Agrociencia

CHIA SEED GERMINATION AND EXTRUSION TO INCREASE NUTRITIONAL VALUE, PHENOLIC COMPOUNDS, GABA, ANTIOXIDANT ACTIVITY, AND *in vitro* ANTIHYPERTENSIVE POTENTIAL

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Citation: Madrigales-Reátiga LF, Castro-Montoya YA, Reyes-Moreno C, Gutiérrez-Dorado R, Salas-López F, Perales-Sánchez JXK. 2024. Chia seed germination and extrusion to increase nutritional value, phenolic compounds, GABA, antioxidant activity, and *in vitro* antihypertensive potential. **Agrociencia.** https://doi.org/ 10.47163/agrociencia.v58i4.3043

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: July 09, 2023.
Approved: May 16, 2024.
Published in Agrociencia: May 29, 2024.

ABSTRACT

A functional ingredient derived from extruded, germinated chia seeds would be an excellent substitute to improve the nutritional and nutraceutical quality of widely consumed foods in Mexico. The aim of this study was to create a functional ingredient with outstanding nutritional and nutraceutical value and microbiological safety by processing chia seeds using a combined germination and extrusion process under optimal conditions. The implementation of optimized germination and extrusion techniques was hypothesized to boost the nutritional value, phenolic compounds, γ-aminobutyric acid (GABA), antioxidant activity, *in vitro* antihypertensive potential, and microbiological stability of the functional flour obtained from chia seeds. Germinated chia flour (GCF) was produced under optimized germination conditions (21 °C for 157 h). To determine the optimal conditions for GCF extrusion, the response surface methodology was used. A central composite rotatable design was applied with two factors [extrusion temperature (ET, 50–160 °C) and screw velocity (SV, 50–240 rpm)] and five levels. Total phenolic compounds (TPC), antioxidant activity (AoxA), and *in vitro* protein digestibility (IVPD) were selected as response variables. The best combination of extrusion process variables to produce extruded germinated chia flour (EGCF) with maximum TPC, AoxA, and IVPD values was ET = 147 °C and SV = 170 rpm. EGCF had higher protein content (88 %), total dietary fiber (13 %), phenolic compounds (57 %), GABA (933 %), and antioxidant activity (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), 110 %; 2,2-diphenyl-1-picrylhydrazyl (DPPH), 114 %) than raw chia flour (RCF). Regarding *in vitro* antihypertensive potential, germinated-extruded chia flour presented the best IC_{50} value. The main findings of this research show that extrusion processing applied in combination with germination is a promising strategy for increasing the nutritional value, bioactive compound content, antioxidant and antihypertensive potential, and microbiological stability of functional flours obtained from pseudocereal seeds.

Keywords: Functional ingredient, optimization, nutraceutical, microbiological stability.

INTRODUCTION

Chia (*Salvia hispanica*) is a flowering herbaceous plant from the Lamiaceae family. Its seeds, which have a high nutritional and nutraceutical value, are considered functional foods. Chia seeds are a good source of proteins (15–24 %) and lipids (25–40 %). They also contain B vitamins and have a higher mineral content than cereals. In addition, due to their dietary fiber content (35 %), they have a positive effect on insulinemic and glycemic responses, reducing cholesterolemia and some chronic disorders (CD), such as cardiovascular diseases. Chia seeds contain bioactive compounds such as polyphenolic compounds (phenolic acids, flavonols, and isoflavones) that are associated with antioxidant properties and prevention against CD (Ghafoor *et al*., 2020; Hernández-Pérez *et al*., 2020).

Germination is an inexpensive and sustainable process suitable for the development of functional foods and ingredients. This process offers several advantages, including improved nutritional value (increased digestibility of nutrients) and increased content of bioactive compounds (γ-aminobutyric acid (GABA) and phenolic compounds) that improve antioxidant activity (Bermejo and Munné-Bosch, 2023). Chia sprouts have enormous potential in the agri-food industry as an ingredient in new food products. Their use is an effective strategy to improve food nutritional and nutraceutical value (Abdel-Aty *et al*., 2021). Germinated seeds are consumed fresh, without any process that eradicates pathogenic organisms that may proliferate due to processing conditions.

The ingestion of sprouts has been associated with the appearance of disease outbreaks transmitted by food consumption. Due to this microbiological problem, it is necessary to apply technologies that ensure microbiological quality and preserve the high content of bioactive compounds and nutraceutical properties generated during seed germination. Among the processing techniques that have this potential, extrusion stands out because, in addition to ensuring the microbiological stability of the sprouts, it improves the nutritional, physicochemical, and techno-functional properties, as well as the palatability of the foods (Paucar-Menacho *et al*., 2022). Extrusion also applies high temperatures, pressure, and shear force for a brief period of time and is extremely effective in reducing energy usage and water pollution.

It is increasingly evident that the adoption of a healthy diet is necessary to prevent the risk of diseases as well as to maintain environmental sustainability and food security. Germination-extrusion has been used as a combined process to produce functional foods, such as functional flours, with better nutritional, nutraceutical, and sensory properties, as well as higher microbiological quality by eliminating the microbial load of the sprouts when applying the extrusion process. The use of germinated-extruded flours for partial or total replacement of common cereals represents an opportunity for the development of new foods (Albarracín *et al*., 2019; Paucar-Menacho *et al*., 2022).

This study had the objective to develop a functional ingredient with high nutritional and nutraceutical value through the combination of germination and extrusion technologies in chia seeds. The working hypothesis was that the combination of procedures (germination and extrusion) used on chia seeds increases nutritional value, bioactive chemicals, antioxidant activity, and *in vitro* antihypertensive potential.

MATERIALS AND METHODS

Chia seed germination and extrusion

Chia seeds were purchased at the "Rafael Buelna" market in Culiacán, Sinaloa, Mexico. Chia germination was carried out according to Gómez-Favela *et al*. (2017). Seed lots weighing 200 g were distributed on sterilized trays and placed in germination chambers (germination temperature: 21 °C, germination time: 157 h). Light and dark periods (50/50 %) were used during the germination process. The sprouts were dried at 50 °C for 8 h) and ground (US 80 mesh) to obtain germinated chia flour (GCF).

To obtain extruded flour, GCF lots weighing 500 g were defatted using an oil press (lipids up to 8 % dry weight (DW)). Water was added to the defatted GCF until a humidity of 17 % was achieved (4–8 °C, 12 h). Extrusion was carried out in a Mod 20 DN single screw extruder (Brabender Instruments, Inc.; NJ, USA). The operating conditions of the extruder were: extrusion temperature (ET) = 50–160 °C and screw velocity (SV) = 50–240 rpm. The extrudates were cooled, ground (80 mesh), and packaged in plastic bags.

Response surface methodology (RSM)

RSM was used to find the best combination of extrusion process variables to obtain extruded germinated chia flour (EGCF) with maximum total phenolic compounds (TPC), antioxidant activity (AoxA), and *in vitro* protein digestibility (IVPD) values. A central composite rotatable experimental design was selected with two factors (ET: 50, 66.1, 105, 143.9, and 160 °C; SV: 50, 77.8, 14.5, 212.2, and 240 rpm) and five levels of variation. The regression procedure was applied, non-significant terms ($p > 0.1$) were eliminated, and a new polynomial was used to obtain a prediction model for each response variable. The conventional graphic method was applied as an optimization technique to obtain maximum TPC, AoxA, and IVPD values. The prediction models were used to graphically represent the system. The contour plots of each variable response were superimposed to obtain a contour plot for the observation and selection of the optimal combination of ET and SV to produce EGCF. For RSM analyses, Design Expert statistical software (Stat-Ease; Minneapolis, MN, USA) was used.

Free and bound phenolic compounds extraction

Free and bound phenolic compounds were extracted in accordance with Salas-López *et al*. (2018). One gram of dry sample was mixed with an ethanol-water solution (80–20 v/v) and agitated for 10 min. The supernatant was recovered by centrifugation (3000 xg, 10 min) (Sorvall RC5C, Sorvall Instruments, Dupont; Wilmington, DE, USA). The pellets from the extraction of free phenolic compounds were hydrolyzed (2 M NaOH) and neutralized (HCl). The final solution was extracted with ethyl acetate and evaporated. Extracts were reconstituted in 2 mL of 50 % methanol and stored at -20 °C. All extractions were performed in triplicate.

Chemical characterization

The chemical composition of chia flour was determined using the AOAC (2012) chemical methods. The TPC content of free and bound phenolic extracts was determined colorimetrically (Folin-Ciocalteu reagent) according to Servín de la Mora-López *et al*. (2018). AoxA was assessed using the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) (Salas-López *et al*., 2018) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays (Servín-de la Mora-López *et al*., 2018). The extracts were evaluated against gallic acid and Trolox standards, respectively. The TPC content results were expressed as mg of gallic acid equivalents (GAE) 100 g^{-1} (DW). The AoxA was expressed as μmol of Trolox equivalents (TE) 100 g^{-1} (DW). The γ-aminobutyric acid (GABA) content was determined using the methodology reported by Watchararparpaiboon *et al*. (2010) and expressed as mg of GABA 100 g^{-1} sample (DW). All measurements were performed in triplicate.

Nutritional properties

The essential amino acid composition, IVPD, and protein efficiency ratio were determined in accordance with Salas-López *et al*. (2018). The essential amino acid composition was determined using an analytical scale (4.6 × 250 mm) 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. IVPD was evaluated using a multienzyme system. The chemical score (CS) was calculated as follows:

$$CS = (\text{Content of the most limiting } EAA/REAAR) \times 100$$

where *EAA* is the essential amino acid, and *REAAR* is the recommended amino acid requirement for three-year old children and older, adolescents, and adults (FAO, 2013). The calculated protein efficiency ratio (C-PER) is based on the IVPD and the essential amino acid composition of the optimized mixture. All determinations were made in triplicate.

Antihypertensive potential (IC_{50})

Angiotensin-converting enzyme (ACE) inhibitory activity, recognized as antihypertensive potential (IC_{50}), was evaluated in free and bound phenolic extracts using the Dojindo ACE Kit-WST test kit (Dojindo Laboratories; Kumamoto, Japan). This method is based on the detection of a colorimetric indicator after a redox reaction at an absorbance of 450 nm measured with a microplate reader (Synergy™ HT Multi-Detection, BioTek, Inc.; Winooski, VT, USA). The ACE inhibitory activity of the phenolic extracts was calculated using the following equation:

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

where Abs_{450} extract is the absorbance of the reaction solution containing phenolic extract, Abs_{450} control is the absorbance of the reaction solution without phenolic extract, and Abs_{450} blank is the absorbance of the reaction solution with enzyme and without substrate and phenolic extract. IC_{50} 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).

Microbiological stability

The evaluation of the microbiological stability of raw chia flour (RCF), GCF, and EGCF required the preparation of a flour suspension in 1 % peptone water (10 g of flour in 90 mL peptone water) for each sample. The count of total coliforms was carried out according to NOM-113-SSA1-1994 (DOF, 1994c). Decimal dilutions up to 10^{-5} were prepared, inoculating 1 mL in violet-red bile glucose agar by the mass seeding method. The plates were incubated at 35 °C for 24 h. The count of aerobic mesophilic microorganisms was carried out according to NOM-092-SSA1-1994 (DOF, 1994a) using plate count agar (Biokar). Plates were inoculated with 1 ml of the corresponding dilutions and incubated at 35 °C for 48 h. The mold count was carried out according to NOM-111-SSA1-1994 (DOF, 1994b); decimal dilutions up to 10^{-3} were prepared, inoculating 1 mL in 10 mL of acidified potato dextrose agar (PDA) by the mass homogenization method. The plates were incubated for 3–4 d at 25 °C. The counts were performed in triplicate, and the results were expressed as colony-forming units per gram (CFU g^{-1}).

Statistical analysis

The experimental results of the chemical composition, physicochemical and functional properties, and microbiological stability of the flours were subjected to a one-way analysis of variance (ANOVA), followed by Tukey's multiple range test ($p \leq 0.05$). These analyses were conducted using Statgraphics Plus 6.0 software.

RESULTS AND DISCUSSION

Prediction models for response variables

The experimental values for total phenolic content (TPC), antioxidant activity (AoxA), and *in vitro* protein digestibility (IVPD) varied from 584 to 650 mg GAE 100 g^{-1} (DW), 9067 to 13 940 μmol TE 100 g^{-1} (DW), and 70 to 78.2 %, respectively. The prediction models included linear, quadratic, and interaction terms of ET and SV ($p \leq 0.05$) for each response variable. The prediction models for each of the response variables were:

$$TPC = 588.34 + 15.19\,(ET) + 9.49\,(SV) + 11.35\,(ET)\,(SV) + 14.53\,(ET)^2 + 13.40\,(SV)^2$$

$$AoxA = 9408.20 + 815.63\ (ET) + 1083.53\ (SV) + 367.50\ (ET)\ (SV) + 1257.65\ (ET)^2 + 1246.65\ (SV)^2$$

$$IVPD = 76.75 + 0.73\ (ET) - 2.15\ (ET)^2 - 2.82\ (SV)^2$$

The regression models explained 95.46, 96.96, and 85.97 % of the total variability ($p \leq 0.0001$) of TPC, AoxA, and IVPD, respectively. The lack of fit ($p > 0.05$) and the relative dispersion of the experimental points of the model predictions (CV) were found to be < 10 %. These values indicated that the experimental models were adequate and reproducible. The TPC values of EGCF increased with ET and SV until reaching values of 682.20 mg GAE 100 g^{-1} (DW) at 150 °C and 240 rpm (Figure 1A). In the same way, AoxA increased as ET and SV increased, obtaining a maximum value of 16 128 µmol

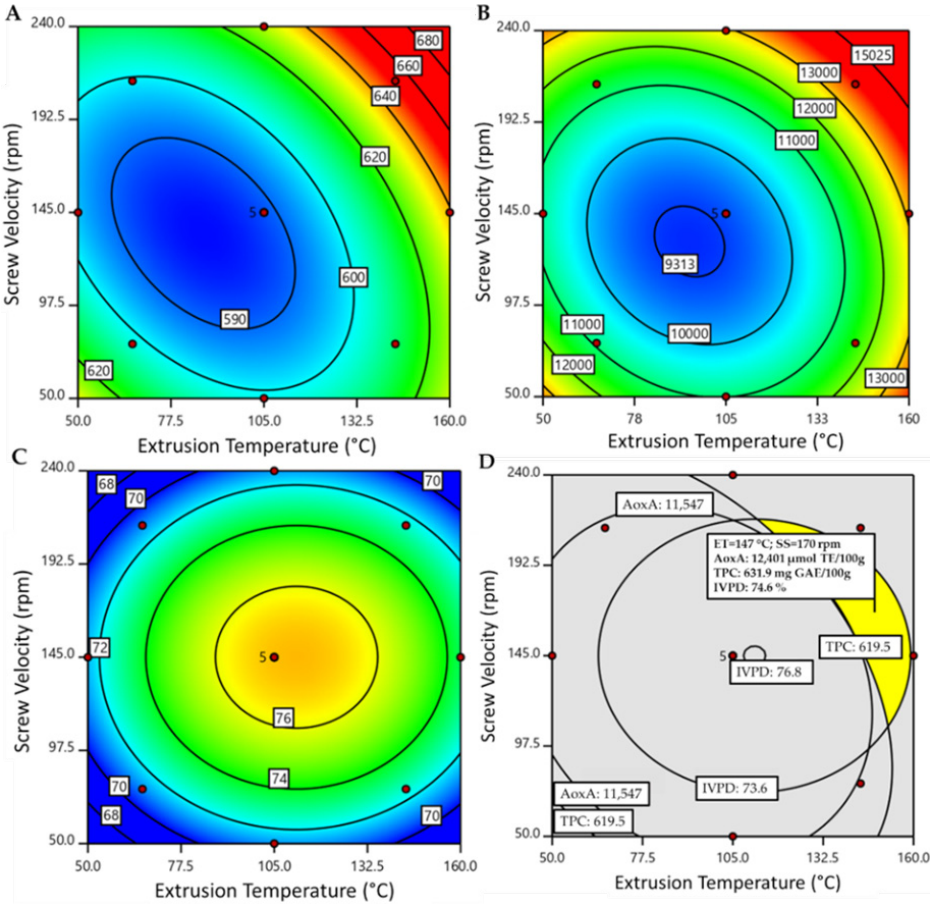

Figure 1. Contour plots showing the effect of the process variables (ET and SV). A: total phenolic compounds; B: antioxidant activity; C: *in vitro* protein digestibility; D: overlay graph showing the region with the best combination of process variables to obtain an optimized extruded germinated chia flour (EGCF).

TE 100 g^{-1} (DW) at 150 °C and 240 rpm (Figure 1B). In the case of IVPD, the highest value (78.2 %) was obtained at 145 °C and 105 rpm (Figure 1C).

AoxA and TPC decreased quadratically with ET and SV (Figures 1A and 1B). The reactions tended to reach a minimum stationary point, as evidenced by the positive sign of the quadratic impact of ET and SV (data not shown); the minimal values of this response are undesirable. The desired values of these responses (AoxA, TPC) were the highest, which were located in a range of 135–160 °C and 145–240 rpm for ET and SV, respectively. It was observed that IVPD increased quadratically with ET and SV. The negative sign of the quadratic effect of ET and SV (data not shown) indicated that the responses tended to reach a maximum stationary point. The maximum values of this response are desired, in a range of 100–120 °C and 120–160 rpm for ET and SV, respectively.

Process condition optimization

The TPC, AoxA, and IVPD values of EGCF are affected by ET and SV (Figures 1A, 1B, and 1C). The superposition of these graphs (Figure 1D) indicates the ideal combination of process variables to produce an EGCF with maximum values for TPC, AoxA, and IVPD. With this combination of process variables (ET = 147 °C and SV = 170 rpm), it is possible to predict the following values: TPC = 631.9 mg GAE 100 g^{-1} of sample (DW), AoxA = 12 401 μmol TE 100 g^{-1} of sample (DW), IVPD = 74.6 %. The experimental values of AoxA, TPC, and IVPD of EGCF were similar to the predicted values. This means that the optimal extrusion conditions were appropriate and reproducible.

Chemical composition and nutritional properties of chia flours

The chemical composition and nutritional properties of RCF, GCF, and EGCF (Table 1) show that germination under optimized conditions led to an increase ($p < 0.05$)

Table 1. Chemical composition of chia flours[†].

Property	Raw chia f lour (RCF)	Germinated chia flour (GCF)	Extruded germinated chia flour (EGCF)
Chemical composition (%, DW)			
Proteins	18.47 ± 0.15^{C}	22.78 ± 0.30^{B}	34.74 ± 0.45^{A}
Lipids	32.64 ± 0.23^{A}	18.48 ± 0.29^{B}	7.71 ± 0.11^{C}
Ashes	3.74 ± 0.12^{C}	5.87 ± 0.17^{B}	6.17 ± 0.08^{A}
Dietary fiber			
Soluble	3.99 ± 0.043^{C}	3.45 ± 0.01^{B}	4.78 ± 0.05^{A}
Insoluble	38.53 ± 0.47^{C}	40.51 ± 0.12^{B}	43.30 ± 0.71^{A}
Total	42.52 ± 0.43^{C}	43.96 ± 0.05^{B}	47.85 ± 0.33^{A}
Carbohydrates	2.82 ± 0.04^{C}	9.35 ± 0.18^{A}	3.6 ± 0.24^{B}

[†] Means with different letters per row are different (Tukey, $p \leq 0.05$). DW: dry weight. A, B, C mean values per column with different letters are statistically different ($p \leq 0.05$).

in protein content (23 %). The result coincides with reports from other researchers on different pseudocereals and legumes (Gómez-Favela *et al*., 2017; Salas-López *et al*., 2018). This is mainly attributed to the loss of nutrients, particularly carbohydrates, through the respiration process to produce CO_2 and water, as well as the activation of enzymes (α-amylase) that break down starch into simple sugars. Extrusion caused an increase in protein content (52 %); however, this is attributed to a concentration effect due to the partial defatting of the GCF before extrusion.

RCF and GCF showed lipid contents of 32.64 and 18.48 % (DW), respectively (Table 1). During germination, lipids are used as an energy source to perform various metabolic activities (synthesis of DNA, RNA, enzymes, and structural proteins) necessary for seedling development using nutrient reserves (starch and lipids). Gómez-Favela *et al.* (2017) and Salas-López *et al*. (2018) reported a decrease in lipid content during the germination of pseudocereals and legumes.

The total dietary fiber content (TDF) of raw and germinated chia flours was 42.52 and 43.96 % (DW), respectively (Table 1). During the germination process, the structure of the polysaccharides of the seed cell wall is modified, producing an increase in hemicellulose, cellulose, and polysaccharides. These results coincide with Salas-López *et al*. (2018). When applying the extrusion process to chia sprouts, the TDF content increased ($p \leq 0.05$), presenting a value of 47.85 %. This is due to the "nutrient concentration" effect of defatting the GCF as pre-extrusion conditioning and to the release of oligosaccharides due to the breaking of the glycosidic bonds of the polysaccharides by mechanical stress, as well as the formation of materials resistant to enzymatic degradation, such as starch and protein-polysaccharide complexes caused by heating.

The essential amino acid (EAA) content of RCF was higher than the standard of EAA recommended by FAO (2013) for children, adolescents, and adults, except for Leu and Lys (Table 2).

Sandoval-Oliveros and Paredes-López (2013) reported similar values in EAA content in chia seeds. The EAA content of GCF and EGCF was higher than that recommended by FAO (2013), with Lys acting as a limiting EAA in both processed seed flours. The extrusion process caused a slight decrease ($p < 0.05$) in the EAA content of the GCF; however, both flours (GCF and EGCF) presented a high chemical score (98.12, 96.88).

The IVPD of the RCF, GCF, and EGCF were 65.1, 72.9, and 83.2 %, respectively (Table 2). Ohanenye *et al*. (2022) reported on the increase in protein digestibility of legume seeds as a consequence of germination. The improvement of IVPD during germination may be due to the hydrolysis of compounds that contain organic phosphates and release inorganic phosphates, which are used for plant growth (Gamel *et al*., 2007). The extrusion increases protein digestibility and favors their denaturation, making them more accessible to the action of proteases. The protein digestibility values of the extruded products are higher than RCF and GCF; this is possible due to the shear cut and high temperatures, causing protein denaturation and the inactivation of anti-nutritional factors that hinder digestion (Salas-López *et al*., 2018).

Table 2. Protein characteristics of chia flours†.

Property	Raw chia flour (RCF)	Germinated chia flour (GCF)	Extruded germinated chia flour (EGCF)	FAO (2013)¶
His	2.14 ± 0.03^{B}	4.07 ± 0.02^{A}	4.05 ± 0.03^{A}	1.6
Ile	3.35 ± 0.03^{C}	6.59 ± 0.03^{A}	6.42 ± 0.04^{B}	3.0
Leu	5.41 ± 0.03^{C}	7.85 ± 0.02^{A}	7.78 ± 0.03^{B}	6.1
Lys	3.49 ± 0.02^{C}	4.71 ± 0.02^{A}	4.65 ± 0.03^{B}	4.8
Met+Cys	2.67 ± 0.02^{C}	5.91 ± 0.03^{A}	5.74 ± 0.04^{B}	2.3
Phe+Tyr	4.51 ± 0.03^{B}	7.28 ± 0.03^{A}	7.26 ± 0.03^{A}	4.1
Thr	2.65 ± 0.02^{B}	3.20 ± 0.02^{A}	3.18 ± 0.04^{A}	2.5
Trp	1.78 ± 0.02^{C}	2.17 ± 0.02^{A}	2.11 ± 0.03^{B}	0.66
Val	4.04 ± 0.02^{B}	6.92 ± 0.03^{A}	6.93 ± 0.03^{A}	4.0
Total	30.04	48.70	48.12	29.06
Chemical score	72.70	98.12	96.88	
Limited EAA	Lys	Lys	Lys	
IVPD	65.1 ± 1.50^{C}	72.9 ± 0.89^{B}	83.2 ± 0.77^{A}	
C-PER	1.10	2.03	2.27	

† Means with different letters per row are different (Tukey, $p \leq 0.05$). A, B, C mean values per column with different letters are statistically different ($p \leq 0.05$). ¶Amino acid requirements for children (three years and older), adolescents, and adults. EAA: essential amino acid; IVPD: *in vitro* protein digestibility; C-PER: calculated protein efficiency ratio.

The combined germination-extrusion processes, both under optimized conditions, increased ($p < 0.05$) the IVPD by 19.81 % (Table 2). El-Hady and Habiba (2003) reported an increase of 6.21 % in the IVPD of legumes (peas, chickpeas, and beans) processed sequentially by soaking and extrusion. Regarding C-PER, its content improved when applying the combined processes (germination and extrusion) due to the increase in IVPD, despite the slight decrease in EAA due to the extrusion process.

Phenolic compounds, gamma aminobutyric acid, and antioxidant properties

The content of free phenolic compounds in ungerminated chia seeds was 489 mg GAE 100 g^{-1} sample (DW) (Table 3). Germination increased ($p < 0.05$) the contents of free (44.24 %), bound (14.48 %), and total (32.92 %) phenolic compounds. Khang *et al*. (2016) reported that the concentration of phenolic compounds increased in soybeans and peanuts after germination; this could be due to the release and biosynthesis of phenolic compounds, such as hydroxycinnamates, that bind to non-starch polysaccharides in the cell walls of grains through ester and ether linkages. The action of cell wall-degrading enzymes (esterases) on these bonds contributes to the release of bound phenolic compounds (Perales-Sánchez *et al*., 2014).

On the other hand, the extrusion caused a 17.84 % increase in TPC levels. This is associated with the destruction of cell walls and the release of phenolic compounds,

Table 3. Content of phenolic compounds, γ-aminobutyric acid (GABA), and antioxidant activity of chia flours[†].

Property	Raw chia flour (RCF)	Germinated chia flour (GCF)	Extruded germinated chia flour (EGCF)
	Phenolic compounds[¶]		
Free	226 ± 3^{C}	326 ± 4^{B}	401 ± 6^{A}
Bound	283 ± 2^{B}	324 ± 2^{B}	365 ± 9^{A}
Total	489 ± 4^{C}	650 ± 3^{B}	766 ± 8^{A}
GABA[§]	13.08 ± 0.55^{C}	138.28 ± 0.82^{A}	135.16 ± 0.75^{B}
	Antioxidant activity[Þ]		
	ABTS		
Free phenolics	2515 ± 92^{C}	9475 ± 137^{B}	13 905 ± 198^{A}
Bound phenolics	8642 ± 167^{C}	12 747 ± 131^{A}	9568 ± 171^{B}
Total phenolics	11 157 ± 182^{C}	22 222 ± 122^{B}	23 473 ± 182^{A}
	DPPH		
Free phenolics	2370 ± 136^{C}	6620 ± 140^{B}	8825 ± 154^{A}
Bound phenolics	4738 ± 117^{C}	7095 ± 123^{A}	6440 ± 134^{B}
Total phenolics	7108 ± 72^{C}	13 716 ± 108^{B}	15 265 ± 121^{A}
	Antihypertensive potential (IC_{50})		
Ace inhibition	0.62 ± 0.03^{A}	0.45 ± 0.07^{B}	0.22 ± 0.03^{C}

[†] Means with different letters per row are different (Tukey, $p \leq 0.05$). A, B, C mean values per column with different letters are statistically different ($p \leq 0.05$). [¶]mg GAE 100 g^{-1} sample (DW). [§]mg γ aminobutyric acid 100 g^{-1} sample (DW). [Þ]μmol TE 100 g^{-1} sample (DW).

as well as the formation of Maillard reaction products that are quantified as phenolic compounds. Some researchers have observed that the combined processes (germination and extrusion) in rice (Albarracín *et al*., 2015) and corn (Gong *et al*., 2018) increase the content of phenolic compounds.

γ-aminobutyric acid (GABA), a non-protein essential amino acid, functions as the predominant inhibitory neurotransmitter in the central nervous system. It is also effective in lowering blood pressure and treating epilepsy. The GABA content in RCF was 13.08 mg 100 g^{-1} (DW); after germination under optimal conditions (21 °C for 157 h), chia seeds showed an increase ($p < 0.05$) in GABA (10.6 times) (Table 3). Germination causes the partial hydrolysis of proteins, increasing the availability of free glutamic acid. GABA is mainly produced by the decarboxylation of L-glutamic acid and is catalyzed by glutamate decarboxylase. These results coincide with what was reported by Paucar-Menacho *et al*. (2022), who reported increases in GABA content during the germination of pseudocereals. The extrusion caused a decrease in GABA content; this can be attributed to the heat and shear used during processing. However, it can be

seen that the GABA content of EGCF is higher than that of unprocessed chia. Zhu *et al*. (2017) reported that extrusion could be a suitable processing technology to produce grain-based products with high GABA content.

The total AoxA was determined using the ABTS and DPPH methodologies. The AoxA evaluated using the ABTS assay was 11 157, 22 222, and 23 473 μmol TE 100 g^{-1} of sample (DW) for RCF, GCF, and EGCF, respectively (Table 3). The increase in AoxA is one of the many metabolic changes that occur when germinating seeds, due to an increase in the content of phenolic compounds caused by the action of endogenous hydrolytic enzymes. Extrusion caused a 46.75 % AoxA increase ($p < 0.05$) in free phytochemicals and a 33.22 % decrease ($p < 0.05$) in bound phytochemicals. The AoxA of TPC increased by 10.13 % (Table 3). The increase in the total hydrophilic AoxA could be the result of the release of antioxidant phenolic compounds during the extrusion, the prevention of oxidation of the phenolic compounds in the extruded product by enzymatic inactivation during processing, and the presence of Maillard reaction products with antioxidant activity generated during the extrusion of raw materials containing amino acids and reducing sugars (Argüelles-López *et al*., 2018).

Combined processes (germination and extrusion) increased the total hydrophilic AoxA of RFC by 110 and 115 % ($p < 0.05$), as evaluated by the ABTS and DPPH methodologies, respectively (Table 3). Albarracín *et al.* (2015) reported an 86 % increase in total AoxA, evaluated by ABTS, in whole rice flours sequentially processed by germination-extrusion. Hegazy *et al.* (2017) reported a 19 % increase in AoxA (DPPH) in extruded germinated maize and chickpea mixtures.

Antihypertensive potential of phenolic extracts

The antihypertensive potential (IC_{50}) is defined as the concentration (mg of extract per mL) required to inhibit 50 % of angiotensin-converting enzyme (ACE) activity. The phenolic extracts of RCF, GCF, and EGCF had antihypertensive potential corresponding to an IC_{50} of 0.62, 0.45, and 0.22 mg extract mL^{-1}, respectively (Table 3). The improvement in the antihypertensive potential of germinated chia seeds is due to the increase in the total content of phenolic compounds and GABA (Salgado *et al*., 2022). The improvement in IC_{50} values during extrusion could be due to the release and formation of bioactive compounds (phenolic compounds, Maillard reaction products) with antihypertensive potential. The degree of inhibition of the ACE activity depends on the absorption and metabolism of these compounds, their mode of action related to the class (subclass), and the structure of the phenolic compound that is used. According to this research, the phenolic compounds present in RCF, GCF, and EGCF are suitable for use as functional food supplements or natural medicines to treat hypertension.

Microbiological stability of chia flours

The RCF is within the NOM-147-SSA1-1996 guidelines (DOF, 1996) since the indicator microorganisms were evidenced in amounts less than those established as the maximum allowed limit (Figure 2).

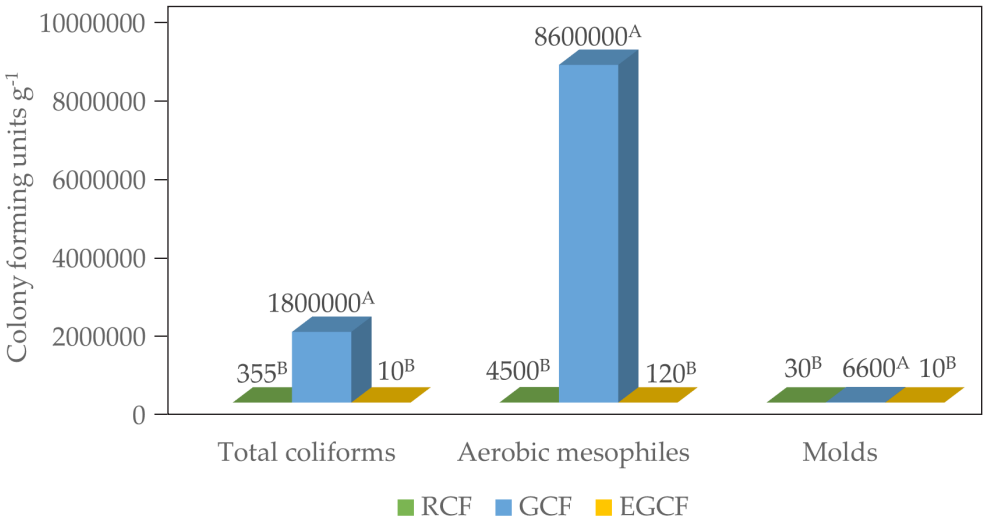

Figure 2. Total coliforms, aerobic mesophiles, and molds in chia flours. Means with different letters for the same type of microorganism present statistical differences (Tukey, $p \leq 0.05$).

The GCF has a low microbiological quality, with total coliforms, aerobic mesophiles, and molds at 18 x 10^5, 86 x 10^5, and 65 x 10^5 CFU g^{-1}, respectively (Figure 2). This represents an increase of 4, 3, and 2 log units, respectively, compared to RCF. These amounts exceed the maximum allowable limit (500, 500 000, and 500 CFU g^{-1} for total coliforms, aerobic mesophiles, and molds, respectively) established by NOM-147-SSA1-1996. This may be due to the characteristic conditions of the germination, such as the high humidity of the seed and the environment, temperature, pH close to neutrality, and availability of carbohydrates and other nutrients, which favor the growth of the bacteria inside or outside the seed (Cava *et al*., 2009).

To improve the microbiological stability of GCF and preserve and/or increase its nutritional and nutraceutical quality, extrusion was applied under optimized conditions as a combined process. As a result, a reduction was observed in the proliferation of microorganisms compared to RCF ($p > 0.05$). Bacterial reductions of up to five logarithms were obtained for total coliforms and aerobic mesophiles (Figure 2). Cava *et al*. (2009) reported an increase of five (coliforms) and six (aerobic mesophiles) logarithms in germinated bean grains compared to ungerminated grains. In the EGCF, the microorganisms were within the specifications of the Official Mexican Standard NOM-147-SSA1-1996 (DOF, 1996).

Microbiological safety is an important factor that determines the quality, shelf life, and effects on human health. In extrusion, thermal processing is designed to kill mesophilic organisms. Shear stress may be involved in reducing the microbial load during the extrusion process, which predicts that mechanical forces may cause cell

rupture. Most of the pathogenic organisms in food can be inactivated by extrusion if the process conditions are previously optimized (Temgire *et al*., 2021).

CONCLUSIONS

The combined germination-extrusion processes of chia seeds under optimized conditions allowed the generation of a new functional ingredient (flour) with nutritional value, high nutraceutical properties, and adequate microbiological stability. The extruded germinated chia flour can be used as a functional ingredient in highly consumed foods to improve their nutritional and nutraceutical quality. This work concluded that it is possible to produce an ingredient with health benefits through the combination of optimized processes in pseudocereals. For the development of new foods that incorporate the use of germinated seed flour, the use of extrusion technology is recommended as an efficient process after germination that ensures the microbiological stability of these flours, maintaining their nutritional and nutraceutical quality.

ACKNOWLEDGEMENTS

To the Mexican *Consejo Nacional de Humanidades, Ciencias y Tecnologías* (CONAHCYT), Convocatoria 2019 - Ciencia de Frontera (Grupal) for the funds supporting this research (ID Number: 263,352). To *Programa de Fomento y Apoyo a Proyectos de Investigación* (PROFAPI) under the *Universidad Autónoma de Sinaloa*, for supplementary funds.

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