. 2002. New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. *European Food Research and Technology*, 215:472-477
- Yolmeh, M. and Jafari, S.M. 2017. Applications of response surface methodology in the food industry processes. *Food and Bioprocess Technology*, 10: 413-433.