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Effect of extrusion conditions and lipoxygenase inactivation treatment on the physical and nutritional properties of corn/cowpea (*Vigna unguiculata*) blends

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Abstract
The influence of lipoxygenase inactivation and extrusion cooking on the physical and nutritional properties of corn/cowpea (*Vigna unguiculata*) blends was studied. Corn was blended in an 80:15 proportion with cowpea flour treated to inactivate lipoxygenase (CI) or non-inactivated cowpea flour (CNI). Extrusion variables were temperature (150°C, 165°C and 180°C) and moisture (15%, 17% and 19%). Based on their physical properties, the 165°C/15% corn:CNI, and 165°C/15% corn:CI, and 150°C/15% corn:CI blends were chosen for nutritional quality analysis. Extrudate chemical composition indicated high crude protein levels compared with standard corn-based products. With the exception of lysine, essential amino acids content in the three treatments met FAO requirements. Extrusion and lipoxygenase inactivation are promising options for developing corn/cowpea extruded snack products with good physical properties and nutritional quality.

Keywords: *Extrusion, corn, cowpea, nutritional quality*

Introduction

Extrusion is a common procedure aimed at improving quality in end-products such as confectionary products, baby foods, snacks, ready-to-eat breakfast cereals and pet foods. Most extruded snacks on the market can be classified as direct-expanded snacks, and the most popular of these are corn-based (Akdogan 1999). Use of corn/legume blends in snack manufacturing is a promising alternative for improving product protein quality (Messina 1999). Pérez-Navarrete et al. (2006) evaluated the effect of extrusion on the nutritional quality of a 50:50 proportion corn/Lima bean

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blend, while Ruiz-Ruiz et al. (2008) did the same for a 50:50 proportion corn/hard-to-cook bean blend. Both reports indicate that the blends had acceptable physical characteristics and better nutritional quality than solely corn-based products.

Legumes are a widespread food resource in Latin America and are well known for their good nutritional value, especially in Mexico and Brazil (Broughton et al. 2003). Some tropical legumes have excellent potential applications, but their use is quite limited. For example, cowpea (*Vigna unguiculata*) is a good, low-cost source of protein, carbohydrates, vitamins and minerals, but receives only limited use in many regions. Incorporating it into diverse products could improve nutritional quality and increase use of this promising resource.

The enzyme lipoxygenase can be found in legumes such as soybeans, peas, peanuts, mung beans, and navy beans, and can have negative effects on the colour, flavour, texture, and nutritional nutritive properties of foods (Addo et al. 1993). Development of off-flavour in legume products is highly dependent on the action of the various endogenous lipoxygenases, since subsequent decomposition of the resulting hydroperoxides produces a rancid flavour. Initial hydroperoxidation of unsaturated fatty acids, which is catalysed by lipoxygenase, may lead to the formation of short-carbon-chain acids, ketones, and aldehydes that are most probably responsible for the beany flavour of legume products (Knorr 1993).

There are several ways of inactivating lipoxygenase, including dry and wet heat, steam heat, heat with pH adjustment and microwave heat. Although heat treatment does effectively inactivate lipoxygenase, it is also denatures and insolubilizes proteins (Indrawati et al. 1999). Legume/cereal flour blends have been shown to greatly improve the nutritious value of extruded products. Previous trials using *in vivo* assays have shown that *Phaseolus lunatus* mixed at up to a 1:1 ratio with corn provides products with notably better nutritional, particularly in terms of the biological use of protein and starch in the extruded mixture (Pérez-Navarrete et al. 2007). The present study aim was to determine the effect of extrusion conditions and lipoxygenase inactivation on the physical and nutritional properties of corn/cowpea (*V. unguiculata*) blends.

Materials and methods

Samples and chemicals

Yellow dent corn (Dekalb 696) was provided by the Santa Fe Food Technology Institute, Argentina, and cowpea (*V. unguiculata* L. Walp) seeds were acquired from harvests in the state of Yucatan, Mexico. All chemical reagents were analytical grade (Sigma Co., St Louis, MO, USA).

Lipoxygenase inactivation treatment

The cowpea seeds were divided into two lots. One was steamed at 1 atm for 10 min to inactivate lipoxygenase (cowpea inactivated [CI]), while the other was not treated (cowpea non-inactivated [CNI]). Both were dried at 50°C to 10% moisture content.

Flour preparation

According to specific previous assays for legumes, the seeds were processed in a Buhler-Miag roller mill (Bühler AG, Uzwil, Switzerland) with a progressive,

successive reduction in roller spacing. After each milling, the flours were sifted through 1.168, 0.833, 0.351 and 0.208 mm screens. The 0.351-mm and 0.208-mm flours were used in the extrusion process. Using a pneumatic separator, particles >1.168 mm and >0.833 mm were divided into two portions, one containing the hull and germ, which was discarded, and the other the flour. This was then milled again, sifted through 1.168, 0.833, 0.351 and 0.208 mm screens to separate the 0.351-mm and 0.208-mm fractions, and these added to the 0.351/0.208 mm flour from the first milling. Corn grains were milled following a milling process developed by Robutti et al. (2002).

Flour blend preparation

Flour moisture content was determined to establish the amount of water to be added to adjust moisture content to required levels. The corn flour was blended with CI or CNI flour in an 85:15 proportion in sufficient quantities to produce 500 g flour blend per treatment. The blends were placed in a planetary mixer (Brabender P600; Duisburg, Germany), homogenized for 10 min, and water added until the required moisture content was attained. Blend preparation was done 1 h before extrusion.

Extrusion procedures

The flour blends were extruded using a Brabender 20 DN single screw extruder with the following specifications: pressure and temperature sensor; two heating zones; 4:1 screw compression ratio; and a 3.5 mm diameter $\times 20$ mm long (3.5×20) die. A 3^2 model plus two central points was used to evaluate extrusion conditions. The evaluated factors and levels were temperature (150°C, 165°C and 180°C) and moisture content (15%, 17% and 19%), with a feed rate of 200 g/min at a fixed screw speed of 150 rpm. After extrusion, the products were placed on trays to cool for 10 min. The product moisture content was conditioned for 24 h at 60°C until reaching 6%, and the products were stored in polyethylene bags until analysis.

Expansion (E) was measured as described by Gujska and Khan (1990); that is, by dividing extrudate diameter by die orifice diameter. The specific weight (SW) was determined following Wang et al. (1993). The extrudate diameter (d), length (l) and weight sample (ws) were measured and then the SW calculated: $SW = ws/\pi(r^2)(l)$.

Chemical composition

Proximate composition was determined using AOAC (1997) methods: moisture content (Method 925.09), ash (Method 923.03), crude fat (Method 920.39), crude protein using a 6.25 nitrogen–protein conversion factor (Method 954.01), and crude fibre (Method 962.09). Carbohydrate content was estimated as the nitrogen-free extract.

In vitro *protein digestibility*

Following Hsu et al. (1977), *in vitro* protein digestibility was determined with a multi-enzymatic solution containing 1.6 mg trypsin (type IX Sigma T-0303 with 13,000–20,000 BAEE units/mg protein), 3.1 mg chemotrypsin (type II Sigma C-4129 with ≥ 40 units/mg powder) and 1.3 mg peptidase (type III grade Sigma P-7500 with

50–100 units/g powder) per millilitre. Changes in pH were measured with a potentiometer after 10 min. Apparent *in vitro* digestibility (Y) was measured using the equation:

$$Y = 210.464 - 18.103X$$

where X is the pH of the protein suspension immediately after digestion with multi-enzymatic solution for 10 min.

Amino acid analysis

Amino acid profiles were determined according to Alaiz et al. (1992), using precolumn derivatization with diethyl ethoxymethylenemalonate and reversed-phase high-performance liquid chromatography (HPLC) with spectrophotometric detection at 280 nm. The HPLC system (Waters; Milford, MA, USA) consisted of a model 600E multi-solvent delivery system, a Wisp Model 712 automatic injector and a model 484 UV–VIS detector. Samples containing D,L-α-aminobutyric acid as an internal standard were dissolved in 6.0 mol/l hydrochloric acid. The solutions were gassed with nitrogen and sealed in hydrolysis tubes under nitrogen, then incubated in an oven at 110°C for 24 h. Formation of *N*-[2,2-bis(ethoxycarbonyl)vinyl] derivatives of sample hydrolysates was done by adding 0.8 μl diethyl ethoxymethylenemalonate to a dried sample hydrolysate (200 μg) in 1 mol/l sodium borate buffer (pH 9.0) (1 ml) containing 0.02% sodium azide. The reaction was carried out at 50°C for 50 min under vigorous shaking. Amino acid derivative resolution was determined using a binary gradient system. The solvents used were 25 mmol/l sodium acetate containing 0.02% sodium azide (pH 6.0) (Solvent A) and acetonitrile (Solvent B). Solvents were injected into the column at a 0.9 ml/min flow rate, as follows: time 0.0–3.0 min, linear gradient from A–B (91:9) to A–B (86:14); 3.0–13.0 min, elution with A–B (86:14); 13.0–30.0 min, linear gradient from A–B (86:14) to A–B (69:31); 30.0–35.0 min, elution with A–B (69:31).

Tryptophan content

Tryptophan levels were determined by HPLC with spectrophotometric detection at 280 nm (Yust et al. 2004). Samples (10 mg) were dissolved in 3 ml of 4 mol eq./l sodium hydroxide, sealed in hydrolysis tubes under nitrogen, and incubated in an oven at 100°C for 4 h. Hydrolysates were cooled on ice, neutralized to pH 7 using 12 mol eq./l HCl, and diluted to 25 ml with 1 mol/l sodium borate buffer (pH 9). Aliquots of these solutions were filtered through 0.45-m Millex filters (Millipore; Bedford, MA, USA) prior to injection. Standard tryptophan solutions were prepared by dilution of a stock solution (0.51 mg tryptophan/ml 4 mol eq./l NaOH) to 3 ml with 4 mol eq./l sodium hydroxide, followed by incubation. Samples of 20 μl were injected into the column. An isocratic elution system was used consisting of 25 mmol/l sodium acetate and 0.02% sodium azide (pH 6)/acetonitrile (91:9) delivered at 0.9 ml/min.

Available lysine

This variable was determined following Booth (1971), using a quantity of sample containing 35 mg nitrogen. To each sample, 8 ml $NaHCO_3$ (8% v/v) and 12.3 ml fluoro-2-4-dinitrobenzene solution (0.3 ml in 12 ml ethanol) were added and the

mixture agitated for 2 h. Excess solvent was evaporated, 200 ml of 8 mol/l HCl added, and the mixture heated for 16 h. The content was filtered, and 2 ml mixture added to two tubes (A and B). The content of tube B was extracted with 5 ml sulphuric ether, the residual ether evaporated in a water bath at 80°C, and phenolphthalein and NaOH 12% (w/v) added until a pink colouration appeared. Then, 2 ml buffer (19.5 g $NaHCO_3 + 1$ g Na_2CO_3 in 250 ml water) and five drops of methyl chloroformate were added, and the tube covered and shaken. Next, 0.75 ml HCl was added, the solution extracted four times with 5 ml sulphuric ether each time, and the content transferred to a 10-ml flask. The content of tube A was extracted three times with ether, and the mixture transferred to a 10-ml flask and measured with 1 mol eq./l HCl. Absorbances were read at 435 nm using a spectrophotometer (Thermospectronic Genesis 10uv, Madison, WI, USA). Lys was determined as dinitrofluorobenzene lysine:

$$\text{g Lysine}/16\text{ g N} = (L \times 250 \times 0.42 \times 100)/(E \times \text{aliquot} \times M \times \%\text{ protein dry basis})$$

where L is the A–B absorbance, 0.42 is the dinitrofluorobenzene lysine to lysine conversion factor, E is the molar coefficient, M is the weight of the sample and the aliquot is 2 ml.

Calculated protein efficiency ratio

The calculated protein efficiency ratio (cPER) was determined following the AOAC (1997) method, using the *in vitro* digestibility value and the amount (g) of amino acid/100 g protein of Lys, Met + Cys, Thr, Ile, Leu, Val, Phe + Tyr and Trp. This assumes that the Cys and Tyr values in the Met + Cys and Phe + Tyr combinations do not surpass 50% of the total of their respective combinations.

Total starch

Total starch (TS) was quantified by AOAC (1999) Method 996.11, using a TS assay (K-TSTA 01/05 Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland). Ethanol (80% v/v) and dimethyl sulphoxide were used to sample solubilization. Hydrolysis was performed using thermostable α-amylase and amyloglucosidase. Glucose oxidase/peroxidase was used for colorimetric determination at 500 nm using a spectrophotometer (Thermospectronic Genesis 10UV). TS was calculated with the equation:

$$\%\text{TS} = \Delta A \times F \times 1,000 \times 1/1,000 \times 100/W \times 162/180$$

where ΔA is the absorbance (reaction) read against the reagent blank, $F = 100$(μg glucose)/absorbance for 100 μg glucose, 1,000 is the volume correction (0.1 ml taken from 100 ml), 1/1,000 is the conversion from micrograms to milligrams, $100/W$ is the factor that expresses 'starch' as a percentage of flour weight, W is the weight in milligrams ('as is' basis) of analysed flour, 162/180 is the adjustment from free glucose to anhydrous glucose. TS (g/kg) (dry basis) = starch (g/kg) × 100/100 − moisture content (g/kg w/w).

Available starch

Available starch (AS) was quantified in the same way as TS (AOAC 1999), with the exception that dimethyl sulphoxide was not added.

Resistant starch

Resistant starch (RS) was calculated by the difference between TS and AS: RS = TS – AS.

Total dietary fibre

Total dietary fibre (TDF) was determined following Prosky (1989). Briefly, 1 g sample was weighed into each of four flasks, and 50 ml of 0.05 mol eq./l phosphate buffer at pH 6 added to each. The flasks were placed in a bath at 100°C, 0.1 ml thermostable α-amylase (Sigma A-3306) added to each and agitated at 60 rpm for 15 min. After cooling, the pH was adjusted to 7.5. The flasks were placed in a bath at 60°C, 0.1 ml protease (Sigma P-3910) added to each and agitated at 60 rpm for 30 min. After cooling, the pH was adjusted to 4.0. The flasks were placed in a bath at 60°C, 0.3 ml amyloglucosidase (Sigma A-9913) added and agitated for 30 min. Finally, 95 g/kg ethanol at 60°C was added at a 1:4 (v/v). In a vacuum, flask content was filtered into crucibles containing celite (Sigma C-8656). The residue in the flask was washed three times with 20 ml of 780 g/kg ethanol, twice with 10 ml of 950 g/kg ethanol and twice with 10 ml acetone. Crude protein was determined for the residue in two crucibles and the residue in another two was burned at 550°C for 4 h.

$$\text{TDF (g/kg)} = [(\text{Residue weight (g)} - \text{protien (g)} - \text{ash (g)} - \text{Black}) \times 1,000]/\text{sample weight (g)}$$

Insoluble dietary fibre

Insoluble dietary fibre (IDF) was determined following Prosky (1989), except that 95 g/kg alcohol at 1:4 was added.

Soluble dietary fibre

Soluble dietary fibre (SDF) was calculated by the difference between TDF and IDF: SDF = TDF – IDF

Statistical analysis

Statistical treatment of the extrusion process results was performed by multivariate analysis following Johnson and Wichern (1992). A multivariate analysis of variance and a means comparison were applied to establish differences using the least significant different (LSD) method, regression analysis and surface response, according to Montgomery (2004). Nutritional change data for starch, protein and fibre was processed using central tendency (mean) and dispersion (standard deviation) measurements. All analyses were carried out with the Statgraphics 5.0 program (Statgraphics.net, Madrid, Spain).

Results and discussion

Inclusion of cowpea in any food system should consider its lipoxygenase activity, which causes hydroxyperoxidation of unsaturated fatty acids immediately after the grain has been broken. This activity can produce a typical 'beany' flavour that can

negatively affect consumer acceptance in countries where beans are not a staple food. Lipoxygenase can be inactivated by heat processing before grinding (Pérez et al. 2008). For example, steaming at atmospheric pressure for 10 min is sufficient to inactivate over 85% of lipoxygenase activity in *V. unguiculata* seeds (Fritz et al. 2006), reducing the enzyme activity to produce satisfactory sensory test results.

Extrusion process and physical evaluation

Multivariate analysis showed that moisture content affected ($P<0.05$) E, and both temperature and moisture content affected ($P<0.05$) the SW. Factor interaction had no effect ($P>0.05$) on any of the response variables. The regression analysis for each response variable showed an adequate fit of the experimental values to first-order polynomial models that can describe E and SW as a function of temperature and moisture content. The mathematical models indicated these behaviours to be represented by Equations (1)–(4) presented in Figures 1–4.

Expansion

Moisture content (X_2) negatively affected ($P<0.05$) extrudate E, in that E increased as the moisture content decreased (Equations (1) and (2)). The regression model response surface graphics (Figures 1 and 2) showed the CI extrudates to have higher E than the CNI. This may be attributed to structural changes in the cowpea protein caused by the lipoxygenase inactivation treatment, which may have led to reduced interaction with corn components, thus allowing the CI blends to have E values near those of corn meal (>3.5). The inverse relationship between expansion and moisture content in the extrudates is consistent with reports for corn meal. Water has a lubricating effect in the extruder, causing less mechanical energy dissipation and therefore less expansion (González et al. 2002, 2007). The effect of temperature on E was less evident. In the CI treatments, increased temperature resulted in lower E at lower moisture content (15%), but higher E at higher moisture contents. For the CNI treatments the relationship was the inverse, with higher temperature leading to lower E.

Specific weight

Temperature and moisture negatively affected ($P<0.05$) the SW (Equations (3) and (4)). The CI extrudates exhibited a wider variation range than the CNI extrudates,

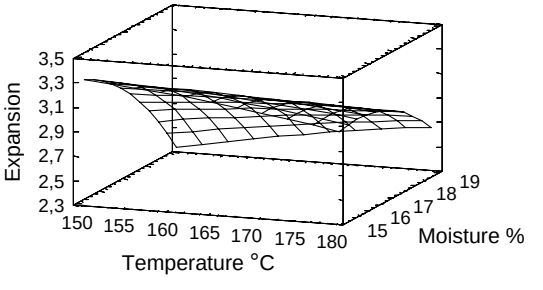

Figure 1. Effect of moisture content (X_2) on expansion in Corn/CI extrudates. IE $=2.97-0.29$ (X_2) (Equation (1)). $R^2=0.5984$.

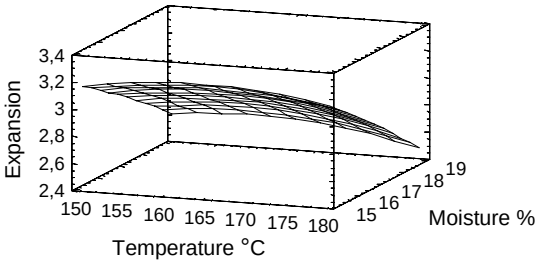

Figure 2. Effect of moisture content (X_2) on expansion in Corn/CNI extrudates. IE = 2.98 − 0. 33 (X_2) (Equation (2)). $R^2 = 0.8503$.

probably due to the behaviour of E (Figures 3 and 4). The lower interaction between the corn and cowpea components (Liu et al. 2000) may have allowed the mixture to behave more like corn meal under the applied extrusion conditions.

Roudaut et al. (2002) emphasized that the acceptability range of directly expanded snack products is quite broad; the E and SW of commercial products are usually about 3.5 and 0.180 g/cm^3, respectively. Based on these values, the corn:CNI 165°C/15% (3.2, 0.197 g/cm^3), corn:CI 165°C/15% (3.1, 0.226 g/cm^3) and corn:CI 150°C/15% (3.0, 0.243 g/cm^3) treatments were chosen for nutritional evaluation.

Chemical composition

The moisture, protein, lipids, crude fibre and ash contents of the three samples did not differ ($P > 0.05$) (Table I), and the protein content was similar between treatments (9.9%). Extrusion does not change the protein content, but the high temperature, pressure and mechanical force of the process change the protein physical and chemical properties (Camire 2000). The crude lipid content was much lower than expected based on corn meal and cowpea lipid contents, probably due to lipid–protein and lipid–amylose interactions during extrusion, which reduce lipid extraction with non-polar solvents (Asp and Björck 1989). Recorded crude fibre content met with expected levels and reflected changes in the relationship between dietary fibre fractions caused by extrusion (Rabe 1999).

In vitro *protein digestibility*

In vitro protein digestibility did not differ ($P > 0.05$) between treatments (84.46% for CNI 165°C/15%; 83.65% for CI 150°C/15%; and 83.29% for CI 165°C/15%).

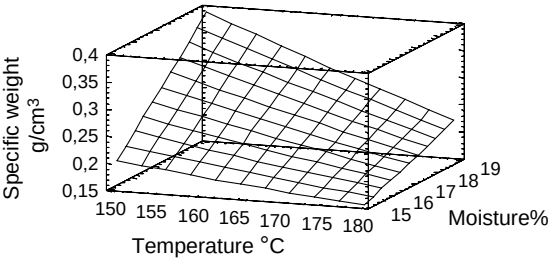

Figure 3. Effects of temperature (X_1) and moisture content (X_2) on specific weight in Corn/CI extrudates. SW = 245.21 − 40.78 (X_1) + 40.78 (X_2) (Equation (3)). $R^2 = 0.9118$.

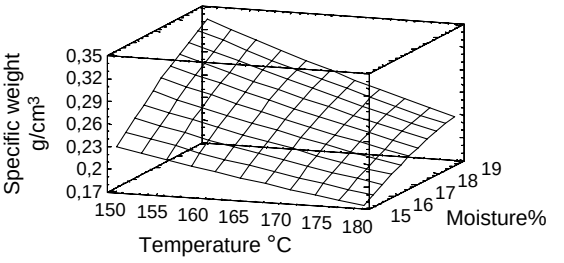

Figure 4. Effects of temperature (X_1) and moisture content (X_2) on specific weight in Corn/CNI extrudates. SW = 237.86 – 54.39 (X_1) + 64.07 (X_2) (Equation (4)). R^2 = 0.8122.

Similar values (83.5%) have been reported for *Phaseolus vulgaris* L. at 160°C and 22% moisture content (Balandran-Quintana et al. 1998). These protein digestibility values may be due to two phenomena caused by thermal treatment during lipoxygenase inactivation and extrusion cooking: protein denaturalization, which may increase exposure of sites susceptible to enzymatic activity (Camire 2000); and inactivation of trypsin and chymotrypsin inhibitors (Alonso et al. 2000).

Amino acid profile and corrected chemical calculation

With the exception of lysine, the essential amino acid content in the three treatments met FAO/WHO (1991) requirements (Table II). Lipoxygenase inactivation and extrusion did not drastically affect most amino acid contents, although the sulphur amino acid content did decrease in the CI 150°C/15% and CI 165°C/15% treatments compared with the CNI 165°C/15% treatment. Alonso et al. (2000) observed that in extruded *Pisum sativum* L. flours, methionine may be unavailable due to the formation of interlaced ligands. The corrected calculation (Table III) showed lysine to be the main limiting amino acid in the three samples, a result of the blends' high legume content; however, the lysine value was still almost 70 in the CNI 165°C/15% treatment. Björck et al. (1983) demonstrated that as the extrusion temperature increases, the lysine, methionine, arginine and tryptophan levels decrease.

Available lysine

No differences ($P > 0.05$) were observed between available lysine (g/16 g N) in the three treatments (4.26 for CNI 165°C/15%; 4.33 for CI 150°C/15%; and 4.43 for CI

Table I. Proximate composition (% dry basis) of blends of CNI and CI flour extruded at 15% moisture content and 150°C or 165°C.

Component	CNI 165°C/15%	CI 150°C/15%	CI 165°C/15%
Moisture	9.89^A	9.92^A	9.90^A
Protein	9.82^A	9.92^A	9.95^A
Fat	0.26^A	0.33^A	0.31^A
Crude fibre	0.48^A	0.53^A	0.50^A
Ash	1.13^A	1.01^A	1.08^A
Nitrogen-free extract	78.42^A	78.29^A	78.26^A

The same uppercase superscript letter in the same column indicates no statistical difference ($P > 0.05$).

Table II. Amino acid content (g/100 g protein) of blends of CNI and CI flour extruded at 15% moisture content and 150°C or 165°C.

Amino acid	CNI 165°C/15%	CI 150°C/15%	CI 165°C/15%	FAO
Lysine	4.80	4.60	4.95	5.8
Tryptophan	1.00	1.00	0.90	1.1
Methionine + cysteine	3.3	2.65	2.45	2.5
Phenylalanine + tyrosine	7.20	7.40	7.15	6.3
Threonine	4.20	4.20	4.30	3.4
Isoleucine	3.20	3.20	3.30	2.2
Leucine	11.05	11.25	11.00	6.6
Valine	4.65	5.00	4.70	3.5
Hystidine	3.25	3.20	3.30	1.9

165°C/15%). Available lysine levels were lower than the lysine levels (Table II), a loss that can be attributed to Maillard reactions between reducing sugars and the protein's free amino groups. Reduction in lysine availability during extrusion is heavily influenced by the processing temperature. Martínez-Flores et al. (2005) reported values of 3.92 g/16 N available lysine in a corn:soybean blend (85:15) extruded at 130°C and 14% moisture content, while Pérez-Navarrete et al. (2006) reported values of 4.1/16 g N for a 75/25 corn/Lima bean blend and 4.55 g/16 g N for a 50/50 blend of the same raw materials. These results are similar to those observed in the present study, indicating that lipoxygenase inactivation and extrusion did not significantly affect available lysine, and confirming that cooking by extrusion has advantages over other thermal treatments (Lasekan et al. 1996).

Calculated protein efficiency ratio

Higher extrusion temperature tended to decrease the cPER in the three treatments, although any differences between them were minimal (1.88 for CNI 165°C/15%; 1.85 for CI 150°C/15%; and 1.83 for CI 165°C/15%). When processing soya flours with an autoclave, Yeoung and Barbeau (1991) observed a decrease in cPER (from 2.6 to 1.97), which they attributed to cysteine degradation. In a study of extruded chickpea flour, Milán-Carrillo et al. (2000) reported cPER values (1.87) similar to those reported in this work. The present results indicate that neither lipoxygenase inactivation nor extrusion significantly affected protein quality.

Table III. Corrected chemical calculation of blends of CNI and CI flour extruded at 15% moisture content and 150°C or 165°C.

Amino acid	CNI 165°C/15%	CI 150°C/15%	CI 165°C/15%
Lysine	69.90	66.34	69.07
Tryptophan	76.78	76.05	72.10
Methionine + cysteine	111.49	88.67	86.81
Phenylalanine + tyrosine	114.29	112.86	112.62
Threonine	104.33	103.33	104.33
Isoleucine	96.53	95.60	98.37
Leucine	118.65	142.58	142.28
Valine	112.21	119.50	115.67

Total starch

The highest TS content was in the CNI 165°C/15% treatment (81.5%), followed by the CI 150°C/15% (72.5%) and CI 165°C/15% (64.6%) treatments (Table IV). These discrepancies between the CNI treatment and the CI treatments (11% and 20%, respectively) were probably due to the double thermal treatment applied in the latter, and the difference between the two CI treatments was due to the extrusion temperature. Starch molecule degradation is a function of extrusion conditions, particularly temperature (Asp and Björck 1989; Camire 2000; Pérez-Navarrete et al. 2006), and lipoxygenase inactivation treatment would have contributed to increasing molecular degradation during extrusion.

Available starch

The highest AS content was in the CNI 165°C/15% (78.8%), followed by the CI 150°C/15% (68.7%) and the CI 165°C/15% (59.5%) (Table IV). Reductions in AS during extrusion may be attributed to transglucosidation reactions, which generate atypical bonds that are not hydrolysable by amylolytic enzymes (Tovar et al. 1990). Molecular degradation during extrusion produces low-molecular-weight fractions in proportion to extrusion condition intensity (González et al. 2007). These would facilitate association with other components (i.e. protein, lipids and fibre), consequently increasing the RS fraction, quantified as the difference between TS and AS. This most probably occurred in the present case since the RS fraction increased as temperature increased. However, the recorded RS changes were only marginal, suggesting they may be due to newly generated chemical bonds that were not quantified by the analytical methods applied here (Tovar and Melito 1996).

Dietary fibre

The TDF content (5.5–5.9%) did not vary ($P > 0.05$) between treatments. Lipoxygenase inactivation and extrusion did not significantly affect TDF content in the treatments. However, the higher extrusion temperature in the CI 165°C/15% treatment produced a decrease in IDF and an increase in SDF versus the CI 150°C/15% treatment. Lipoxygenase inactivation also apparently affected the fibre proportion since it lowered the insoluble fibre content and raised the soluble fibre content in the CI 165°C/15% treatment versus the CNI 165°C/15% treatment. In a study of wheat and okra flour extrudates, Rinaldi et al. (2000) reported that as the extrusion temperature increased, the IDF decreased (8.50–7.3%) and SDF increased

Table IV. Starch and fibre contents of blends of CNI and CI flour extruded at 15% moisture content and 150°C or 165°C.

Component	CNI 165°C/15%	CI 150°C/15%	CI 165°C/15%
Total starch (%)	81.5^{A}	72.5^{B}	64.6^{C}
Available starch (%)	78.8^{A}	68.7^{B}	59.5^{C}
Resistant starch (%)	2.6^{A}	3.8^{B}	4.7^{C}
Total dietary fibre (%)	5.5^{A}	5.9^{A}	5.7^{A}
Insoluble dietary fibre (%)	3.4^{A}	3.8^{A}	2.9^{B}
Soluble dietary fibre (%)	2.1^{A}	2.1^{A}	2.8^{B}

Different uppercase superscript letters in the same row indicate statistical difference ($P < 0.05$).

(2.22–3.19%). A reduction in IDF was also reported by Lue et al. (1991) for a corn and beet fibre blend (70:30) extrudate. In both studies, solubilization of IDF components was attributed to thermomechanical treatment during extrusion causing the formation of lower molecular weight, soluble fragments. Fibre values similar to those observed in the present study were reported by Berglund et al. (1994) for a barley:rice blend (50:50) extrudate (TDF = 5.82%; IDF = 3.04%; SDF = 2.78%). The dietary fibre content of the analysed samples was not high, but is worth noting since dietary fibre is necessary for a normal diet (Villarroel et al., 2003).

Conclusions

Extrusion of corn-cowpea (*V. unguiculata*) flour blends (85:15 proportion) with 15% moisture content at temperatures of 150°C and 165°C produced extrudates with good physical and nutritional characteristics. Replacement of 15% of the corn flour with cowpea flour increased the protein content 40%, and improved quality. At this replacement level, lipoxygenase inactivation and extrusion did not affect protein digestibility, tryptophan content or available lysine content. The TS and AS content decreased in both the CI treatments, although the RS content changed little, probably because of new chemical bonds underestimated by the methods used. The TDF content was not affected, but lipoxygenase inactivation apparently helped to decrease the insoluble fibre content and increase the soluble fibre content in the CI 165°C/15% sample versus the CNI 165°C/15% sample. When processed with lipoxygenase inactivation, this corn–cowpea blend is a promising option for development of extruded snack products.

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References

Addo K, Burton D, Stuart MR, Burton HR, Hildebrand DF. 1993. Soybean flour lipoxygenase isozyme mutant effects on bread dough volatiles. J Food Sci 58:583–585.

Akdogan H. 1999. High moisture food extrusion. Int J Food Sci Technol 343:195–207.

Alaiz J, Navarro L, Girón J, Vioque E. 1992. Amino acid analysis by high-performance liquid chromatography after derivatization with diethyl ethoxymethylenemalonate. J Chromatogr 591:181–186.

Alonso R, Grant G, Dewey P, Marzo F. 2000. Nutritional assessment in vitro and in vivo of raw and extruded peas (*Pisum sativum* L.). J Agric Food Chem 48:2286–2290.

AOAC. 1997. Official methods of analysis of the Association of Official Analytical Chemists. 16th ed. Washington, DC: William Horwitz.

AOAC. 1999. Official methods of analysis of the Association of Official Analytical Chemists. 16th ed. Washington, DC: William Horwitz.

Asp NG, Björck I. 1989. Nutritional properties of extruded foods. In: Mercier C, Linko, P, Harper JM, editors. Extrusión cooking. St. Paul, MN: American Association of Cereal Chemists. pp 398–415.

Balandrán-Quintana RR, Barbosa-Cánovas GV, Zazueta-Morales JJ, Anzaldúa-Morales A, Quintero-Ramos A. 1998. Functional and nutritional properties of extruded whole pinto bean meal (*Phaseolus vulgaris* L.). J Food Sci 63:113–116.

Berglund PT, Fastnaught CE, Holm ET. 1994. Physicochemical and sensory evaluation of extruded high-fiber barley cereals. Cereal Chem 71:91–95.

Björck I, Noguchi A, Asp NG, Cheftel JC, Dahlqvist A. 1983. Protein nutritional value of biscuit processed by extrusion-cooking: Effects on available lysine. J Agric Food Chem 31:488–492.

Booth VH. 1971. Problems in the determination of FDNB-avaliable lisien. J Food Sci Agric 22:6584665.

Broughton WJ, Hernandez G, Blair M, Belbe S, Gepts P, Vanderleyden J. 2003. Beans (Phaseolus spp.) model food legumes. Plant Soil 252:554128.

Camire ME. 2000. Chemical and nutritional changes in food during Extrusion. In: Riaz MN, editor. Extruders in food application. Boca Raton, FL: CRC PRESS. pp 101–105.

FAO/WHO. (1991). Protein quality evaluations. Report of joint FAO/WHO expert consultation. Food and nutrition. Paper No. 51. Rome: Food Agriculture Organization and the World Health Organization.

Fritz M González RJ, Carrara C, De Greef DM, Torres RL, Chel L 2006. Selección de las condiciones de extrusión, para una mezcla maíz-frijol: aspectos sensoriales y operativos. Braz J Food Technol Edición Especial III JIPCA: 3–7.

González R, Torres R, Degreef D. 2002. Extrusión-cocción de cereales. Bol SBCTA Campinas 36(2):104–115.

González R, Carrara C, Tosi E, Añón MC, Pilosof A. 2007. Amaranth starch-rich fraction properties modified by extrusion and fluidized bed heating. Lebensm Wiss U Technol 40:136–143.

Gujska EY, Khan K. 1990. Effect of temperature on properties of extrudates from high starch fractions of navy, pinto and garbanzo beans. J Food Sci 55:466–469.

Hsu H, Vavak D, Satterlee L, Miller G. 1977. A multienzyme technique for estimating protein digestibility. J Food Sci 42:1269–1279.

Indrawati, Van Loey AM, Ludikhuyze LR, Hendrickx ME. 1999. Soybean lipoxygenase inactivation by pressure at subzero and elevated temperatures. J Agric Food Chem 47:2468–2474.

Johnson AR, Wichern DW. 1992. Applied multivariate statistical analysis. Englewood Cliffs, NJ: Prentice Hall.

Knorr D. 1993. Effects of high-hydrostatic-pressure processes on food safety and quality. Food Technol 13:532–538.

Lasekan OO, Lasekan E, Idowu MA, Ojo OA. 1996. Effect of extrusion cooking conditions on the nutritional value, storage stability and sensory characteristics of a maize-based snack food. J Cereal Sci 24:79–85.

Liu H, Corke H, Ramsden L. 2000. The effect of autoclaving on the acetylation of ae, wx, and normal maize starches. Starch/Stärke 52:353–360.

Lue S, Hsieh F, Huff HE. 1991. Extrusion cooking of corn meal and sugar beet fibre: Effects on expansion properties, starch gelatinization, and dietary fibre content. Cereal Chem 68:227–234.

Martinez-Flores HE, Cruz MC, Larios SA, Jiménez GE, Figueroa GD, Gómez-Aldapa CA. 2005. Sensorial and biological evaluation of an extruded product made from corn supplemented with soybean and safflower pastes. Int J Food Sci Technol 40:517–524.

Messina MJ. 1999. Legumes and soybeans: Overview of their nutritional profile and health effects. Am J Clin Nutr 70:439–450.

Milán-Carrillo J, Reyes-Moreno C, Ermienta-Rodelo E, Carábez-Trejo A, Mora-Escobedo R. 2000. Physicochemical and nutritional characteristics of extruded flours from fresh and hardened chickpeas (*Cicer arietinum* L). Int J Food Sci Technol 33:117–123.

Montgomery D. 2004. Diseño y análisis de experimentos. México: Editorial Limusa S.A. de C.V. pp 218–276.

Pérez A, Drago SR, Carrara C, De Greef DM, Torres RL, González RJ. 2008. Extrusion cooking of a maize-soybean mixture: Factors affecting expanded product characteristics and flour dispersion viscosity. J Food Eng 87(3):333–340.

Pérez-Navarrete C, González R, Chel-Guerrero L, Betancur-Ancona D. 2006. Effect of extrusion on nutritional quality of maize and Lima bean flour blends. J Sci Food Agric 86:2477–2484.

Pérez-Navarrete C, Betancur-Ancona D, Casotto M, Carmona A, Tovar J. 2007. Efecto de la extrusión sobre la biodisponibilidad de proteína y almidón en mezclas de harinas de maíz y frijol lima. Arch Latinoam Nutr 57(3):278–286.

Prosky L. 1989. Determination of insoluble, soluble and total dietary fiber in food and food products: Interlaboratory study. AOAC 71:1017–1013.

Rabe E. 1999. Effect of processing on dietary fiber in foods. In: Cho S, Prosky L, Deher ML, editors. Complex carbohydrates in foods. New York: Marcel Dekker. pp 395–409.

Rinaldi VEA, Ng PK, Bennink MR. 2000. Effects of extrusion on dietary fiber and isoflavone contents of wheat extrudates enriched with wet okara. Cereal Chem 77:237–240.

Robutti JL, Borrás FS, González RJ, Torres RL, De Greef DM. 2002. Endosperm properties and extrusion cooking behavior of maize cultivars. Food Sci Tech 35:663–669.

Roudaut G, Dacremont C, Valles-Pamies B, Colas B, Le Mestre M. 2002. Crispness: A critical review on sensory and material science approaches. Food Sci Technol Int 13:217–227.

Ruiz-Ruiz J, Martínez-Ayala A, Drago S, González R, Betancur-Ancona D, Chel-Guerrero L. 2008. Extrusion of a hard-to-cook bean (*Phaseolus vulgaris* L.) and quality protein maize (*Zea mays* L.) flour blend. LWT Food Sci Technol 41:1799–1807.

Tovar J, Melito C. 1996. Steam-cooking and dry heating produce resistant starch in legumes. J Agric Food Chem 44:2642–2645.

Tovar J, Björck IM, Asp NG. 1990. Starch content and α-amylolysis rate in precooked legumes flours. J Agric Food Chem 38:1818–1823.

Villarroel M, Acevedo C, Yánez E, Biolley E. 2003. Propiedades funcionales de la fibra del musgo Sphagnum magellanicum y su utilización en la formulación de productos de panadería. Arch Latinoam Nutr 53:400–407.

Wang W, Klopfenstein Y, Ponte J. 1993. Effects of twin-screw extrusion on the physical properties of dietary fiber and other components of whole wheat bran and on the baking quality of the wheat bran. Cereal Chem 76:707–711.

Yeoung AK, Barbeau W. 1991. Changes in the nutritive value of soy protein concentrate during autoclaving. Plant Foods Hum Nutr 41:179–192.

Yust MM, Pedroche J, Girón-Calle J, Vioque J, Millán F, Alaiz M. 2004. Determination of tryptophan by high-performance liquid chromatography of alkaline hydrolysates with spectrophotometric detection. Food Chem 85:317–320.

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