Nutraceutical Beverage from a High Antioxidant Activity Mixture of Extruded Whole Maize and Chickpea Flours

Jesús J.Rochín-Medina[a], Roberto Gutiérrez-Dorado[a,b], Saraid Mora-Rochín[b], Sergio Medina-Godoy[c], ÁngelValdez-Ortiz[a,b], José A.López-Valenzuela[a,b], Francisco Delgado-Vargas[a,b], Jorge Milán-Carrillo[a,b], Cuauhtémoc Reyes-Moreno[a,b,]*

[a]Maestría en Ciencia y Tecnología de Alimentos, Facultad de Ciencias Químico Biológicas (FCQB), Universidad Autónoma de Sinaloa (UAS), Ciudad Universitaria, A.P. 1354, CP 80000, Culiacán, Sinaloa, México.

[b]Programa Regional del Noroeste para el Doctorado en Biotecnología, FCQB-UAS, Ciudad Universitaria, A.P. 1354, CP 80000, Culiacán, Sinaloa, México.

[c] Departamento de Biotecnología Agrícola, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional-Unidad Sinaloa del IPN, CP 81101, Guasave, Sinaloa, México.

*Corresponding author: Cuauhtémoc Reyes Moreno, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, México. Postal address: Lichis # 1986, La Campiña, CP 80060, Culiacán, Sinaloa, México.Tel/Fax: +52 (667) 7157641. E-mail: creyes@uas.uasnet.mx

Abstract

The objective of this investigation was to determine the best combination of extrusion process variables for the production of a high antioxidant capacity mixture of extruded whole maize (EMF) and chickpea (ECF) flours suitable to elaborate a nutraceutical beverage. Extruder operation conditions were extrusion temperature (ET, 120-170ºC) and screw speed (SS, 120-200 rpm). The desirability method was applied to obtain optimum maximum values for the two response variables (AoxA=Antioxidant activity, A=Aceptability). The best combinations of extrusion process variablesfor producing EMF and ECF to prepare anoptimized mixture [(60g EMF+40g ECF)(100g)$^{-1}$ mixture]were: ET=109°C/SS=158 rpm; and, ET=127°C/SS=151rpm, respectively. The optimized mixture had $A_{ox}A$=9,972 µmol Trolox equivalent (TE)(100 g)$^{-1}$ sample (dw), and acalculated protein efficiency ratio (C-PER)=2.24. A 200 mL portion of the beverage prepared with the optimized mixture had$A_{ox}A$=1,994 µmol TE, and A=85. The nutraceutical beverage could be used for health promotion and disease prevention.

Keywords: Antioxidant activity, extruded whole maize/chickpea, optimization, nutraceutical beverage

1. Introduction

Whole grains consumption has been associated with the prevention of cardiovascular disease (CVD), type 2 diabetes, and some cancers (Dixit et al., 2011). The USDA Dietary Guidelines for Americans emphasizes the need for consumption of whole grains (USDA, 2010). Maize (*Zea mays* L) is the most important cereal with a global production that exceeds 819 milliontonnes (Mt) (FAOSTAT, 2011). This cereal provides about 50% of the proteins and calories in the diet of developing countries. Maize kernel contains 69.6–74.5% (dw) carbohydrates, 7.7–13.6 % (dw) proteins, 3.2-7.7% (dw) fats, and some vitamins (B complex)and unsaturated fatty acids (oleic, linoleic). The maize proteins are deficient in the essential amino acids lysine and tryptophan. Phytochemicals such as phenolics compounds, amongst others have also been reported on several maize genotypes (López-Martínez et al., 2009). Maize has a higher antioxidant activity when compared to wheat, oat, and rice (Adom and Liu, 2002). The antioxidant properties of maize have been associated with anticarcinogenic effects (Liu, 2007).

Chickpea (*Cicer arietinum* L) is one of the most important grain-legume crops in the world, with a world production of 10.4 Mt (FAOSTAT, 2011). Chickpea seedscontain high levels of proteins (21.7-24.5%, dw), carbohydrates (61.1 - 67.4%, dw), and vitamins like thiamine and niacin as well as unsaturated fatty acids (oleic, linolenic). The chickpea proteins are deficient in sulfur amino acids (Met+Cys). Chickpea grains contain a wide range of phenolic compounds such as flavonols, flavone glycosides, and oligomeric and polymeric proanthocyanidinswhich could be considered as bioactive compounds due to their antioxidant capacity (Han and Baik, 2008).

When mixed together, the proteins of maize and chickpea are complementary; the blend shows a better quality protein profile characterized by an improved balance of essential amino acids (Alarcón-Valdez et al., 2005). Several studies have demonstrated that the mixture of cereal and legume improves significantly the protein efficiency ratio (PER). Paredes-López et al. (2006) reportedthe highest PER value for a 60:40 mixture of maize:common bean; the PER value increased from 1.0 in maize and 1.4 in common bean to 2.4 in the mixture.

Extrusion is a high temperature/short time technology that offers numerous advantages including versatility, high productivity,low operatingcosts, energy efficiency, high quality of resulting products and an improvement in digestibility and biological value of proteins(Gutiérrez-Dorado et al., 2008).The versatility of the extrusion process has allowed its use to elaborate several food products, including breakfast cereals, snacks, and precooked flours. The use of extruded flours to elaborate some food products has several advantages, since the extrusion process is accompanied by pre-gelatinization of starch granules, resulting in loss of the molecular order and the complete degradation of polymers with the formation of highly soluble fragments. Therefore, suspensions of flours precooked by extrusion are able to increase their viscosity rapidly, with a low tendency to form lumps, since starch granules have been modified showing high swelling capacity under both cold and hot conditions, which makes extruded flours highly recommended for preparation of instant food products (Vasanthan et al., 2001) such as beverages.

Some researchers (Serna-Saldívar et al., 1988; Milán-Carrillo et al., 2006) have extruded maize using lime to produce instant flours to elaborate tortillas as an alternative to the traditional nixtamalization process.In Mexico, instant flours from nixtamalized maize, as well as flours from raw, roasted, germinated, and fermented maize are used to elaborate beverages traditionally consumed as atole, pinole, tesgüino and pozol (Paredes-López et al., 2006). Precooked chickpea flours have also been produced using the extrusion technology (Milán-Carrillo et al., 2002); fragmented chickpeas are conditioned with salt solutions (softening) prior to extrusion in order to decrease the cooking time and the levels of some antinutritional factors.

Nutraceutical beverages represents one of the fastest annual growing markets worldwide,reaching a compound annual growth rate of 13.6 % between 2002 and 2007 (Heckman et al., 2010). The maincriterion for acceptance of this kind of drinks is the taste and acceptability; thus, formulation of high quality beverages with good taste is important for their adequate levels of consumption, which are needed for health promotion and disease prevention.

The objective of this investigation was to determine the best combination of extrusion process variables for the production of a high antioxidant capacity mixture from extruded whole maize and chickpea flours suitable to elaborate a nutraceutical beverage of good acceptability.

2. Materials and methods

2.1. Chemicals

The 2,2'-Azobis (2-amidinopropane) reagent was obtained from Sigma Chemical Co. (St Louis, MO, USA). Sodium hydroxide, hexane, methanol, ethanol and ethyl acetate were purchased from DEQ (Mexico). All reagents used were of analytical grade.

2.2. Grains

Maize hybrid 30P49 from Pioneer and chickpea var. Blanco Sinaloa 92 were provided by the National Research Institute for Forestry, Agriculture and Livestock (INIFAP), Culiacán, Sinaloa, México.

2.3. Production of extruded maize (EMF) and chickpea (ECF) flours

The flours were prepared as recommended byMilán-Carrillo et al. (2006).Whole maize or chickpea kernels (1 kg lots) were placed in a domestic blender at high velocity to obtaingritsthat passed through a 40-US mesh (0.425 mm) screen. Maize grits were mixed with lime [0.21g lime(100 g)$^{-1}$ grits] and water, while chickpea grits were mixed with a salt solution [(2.5g NaCl + 7.5g $NaHCO_3$)L^{-1} distilled water], in both cases to reach a moisture content of 28gH_2O (100 g)$^{-1}$wet grits. Each lot was packed in a polyethylene bag and stored for 12 h at 4ºC. Prior to extrusion, grits were tempered at 25ºC for 1 h. Extrusion cooking was done using a single-screw extruder Brabender 20 DN (CW Brabender Instruments, Inc, NJ, USA) with a 19 mm screw diameter, length-to-diameter 20:1, nominal compression ratio 1:1, and die opening of 3 mm. The single screw extruder operation conditions were selected from a factorial combination of process variables: extrusion temperature (ET, 120-170ºC) and screw speed (SS, 120-200 rpm). Extrudates were cooled, equilibrated at environmental conditions (25ºC, 65% RH), milled to pass through an 80-US mesh (0.180 mm) screen, and packed in plastic bags. Extruded wholemaize (EMF) and chickpea (ECF) flours were stored at 4ºC until use.

2.4. Beverage preparation

Each flour mixture (22 g) was added with sucrose (3.2 g), low calorie sweetener (Azúcar BC Metco®, containing a mixture of sucrose, high intensityedulcorants, inulin, and fructo-oligosacarides with two times the edulcorant power of sucrose) (4.8 g), powder vanillin (1 g) and purified water (168 mL); the suspension was stirred in a domestic shaker (medium velocity), refrigerated (8-10ºC) and sensory evaluated for acceptability (*A*). All determinations were made by triplicate.

2.5. Proximate composition

The following Association of Official Analytical Chemists (AOAC, 1999)methods were used to determine proximate composition: Drying at 105ºC for 24 h, for moisture (method 925.09B); incineration at 550ºC, for ashes (method 923.03); defatting in a Soxhlet apparatus with petroleum ether, for lipids (method 920.39C); and microKjeldahl for protein (Nx6.25) (method 960.52). Carbohydrate content was estimated by difference. All determinations were made by triplicate.

2.6. Extraction of free phenolic

Free phenolic compounds in ground samples were extracted as previously reported by Dewanto et al. (2002) with minor changes. A dry ground sample of 1.0 g was mixed with 10 mL of chilled ethanol–water (80:20, v/v) for 10 min in a shaker at 50 rpm. Then, blends were centrifugated (3000*g*, 10 min) (Sorvall RC5C, Sorvall Instruments, Dupont, Wilmington, DE, USA) in order to recover the supernatant. The extracts were concentrated to 2 mL at 45ºC using a vacuum evaporator (Savant

SC250 DDA Speed Vac Plus centrifugal, Holbrook, NY, USA) and stored at -20°C until use.All extractions were made by quadruplicate.

2.7.Extraction of boundphenolic

Bound phenolic compounds in maize samples were extracted according to the method recommended by Adom andLiu (2002) with minor modifications. After extraction of free phenolic compounds, the pellet was resuspended in 10 mL of 2 mol L^{-1} NaOH at room temperature and nitrogen was flushed to displace air present in the tube headspace before digestion. Samples were hydrolyzed at 95ºC and 25ºC in a shaking water bath oscillating at 60 rpm for 30 and 60 min, respectively. The hydrolyzed was neutralized with an appropriate amount of HCl before removing lipid with hexane. The final solution was extracted five times with 10 mL of ethyl acetate and the pool was evaporated to dryness. Bound phenolic compounds were reconstituted in 2 mL of 50% methanol and stored at -20ºC until use. All extractions were made by quadruplicate.

***2.8.*Total antioxidant activity (*AoxA*)**

Free and bound hydrophilic antioxidant activities were determined using the oxygen radical absorbance capacity (ORAC) assay. Extracts were evaluated against a standard of Trolox with Fluorescein as a probe as described initially by Cao et al. (1993) and later modified by Ou et al. (2001). Peroxyl radicals were generated by 2,2-azobis (2-amidinopropane) dihydrochloride, and fluorescent loss was monitored in a Micro- plate Reader (SynergyTM HT Multi-Detection, BioTek, Inc., Winooski, VT). The absorbance of excitation and emission was set at 485 and 538 nm, respectively. Data was expressed as micromoles of Trolox equivalents (TE) per 100 g of dry weight (dw) sample.All measurements were made byquadruplicate.

***2.9.In vitro* protein digestibility (IVPD)**

The IVPD was determined according to Hsu et al. (1977) using a multi-enzyme system, consisting of a mixture of porcine pancreatic trypsin type IX, bovine pancreatic chymotrypsin type II and porcine intestinal peptidase grade III (Sigma). A 50 mL aqueous protein suspension (6.25 g of proteinL^{-1}), pH 8.0, was prepared while stirring in a water bath at 37ºC. Five milliliteraliquots of the multi-enzyme solution were added to the protein suspension with stirring at 37ºC. The rapid pH drop was recorded automatically over a 10 min period using a pH meter. IVPD was calculated from the equation IVPD = 210.46 – 18.10 X, when X = pH after 10 min. All determinations were made by triplicate.

2.10. Essential amino acid analysis

Essential amino acid composition was determined using the method described by Lopez-Cervantes et al.(2006). Fiftymg of sample flour were mixed with 10 mL of 6 mol L^{-1} HCl and incubated for 24 h at 100ºC. The hydrolyzed sample was filtered and the extract diluted 200 times with milliQ water. A 300 µL aliquot of the extract was dried and derivatized with 300 µL of 9 fluorenymethyl-chloroformate (FMOC). A 20 µL aliquot was analyzed using an analytical scale (4.6 mm x 250 mm) SGE Hypersil ODS C18 column (SGE, Dandenong, Australia) kept at 38ºC and connected to an HPLC system (GBC, Dandenong, Australia) equipped with a fluorescence detector LC 5100. The mobile phases used were as follows: (A) 30 nmol L^{-1} ammonium phosphate (pH 6.5) in 15:85 (v/v) methanol/water; (B) 15:85 (v/v) methanol/water; and (C) 90:10 (v/v) acetonitrile/water. The flow rate was 1.2 m$Lmin^{-1}$ and the gradient programme used was reported in Table 1 by López-Cervantes et al. (2006). Fluorescence detection was at 270 nm and 316 for excitation and emission, respectively. A calibration curve was constructed using a mix of standard amino acids.

Tryptophan levels were determined using an alkaline hydrolysis. 25 mg of sample were mixed with 3 mL of 4.2 mol L^{-1} NaOH and incubated in sealed tubes (N_2 athmosphere) at 120ºC for 4 h. After hydrolysis, the sample was adjusted to pH 9, washed with borate buffer (pH 9), vaccum filtered and then diluted to 50 mL with borate buffer. After centrifugation, the supernatant was filtered (0.45 µm) and then a 20 µL aliquot was analyzed as described above. Tryptophan was detected at 280 nm with an ultraviolet detector. All determinations were made by triplicate.

2.11. Chemical score (CS)

The chemical score is a measure of protein quality based on the amino acid composition. The content of each of the essential amino acids in the sample was compared with that recommended for children 10 to 12 years old (FAO/WHO/UNU, 1985) to identify the most limiting amino acid. The chemical score was calculated as follows:

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

Where CS is the chemical score; EAA is the essential amino acid and REAAR is the recommended amino acid requirement. All determinations were made by triplicate.

2.12. Calculated protein efficiency ratio(C-PER)

C-PER was calculated as decribed by Satterlee et al. (1982)and summarised by the AOAC (1999). This procedure is based on the IVPD and the essential amino acids (EAA) composition of the optimized mixture. All determinations were made by triplicate.

2.13. Sensory evaluation

The sample volume used for sensory evaluation of each beverage was 20 liters and the total number of different beverages tested was 30, based on the different extrusion conditions used (Table 1). A different beverage was prepared and evaluated every day. The beverages were evaluated after 30 min of preparation, at room temperature. Sensory evaluation of each beverage was done using a panel of 60 judges.Panelists were seated in individual booths in a laboratory with controlled temperature (25°C) and humidity (50-60%), and day-light fluorescent lights.Samples were rated on a 100 mm bidirectional scale LAM(labeled affectivemagnitude) [-100 (maximum valueofimaginable dislike) to+100 (maximum value of imaginable like); zero(I do not like, nor dislike) is thecentralpoint] for one attribute: Acceptability (*A*). Subsequentlythesevaluesweretransformedtoa scalefrom zero to 100 [0 =maximumvalueof imaginabledislike, 100 =maximumvalue of imaginable like; 50=I do not like, nor dislike]. Thistransformationwasperformedtoobtainpredictivemathematical modelfor*A*(Cardello and Schutz, 2004).

2.14. RSM experimental design and statistical analysis

Response surface methodology (RSM) was applied to determine the best combination of extrusion process variables[for extruded whole maize (EMF) and chickpea (ECF) flours] to produce a mixture [(60g EMF + 40g ECF)(100 g)$^{-1}$ mixture] with high antioxidant activity and suitable to elaborate a beverage with high acceptability.The independent process variables were: Extrusion temperature (ET, 120-170ºC) and screw speed (SS, 120-200 rpm). The dependent response variables chosen were antioxidant activity ($A_{ox}A$) of the mixtures and acceptability (*A*) of the beverages produced from them. A central composite experimental design with four factors (ETM = Extrusion temperature for maize, SSM = Screw speed for maize, ETC = Extrusion temperature for chickpea, SSC = Screw speed for chickpea) and five variation levels was chosen. The following empirical "black box" modeling represents the relationships among process (ETM, SSM, ETC, SSC) and response (*AoxA, A*) variables:

Process variablesProcess Response variables

X_1= ETM
X_2=SSM Y_1 = *AoxA*
X_3=ETC Y_2 = *A*
X_4= SSC

$$Y_k = \beta_{k0} + \sum_{i=1}^{4} \beta_{ki} X_i + \sum_{i=1}^{4} \beta_{kii} X_i^2 + \sum_{i=1}^{4} \sum_{j=1+1}^{4} \beta_{kij} X_i X_j + \epsilon$$

The expression inside the "black box" represents $A_{ox}A$ and A when the value of k is changed from 1 to 2;k_0,k_{ii} and k_{ij} represent the constant and coefficients of linear, quadratic and interaction effects, respectively; X_i, X_{ii}^2 and X_iX_j represent the linear, quadratic and interaction effect of the independent variables, respectively,whileεis the random error primarily to account for the inability to determine the true model. Applying the stepwise regression procedure, non-significant terms (P> 0.1) were deleted from the second order polynomial and a new polynomial was recalculated to obtain a predictive model for each response variable (Khuri and Cornell, 1987). The statistical analysis system software Design Expert version 7.0.0.was used for analysis and evaluation (Design Expert, 2005).

2.15. Optimization

The desirability method described by De la Vara andDomínguez (2002) was used to find the best combination of extrusion process variables[extrusion temperature for maize (ETM)/screw speed for maize (SSM) –extrusion temperature for chickpea (ETC)/screw speed for chickpea (SSC)]that will result in a mixture [(60g EMF + 40g ECF)(100 g)$^{-1}$ mixture] with optimum values for the two dependent variables ($A_{ox}A$, A). The two fitted models can be evaluated at any point X = (X_1, X_2, X_3, X_4) of the experimental zone and as a result two values were predicted for each model, namely $\hat{Y}_1$ (X) and $\hat{Y}_2$ (X). Then each $\hat{Y}_i$ (X) is 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 intented to be reached. In this research, we wanted all response variables to be as high as possible. Thus, the transformation is:

$$d_i(x) = \begin{cases} 0 & \text{if } \hat{Y}_i(x) \le Y_{i*} \\ \dfrac{\hat{Y}_i(x) - Y_{i*}}{Y_i^* - Y_{i*}} & \text{if } Y_{i*} \le \hat{Y}_i(x) \le Y_i^* \\ 1 & \text{if } \hat{Y}_i(x) \ge Y_i^* \end{cases}$$

Where: d_i(X) = Value of the desirability of the ith response variable, $\hat{Y}_i$(X) = Estimated response variable, Y_i* = Maximum acceptable value of the ith response variable, Y_{i*} = Minimum acceptable value of the ith response variable. Once the two individual desirabilities were calculated, the next step was to obtain the global desirability for the two response variables, using the mathematical function of transformation $D = (d_1d_2)^{1/2}$, where the ideal optimum value is D = 1; an acceptable value for D can be between 0.6 and 0.8 (0.6 < D < 0.8). This acceptable value was found by using the Design Expert program version 7.0.0. (Design Expert, 2005).

2.16. Statistical analysis

The resultsforantioxidant activity of the unprocessed mixture and optimized extruded mixture were analysed using one-way analysis of variance (ANOVA) followed by Duncan´s multiple range test comparisons among means with significance level of 5%.

3. Results and discussion

3.1.Predictive model for antioxidant activity ($A_{ox}A$)

The $A_{ox}A$ values of the mixtures [(60gEMF + 40gECF)(100 g)$^{-1}$ mixture] varied from 6,509 to 10,369 μmol TE (100 g)$^{-1}$ of mixture (dw) (Table 1). Analysis of variance showed that $A_{ox}A$ was significantly dependent on linear terms of ETM (P<0.0001), and ETC (P=0.0122), quadratic terms of ETM, SSM and SSC [$(ETM)^2$ (P=0.0015); $(SSM)^2$ (P=0.0405); $(SSC)^2$ (P=0.0741)] and SSM-ETC interaction [(SSM)(ETC) (P<0.0001)]. Predictive model using original variables for the $A_{ox}A$ of the mixtures was:

$A_{ox}A$ = - 14344.67 + 116.47 ETM + 121.83 SSM+ 84.87 ETC + 32.46 SSC – 0.51 $(ETM)^2$ – 0.12 $(SSM)^2$ – 0.10 $(SSC)^2$ – 0.59 (SSM)(ETC)

The regression model explained 85.23% of the total variability (P< 0.0001) in the $A_{ox}A$ of the mixtures (Table 2).

3.2. Predictive model for acceptability (*A*)

The *A* values of beverages obtained from the mixtures [(60gEMF + 40g ECF)$(100\ g)^{-1}$ mixture] varied from40 to 90 (Table 1). Analysis of variance showed that *A*was significantly dependent on linear terms of ETM (P=0.066), and ETC (P<0.0001), quadratic terms of SSM and ETC [$(SSM)^2$ (P=0.0006); $(ETC)^2$ (P<0.0001)] and ETM-SSM, and SSM-ETC interactions [(ETM)(SSM) (P=0.0160); (SSM)(ETC) (P=0.0754)].Predictive model using original variables for the *A* of the beverages was:

A = 54.71 – 0.48 ETM – 0.037SSM – 2.80 ETC – 0.019 SSC – 1.90 $(SSM)^2$ – 0.012 $(ETC)^2$ + 0.0026 (ETM)(SSM) + 0.0019 (SSM)(ETC)

The regression model explained 90.35% of the total variability (P<0.0001) in the *A* of the beverages prepared from the mixtures (Table2).

3.3. Optimization

The common maximum values for the two dependent response variables were obtained at a global desirability (D) valueof 0.907, as a result ofthe best combination of extrusion process variables for the production of extruded maize (EMF: ETM=109ºC/ SSM=158 rpm) and chickpea (ECF: ETC= 127ºC / SSC = 151 rpm) flours to prepare a mixture [(60g EMF + 40g ECF)$(100\ g)^{-1}$ mixture]with high antioxidant activity and suitable to elaborate a beverage with high acceptability (Fig 1). The D value obtained was higher than that considered to be acceptable (0.6 < D < 0.8) (De la Vara and Domínguez, 2002). This mixture was recognized as the optimized mixture and it was used to determine its chemical, nutritional and antioxidant properties.

3.4. Chemical composition and nutritional propertiesof the optimizedmixture

The optimized mixture contained 16.57 g proteins, 3.87 g lipids, 76.24 g carbohydrates, and 3.32 g ashes $(100\ g)^{-1}$of sample(dw) (Table 3).

The EAA content of the optimized mixture was found to be higher than the requirements for children 10 -12 years old, its EAA score was 100, and did not present limiting EAA. The optimized mixture had *in vitro* protein digestibility (IVPD) and calculated protein efficiency ratio (C-PER) values of 89.1% and 2.24, respectively (Table 3). Serna-Saldívar et al. (1999) reported, for wheat bread fortified with defatted soybean and sesame meals IVPD´s and C-PER´s of 83.1-84.87% and 1.22-1.35, respectively. Alarcón-Valdez et al. (2005)prepared an infant food based in a mixture of nixtamalized maize (26.7%) and extruded chickpea (73.3%) flours; this food had values of IVPD and C-PER of 87.9% and 1.86, respectively. According to Serna-Saldívar et al. (1999) the C-PER technique predicted the same differences observed in the rat bioassay. Therefore, it is recommended to use the *in vitro* techniques as fast, accurate indicators of protein digestibilities and PER´s.

3.5. Total antioxidant activity of the optimized mixture

The optimized mixture produced from extruded whole maize and chickpea flours retained more than 91% the antioxidant activity measured by ORAC in the unprocessed mixture [9,972 vs 10,897 µmol Trolox equivalent (TE) $(100\ g)^{-1}$ sample (dw)] (Table 4). It was observed that the ORAC value of free phenolic and bound phenolic significantly increased (p<0.05) and decreased (p<0.05) in extruded optimized mixture, respectively, when compared with unprocessed mixture (Table 4). This behavior could be attributed to (i) breaking of conjugated phytochemicals and release free phytochemicals

(Dewato et al., 2002), (ii) prevention of enzymatic oxidation and, (iii) darker color of the extruded optimized mixture indicating formation of Maillard reaction products having antioxidant properties (Fares and Menga, 2012). Our results show that the bound phytochemicals were the primary contributors (72.97-81.00 %) to ORAC value in both unprocessed and extruded optimized mixture (Table 4). Bioactive phytochemicals exist in free, soluble conjugated, and bound form; bound phytochemicals, mostly in cell wall materials, are difficult to digest in the upper gastrointestine and may be digested by bacteria in the colon to provide health benefits and reduce the risk of colon cancer (Adom and Liu, 2002; Liu, 2007).

O"zer et al.(2006)studied the effect of screw speed, feed moisture content and feed rate on the total phenolics concentration and antioxidant activity of an extruded snack (chickpea + corn + oat + corsnstarch + carrot + raw hazelnut); they used an extrusion temperature of 110ºC. These researchers found that the extrusion conditions did not change the total phenolics concentration in the samples but the total antioxidant activity decreased (33.8-45.11%)as the screw speed increased (280-330 rpm) and the moisture content decreased (13-11 %). Delgado-Licon et al. (2009) evaluated the influence of extrusion on the bioactive compounds and the antioxidant activity ofbean+corn mixtures. Extrusion was performed using different moisture and temperature conditions. The best extrusion procedure was achieved using feed moisture content, extrusion temperature and screw speed of 16.3%, 142ºC and 90 rpm, respectively, resulting in the highest contents of polyphenols and flavonoids and antioxidant activity.

3.6. Formulation, nutrimental composition, energy content and antioxidant activity of the nutraceutical beverage

The formulation of a 200 mL portion of the 30 different beverages prepared from different flour mixtures (Table 1) and the beverage prepared from the optimized flour mixture was based on those of traditional beverages widely consumed in Mexico, which are produced from different grain flours (for example, rice, barley), as well as sensorial tests to define the proper amounts for each ingredient (data not shown). The Mexican norm NMX-F-439-1983 for foods and non-alcoholic beverages was also considered. This norm defines a nutritious beverage when it contains at least 1.5% protein or protein hidrolyzates with a quality equivalent to that of Casein; it also establishes that the beverage must contain 10 to 25% of the main ingredientused to prepare it; these beverages can also contain up to 2% ethanol, edulcorants, flavouring agents, carbon dioxide, juices, fruit pulp, vegetables or legumes and other additives authorized by the Health and Assistance Secretary of Mexico. All the formulations used in this study contained 11% of the flour mixture and 1.66 % proteins of good quality. Besides, these beverages contained both sucrose and a low calorie sweetener for two reasons: 1) to satisfy the recommendations of the Health and Assistance Secretary of Mexico, regarding the fact that a 200 mL portion of a beverage (food) must contain no more than 100 kcal, and 2) to maintain a high sensorial acceptability.

The 200 mL portion of the beverage prepared with 22 g of the optimized mixture contained 3.31 g proteins, 0.77 g lipids, 19.5 g carbohydrates and 98 kcal. This portion covers 26.36% and 17.65% of the daily protein requirements forchildren 1-3and4-8years old, respectively.This nutraceutical beverage (200 mL) showed a total antioxidant activityof 1,994 μmol TE, which contributes with 39.80-66.44% ofthe recommended (3,000 to 5,000μmolTE) daily intake for antioxidants (USDA, 2010). The semi-trained panelists asigned an average value of 85 in acceptablility to the beverage (level of satisfaction between "I like it" and "I like it extremely"). It is expected that this acceptability allows an adequate consumption to provide health benefits.

The high nutritional, antioxidant and sensory value of this beverage can be attributed to the use of whole maize and chickpea grains mixture and the application of optimum extrusion processing conditions. This nutraceutical beverage could be usedfor health promotion and disease prevention as an alternative to beverageswith low nutritional / nutraceutical value.

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References

Adom, K. K., & Liu, R. H. (2002). Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry*, 50, 6182-6187.

Alarcón-Valdez, C., Milán-Carrillo, J., Cárdenas-Valenzuela, O. G., Mora-Escobedo, R., Bello-Pérez, A., & Reyes-Moreno, C. (2005). Infant food from quality protein maize and chickpea: Optimization for preparing and nutritional properties. *International Journal of Food Sciences and Nutrition*, 56, 273-285.

Association of Official Analytical Chemists (AOAC). (1999). *Official Methods of Analysis*(16th ed). Association of Official Analytical Chemists, Washington, DC, USA.

Cao G, Alessio HM, Culter R. (1993). Oxygen-radical absorbance capacity assays for antioxidants. *Free Radical Biology & Medicine*, 1, 303-311

Cardello, A. V., & Schutz, H. G. (2004). Research note numerical scale-point locations for constructing the LAM (labeled affective magnitude) scale. *Journal of Sensory Studies*,19, 341-346.

Delgado-Licon, E., Martínez- Ayala, A. L., Rocha-Guzmán, N. E., Gallegos-Infante, J. A., Atienzo-Lazos, M., Drzewiecki, J., Martínez-Sánchez, C. E., & Gorinstein, S. (2009). Influence of extrusion on the bioactive compounds and the antioxidant capacity of the bean/corn mixtures. *International Journal of Food Science and Nutrition*, 60, 522-532.

De la Vara, S. R., & Domínguez, D. J. (2002). Métodos de Superficie de Respuesta; un Estudio Comparativo. *Revista de Matemáticas: Teoria y Aplicaciones*,1, 47-65.

Design Expert. (2005). *Version 7.0.0.* by *Stat-Ease*. Minneapolis, MN, USA, Design Expert Inc.

Dewato, V., Wu, X., & Liu, R. H. (2002). Processed sweet corn has higher antioxidant activity. *Journal of Agricultural and Food Chemistry*, 50, 4959-4964.

Dixit, A. A., Azar, K. M. J.,Gardner, C. D.,&Palaniappan, L. P. (2011). Incorporation of whole, ancient grains into a modern Asian Indian diet to reduce the burden of chronic disease. *Nutrition Reviews*,69, 479-488.

FAOSTAT. (2011). Statistical database; Online referencehttp://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor.Accessed 28/10/2011.

FAO/WHO/UNU Expert Consultation. (1985). Energy and protein requirements. WHO Tech Rep Ser No 724. Word Health Organization: Geneva.

Fares, C.,& Menga, V. (2012). Effects of toasting on the carbohydrate profile and antioxidant properties of chickpea (*Cicer arietinum* L) flour added to durum wheat. *Food Chemistry,* 131, 1140-1148.

Gutiérrez-Dorado, R., Ayala-Rodríguez, A. E., Milán-Carrillo, J., López-Cervantes, J. A., Garzón-Tiznado, J. A., López-Valenzuela, J. A., Paredes-López, O., & Reyes-Moreno, C. (2008).

Technological and nutritional properties of flours and tortillas from nixtamalized and extruded quality protein maize (*Zea mays* L). *Cereal Chemistry*, 85, 808-816.

Han, H. & Baik, B.K. 2008. Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*), and their quantitative changes during processing. *International Journal of Food Science and Technology*, 43**,** 1971–1978.

Heckman, M. A., Sherry, K., & González de Mejía, E. (2010). Energy drinks: an assessment of their market size, consumer demographics, ingredient profile, funcionality, and regulations in the United States. *Comprehensive Reviews in Food Science and Food Safety*, 9, 303-317.

Hsu, H. W., Vavak, D. I., Satterlee, I. D., & Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science*, 42, 1269-1273.

Khuri, A. A., & Cornell, J. A. (1987). *Response Surfaces: Designs and Analyses*. Pp 1-17, 254. New York, NY, USA: Marcel Dekker Inc.

Liu, R. H. (2007). Whole grain phytochemicals and health. *Journal of Cereal Science*, 46, 207-219.

López-Cervantes, J., Sánchez-Machado, D. I., & Rosas-Rodríguez, J. A. (2006). Analysis of free amino acids in fermented shrimp waste by high-performance liquid cromatograph. *Journal of Chromatography*, 1105, 106-110.

López-Martínez, L. X., Oliart-Ros, R. M., Valerio-Alfaro, G., Lee, C. H., Parkin, K. L., & García, H. S. (2009). Antioxidant activity, phenolic compounds and anthocyanins content of eighteen strains of mexican maize. *Food Science and Tehcnology*,42, 1187 – 1192.

Milán-Carrillo, J., Reyes-Moreno, C., Camacho-Hernández, I. L., & Rouzaud-Sandez, O. (2002). Optimization of extrusion process to transform hardened chickpeas (*Cicer arietinum* L) into a useful product. *Journal of the Science of Food and Agriculture*, 82, 1718-1728.

Milán-Carrillo, J., Gutiérrez-Dorado, R., Perales-Sánchez, J. X. K., Cuevas-Rodríguez, E. O., Ramírez-Wong, B., & Reyes-Moreno, C. (2006). The optimization of the extrusion cooking process when using maize flour with a modified amino acid profile for making tortillas. *International Journal of Food Science and Technology*, 41, 727-736.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry*, 49, 4619-4626.

O"zer, E. A., Herken, E. N., Güzel, S., Ainsworth, P., & İbanoğlu, S. (2006). Effect of extrusion process on the antioxidant activity and total phenolics in a nutritious snack food. *International Journal of Food Science and Technology*,41, 289-293.

Paredes-López, O., Guevara-Lara, F., & Bello-Pérez, L. A. (2006). Los alimentos mágicos de las culturas indígenas mesoamericanas. Fondo de Cultura Económica. Pp. 32-34, 81-88. México, DF. ISBN 968-16-7567-3.

Satterlee, L. D., Kendrick, J. G., Marshall, H. F., Jewell, D. K., Alí, R. A., Heckman, M. M., Fred-Steinke, H., Larson, P., Phillips, R. D., Sarwar, G., & Slum, P. (1982). *In vitro* assay for predicting protein efficiency ratio as mesured by rat bioassay: Collaborative study. *Journal of the Association of Official Analytical Chemists,* 65, 798-809.

Serna-Saldívar, S. O., Canett, R., Vargas, J., González, M., Bedolla, S., & Medina, C. (1988). Effect of soybean and sesame addition on the nutricional value of maize and decorticated sorghum tortillas produced by extrusion cooking. *Cereal Chemistry*, **65**, 44-48.

Serna-Saldívar, S. O., Abril-Domínguez, J. R., López-Ahumada, G., & Ortega-Ramírez, R. (1999). Nutritional evaluation of table bread fortified with defatted soybean and sesame meals. *Archivos Latinoamericanos de Nutrición*, 49, 260-264.

USDA. (2010). Antioxidants and Heatlh. ACES publications. 4 pp.
Vasanthan, T., Yeung, J., & Hoover, R. (2001). Dextrinization of starch in barley flours with thermostable alpha-amylase by extrusion cooking. *Starch/Stârke*, **53**, 616-622.

Table & Figure

Table 1. Combination of extrusion process variables used to produce whole maize and chickpea flours mixtures [(60g EMF + 40g ECF)$(100\ g)^{-1}$ mixture] and experimental results for response variables (*AoxA*, *A*).

Treatment[1]	**Process variables**				**Response variables**	
	ETM[2] **(°C)**	**SSM**[2] **(rpm)**	**ETC**[2] **(°C)**	**SSC**[2] **(rpm)**	$A_{ox}A$[3] **[µmol TE (100g)$^{-1}$ sample, dw]**	*A*[4]
1	120	120	120	120	8,572	**90**
2	170	120	120	120	7,949	76
3	120	200	120	120	9,618	74
4	170	200	120	120	8,682	80
5	120	120	170	120	9,402	66
6	170	120	170	120	7,838	62
7	120	200	170	120	8,282	66
8	170	200	170	120	**6,506**	70
9	120	120	120	200	8,999	82
10	170	120	120	200	7,451	78
11	120	200	120	200	10,340	74
12	170	200	120	200	8,088	84
13	120	120	170	200	**10,369**	60
14	170	120	170	200	8,137	60
15	120	200	170	200	8,024	64
16	170	200	170	200	7,167	64
17	95	160	145	160	10,174	88
18	195	160	145	160	6,710	70
19	145	80	145	160	9,096	68
20	145	240	145	160	8,805	70
21	145	160	95	160	9,833	64
22	145	160	195	160	8,708	**40**
23	145	160	145	80	9,596	80
24	145	160	145	240	8,518	80
25	145	160	145	160	9,735	82
26	145	160	145	160	9,341	78
27	145	160	145	160	9,060	80
28	145	160	145	160	9,792	84
29	145	160	145	160	9,307	88
30	145	160	145	160	9,635	86

1Does not corresponded to order of processing; **2**ETM= Extrusion temperature for maize, SSM= Screw speed for maize; used for producing extruded whole maize flour (EMF), ETC= Extrusion temperature for chickpea, SSC= Screw speed for chickpea; used for producing extruded whole chickpea flour (ECF);**3**$A_{ox}A$ = Antioxidant activity;**4***A* = Acceptability.

Table 2. Regression coefficients and analyses of variance of the second – order polynomial models showing the relationships among response variables (Y_k) and process variables (X).

Coefficient	***AntioxidantActivity*** ***($Y_{AoxA}{}^{1}$)***	***Acceptability*** ***($Y_A{}^{1}$)***
Intercept		
β_0	9330.14	81.88
Lineal		
β_1	-779.76***	-1.58*
β_2	-107.97^{NS}	0.25^{NS}
β_3	-259.35**	-7.25***
β_4	-17.97^{NS}	-0.75^{NS}
Quadratic		
β_{11}	-318.23***	-
β_{22}	-191.08**	-3.05***
β_{33}	-	-7.30***
β_{44}	-164.53**	-
Interactions		
β_{12}	-	2.63**
β_{23}	-595.27***	1.88*
P	<0.0001	<0.0001
R^2	0.8523	0.9035

[1] $A_{ox}A$ = Antioxidant activity, A= Acceptability

* Significant at P≤0.10 level, ** Significant at P≤0.05 level, *** Significant at P≤0.01 level; ^{NS} Not Significant (P > 0.10 level).

Table 3. Chemical composition and nutritional properties of the optimized mixture.

Property	**Optimized mixture**[1]	**Requirements for children 10 -12 years old** [2]
Chemical composition [g (100 g)$^{-1}$ sample, dw]		
Proteins	16.57±0.14	
Lipids	3.87±0.05	
Ashes	3.32±0.09	
Carbohydrates	76.24±1.02	
Nutritional		
EAA[3](g kg^{-1} protein)		
Ile	2.88±0.04	2.8
Leu	8.32±0.07	4.4
Lys	4.51±0.02	4.4
Met+Cys	3.80±0.05	2.5
Phe+Tyr	7.43±0.06	2.2
Trp	0.91±0.01	0.9
Thr	3.51±0.06	2.8
Val	4.38±0.03	2.5
Chemical score	100	---
Limiting EAA	ND[4]	---
IVPD[3] (%)	89.1±1.01	---
C-PER[3]	2.24±0.03	---

[1]Optimized mixture = (60g EMF + 40g ECF)(100 g)$^{-1}$ mixture; EMF = Extruded whole maize flour; ECF = Extrudedwhole chickpea flour;[2]FAO/WHO/UNU (1985)
[3]EEA = Essential aminoacids, IVPD = *in vitro* protein digestibility, C-PER = Calculated protein efficiency ratio.

Table 4. Total hydrophilic antioxidant activity of the optimized mixture.

Property	Unprocessed mixture[1]	Optimized mixture[2]
Antioxidant activity [µmol TE/ 100 g (dw)]		
Free phenolic	2,070±105[b]	2,695±135[a]
Bound phenolic	8,827±238[a]	7,277±240[b]
Total	10,897±387[a]	9,972±295[b]

[a-b] Means with different superscripts in the same row are significantly different (Duncan, $p \leq 0.05$); [1]Unprocessed mixture = (60g raw whole maize flour+40g raw whole chickpea flour)(100 g)$^{-1}$ raw mixture; [2]Optimized mixture = (60g EMF + 40g ECF) (100 g)$^{-1}$ mixture; EMF = Extruded whole maize flour;ECF = Extruded whole chickpea flour.

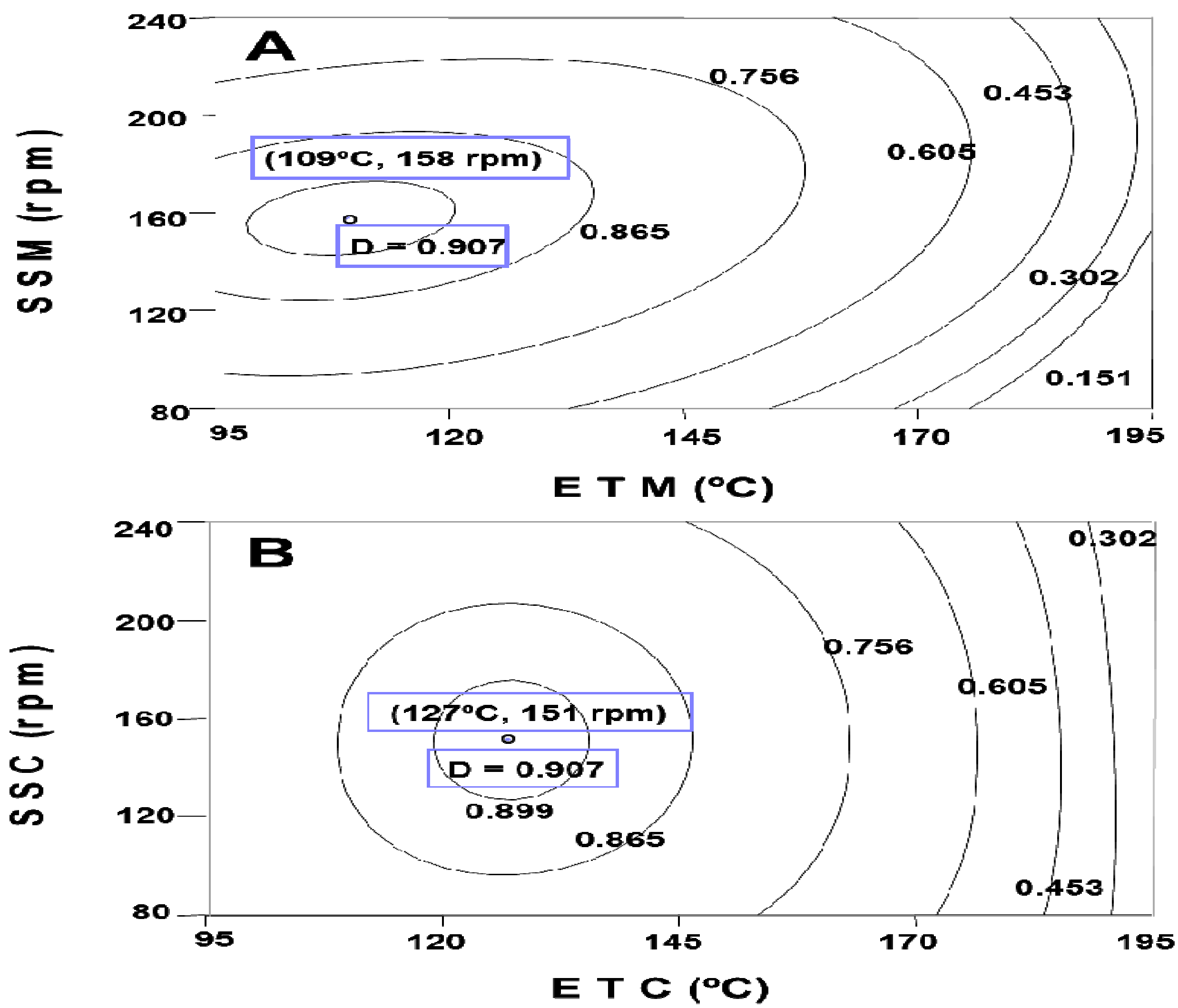

Figure 1. Desirability graph showing the best combination of extrusion process variables to prepare a extruded whole maize (60%) / chickpea (40%) flours mixture with high antioxidant activity and suitable to elaborate a beverage with high acceptability [Global desirability (D) = 0.907]. **(A)**: Extruded maize flour (EMF) (ETM = 109ºC / SSM = 158 rpm) and **(B)** Extruded chickpea flour (ECF) (ETC= 127ºC / SSC = 151 rpm).