ORIGINAL PAPER



Healthy Ready-to-Eat Expanded Snack with High Nutritional and Antioxidant Value Produced from Whole Amarantin Transgenic Maize and Black Common Bean

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Published online: 12 May 2016 © Springer Science+Business Media New York 2016

Abstract The snack foods market is currently demanding healthier products. A ready-to-eat expanded snack with high nutritional and antioxidant value was developed from a mixture (70:30) of whole amarantin transgenic maize (*Zea mays* L.) and black common bean (*Phaseolus vulgaris* L.) by optimizing the extrusion process. Extruder operation conditions were: feed moisture content (FMC, 15–25 %, wet basis), barrel temperature (BT, 120–170 °C), and screw speed (SS, 50– 240). The desirability numeric method of the response surface methodology (RSM) was applied as the optimization technique over four response variables [expansion ratio (ER), bulk density (BD), hardness (H), antioxidant activity (AoxA)] to obtain maximum ER and AoxA, and minimum BD, and H values. The best combination of extrusion process variables for producing an optimized expanded

Electronic supplementary material The online version of this article (doi:10.1007/s11130-016-0551-8) contains supplementary material, which is available to authorized users.

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snack (*OES*, healthy snack) were: FMC = $15 \%/BT = 157 \circ C/$ SS = 238 rpm. The OES had ER = 2.86, BD = 0.119 g/cm³, H = 1.818 N, and AoxA = 13,681 µmol Trolox equivalent (TE)/ 100 g, dry weight. The extrusion conditions used to produce the OES increased the AoxA (ORAC: +18 %, ABTS:+20 %) respect to the unprocessed whole grains mixture. A 50 g portion of OES had higher protein content (7.23 vs 2.32 g), total dietary fiber (7.50 vs 1.97 g), total phenolic content (122 vs 47 mg GAE), and AoxA (6626 vs 763 µmol TE), and lower energy (169 vs 264 kcal) than an expanded commercial snack $(ECS = Cheetos^{TM})$. Because of its high content of quality protein, dietary fiber and phenolics, as well as high AoxA and low energy density, the OES could be used for health promotion and chronic disease prevention and as an alternative to the widely available commercial snacks with high caloric content and low nutritional/nutraceutical value.

Keywords Expanded snack · Amarantin transgenic maize · Black common bean · Extrusion · Optimization

Introduction

Several researchers [1, 2] recommend the consumption of whole grains their phytochemical content might be associated with lower risk of some types of cancer, type 2 diabetes and cardiovascular disease (CVD). Maize (*Zea mays* L) is the most highly produced cereal in the world and a major source of energy, proteins and other nutrients for both human and live-stock [3]. However, maize proteins are deficient in lysine and tryptophan (EAA = essential amino acids) providing only about one half of the recommendations for these EAA [4]. Rascón-Cruz et al. [5] expressed the main seed storage protein of

amaranth (Amarantin) in the kernel of common maize: they obtained a transgenic maize with increased amounts of lysine (+18 %) and tryptophan (+22 %). Recombinant Amarantin expressed in maize kernels was digested by simulated gastric fluid treatment. Amarantin transgenic maize did not induce important levels of specific IgE antibodies in BALB/c mice; it is not an allergenicity inducer [6]. Additional physicochemical, functional and nutritional studies on this transgenic maize and its products (flours, tortillas, snacks, etc.) are required to determine its potential industrial use and impact on human nutrition. Phytochemicals such as phenolic compounds (e.g. phenolic acids, flavonoids) have been reported in several maize genotypes; these phytochemicals have been related with anticarcinogenic effects [7] and antioxidant activities [8, 9].

Common bean (*Phaseolus vulgaris* L.) plays an important role in the diet of Latin American people, providing 20–40 % proteins, essential fatty acids, complex carbohydrates, vitamins and minerals; its consumption has been associated with reduced risk of cancer and heart diseases. These physiological effects might be related with the presence of some phenolic compounds (*e.g.* phenolic acids, flavonoids) [10, 11].

Cooking extrusion of starchy materials has become a technique applied to produce a wide range of food products for human consumption (instant flours, breakfast cereals, snacks) [12, 13], and the market of expanded products (snack foods) is increasing rapidly worldwide. These snacks are usually elaborated with maize grits because of its starch content, but they are considered high energy density products that promote weight gain and certain illnesses such as obesity and other related diseases (metabolic syndrome, cardiovascular events, hypertension, cancer) [14, 15]. This makes necessary some technological innovations that allow the production of healthy snack products with less carbohydrate, dyes, saturated fats and energy density, and improved nutraceutical quality; the use of whole grains, with the health benefits mentioned above, has been previously suggested for this purpose [14]. The nutritional value of cereal-based snack foods is low because of their poor protein quality. The incorporation of legumes represents an economic way to improve the biological value of proteins from cereal based foods; the proteins of maize and common bean complement one another by providing to each other significant amounts of the respective limiting amino acids [16]. Paredes-López et al. [17] evaluated the highest protein efficiency ratio (PER) value for a 60 % maize +40 % common bean mixture; the PER value increased from 1.4 in common bean and 1.0 in maize to 2.4 in the mixture. The nutritionally improved product could then be launched into the market on the basis of adding some utilities such as variety and novelty, which are important to modern consumers [18]. However, the use of combinations of cereals and legumes to produce snacks requires changes in the processing conditions to obtain products with optimum physical and functional characteristics.

The aim of this research was to optimize the extrusion process to develop a ready-to-eat expanded snack with high nutritional and antioxidant value from a mixture (70:30) of whole amarantin transgenic maize (*Zea mays* L.) and black common bean (*Phaseolus vulgaris* L.).

Materials and Methods

Materials

Kernels of Amarantin transgenic maize (genetically modified with the cDNA of Amarantin) line 1041/1.7k were obtained from T5 plants grown at the greenhouse of the Research and Advanced Studies Center of the National Polytechnic Institute (CINVESTAV-IPN), Campus Guanajuato, Mexico. The black beans (*Phaseolus vulgaris* L., var. Jamapa Black) grains and expanded commercial snacks (ECS = CheetosTM, crunchy cheese flavored snacks) were purchased at a local market in Culiacán, Sinaloa, México.

Methods

Expanded Snacks (ES) Preparation

Whole Amarantin transgenic maize or common bean kernels (1 kg lots) were grinded to obtain grits that passed through a 40-US mesh (0.425 mm) screen. Amarantin transgenic maize (ATMG) and common bean (CBG) grits were mixed to obtain lots of 250 g (175 g ATMG + 75 g CBG). These lots were conditioned with purified water until they reached moisture contents of 15-25 g H₂O/100 g wet grits according to the selected experimental design (Table 1); this moisture range was selected based on the literature and preliminary experiments. Each lot was packed in a polyethylene bag and stored at 4 °C for 12 h, followed by 1 h at 25 °C after extrusion. Extrusion cooking was carried out in a single screw laboratory extruder Model 20 DN (CW Brabender Instruments, Inc., NJ, USA) equipped with a 19 mm diameter screw, 20:1 length/ diameter, 3:1 nominal compression ratio, and 3 mm die opening. Extrusion conditions were selected from an axial combination of process variables: feed moisture content (FMC, 15-25 %), barrel temperature (BT = 120-170 °C) and screw speed (SS = 50-240 rpm) (Table 1). ES were cooled, equilibrated $(25^{\circ}C, RH = 65 \%)$, packed in hermetic plastic bags, and evaluated for expansion ratio (ER), bulk density (BD),

Assay	Process variables			Response variables			
	FMC (%, wb)	BT (°C)	SS (rpm)	Expansion ratio (ER)	Bulk density (BD) (g/cm ³)	Hardness (H) (N)	AoxA μmol TE/100 g, dw
1	17.0	130.1	88.5	2.42	0.320	21.26	10,572
2	23.0	130.1	88.5	2.08	0.575	32.73	12,657
3	17.0	159.9	88.5	2.46	0.178	8.16	12,276
4	23.0	159.9	88.5	2.00	0.306	45.33	13,047
5	17.0	130.1	201.5	2.84	0.197	20.42	11,577
6	23.0	130.1	201.5	2.22	0.489	5.28	12,020
7	17 0	159.9	201.5	2.50	0.148	31.05	12,713
8	23.0	159.9	201.5	2.22	0.351	29.45	10,614
9	15.0	145.0	145.0	2.76	0.162	9.69	11,820
10	25.0	145.0	145.0	2.18	0.420	21.85	12,228
11	20.0	120.0	145.00	2.22	0.463	6.99	12,203
12	20.0	170.0	145.0	2.20	0.254	21.69	13,242
13	20.0	145.0	50.0	2.02	0.401	39.80	12,403
14	20.0	145.0	240.0	2.67	0.236	38.11	11,578
15	20.0	145.0	145.0	2.37	0.267	14.40	11,587
16	20.0	145.0	145.0	2.32	0.316	14.58	11,311
17	20.0	145.0	145.0	2.35	0.295	17.11	11,703
18	20.0	145.0	145.0	2.20	0.312	17.78	11,432
19	20.0	145.0	145.0	2.18	0.257	14.15	11,325
20	20.0	145.0	145.0	2.30	0.312	17.60	11,981

 Table 1
 Experimental design used to obtain different combinations of feed moisture content (FMC)/extrusion temperature (ET)/screw speed (SS) for production of expanded snacks and experimental results for the response variables

hardness (H) and antioxidant activity (AoxA). These experimental values were used for optimizing the extrusion process.

Expansion Ratio (ER), Bulk Density (BD) and Hardness (H)

The ER was calculated as the cross-sectional diameter of the *ES* divided by the diameter of the die opening. The BD of the *ES* was determined using the equation: $BD = (4)(m)/[(\pi)(d)^2(L)]$; where: m = mass of the *ES* (g), d = diameter (cm) of *ES*, and L = length of *ES* (cm). The *H* was measured as the force (N) employed to penetrate 60 % of the *ES* diameter using a Texturometer Mod 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. ER, BD and *H* were measured over 100 *ES* pieces of 5 cm long.

Extraction of Free and Bound Phenolic Compounds

Free and bound phenolic compounds were extracted according Reyes-Moreno et al. [16], using 80 % chilled ethanol and ethyl acetate as solvents, respectively. All extractions were made by quadruplicated.

Antioxidant Activity (AoxA)

AoxA of free and bound phenolic extracts was determined using the ORAC and ABTS assays, using methodologies reported by Reyes-Moreno et al. [16] and Perales-Sánchez et al. [19], respectively. The results of AoxA were expressed as μ mol of Trolox equivalents (TE)/100 g sample (dry weight, dw). All measurements were made by triplicate.

Total Phenolic Content (TPC)

The *TPC* of free and bound extracts was determined according Reyes-Moreno et al. [16]. *TPC* was expressed as mg of gallic acid equivalents (GAE)/100 g sample (dw). All measurements were made by triplicated.

Proximate Composition

The official AOAC [20] methods were used to determine proximate composition. Dietary fiber and resistant starch (RS) were evaluated using Megazyme RS kit and Sigma-Aldrich (TDF 100A) kit, respectively. All measurements were made by triplicated.

Response Surface Methodology (RSM) Experimental Design, Statistical Analysis and Optimization

A central composite experimental design was chosen for RSM, with three factors [feed moisture content (FMC), barrel temperature (BT), and screw speed (SS)] and five variation levels (FMC = 15, 17, 20, 23, 25; BT = 120, 130, 145, 160, 170°C; SS = 50, 89, 145, 202, 240 rpm) (Table 1). Applying the stepwise regression procedure, non-significant terms (p > 0.1) were deleted from a second order polynomial and a new polynomial was used to obtain a predictive model for each response variable. The desirability method of the RSM was applied to determine the best combination of extrusion process variables (FMC, BT, SS). The four fitted models for the four dependent variables [expansion ratio (ER), bulk density (BD), hardness (H), antioxidant activity (AoxA)] were evaluated at any point $X = (X_1, X_2, X_3)$ of the experimental zone; four values were predicted for each model, \hat{Y}_1 (X), \hat{Y}_2 (X), \hat{Y}_3 (X) and \hat{Y}_4 (X). Each \hat{Y}_i (X) was transformed into a value $d_i(X)$, which falls in the range (0, 1) and measures the desirability degree of the response in reference to the optimum value intended to be reached. In this case, we wanted ER and AoxA to be as high as possible, as well as the minimum values for BD and H. The global desirability for the four response variables was determined from the four individual desirabilities with the mathematical function $D = (d_1d_2d_3d_4)^{1/4}$, 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 version 7.0.0 (Stat-Ease, Minneapolis, MN, USA) was used for the RSM analyses.

Results of chemical composition, AoxA and *TPC* of the optimized expanded snack (*OES*) and unprocessed whole grains mixture (70 % amarantin transgenic maize +30 % black bean) or expanded commercial snack (*ECS* = CheetosTM) were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test comparison among means with 5 % of significance level.

Results and Discussion

Predictive Models for Expansion Ratio (ER), Bulk Density (BD), Hardness (H) and Antioxidant Activity (AoxA) of Expanded Snacks (ES)

The ER, BD, H and $A_{ox}A$ experimental values of the *ES* varied from 2.00 to 2.84, 0.148 to 0.575 g/cm³, 5.28 to 45.33 N, and 10,572 to 13,242 µmol TE/100 g sample (dw), respectively (Table 1). Analysis of variance showed that BD, H and $A_{ox}A$ were significantly (p < 0.1) dependent on linear terms of FMC, BT and SS, while ER significantly depended of linear terms FMC and SS; interaction terms FMC-BT, FMC-

SS and BT-SS significantly affect $A_{ox}A$, while BD and *H* were significantly (p < 0.1) dependent on interaction terms FMC-BT and BT-SS. Quadratic term of FMC only affected the *ER* response, while quadratic term of BT affected the BD and AoxA responses; quadratic term of SS significantly not affected the responses restudied. Predictive models using uncoded variables for the response variables (*ER*, *BD*, *H*, $A_{ox}A$) were:

$$Y_{ER} = 6.18 - 0.36(FMC) - 2.48E^{-03}(SS) + 7.35E^{-03}(FMC)^2$$

$$Y_{BD} = 1.38 + 0.12(FMC) - 0.025(BT) - 0.005(SS) - 0.62E^{-03}$$
$$(FMC)(BT) + 3.29E^{-05}(BT)(SS) + 9.78E^{-05}(BT)^2$$

$$Y_{H} = -160.71 + 18.18 (FMC)$$

+ 0.83 (BT)-0.65 (SS)-0.10(FMC) (BT)
+ 4.07E^{-03}(BT) (SS)

$$Y_{AoxA} = -9307.71 + 2114.85 (FMC) - 136.39 (BT) + 114.28$$
$$(SS) - 10.90 (FMC) (BT) - 3.35 (FMC) (SS) - 0.35$$
$$(BT) (SS) + 1.45 (BT)^{2}$$

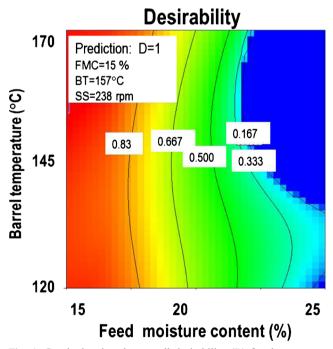


Fig. 1 Graph showing the overall desirability (D) for the response variables [expansion ratio (ER), bulk density (BD), hardness (H), antioxidant activity (AoxA)] to optimize the extrusion process and to find the best combination of process variables for producing an optimized expanded snack (OES = healthy snack) from a whole grains mixture (70 % rough whole amarantin transgenic maize +30 % rough whole black common beans)

The regression models explained 85, 94, 96 and 85 of the total variability (p < 0.0001) in ER, BD, H and A_{ox}A of ES, 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.

Optimization of Extrusion Process Variables for Producing an Optimized Expanded Snack (OES) with High ER/AoxA and Low BD/H Values

The overall desirability (**D**) value obtained during optimization of the extrusion process for producing OES was 1.0 (maximum possible value) which corresponds to the extrusion conditions where ER and AoxA showed the maximum possible

values, while *BD* and *H* had the minimum possible values (Fig. 1). Desirability values in the range of 0.7–1.0 provide a good and acceptable product [16]. The best combination of extrusion process variables associated with the maximum overall desirability was: FMC = $15 \%/BT = 157 \degree C/SS = 238 \text{ rpm}$. The predicted values of ER, BD, H and AoxA (ORAC), using the predictive models of each response variable and the optimal conditions of extrusion, were 2.94, 0.128 g/cm^3 , 2.76 N y 13, 078 µmol TE/100 g, dw, respectively. OES (healthy snack) was produced applying the best combination of extrusion process variables. The experimental values of ER, BD, H and $A_{ox}A$ of OES [ER = 2.86/BD = 0.119 $g/cm^{3}/H = 1.818 N/AoxA = 13, 681 \mu mol TE/100g$ sample (dw)] were similar to the predicted values, above mentioned, indicating that the optimal conditions of extrusion process were appropriated and reproducible.

 Table 2
 Chemical composition, antioxidant activity, and phenolics content of optimized expanded snack (healthy snack)^{a,b}

Property	Unprocessed whole grains mixture ^c	Optimized expanded snack ^d
Chemical composition (%, dw)		
Protein	$14.13\pm0.41^{\rm A}$	$14.59\pm0.65^{\rm A}$
Lipids	$2.44\pm0.11^{\rm A}$	$2.09\pm0.08^{\rm B}$
Ashes	$2.40\pm0.10^{\rm B}$	$2.63\pm0.06^{\rm A}$
Carbohydrates	$81.03\pm0.3^{\rm A}$	$80.69\pm0.9^{\rm A}$
Resistant starch	$0.85\pm0.07^{\rm B}$	$3.60\pm0.02^{\rm A}$
Dietary fiber		
Soluble	$1.93\pm0.02^{\rm B}$	$2.88\pm0.10^{\rm A}$
Insoluble	$11.24\pm0.01^{\rm B}$	$12.02\pm0.07^{\rm A}$
Total	$13.17\pm0.03^{\rm B}$	$14.95\pm0.03^{\rm A}$
Antioxidant activity (ORAC) ^e		
Free phenolics	$2846\pm173^{\rm B}$	$3409\pm185^{\rm A}$
Bound phenolics	$8755\pm705^{\rm B}$	$10,272 \pm 510^{\rm A}$
Total	$11,601 \pm 727^{\rm B}$	$13,\!681\pm 696^{\mathrm{A}}$
Antioxidant activity $(ABTS)^e$		
Free phenolics	$1200\pm98^{\rm B}$	$1888 \pm 113^{\rm A}$
Bound phenolics	$8293\pm498^{\rm B}$	$9499\pm534^{\rm A}$
Total	$9494\pm432^{\rm B}$	$11,\!362\pm557^{\rm A}$
Phenolic compounds ^f		
Free phenolics	$18\pm2.01^{\rm B}$	$34\pm0.99^{\rm A}$
Bound phenolics	$123 \pm 4.95^{\mathrm{B}}$	$214\pm17.2^{\rm A}$
Total	$140\pm5.01^{\rm B}$	$244\pm16.5^{\rm A}$

^a Data are expressed as means + standard deviations

^{b A-B} Means with different superscripts in the same row are significantly different (Duncan, $p \le 0.05$)

^c Unprocessed whole grains mixture (UWGM = 70 % rough whole amarantin transgenic maize flour + 30 % rough whole black common bean flour)

^d Optimized expanded snack (*OES*, healthy snack) obtained from a mixture (70 % amarantin transgenic maize +30 % black common bean) processing by extrusion at optimum conditions (FMC = 15 %, ET = 157°C, SS = 238 rpm)

^e µmol Trolox equivalents (TE)/100 g sample, dw

fmg Galic acid equivalents (GAE)/100 g sample, dw

Effect of Extrusion on Chemical Composition, AoxA and TPC of OES (Healthy Snack)

Unprocessed whole grains mixture (UWGM) (70 % rough whole amarantin transgenic maize +30 % rough whole black beans) was used as reference. The protein contents of UWGM and *OES* were similar (p > 0.05), while UWGM had higher lipids and lower ashes content (p < 0.05) than OES (Table 2). The soluble (SDF), insoluble (IDF) and total (TDF) dietary fiber increased by 49.2, 6.9 and 13.5 %, respectively, as a result of the extrusion process (Table 2). The increase in SDF may be caused by the release of oligosaccharides due to breakage of glycosidic bounds of polysaccharides by mechanical stress, while the increase in IDF can be due to the formation of materials resistant to enzymatic degradation such as starch (Table 2) and protein-polysaccharides complexes originated by heating-cooling cycles and the Maillard reaction, respectively [21]. The lectins activity (LA) of raw samples and OES was determined according to González de Mejia et al. [22] since dietary lectins have generally been considered to be toxic and antinutritional. LA was insignificant in OES [10 hemagglutinin activity units (HAU)/g of OES]. LA of black common bean (2560 HAU/g sample) was significantly reduced in the mixture (640 HAU/g mixture) with transgenic maize (LA non-detected), while this property was reduced 98.4 % by extrusion of the grains mixture.

The AoxA of OES evaluated in free, bound and total phenolics using the ORAC assay, increased by 19.8, 17.3 and 17.9 %, respectively, when compared with UWGM. When AoxA of OES was assessed by the ABTS assay in free, bound and total phenolics, the values were 57.3, 14.5 and 19.7 % higher than those from UWGM, respectively (Table 2). Xu and Chang [23] evaluated the AoxA (ORAC assay) of extruded green and yellow peas, and chickpea, and compared the values with those of raw materials; extrusion process increased the AoxA in 27-114, 12-67 and 25-40 %, respectively. The increase in AoxA could be a result of: i) release of antioxidant phenolic compounds during extrusion process [24, 25], ii) prevention of oxidation of phenolic compounds in the extruded product by enzymatic inactivation during the processing, and iii) the presence of Maillard reaction products with antioxidant activity, generated during extrusion of raw materials that contain amino acids and reducing sugars, such as maize and beans [26-29].

The extrusion process increased free, bound and total phenolic content in *OES* by 88.9, 74.0 and 74.3 %, respectively (Table 2), which may explain the improvement in *AoxA*. The increase of total phenolic content during the extrusion process may be due to (i) destruction of cell walls and the release of phenolic compounds, and ii) formation of Maillard reaction products that are quantified as phenolic compounds [24, 25, 27, 28]. Likewise, Zieliński et al. [24] reported that during the extrusion processing of grains the changes in the content of free phenolic acids were more intensive than those of ester bound phenolic acids. Similar results were found in the present study where the increase in the content of free phenolic compounds in *OES* was higher than that of bound phenolic compounds. Korus et al. [25] also reported that the effect of the extrusion process on phenolic compounds content and antioxidant activity depends significantly on the type and variety of grain used. They also found that the content of polyphenols and antioxidant activity of extruded material is highly dependent on the moisture content of raw material and extrusion temperature. The results of the present study also showed that the antioxidant activity of expanded snacks (*ES*) was affected by the extrusion parameters FMC, BT and SS (**Suppl. Fig. 2**).

Proximate Composition and Antioxidant Activity of OES

Fifty grams portions of *OES* or an expanded commercial snack (*ECS* = ChetosTM) produced mainly with corn starch contained 7.23 g and 2.32 g of proteins, respectively; this *OES* portion covers 55.6 and 38.05 % of the daily protein requirements for children 1–3 and 4–8 years, respectively. The higher content (p < 0.05) of proteins in the *OES* is due to the amarantin transgenic maize and its supplementation with common bean.

A 50 g portion of *OES* and CheetosTM contains 1.04 and 16.76 g of lipids, 32.83 and 25.91 g carbohydrate, 7.50 and 1.97 g of total dietary fiber, and 169 and 264 kcal, respectively. A source of fiber must contain at least 3 g or 3 % of the daily food intake, while the requirement for high fiber food is 6 g or 6 % of the recommended daily intake [30]. A 50 g portion of *OES* provides 7.5 g of total dietary fiber.

A 50 g portion of *OES* and CheetosTM had an antioxidant activity of 6626 and 763 µmol TE; where the portions of *OES* and CheetosTM contributes with 220–132 % and 25–15 % of the daily requirements of antioxidants (3000–5000 µmol ET), respectively [2].

Conclusions

The best combination of extrusion process variables produced an expanded snack with good expansion ratio, bulk density and hardness, which are quality indicators for expanded snacks, as well as high antioxidant activity (AoxA). The optimized extruded snack (OES) had more proteins, total dietary fiber, *AoxA* and total phenolic content, as well as lower energy than an expanded commercial snack (*ECS* = ChettosTM) produced mainly of corn starch. The *OES* with its high content of quality protein, dietary fiber and phenolics, as well as high AoxA and low energy density could be used to promote health and prevent chronic diseases, and as an alternative to commercial snacks with high-calories and low nutritional/ nutraceutical value that dominate the market.

Acknowledgments This research was supported by grants from Programa de Fomento y Apoyo a Proyectos de Investigación -Universidad Autónoma de Sinaloa (2013, 2014).

Compliance with Ethical Standards

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

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