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Gluten-free healthy snack with high nutritional and nutraceutical value elaborated from a mixture of extruded underutilized grains (quality protein maize/tepary bean)

Botana saludable libre de gluten con alto valor nutricional y nutracéutico elaborada con una mezcla de granos extrudidos subutilizados (maíz de calidad proteínica/frijol tépari)

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Abstract

An optimized gluten-free healthy snack (OGFHS) was developed from a mixture of protein quality maize/tepary bean (70:30), applying extrusion-cooking. The extrusion conditions were obtained from the axial combination of process variables (feed moisture content [FMC = 15%-25%]/extrusion temperature [ET = 120 °C-170 °C]/screw speed [SS = 50 rpm-240 rpm]). The desirability function response surface methodology was applied as an optimization technique in three response variables (expansion index [EI]/apparent density [AD]/hardness [H]) to obtain maximum EI and minimum AD and H. The best combination to produce OGFHS was FMC = 16.4%/ET = 137 °C/VT = 237 rpm. The optimized conditions to obtain OGFHS caused little change in the nutritional and nutraceutical properties of the unprocessed grains mixture. OGFHS (50 g) was compared with two commercial snacks (Cheetos[MR], Totis[MR]), showing better protein content, total dietary fiber and AoxA, as well as high acceptability and lower energy content than commercial snacks. Due to its great nutritional properties and AoxA, OGFHS could be used for health promotion and as an alternative to commercial snacks with low nutritional value and high energy content.

Keywords: Healthy snack; gluten-free; quality protein maize; tepary bean; extrusion.

Resumen

Se desarrolló botana saludable libre de gluten optimizada (BSLGO) usando una mezcla de maíz de calidad proteínica/frijol tépari (70:30), aplicando extrusión-cocción. Las condiciones de extrusión se obtuvieron de la combinación axial de variables de proceso (contenido de humedad de alimentación [CHA = 15%-25%]/temperatura extrusión [TE = 120 °C-170 °C]/velocidad tornillo [VT = 50 rpm-240 rpm]). Se aplicó la función de deseabilidad metodología de superficie de respuesta como técnica de optimización, en tres variables de respuesta (índice de expansión [IE]/densidad aparente [DA]/dureza [Du]) para obtener un máximo de IE y mínimos de DA y Du. La mejor combinación para producir BSLGO fue CHA = 16.4%/TE = 137 °C/VT = 237 rpm. Las condiciones optimizadas para obtener BSLGO ocasionaron pocos cambios en las propiedades nutricionales y nutracéuticas de la mezcla de granos sin procesar. Se comparó BSLGO (50 g) contra dos botanas comerciales (Cheetos[MR], Totis[MR]). Dicha mezcla mostró mejor contenido proteínico, fibra dietaria total y AAox, así como alta aceptabilidad y menor contenido energético en comparación con las botanas comerciales. Por sus buenas propiedades nutricionales y AAox, la BSLGO podría utilizarse para promover la salud y como alternativa a botanas comerciales de bajo valor nutricional y alto contenido energético.

Palabras clave: Botana saludable; libre de gluten; maíz calidad proteínica; frijol tépari; extrusión.

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Introduction

Celiac disease (CD), or gluten-sensitive enteropathy, is a generalized autoimmune disease characterized by chronic inflammation and atrophy of the small intestine's mucosa, caused by exposure to dietary gluten, which affects genetically predisposed individuals (Kamel *et al.*, 2020). Gluten is a protein component mainly found in wheat and some other cereal grains such as rye and barley (Rosell *et al.*, 2014). The prevalence of CD in the general population is approximately 1%, with female predominance. The treatment for CD is a life-long, strict gluten-free (GF) diet (excluding dietary wheat, rye, barley, and hybrids like Kamut and triticale), leading to improvement in life quality, preventing the occurrence of refractory celiac disease, small intestinal adenocarcinoma, and ulcerative jejunoileitis (Caio *et al.*, 2019).

GF food products existing in the market today are more expensive than gluten-containing food products. In addition, these are known to have lower nutritional value than the critical needs of celiac patients. It is necessary to develop cheap, nutritionally rich gluten-free food products to promote a healthier lifestyle in people who have the CD (Jnawali *et al.*, 2016). Some of the GF and nutritious grains include cereals (corn, rice), pseudo-cereals (buckwheat, amaranth, chia, quinoa), millets (pearl millet, finger millet, Kodo millet, foxtail millet, pearl millet), and pulses (soybean, common bean, pea, chickpea, Bengal gram), which can be used for the preparation of GF functional foods (Kumar, 2018).

Snacks are defined as small servings of simple, convenient, easy-to-prepare food that can be consumed between regular meals, whereas snacking is described as the act of eating a snack, regardless of whether healthful choices or snack foods are consumed (Mielmann & Brunner, 2019; Warde & Yates, 2017). Enjoying a special occasion, gaining energy, and opportunity-induced eating are the main reasons for unhealthy snacking (Verhoeven *et al.*, 2015). The excessive consumption of snacks has been related to the incidence of overweight and obesity and the first apparition of chronic-degenerative diseases (diabetes, hypertension, cancer). To avoid obesity and other related diseases, establishing healthy snacking habits and evaluating the nutritional quality of products can help (de Vlieger *et al.*, 2017). Nutritious snacks can increase access to healthy, low energy food choices. A nutritious snack can be obtained by supplementing it with legumes, which have high protein content and provide insoluble and soluble dietary fibers, vitamins, and minerals (Kumari & Sangeetha, 2017).

Mixtures of cereals and legumes lead to products with better protein content with high biological value. While legumes are deficient in sulfurous amino acids and rich in lysine, cereals are deficient in lysine and relatively abundant in sulfur-containing amino acids. Therefore, cereals and legumes are considered complementary, offering nutritional quality (Algarni *et al.*, 2019). Several kinds of research have shown the importance of processing (germination, fermentation, extrusion) and combining legume with cereals to generate new food products to increase nutritional and nutraceutical quality (Argüelles-López *et al.*, 2018; Espinoza-Moreno *et al.*, 2016; Milán-Carrillo *et al.*, 2017). Besides, other researchers have identified that the production of nutritious snack products is possible by blending protein sources (peas, lentils, common beans) with starch sources (wheat, corn, barley and rice) (Boye *et al.*, 2010a; Boye *et al.*, 2010b; Félix-Medina *et al.*, 2020).

Maize (*Zea mays* L.) is the most highly produced cereal globally, representing a significant source of energy, proteins, and other nutrients for both humans and livestock. The quality of maize proteins is reduced because they are deficient in the essential amino acids lysine (Lys) and tryptophan (Trp). Maize breeders of the International Maize and Wheat Improvement Center (CIMMyT) and the National Research Institute for Forestry, Agriculture, and Livestock (INIFAP, from its Spanish acronym) in Mexico developed 26 hybrids and cultivars of quality protein maize (QPM), which have yields and other important agronomic traits similar to those of a normal maize. However, they almost double the Lys and Trp content and,

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therefore, the protein quality (Gutiérrez-Dorado *et al.*, 2008). Whole grain maize has a wide range of phytochemicals, exhibiting the health benefits of lowering chronic disease risk. Whole maize grains have unique profiles of nutrients and phytochemicals when compared with other grains. Corn nutrients and phytochemicals include minerals (Mg, P, K), vitamins (A, B, E, K), flavonoids (anthocyanins), phenolic acids (ferulic, coumaric, syringic), carotenoids, and dietary fiber. Regular consumption of whole grain maize lowers the risk of developing chronic degenerative diseases (type 2 diabetes, obesity, and cardiovascular disease) and improves digestive health (Sheng *et al.*, 2018; Zhang *et al.*, 2019).

Tepary bean (*Phaseolus acutifolius*) is a drought-resistant legume from the semi-arid environment and the deserts of southwestern US and northwestern Mexico. This bean has high adaptation to warm climates (i.e., it is heat tolerant), low moisture requirements and is resistance to many diseases. The mature seeds of tepary bean contain 21%-31.9% proteins, 0.9%-1.17% lipids, and 65.3%-69.1% carbohydrates (Salas-López *et al.*, 2018). Currently, tepary bean seeds are scarcely consumed, and their high proteins and carbohydrates content are underutilized. Legumes contain several considered natural antioxidants, representing a significant group of bioactive compounds in foods. They have been associated with reducing the risks of diabetes, heart disease, cancer, inhibition of plasma platelet aggregation, histamine release, and *in vitro* antiviral, antibacterial, antiallergic, and anti-inflammatory activities. The benefits of many of these conditions come partly from the antioxidant characteristic of phenols (Bosi *et al.*, 2019; Singh *et al.*, 2017).

Extrusion technology has a broad scope in developing various food products such as cereal-based snacks, ready-to-eat breakfast cereal, functional foods, and novel ingredients. Extrusion cooking uses high-temperature, high-pressure, and high shear conditions quickly, resulting in chemical reactions and molecular transformation within the extruded products. Extrusion cooking improves starch and protein digestibility, increases the retention of bioactive compounds and soluble dietary fiber, and causes lipid modifications, inactivation of enzymes and microorganisms, and volatile flavor components. It is also a highly efficient alternative technology, which minimizes energy consumption and water pollution (Grasso, 2020; Patil & Kaur, 2018).

This research aimed to find the best conditions of the extrusion process to obtain a gluten-free healthy expanded snack with high nutritional/nutraceutical value, acceptable sensory, from a blend of 70% quality protein maize (QPM) and 30% of tepary bean.

Materials and methods

Materials

The tepary bean seeds were purchased in the Rafael Buelna Market, Culiacan, Sinaloa, México. The quality protein maize (*Zea mays* L.) (QPM) seeds were supplied by Dr. Ricardo Ernesto Preciado Ortiz from INIFAP, Celaya, Guanajuato, México. Both the tepary bean and maize seeds were cleaned and stored in tightly sealed containers at 4 °C until use.

Preparation of gluten-free healthy snacks (GFHS)

The methodology reported by Espinoza-Moreno *et al.* (2016) was used to obtain GFHS. Lots (1 kg) of whole quality protein maize (QPM) or tepary bean (TB) kernels were ground to obtain grits that passed through a 40-US mesh (0.425 mm) screen. QPM and TB grits were mixed to obtain 250 g batches (175 g QPMG+75 g TBG). These lots were conditioned with purified water until they reached moisture contents of 15 g H_2O/100 g−25 g H_2O/100 g wet grits (table 1). Extrusion cooking was carried out in a laboratory extruder model 20

DN (CW Brabender Instruments, Inc., NJ, USA). Extrusion conditions were selected from an axial combination of process variables: feed moisture content (FMC, 15%–25%), extrusion temperature (ET = 120 °C–170 °C), and screw speed (SS = 50 rpm–240 rpm) (table 1). GFHS were cooled, equilibrated (25 °C, RH = 65%), packed in hermetic plastic bags, and evaluated for expansion index (EI), apparent density (AD), and hardness (H). These experimental values were used for optimizing the extrusion process.

Expansion index (EI), apparent density (AD) and hardness (H)

The EI was calculated as the cross-sectional diameter of the GFHS divided by the die opening diameter. The AD of the GFHS was determined using the equation $AD = (4)(m)/[(\pi)(d)^2(L)]$, where m = mass of the GFHS (g), d = diameter (cm) of GFHS, and L = length of GFHS (cm). H was measured as the force (N) employed to penetrate 60% of the GFHS diameter using a texturometer model 3342 (Instron Corporation, Norwood, MA, USA). A cell of 500 N, a descent speed of 10 mm/s, and a penetration depth of 2 mm were used. EI, AD, and H were measured over 100 GFHS pieces of 5 cm long.

Response surface methodology (RSM), experimental design, statistical analysis and optimization

A rotatable central composite experimental design was chosen for response surface methodology (RSM), with three factors (process variables) (feed moisture content [FMC], extrusion temperature [ET], and screw speed [SS]) and five variation levels (two factorials [coded: -1; +1], two axials [coded: -1.682 (-α); +1.682 (+α)], and one central [coded: 0]]. Table 1 shows the experiment design and original levels attributed to each variable. The α value for the axial points was selected so that the experimental design had the property of rotatable. Rotability is a reasonable basis for selecting a response surface design. Since the purpose of RSM is optimization, and the location of the optimum is unknown before running the experiment, it makes sense to use a design that provides equal estimation precision in all directions. Despite that this experimental design is not exactly orthogonal, this property does not significantly affect the multicollinearity (correlation) of the coefficients of the regression models and of the design factors; therefore, they do not present a significant increase in the variance in each one of the coefficients of the analyzed models due to the lack of orthogonality in the design. EI, AD, and H of the GFHS were selected as response variables. The stepwise regression procedure was applied; non-significant terms ($p > 0.1$) were deleted from a second-order polynomial. A new polynomial was used to develop a predictive model for each response variable. The desirability method of the RSM was applied to determine the best combination of extrusion process variables. The three fitted models for the three response variables (EI, AD, H) were evaluated on one point ($X = X_1, X_2, X_3$) of the experimental zone, and three values were obtained [$\hat{Y}_1(X_1)$, $\hat{Y}_2(X_2)$, $\hat{Y}_3(X_3)$]. Each $\hat{Y}_i(X_i)$ was transformed into a value di(X) (individual desirability), which falls within the range (0-1) and measures the desirability degree of the response about the optimum value intended to be reached. In this research, it was desired to obtain the highest possible EI and minimum values for AD and H. The global desirability (D) for the three response variables were determined from individual desirabilities with the mathematical function $D = [(d_1)(d_2)(d_3)]^{1/3}$, where the ideal optimum value is D = 1; an acceptable value for D can be between 0.6 and 0.8. The statistical software Design Expert 7.0.0 (Stat-Ease, Minneapolis, MN, USA) was used for the RSM analyses.

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

The following methods of the Association of Official Analytical Chemists (AOAC, 2012) were used to evaluate proximate composition: moisture (925.09B): drying at 130 °C; lipids (method 920.39C): defatting in a Soxhlet with petroleum ether; protein (method 960.52): micro-Kjeldahl (Nx6.25). Soluble and insoluble dietary fiber (SDF/IDF) was evaluated according to the enzymatic-gravimetric method for total dietary fiber

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(TDF) (method 985.29) (AOAC, 2012). Carbohydrates content was calculated by difference. All determination was made by triplicate.

Physicochemical properties

pH was determined according to AOAC (2012) methodology, the total color difference (∆E) was measured with a Minolta Chromameter mod. CR-210 colorimeter, and the water activity (a_w) was evaluated with an Aqualab mod CX2 equipment. All determinations were made by triplicate.

Essential amino acid (EAA), *in vitro* protein digestibility (IVPD), chemical score (CS) and calculated protein efficiency ratio (C-PER)

The EAA composition was determined using an analytical scale (4.6 mm×250 mm) hypersil ODS C18 column (SGE, Dandenong, Australia) kept at 38 °C. It connected to an HPLC system (GBC, Dandenong, Australia) equipped with a fluorescence detector >LC 5100 set at 270 nm and 316 nm for excitation and emission, respectively (López-Cervantes *et al.*, 2006). Tryptophan was detected at 280 nm with an ultraviolet detector. Other nutritional properties (IVDP, CS and C-PER) were evaluated according to Salas-López *et al.* (2018). The IVPD was considered using a multi-enzyme system. The chemical score (CS) was calculated as follows: CS = (content of the most limiting EAA/REAAR) x 100, EAA = essential amino acid and REAAR = recommended amino acid requirements for three years old children and older, adolescents, and adults (Food and Agriculture Organization of the United Nations [FAO], 2013). C-PER was calculated based on the IVPD and the EAA composition of the sample. All determinations were carried out in triplicate.

Antioxidant activity (AoxA), total phenolic content (TPC) and total flavonoids (TF)

The AoxA of free and bound phenolic extracts was evaluated using the ORAC assay. Aliquots of 25 μL of diluted extracts were mixed with 150 mL of fluorescein (0.1 mM) and 25 μL of the peroxyl radical AAPH (200 mM). After 30 min, fluorescence (485 nm for excitation and 538 nm for emission) was measured (37 °C) at 2 min intervals for 60 min, using a Synergy Microplate Reader (Synergy™ HT Multi-Detection, BioTek, Inc., Winooski, VT) (Mora-Rochín *et al.*, 2010). The ORAC assay results were expressed as μmol Trolox equivalents (TE)/100 g (DW). The TPC of free and bound extracts was determined using 20 mL of appropriate dilutions of extracts, oxidized with 180 mL of Folin-Ciocalteu reagent (Singleton *et al.*, 1999). After 20 min, the blue color's absorbance measured at 750 nm using the Synergy Microplate Reader. Total phenolic content was expressed as mg Gallic acid equivalent (GAE)/100 g (DW). The total flavonoids (TF) of free and bound extracts were determined using 5% $NaNO_2$ and 10% $AlCl_3$ reagents (Xu & Chang, 2007). The absorbance was measured at 510 nm using a UV-visible spectrophotometer, and the results were expressed as mg catechin equivalents (CAE)/100 g (DW). Condensed tannins were evaluated on extract of 80% acetone/water using 4% methanol vainillin solution and concentrated hydrochloric acid, according to Xu & Chang (2007). The absorbance was measured at 500 nm using a UV-visible spectrophotometer, and the results were expressed as mg catechin equivalents (CAE)/100 g (DW). All measurements were carried out in triplicate.

Sensory analysis

A panel of 50 untrained judges carried out the sensory analysis of OGFHS). A hedonic scale of 11 points (1 = Value maximum of imaginable disagree; 11 = Value maximum of imaginable agree) for expanded diameter, texture, and global acceptability parameters was used. In aleatory order, as well as with daylight, the samples were evaluated. All evaluations were made three times in four different days.

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Statistical analysis

To perform the data analysis of OGFHS and expanded commercial snacks, a one-way analysis of variance (Anova) followed by Duncan's multiple range test comparisons among means with a 5% significance level were carried out. The t-student test ($p \leq 0.05$) was used for data analysis of **OGFHS** and unprocessed grains mixture.

Results

Predictive models of response variables (EI, AD and H)

The EI, AD, and H experimental values of the GFHS varied from 2.01 to 2.77, 0.152 g/cm^3 to 0.518 g/cm^3, and 1.82 N to 38.19 N, respectively (table 1). Regression analysis was carried out on these experimental results, obtaining prediction models for each response variable. Contour and surface plots showing the effect of process variables on response variables were obtained from these prediction models. Analysis of variance showed that EI and AD were significantly ($p < 0.01$) dependent on linear terms of FMC, ET, and SS; interaction terms (FMC)(SS), (ET)(SS), significantly affect EI, while BD was significantly ($p < 0.01$) dependent on interaction terms of (ET)(SS). The quadratic term of FMC affected the EI, AD and H responses; the quadratic term of SS only affected the EI response. The prediction models using uncoded variables for the response variables (EI, AD and H) were:

$$Y_{EI} = 2.31 - 0.13(FMC) - 0.048(ET) + 0.059(SS) + 0.064(FMC)(ET) - 0.10(FMC)(SS) - 0.12(ET)(SS) + 0.070(FMC)^2 - 0.050(SS)^2$$

$$Y_{AD} = 0.25 + 0.055(FMC) - 0.059(ET) - 0.05(SS) + 0.054(ET)(SS) + 0.04(FMC)^2$$

$$Y_{H} = 9.01 + 6.7(FMC) - 4.17(ET) - 4.45(SS) + 5.59(ET)(SS) + 3.76(FMC)^2$$

The regression models explained 89.05%, 93.27%, and 82.43% of the total variation of values from EI, AD and H. The lack of fit was not significant ($p > 0.05$); the CV (relative dispersion of the experimental points from the prediction models) was found to be <10%. According to these values, the predictive models were adequate and reproducible.

Table 1. The experimental design was used to obtain different combinations of process variables to elaborate gluten-free healthy snacks from a quality protein maize-tepary bean (70% QPM + 30% TB) mixture and experimental results of response variables.

Assay[a]	Process variables			Response variables		
	Feed moisture content (FMC) (%)	Extrusion temperature (ET) ºC	Screw speed (SS) (rpm)	Expansion index (EI)	Apparent density (AD) (g/cm^3)	Hardness (H) (N)
1	17.0	130.1	88.5	2.30	0.457	25.39
2	23.0	130.1	88.5	2.10	**0.518**	**38.19**
3	17.0	159.9	88.5	2.30	0.183	2.81
4	23.0	159.9	88.5	2.41	0.297	13.81
5	17.0	130.1	201.5	**2.77**	0.194	4.10
6	23.0	130.1	201.5	2.21	0.321	13.54
7	17 0	159.9	201.5	2.37	**0.152**	**1.82**
8	23.0	159.9	201.5	**2.01**	0.302	13.60
9	15.0	145.0	145.0	2.75	0.248	3.88
10	25.0	145.0	145.0	2.34	0.430	31.51
11	20.0	120.0	145.00	2.40	0.330	8.61
12	20.0	170.0	145.0	2.19	0.180	4.01
13	20.0	145.0	50.0	2.03	0.297	10.02
14	20.0	145.0	240.0	2.38	0.183	1.90
15	20.0	145.0	145.0	2.29	0.240	13.54
16	20.0	145.0	145.0	2.46	0.232	5.23
17	20.0	145.0	145.0	2.41	0.244	4.89
18	20.0	145.0	145.0	2.29	0.200	5.59
19	20.0	145.0	145.0	2.20	0.238	17.20
20	20.0	145.0	145.0	2.30	0.250	12.00

Central composite rotatable design (two factors, five levels); 20 assays.
[a] does not correspond to order of processing.
Source: Author's own elaboration.

Optimization of extrusion process variables for producing optimized gluten-free healthy snack (OGFHS)

The global desirability (D) value obtained during the optimization of the extrusion process for producing OGFHS was 1.0 (maximum possible value), which corresponds to the extrusion conditions, where EI showed the maximum possible value. At the same time, AD and H had the minimum possible values (figure 1). Desirability values in the range of 0.7–1.0 provide an excellent and acceptable product. The best combination of extrusion process variables associated with the maximum global desirability was FMC = 16.4%/ET = 137 °C/SS = 233 rpm. The predicted values of EI, AD, and H, using each response variable's predictive models and the optimal extrusion conditions, were 2.9, 0.147 g/cm^3 and 2.90 N, respectively. OGFHS was produced by applying the best combination of extrusion process variables. The experimental values of EI, AD, and H of OGFHS (EI = 2.90/AD = 0.142 g/cm^3 /H = 2.76 N) were similar to the predicted values mentioned above, indicating that the optimal conditions of the extrusion process were appropriate and reproducible.
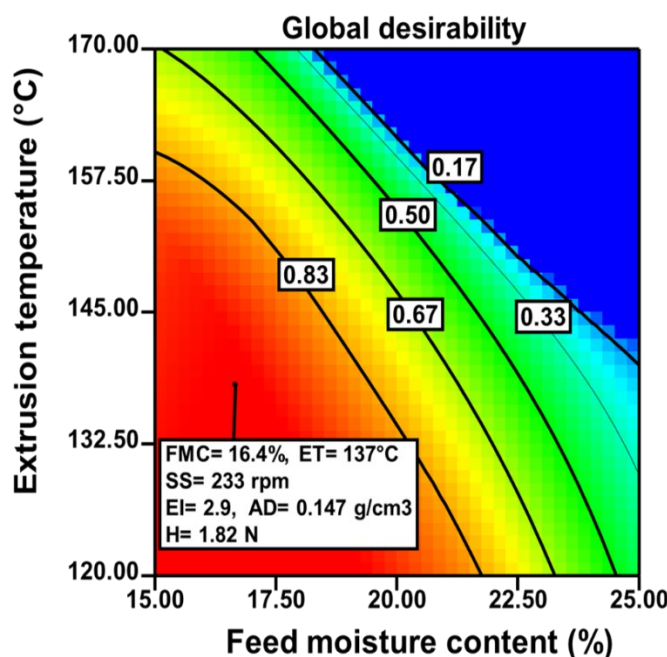

Figure 1. Global desirability plot showing the region of the best process variable combination (FMC, ET, SS) to obtain an optimized gluten-free healthy snack (OGFHS) with maximum EI, and AD and H minimums.
Source: Author's own elaboration.

Proximate composition and nutritional properties

Unprocessed grains mixture (UGM) (70% QPM+30% TP) was used as a reference. The protein contents of UGM and optimized gluten-free healthy snack (OGFHS) were similar ($p > 0.05$), whereas UGM had higher lipids and lower ashes content ($p < 0.05$) than OGFHS (table 2). The soluble (SDF), insoluble (IDF), and total dietary fiber (TDF) increased by 2.04%, 16.6%, and 12.96%, respectively, as a result of the extrusion process (table 2); while the lipid content in the OGFHS decreased 50.3% with respect to the unprocessed mixture.

Table 2. Nutrimental composition and physicochemical properties of optimized gluten-free healthy snack (OGFHS) elaborated from quality protein maize-tepary bean (70% QPM+30% TB) mixture.

Property	Unprocessed grains mixture	OGFHS
Chemical composition (%, DW)		
Proteins	13.71 ± 0.21^A	13.58 ± 0.18^A
Lipids	4.25 ± 0.05^A	2.11 ± 0.06^B
Minerals	2.37 ± 0.10^B	2.59 ± 0.02^A
Dietary fiber		
Soluble	4.90 ± 0.05^B	5.00 ± 0.04^A
Insoluble	14.91 ± 0.17^B	17.38 ± 0.21^A
Total	19.81 ± 0.17^B	22.38 ± 0.22^A
Carbohydrates	59.86 ± 0.83^A	59.34 ± 0.91^A
Physicochemical		
Total difference of color (ΔE)	21.57 ± 1.01^B	33.42 ± 0.87^A
Water activity (a_w)	0.57 ± 0.01^A	0.38 ± 0.01^B
pH	6.32 ± 0.06^B	6.57 ± 0.05^A

Data are expressed as means ± SD. In the same row, means with different superscripts (A-B) are different (t-student test, $p \leq 0.05$)
Source: Author's own elaboration.

The UGM and OGFHS showed higher His, Leu, Phe+Tyr, Met+Cys, Thr, Trp, and Val contents than those recommended by FAO (2013) for three year old children, adolescents, and older adults. Only OGFHS had a limiting essential amino acid (Isoleucine) (table 2). The OGFHS and UGM recorded chemical scores of 98% and 100%, respectively. The *in vitro* protein digestibility (IVPD) and calculated protein efficiency ratio (C-PER) values were 76.37% and 2.07, and 78.41% and 2.05 for the UGM and the OGFHS, respectively (table 3).

Table 3. Nutritional properties of optimized gluten-free healthy snack (OGFHS) from quality protein maize-tepary bean (70% QPM+30% TB) mixture.

Property	Unprocessed grains mixture	OGFHS	>3 years old children, adolescents, and older adults (FAO, 2013)
Nutritional			
EAA (g/100 g of protein)			
His	2.92 ± 0.02^B	3.04 ± 0.03^A	1.60
Ile	3.10 ± 0.03^A	2.95 ± 0.04^B	3.00
Leu	7.98 ± 0.04^A	7.79 ± 0.05^B	6.10
Lys	4.95 ± 0.02^A	4.78 ± 0.03^B	4.80
Phe + Tyr	7.22 ± 0.05^B	7.59 ± 0.02^A	4.10
Met + Cys	4.66 ± 0.04^A	4.14 ± 0.07^B	2.30
Thr	3.49 ± 0.03^A	3.46 ± 0.02^A	2.50
Trp	1.02 ± 0.02^A	0.92 ± 0.03^B	0.66
Val	5.26 ± 0.01^A	4.95 ± 0.05^B	4.00
Total	40.6	39.62	
Chemical score	100	98	
Limited EAA	None	Isoleucine	
In vitro protein digestibility (IVPD)	76.37 ± 0.15^B	78.41 ± 0.13^A	
Calculated protein efficiency ratio (C-PER)	2.07	2.05	

Data are expressed as means ± SD. In the same row, means with different superscripts (A-B) are different (t-student test, $p \leq 0.05$).
Source: Author's own elaboration.

Antioxidant activity (AoxA), total phenolic compounds (TPC), total flavonoids (TF) and condensed tannins (CT)

Table 4 shows the values obtained for AoxA, TPC, TF and CT from unprocessed grains (UGM) and optimized gluten-free healthy snacks (OGFHS). The AoxA values of the UGM were 2835 μmol TE/100 g, 8006 μmol TE/100 g and 10 841 μmol TE/100 g of sample (DW) for free, bound and total phenolic fractions, respectively. Simultaneously, the OGFHS showed AoxA values of 2891 μmol TE/100 g, 8274 μmol TE/100 g, and 11 165 μmol TE/100 g of sample (DW) for free, bound, and total phenolic fractions, respectively. The extrusion process did not show a significant ($p > 0.05$) effect on AoxA of free, bound, and total phenolic fractions. On the other hand, the extrusion process significantly ($p < 0.05$) increases the phenolic compounds content 62%, 8.2%, and 25.2% on the free, bound, and total phenolic fractions, respectively. Likewise, the free and total flavonoid fractions significantly ($p < 0.05$) increased by 27.3% and 3.5%, respectively, by the effect of the extrusion process (table 4).

The extrusion process decreases ($p < 0.05$) the bound flavonoid and condensed tannins contents in 11.4% and 56.1%, respectively (table 4).

Table 4. Antioxidant activity and phytochemicals of optimized gluten-free healthy snack (OGFHS) from quality protein maize–tepary bean (70%QPM+30%TB) mixture.

Property	Unprocessed grains mixture	OGFHS
Antioxidant activity (ORAC)		
Free phenolic	2835±89[A]	2891±289[A]
Bound phenolic	8006±341[A]	8274±190[A]
Total	10 841±395[A]	11 165±315[A]
Phytochemicals		
Phenolic compounds		
Free	50±2.85[B]	81±2.48[A]
Bound	109±2.04[B]	118±2.26[A]
Total	159±4.88[B]	199±4.98[A]
Flavonoids		
Free	22±0.75[B]	28±0.51[A]
Bound	35±1.21[A]	31±0.94[B]
Total	57±1.55[A]	59±1.41[A]
Condensed tannins	224.69±4.37[A]	98.67±2.31[B]

Data are expressed as means ± SD. In the same row, means with different superscripts (A-B) are different (t-student test, $p \leq 0.05$).
Source: Author's own elaboration.

Nutrimental and energetic content, antioxidant activity and sensory analysis

Table 5 shows the protein, dietary fiber, caloric content, AoxA, and sensory evaluation made of a fifty-gram portion of optimized gluten-free healthy snack (OGFHS) and two commercial snacks (Cheetos[MR] and Totis[MR]). The OGFHS portion showed, in dry weight, 6.24% proteins, 0.97% lipids, 1.19% minerals, 10.29% total dietary fiber, and158 kcal of caloric content. The protein, and total dietary fiber contents of OGFHS were higher than those of the commercial snacks (Cheetos[MR] and Totis[MR]). In addition, the OGFHS showed lower lipids and caloric content than Cheetos[MR] and Totis[MR]; therefore, according to those mentioned above, the OGFHS can be considered a better nutritious snack than the commercial snacks (Cheetos[MR] and Totis[MR]). Moreover, the AoxA of OGFHS was 6.7-6.8 folds higher than the evaluated commercial snack (table 5). Some parameters as expansion diameter, snack hardness and global acceptability (with 11 points hedonic scale, where 1 = Value maximum of imaginable dislike and 11 = Value maximum of imaginable like) were evaluated. The Totis[MR] snack showed lower values for the three parameters than Cheetos[MR] and OGFHS. The global acceptability values from OGFHS, Cheetos[MR], and Totis[MR] were 7.34, 7.81 and 5.66, respectively (table 5). These values are between *Either like neither dislike* and *I like very much*.

Table 5. Nutrimental and nutritional properties, antioxidant activity, and sensorial analysis of a fifty-gram portion of optimized gluten-free healthy snack (OGFHS) and expanded commercial snacks (Totis[MR], Cheetos[MR]).

Property	OGFHS	Totis[MR]	Cheetos[MR]
Chemical composition (%, DW)			
Proteins	6.24 ± 0.08^A	2.58 ± 0.02^B	2.30 ± 0.04^C
Lipids	0.97 ± 0.03^C	9.72 ± 0.27^B	16.80 ± 0.32^A
Minerals	1.19 ± 0.01^B	1.89 ± 0.03^A	1.05 ± 0.02^C
Total dietary fiber	10.29 ± 0.10^A	2.18 ± 0.02^B	1.97 ± 0.03^C
Carbohydrates	31.31 ± 0.42^B	33.63 ± 0.30^A	25.87 ± 0.33^C
Energy (kcal)	158^C	223^B	260^A
Nutraceutical			
Antioxidant activity	$5,136\pm145^A$	745 ± 45^B	755 ± 52^B
Physical properties			
Expanded diameter	7.38 ± 0.11^A	5.70 ± 0.62^B	7.25 ± 0.37^A
Hardness	7.41 ± 0.10^A	5.19 ± 0.58^B	7.66 ± 0.20^A
Sensorial analysis			
Global acceptability	7.34 ± 0.11^A	5.66 ± 0.47^B	7.51 ± 0.19^A

Data are expressed as means ± SD. In the same row, means with different superscripts (A-B) are different (Duncan, $p \leq 0.05$).
Source: Author's own elaboration.

Discussion

Regression analysis and predictive models of response variables (EI, AD, H)

To obtain the best extrusion conditions through the response surface methodology in this research, ranges of 15%-25%, 120 °C-170ºC and 50 rpm-140 rpm for the feed moisture content, temperature, and speed screw (respectively) were selected according to what was reported by Espinoza-Moreno *et al.* (2016) and preliminary tests in the laboratory. Likewise, to define the experimental region of exploration, the ranges of the process variables were selected as wide as possible within the region of operation of the process; that is, the values of the levels of the process variables were chosen in such a way that the limits were as wide as possible while all the experiments were feasible. This was for the purpose of fitting an adequate and reproducible second-order regression model for each of the response variables studied (EI, AD, H). Based on the experience with the optimization of different processes in the food area, it has been observed that when a wide range of operating conditions is selected from the process variables, more robust experimental mathematical models (with better statistical parameters) are obtained.

Meng *et al.* (2010) reported that the temperature plays a significant role in the rheological properties of extrudates affecting the expansion degree. The expansion decrement at a high temperature can result from an axial or longitudinal expansion increment, and high temperatures favor the axial expansion. Félix-Medina *et al.* (2020) reported that the higher expansion ratio is found at temperatures between 150 °C and 170 °C, and that the expansion decrease when protein and dietary fiber content increase in the samples affecting the gelatinization of starch by binding water more tightly due to the effect of non-starch polysaccharides from dietary fiber and the type of protein globulins than starch during the extrusion process. Stojceska *et al.* (2008) reported that AD values are highly related to moisture content and EI. Meng *et al.* (2010) observed that the AD decreases as the screw speed and temperature increase. Concerning the hardness of the GFHS, the present investigation results agree with those reported by other researchers (Altan *et al.*, 2008; Félix-Medina *et al.*, 2020), who observed a high correlation between the AD and the hardness of the extrudates. Low moisture contents in foods, low to intermediate temperatures, and high screw speed are recommended to obtain extrudates with low hardness; these same conditions are recommended to achieve a higher EI.

Proximate composition and nutritional properties

Cereals present a high starch content, which forms complexes with lipids during the extrusion process. This behavior could explain the lipid decrement in extruded products. Félix-Medina *et al.* (2020) elaborated a second-generation snack using whole maize-common bean mixture finding a decrease in the lipid content after extrusion of mixture. They reported that the extrusion conditions used, such as pressure, barrel temperature, cutting force and screw speed, could cause the complex formation between fatty acids and amylose of the starch of the material, making lipid extraction more difficult, showing an apparent decrease in this parameter.

Some authors (Espinoza-Moreno *et al.*, 2016; Maseta *et al.*, 2017; Reyes-Moreno *et al.*, 2012) have reported that a combination of maize and bean results in an enhancement of protein amino acid balance, finding relations of 78:22 of QPM maize and common bean (Mora-Avilés *et al.*, 2007), from 75:25 to 95:5 of maize and white common bean (Cuevas-Martínez *et al.*, 2010), 60:40 of QPM maize and common bean (Reyes-Moreno *et al.*, 2012), 70:30 amarantin transgenic maize and black common bean (Espinoza-Moreno *et al.,* 2016), and from 86:14 to 30:70 of maize and common bean (Félix-Medina *et al.,* 2020). They reported that these relations of flours covered at 100% the FAO essential amino acid pattern, excepting blends reported by Cuevas-Martínez *et al.* (2010). In the present work, the inclusion of 30% of tepary bean in

expanded snacks was based on Espinoza-Moreno *et al.* (2016) to obtain a product with an improved essential amino acids balance and without significantly sacrificing its physical properties.

Gutiérrez-Dorado *et al.* (2008) reported an *in vitro* protein digestibility (IVPD) value of 75.02% for extruded quality protein maize (QPM) flour. Moreover, they reported no significant difference between the IVPD values of unprocessed and extruded QPM flours. In contrast, the QPM extruded flour showed higher apparent IVPD values than unprocessed QPM flour. In the current research, it could be inferred that the extrusion process effect on QPM flour was minimal, since the limited EAA value (Isoleucine) was near to the FAO reference pattern value (2.95 *vs.* 3.0, respectively); however, the IVPD value was better after the extrusion process (76.37% *vs.* 78.41%, respectively).

Antioxidant activity (AoxA), total phenolic compounds (TPC), total flavonoids (TF) and condensed tannins (CT)

Gumul *et al.* (2010) and Félix-Medina *et al.* (2020) reported that the extrusion effect on AoxA depends on moisture content and processing temperature. The extrusion process induces the phytochemical release present in cellular walls, which are more available to quantify those (Garzón *et al.*, 2013).

Espinoza-Moreno *et al.* (2016) reported an increase in antioxidant activity (AAox) and total phenolic compounds (TPC) in a whole amarantin transgenic maize: black common bean blend after the extrusion process. They suggest that the increment in AAox could be related to the used extrusion temperature (157 °C), where i) antioxidant phenolic compounds could be released by destruction of cell wall during extrusion process; ii) inactivation of enzymes favor the prevention of phenolic compounds oxidation in the extruded product by processing; and iii) Maillard reaction products with antioxidant activity are formed during extrusion process from raw material containing amino acids and reducing sugars . Stojceska *et al.* (2008) elaborated extruded snacks from cauliflower-wheat-maize starch flour mixtures. They reported an increase of total phenolic compounds by the extrusion process's effect, according to the current research results. They indicated that this increment could be the consequence of water-stress, wounding, and high temperatures, inducing the enzymes synthesis responsible for phenolic compounds increment in the metabolic pathway. Félix-Medina *et al.* (2020) reported an increment in the phenolic compounds content in a maize-common bean second-generation snack, indicating that extrusion process produces Maillard reaction products, which absorbed light at the same wavelength than phenolic compounds and counted within the phenolic content value.

A daily intake of 3000 µmol-5000 µmol TE is recommended to keep an adequate level of antioxidants in our body (Keith, 1999). According to this recommended antioxidant intake, fifty-gram portions of OGFHS contribute with 62%-103% of recommended daily consumption (Keith, 1999).

On the other hand, James & Nwabueze (2013) reported a decrease in tannins content in snacks elaborated from an African breadfruit-soybean-maize blend, mentioning that this decrease notably favors the bioavailability of macromolecules such as protein, increases the palatability, reduces pathogenesis of cancer development, and reduces the injury on the intestinal tract. Likewise, Jadhav & Annapure (2013) reported a reduction above of 45% on tannins content of extruded snacks using different sorghum varieties with respect to unprocessed samples, suggesting that the tannins form complexes with other molecules as protein, carbohydrates, or minerals, which made them very hard to extract and quantify.

Nutrimental and energetic content, antioxidant activity and sensory analysis

Espinoza-Moreno *et al.* (2016) evaluated a comparison between their second-generation snacks, obtained from a whole amarantin transgenic maize and black common bean blend by extrusion process, and a commercial expanded snack (Cheetos[TM]). They evaluated chemical characteristics, as well as the antioxidant activity, finding that snack obtained from the flour blend showed higher values of protein, dietary fiber, and antioxidant activity than commercial snacks. They mentioned that this difference was due to the commercial snacks, which are mainly elaborated with corn starch while they used whole grains, thus, enriching the snack produced. The snack obtained in this work was compared with two commercial expanded snacks (Cheetos[MR] and Totis[MR]), evaluating the same parameters as mentioned above, finding a similar behavior. In addition, the OGFHS showed excellent physical parameters (expanded diameter and hardness) as well as good acceptability, similar to Cheetos[MR] commercial snack. This comparison with commercial snacks sparked the idea to compete in the snack market offering a healthy snack.

Conclusions

The best combination of extrusion process variables produced an optimized gluten-free healthy snack (OGFHS) with good expansion index, apparent density, hardness, and quality indicators for expanded snacks. The OGFHS had more proteins, total dietary fiber, AoxA, and total phenolic content and lower caloric content than expanded commercial snacks produced mainly from corn starch. The OGFHS, with its high content of quality protein, dietary fiber, phenolics, and high AoxA and low caloric content, could be used to promote health and prevent chronic diseases, as well as an alternative to commercial gluten-free food products with low nutritional/nutraceutical value.

Acknowledgments

Conflicts of interest

The authors declare no conflict of interest.

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