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ORIGINAL PAPER

Improvement of Chia Seeds with Antioxidant Activity, GABA, Essential Amino Acids, and Dietary Fiber by Controlled Germination Bioprocess

Mario Armando Gómez-Favela [1] · **Roberto Gutiérrez-Dorado** [1,2] · **Edith Oliva Cuevas-Rodríguez** [1,2] · **Vicente Adrián Canizalez-Román** [1,3] · **Claudia del Rosario León-Sicairos** [1] · **Jorge Milán-Carrillo** [1,2] · **Cuauhtémoc Reyes-Moreno** [1,2,4]

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Abstract Chia (*Salvia hispanica* L.) plant is native from southern Mexico and northern Guatemala. Their seeds are a rich source of bioactive compounds which protect consumers against chronic diseases. Germination improves functionality of the seeds due to the increase in the bioactive compounds and associated antioxidant activity. The purpose of this study was to obtain functional flour from germinated chia seeds under optimized conditions with increased antioxidant activity, phenolic compounds, GABA, essential amino acids, and dietary fiber with respect to un-germinated chia seeds. The effect of germination temperature and time (GT = 20–35 °C, Gt = 10–300 h) on protein, lipid, and total phenolic contents (PC, LC, TPC, respectively), and antioxidant activity (AoxA) was analyzed by response surface methodology as optimization tool. Chia seeds were germinated inside plastic trays with absorbent paper moisturized with 50 mL of 100 ppm sodium hypochlorite dissolution. The sprouts were dried (50 °C/8 h) and ground to obtain germinated chia flours (GCF). The prediction models developed for PC, LC, TPC, and AoxA showed high coefficients of determination, demonstrating their adequacy to explain the variations in experimental data. The highest values of PC, LC, TPC, and AoxA were obtained at two different optimal conditions (GT = 21 °C/Gt = 157 h; GT = 33 °C/Gt = 126 h). Optimized germinated chia flours (OGCF) had higher PC, TPC, AoxA, GABA, essential amino acids, calculated protein efficiency ratio (C-PER), and total dietary fiber (TDF) than un-germinated chia seed flour. The OGCF could be utilized as a natural source of proteins, dietary fiber, GABA, and antioxidants in the development of new functional beverages and foods.

Keywords Chia · Germination · Optimization · Antioxidant activity · Phenolic compounds · GABA

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✉ Cuauhtémoc Reyes-Moreno
creyes@uas.edu.mx

1 Programa Regional de Posgrado en Biotecnología, Facultad de Ciencias Químico Biológicas (FCQB), Universidad Autónoma de Sinaloa (UAS), Ciudad Universitaria, A.P. 1354, CP 80000 Culiacán, Sinaloa, Mexico

2 Programa de Posgrado en Ciencia y Tecnología de Alimentos, FCQB-UAS, Ciudad Universitaria, A.P. 1354, CP 80000 Culiacán, Sinaloa, Mexico

3 Departamento de posgrado, Facultad de Medicina, UAS, Ave. Cedros y Calle Sauces S/N, Fraccionamiento los Fresnos, CP 80019 Culiacán, Sinaloa, Mexico

4 Calle Lichis Ote 1986, Col La Campiña, CP 80060 Culiacán, Sinaloa, Mexico

Introduction

Chia (*Salvia hispanica* L.) is a plant native from southern Mexico and northern Guatemala. It possesses small seeds ranging from white, brown and dark black in color, and their major chemical components are proteins (around 19% of the total weight) of high biological value, dietary fiber (over 30% of the total weight), and lipids (about 40% total weight of the seed) [1, 2].

Functional foods have received substantial attention in recent years as components of healthy lifestyle changes. Many new foods contain bioactive functional compounds (fibers, prebiotics, probiotics, phytochemicals, and antioxidants) that confer functional properties or beneficial effects on human health. Natural antioxidants prevent lipid oxidation in foods, protect the human body against free radicals, and inhibit many chronic diseases. Functional foods may have potential roles in reducing the risk of chronic degenerative diseases [3].

Chia seed might be considered a functional food. It is a promising source of antioxidants due to the presence of polyphenols such as phenolic acids (rosmarynic, chlorogenic, protocatechuic, gallic, ferulic and caffeic acids), flavonols (myricetin, quercetin and kaempferol), and isoflavones (genistin, genistein, daidzin, daidzein, glycitin, glycitein) [4–6]. These phytochemicals not only effectively prevent the oxidation in foods, but also several biological activities and beneficial properties (antiallergic, antiinflammatory, antiproliferative, antimutagenic, anticarcinogenic, antioxidant, regulation of cell cycle arrest, and apoptosis) have been associated with dietary polyphenols [7]. Therefore, these polyphenols protect consumers against several chronic degenerative diseases.

Additionally, chia seeds contain the highest fiber content out of any food, being beneficial for changes in intestinal function, modification of the insulinemic and glycemic responses, reduction of cholesterolemia, and lowering some chronic disorders such as cardiovascular diseases and colon cancer [8].

Germination is considered a straightforward and economic bioprocess to improve the nutritional and nutraceutical value of the seeds by causing desirable changes in the chemical composition, vitamins and minerals availability, increasing the levels of free amino acids, dietary fiber, and other components. Germination also improves the functionality of the seeds due to the subsequent increase in the bioactive compounds and associated antioxidant activity [9–11].

The effects of germination bioprocess on biochemical constituents, chemical composition, phytochemicals content, antioxidant activity of bioactive compounds, and antinutritional factors of the seeds depend on germination conditions. These conditions include soaking time, relative humidity, light, germination temperature and time, and presence of elicitors [10, 12, 13].

Among the bioactive compounds that increase during germination are polyphenols and γ-aminobutyric acid (GABA). GABA is a four carbon non-proteinaceous amino acid and is one of the major inhibitory neurotransmitters in the central nervous system. This compound has been related to several health beneficial effects as antidiabetic, anti-inflammatory, hypocholesterolemic and hypotensive effects, depression and anxiety reduction, antiproliferative action against cancer cells, and its administration could be capable of inhibiting the metastasis of cancer cells [14, 15]. Kanehira et al. [16] suggested that intake of 50 mg GABA-containing beverages may help reduce both physical and psychological fatigue and improve task-solving ability.

In literature, there is only one report about germination of chia seeds, and which was realized by Pereira de Paiva et al. [17]. This research was carried out only to evaluate the effect of different light regimes and temperatures of germination on agronomic parameters of chia seeds; they used a germination time of five days. However, there is not any research which has been studied the increase of the nutritional and nutraceutical value of chia seeds through of germination bioprocess. Therefore, the purpose of this research was to enhance antioxidant activity, phenolic compounds, GABA, essential amino acids, and dietary fiber of chia seeds by germination bioprocess under optimized conditions.

Materials and Methods

Chia (*Salvia hispanica*) seeds were acquired from the local market "Rafael Buelna", Culiacán, Sinaloa, México. Material and methods section are provided as supplementary section.

Results and Discussion

Predictive Models for Protein Content (PC), Lipid Content (LC), Total Phenolic Content (TPC), and Antioxidant Activity ($A_{ox}A$) of Germinated Chia Flours (GCF)

The PC, LC, TPC and AoxA experimental values of the GCF varied from 16.9 to 27.1% (dw), 5.8 to 39.3% (dw), 473.1 to 630.1 mg GAE/100 g sample (dw), and 13,825 to 27,868 μmol TE/100 g sample (dw) (ABTS method), respectively (Table 1). Regression coefficients and analyses of variance of the second order polynomial model were carried out showing the relationships among PC, LC, TPC and AoxA and process variables. Predictive models using uncoded variables for the response variables (PC, LC, TPC, AoxA) were:

$$PC = 20.64 + 1.14(X_1) + 2.91(X_2) + 1.82(X_1)^2$$

$$LC = 10.44–1.46(X_1)–11.84(X_2) + 2.28(X_1)(X_2) + 2.08(X_1)^2 + 6.42(X_2)^2$$

$$TPC = 603.32 + 4.33(X_1) + 46.76(X_2) + 9.40(X_1)^2–26.94(X_2)^2$$

$$AoxA = 23,865.26 + 1,124.34(X_1) + 4,567.48(X_2)–1,685.17(X_2)^2$$

Table 1 Experimental design used to obtain different combinations of germination temperature/germination time for producing germinated chia flours, and experimental results for response variables

Assay[a]	Process variables		Response variables			
	Germination temperature (X_1) (°C)	Germination time (X_2) (h)	Protein content[b]	Lipids content[b]	Total phenolic content[c]	Antioxidant activiy[d]
1	22.2	52.5	19.0	35.4	538.2	16,272
2	32.8	52.5	20.8	27.6	553.0	19,045
3	22.2	257.5	23.4	7.2	618.6	25,511
4	32.8	257.5	25.3	8.5	624.7	26,486
5	20.0	155.0	22.7	15.7	619.4	22,301
6	35.0	155.0	26.5	12.1	629.1	26,011
7	27.5	10.0	16.9	39.3	473.1	13,825
8	27.5	300.0	27.1	5.8	630.1	27,868
9	27.5	155.0	19.0	10.0	623.4	23,834
10	27.5	155.0	20.9	10.8	611.9	24,983
11	27.5	155.0	21.5	9.9	595.8	22,963
12	27.5	155.0	19.6	11.1	601.3	23,659
13	27.5	155.0	20.2	10.4	584.2	24,009

Central composite rotatable design with two factors and five levels; 13 assays

[a] Does not correspond to order of processing

[b] %, dw

[c] mg Gallic acid equivalents (GAE)/100 g sample (dw)

[d] ABTS method, μmol Trolox equivalents (TE)/100 g (dw)

The regression models explained 88.61, 99.64, 93.81, and 97.34% of the total variability ($p \leq 0.0001$) in PC, LC, TPC, and AoxA, respectively. The lack of fit was not significant ($p > 0.05$) and the relative dispersion of the experimental points from the predictions of the models (CV) was found to be <10%. These values indicated that the experimental models were adequate and reproducible. In general, PC, TPC, and AoxA of germinated chia flours (GCF) increased with Gt, while LC decreased with this process variable (Fig. 1). In the case of PC, this response had two regions with the highest values (27–30%, dw), at 20 and 35 °C, both treatments germinated by 300 h (Fig. 1a). LC presented the highest values (37–51%, dw) throughout the temperature range 20–35 °C and 10 h of germination (Fig. 1b). In the case of the TPC response, a saddle behavior (Fig. 1c) was obtained and located the stationary point at 26 °C and 244 h [TPC = 623 mg GAE/100 g sample (dw)]. In Fig. 1c, it could be observed that the surface curves up in temperature direction, and curves down in time course. The highest values of TPC [636–649 mg GAE/100 g sample (dw)] was found at 20 and 35 °C with Gt of 245 h in both cases. The response AoxA increased with GT and Gt until reaching a maximum value [28,540 μmol TE/100 g sample (dw); ABTS method] at 35 °C and 300 h (Fig. 1d).

Optimization

Figure 1e was obtained by superimposition of contour plots (Fig 1a–d) and utilized it to determine the best combination of process variables for production of optimized germinated chia flour (OGCF). The central point of the optimization regions in Fig. 1e corresponds to the optimum combinations of bioprocess variables (GT = 21 °C/Gt = 157 h; GT = 33 °C/Gt = 126 h) for producing two OGCF with the highest PC, LC, TPC and AoxA values. The predicted values of PC, LC, TPC, and AoxA, using the prediction models of each response variable and the two selected optimal conditions of germination were 22.07% (dw), 15.09% (dw), 613.31 mg GAE/100 g sample (dw), 22,577 μmol TE/100 g sample (dw) (ABTS method), and 23.15% (dw), 14.45% (dw), 603.5 mg GAE/100 g sample (dw), 23,651 μmol TE/100 g sample (dw) (ABTS method), for the 1 and 2 optimal conditions, respectively (Fig. 1e). The OGCF was produced by applying the optimal conditions of germination bioprocess. However, only the OGCF created at GT = 21 °C/Gt = 157 h was selected to evaluate experimental values of response variables, due to the germinated samples at GT = 32 °C/Gt = 126 h showed fungal growth tendency and a few number of germinated seeds. The experimental values of PC, LC, TPC, and AoxA of the OGCF produced at the selected germination optimum conditions (Tables 2 and 3) were similar to the predicted

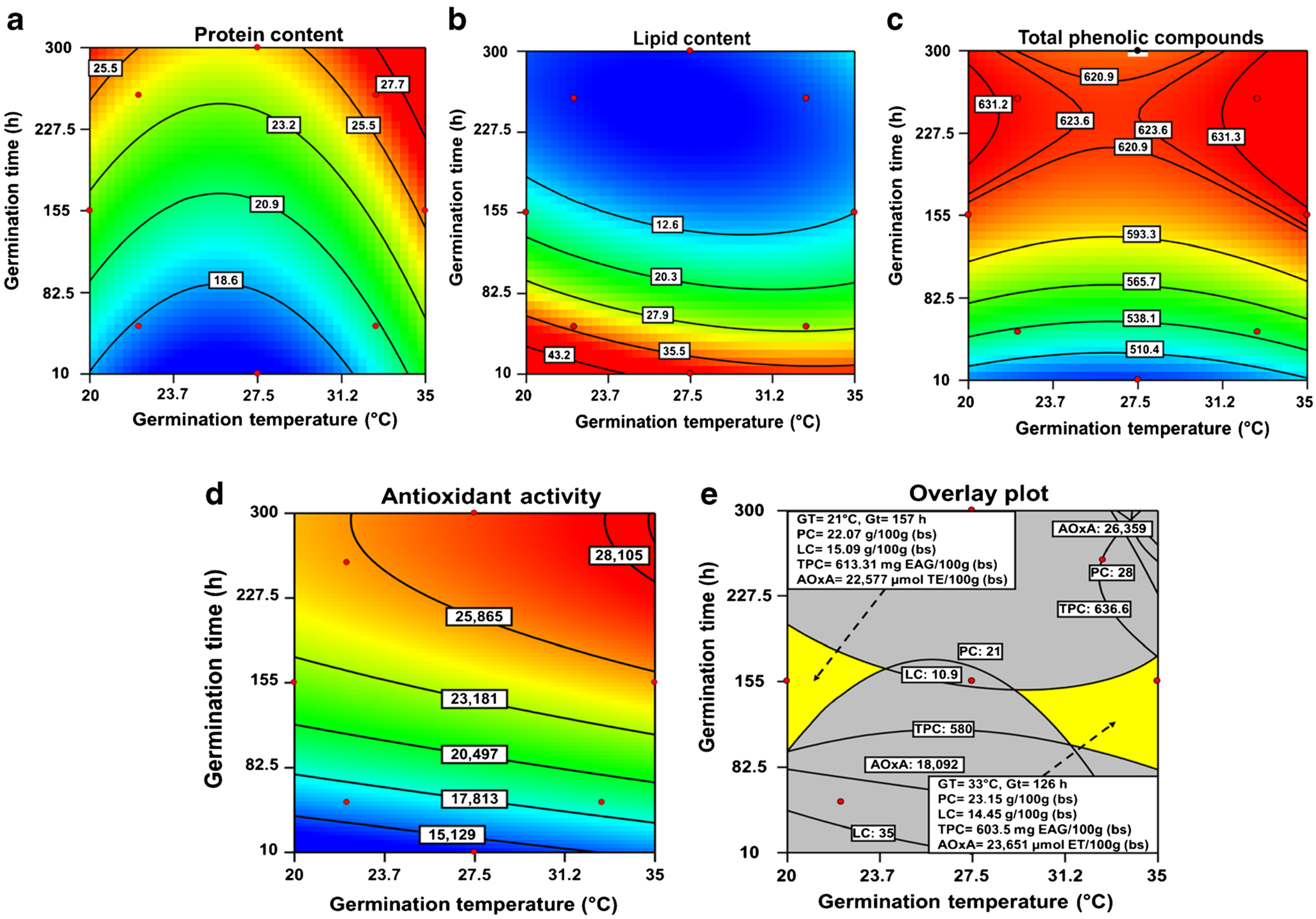

Fig. 1 Contour plots of four response variables **a** protein content (PC), **b** lipid content (LC), **c** total phenolic compounds, **d** antioxidant activity, and **e** overlay graphic with optimal combination of chia seeds germination temperature and time, and predicted values of the response variables

values, above mentioned, indicating that this optimal condition of chia seeds germination was appropriated and reproducible.

Chemical Composition and Nutritional Properties of Optimized Germinated Chia Flour (OGCF)

The protein content (dw) (18.48%) of un-germinated chia flour (Table 2) was lower than Mexican (22.7%) and Chilean (25.32%) chia seeds, and similar to seeds from other *Salvia hispanica* L. genotypes [18–20]. As shown in Table 2, following germination at optimal conditions (GT = 21 °C/ Gt = 157 h) a significant increase (20.89%) in chia protein content was observed. Herchi et al. [21] reported an increase in protein content after germination in flaxseed, other oilseeds. The increasing protein content by germination could be attributed to losses in dry weight; these losses can be accounted mainly as a loss in lipids and carbohydrates (mostly sugars) during respiration due to the production of water and carbon dioxide [9].

The fat content (33.7%) of un-germinated chia seeds was found in the range (32–39%) reported by Ayerza [20] for different varieties of *Salvia hispanica* L. The lipid content of chia seeds after germination at optimal conditions (GT = 21 °C/Gt = 157 h) had a trend toward a significant ($p < 0.05$) decline (−55.31%) (Table 2). Herchi et al. [21] studied the effect of the germination of flaxseed on the lipid content; they found that the oil content of flaxseed progressively decreased with the germination time. The reduction in oil content of seeds during germination can be attributed to the production of energy required for metabolic activity, such as synthesis of DNA, RNA, enzymes, structural proteins, and other biological molecules [22].

The un-germinated chia seeds contain moderate amounts of carbohydrates (44.62%) and minerals (3.2%), as well as a significant amount of total dietary fiber (42.52%) (Table 2). The total dietary fiber content is higher than that of traditional sources of fiber such as whole legume [chickpea (13.9%), pea (22.03%), lentil (20.44%), common bean (22.32%)], and cereal [oat (10.4%), corn (12.8%), wheat (14.2%), barley (15.4%)] grains [23, 24]. This fact confirms that chia seed is an outstanding source of dietary fiber as compared to most known sources [18]. Table 2 shows the effect of germination bioprocess on dietary fiber content in chia seeds; insoluble

Table 2 Proximate composition and nutritional properties of chia flours

Property	Un-germinated chia flour (UCF)	Optimized germinated chia flour (OGCF)	(FAO, 2013)[a]
Chemical composition (%, dw)			
Proteins	18.48 ± 0.76^{B}	22.34 ± 0.51^{A}	–
Lipids	33.7 ± 0.16^{A}	15.06 ± 0.60^{B}	–
Minerals	3.2 ± 0.3^{B}	5.1 ± 0.2^{A}	
Carbohydrates	44.62 ± 0.4^{B}	57.5 ± 0.6^{A}	
Dietary fiber			–
Soluble fiber	3.99 ± 0.043^{A}	3.45 ± 0.01^{B}	–
Insoluble fiber	38.53 ± 0.47^{B}	40.51 ± 0.12^{A}	–
Total fiber	42.52 ± 0.43^{B}	43.96 ± 0.05^{A}	–
Nutritional			
EAA[b] (g/100 g protein)			
His	2.10 ± 0.02^{B}	4.29 ± 0.03^{A}	1.6
Ile	3.24 ± 0.03^{B}	6.66 ± 0.04^{A}	3.0
Leu	5.30 ± 0.04^{B}	7.85 ± 0.05^{A}	6.1
Lys	3.52 ± 0.02^{B}	4.61 ± 0.03^{A}	4.8
Met + Cys	2.75 ± 0.05^{B}	5.86 ± 0.02^{A}	2.3
Phe + Tyr	4.49 ± 0.04^{B}	7.53 ± 0.07^{A}	4.1
Thr	2.49 ± 0.03^{B}	3.05 ± 0.02^{A}	2.5
Trp	1.80 ± 0.02^{B}	2.10 ± 0.03^{A}	0.66
Val	3.73 ± 0.01^{B}	6.58 ± 0.05^{A}	4.0
Total	29.42	48.53	29.06
EAA[b] chemical score (%)	73	96	100
Limiting EAA[b]	Lys	Lys	–
IVPD[c] (%)	79.1 ± 1.50^{B}	82.9 ± 0.89^{A}	–
C-PER[d]	1.51	2.22	–

Data are expressed as means ± standard deviation. Means with different superscripts (A-B) in the same row are different (Duncan, $p \leq 0.05$)

[a] Requirements of amino acids for older child, adolescent, and adult (3 years and older) according FAO (2013)

[b] EAA = Essential amino acid

[c] IVPD = *in vitro* protein digestibility (%)

[d] C-PER = Calculated protein efficiency ratio

(+5.14%) and total dietary (+3.39%) fiber contents in chia seeds increased after germination, while soluble (−13.53%) dietary fiber decreased by germination effect. It can be due to the loss of mucilage when the seeds are in contact with hydration solution at the beginning of the germination process. When comparing these findings about the increase in total dietary fiber content in germinated seed with other studies, in this study only a slightly effect on these compounds was found; for example, Tiansawang et al. [25] and Perales-Sánchez et al. [9] reported an increase of total dietary fiber up to 30 and 120%, after germination of sesame and amaranth seeds, respectively, meanwhile in this research only an increase of 3.39% was found. Generally, germination produces and increases hemicellulose, cellulose, and pectic polysaccharides. The germination process tends to modify the structure of cell wall polysaccharides of the seeds, probably by affecting the intactness of tissue histology and disrupting the protein-carbohydrate interaction [26]. The results obtained in this work for dietary fiber are not according with the tendencies reported in other seeds as above mentioned; this indicate that under these conditions the chia sprouts have not yet synthesized a high amount of hemicellulose, cellulose, and pectic polysaccharides. However, considering the absolute values of dietary fiber content of germinated chia seeds, these resulted be a better source of dietary fiber than germinated seeds reported by authors above mentioned. Dietary fiber significantly influences gut physiology and is a crucial component of a healthy diet. Some studies have reported the effect of fiber in lowering risk of develop cardiovascular diseases, hypertension, diabetes, obesity, dyslipidemia, gastrointestinal diseases, and colorectal cancer [27].

The essential amino acids (EAA) content of un-germinated and germinated chia flour (OGCF) is shown in Table 2. In general, the EAA content of un-germinated chia flour was

Table 3 Antioxidant activity and phytochemicals content of chia flours

Property	Un-germinated chia flour (UCF)	Optimized germinated chia flour (OGCF)
Antioxidant activity ORAC[a]		
Free phenolics	6961 ± 230[B]	19,765 ± 858[A]
Bound phenolics	12,169 ± 694[B]	13,427 ± 918[A]
Total phenolics	19,130 ± 432[B]	33,192 ± 890[A]
Antioxidant activity ABTS[a]		
Free phenolics	2412 ± 83[B]	10,505 ± 970[A]
Bound phenolics	9843 ± 316[B]	13,596 ± 275[A]
Total phenolics	12,255 ± 232[B]	24,101 ± 580[A]
Phenolic content[b]		
Free phenolics	190.8 ± 10.3[B]	338.1 ± 2.6[A]
Bound phenolics	224.4 ± 16.2[B]	273.9 ± 2.6[A]
Total phenolics	415.2 ± 6.3[B]	612.0 ± 7.9[A]
GABA content[c]	9.51 ± 0.78[B]	117.66 ± 0.36[A]

Data are expressed as means ± standard deviation. Means with different superscripts (A-B) in the same row are different (Duncan, $p \leq 0.05$)

[a] μmol Trolox equivalents (TE)/100 g (dw)

[b] mg Gallic acid equivalents (GAE)/100 g sample (dw)

[c] mg Gamma-aminobutyric acid (GABA)/ 100 g sample (dw)

higher than those of the suggested pattern of EAA requirements for older children, adolescents and adults (3 years and older) [28], except for Leu, Lys, and Val. Sandoval-Oliveros and Paredes-López [18] and Nitroyová et al. [29] reported similar results for EAA content in *Salvia hispanica* L. The EAA content of chia seeds significantly increased after germination bioprocess. The final EAA content of OGCF was higher than those of the mentioned pattern of amino acid, except for Lys; thus, in proteins of OGCF, the limiting EAA was Lys, with EAA score of 96. There aren't reports in the literature about germination process effect on EAA content of chia seeds. Also, the germination bioprocess significantly increased the *in vitro* protein digestibility (IVPD) and the calculated protein efficiency ratio (C-PER) of chia seeds (Table 2).

Total Phenolic and GABA Content, and Antioxidant Properties of Optimized Germinated Chia Flour (OGCF)

The amount of free phenolic compounds in un-germinated chia seeds was 190.8 mg GAE/100 g sample dw (Table 3). Martínez-Cruz and Paredes-López [6] reported similar values of free phenolic compounds (163.98 mg GAE / 100 g, dw) in chia seeds. As shown in Table 3, the germination bioprocess increased ($p < 0.05$) free (+77.20%), bound (+22.06%), and total (+47.40%) phenolic contents. Khang et al. [30] reported that the phenolic concentration dramatically increased ($p < 0.05$) in peanuts and soybeans after germination (106 and 133%, respectively); this could be due to the release and biosynthesis of phenolic compounds. The phenolic compounds such as hydroxycinnamates are bound to nonstarch polysaccharides in grain cell walls through associations such as ester and ether bonds. The action of cell wall-degrading enzymes (mainly esterases) on these bonds contributes to the release of bound phenolic compounds. On the other hand, the activation of phenylalanine ammonia lyase (a key enzyme in phenolic biosynthesis) during germination of seeds has been reported [9].

The γ-aminobutyric acid (GABA) content (dw) in un-germinated chia flour was 9.51 mg/100 g dw (Table 3). Tiansawang et al. [25] reported GABA concentrations of 13.25, 12.22, 4.38, and 9.07 mg/100 g dw, in raw mung bean, soybean, black bean, and sesame grains, respectively. After germination in optimal conditions (21 °C/157 h) chia seeds showed a significant increase ($p < 0.05$) in GABA (11.4 fold). Paucar-Menacho et al. [13] observed that after germination of amaranth (*Amaranthus caudatus*) grains at optimal germination conditions (26 °C/63 h) the GABA content increased 29.1 fold. GABA is produced primarily by the decarboxylation of L-glutamic acid and catalyzed by glutamate decarboxylase (GAD) during seed germination, and has biological activities against diabetes, hypercholesterolemia, hypertension, inflammation, and depression, as well as anti-proliferative action against cancer cells [14].

The total hydrophilic antioxidant activity (H-ORAC) of un-germinated chia seeds was 19,130 μmol TE/100 g (Table 3). Marineli et al. [19] reported that Chilean chia seeds have antioxidant activities of H-ORAC = 51,730 μmol TE/100 g to free fraction of defatted sample. The ORAC value for ungerminated chia flour was lesser than the reported by Marineli et al. [19]. This difference could be due to several agronomics factors such as soil type, harvest time, weather conditions, and others; which influence on secondary metabolites in plants leading to the increase or decrease of phenolic compounds with antioxidant activity and in this way, increase or decrease the antioxidant activity of seeds. The high antioxidant activities in chia seeds could be due to a high content of polyphenolic compounds. In this research, in general, H-ORAC of chia seeds increased after bioprocessing at optimal germination conditions (21 °C/157 h) (Table 3). The H-ORAC for free (+ 183.9%), bound (+ 10.3%) and total (+ 73.5%) phenolics increased after germination (Table 3). Increases in antioxidant activities during germination evaluated by ABTS method (+335.5, +38.1, +96.7 to free, bound, and total phenolics, respectively) were higher than ORAC method. Perales-Sánchez et al. [9] studied the changes of antioxidant activity of germinated amaranth seeds; they observed that, after germination, the H-ORAC for free, bound and total phytochemicals, increased in 386, 235, and 300%, respectively, while free, bound and total antioxidant activity, evaluated by ABTS method, increased in 756, 261, and 470%, respectively. Paucar-Menacho et al. [13] reported that after germination of *Amaranthus caudatus* grains at optimal germination temperature and time (26 °C/63 h) the antioxidant activity increased 542.4%. The increase in phenolic compounds with antioxidant capacity after the germination bioprocess is one of the many metabolic changes that take place upon germination of seeds, mainly due to an increase in the activity of the endogenous hydrolytic enzymes. The present study indicated that AoxA had a stronger correlation (r = 0.939, $p < 0.001$) with TPC content. Cevallos-Casals and Cisneros-Zevallos [31] reported that the increase of phenolic compounds after seeds germination has a potentially important role in the enhance of antioxidant activity, as well as the increase of the nutraceutical potential of seeds.

Conclusions

The best combination of germination bioprocess variables produced a functional flour of germinated chia with high protein content, lipid content, total phenolic content and antioxidant activity. The optimized germination process is an effective strategy to increase the content and quality of proteins, total phenolic content, antioxidant activity, and γ aminobutyric acid content of chia seeds. Therefore, the optimized germinated chia flour (OGCF) could be used as a source of natural antioxidants, GABA, protein, and dietary fiber in the development of new functional foods and beverages.

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Compliance with Ethical Standards

Conflict of Interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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