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Extrusion of a hard-to-cook bean (*Phaseolus vulgaris* L.) and quality protein maize (*Zea mays* L.) flour blend

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Abstract

Heated extrusion was tested as an alternative process for incorporating "hard-to-cook" beans into food products. A 3^2 factorial design was used to evaluate extrusion conditions for a 40/60 (w/w) blend of "hard-to-cook" beans and quality protein maize. Tested extrusion variables were temperature (155, 170 and 185 °C) and moisture content (15.5, 17.5 and 19.5 g/100 g). Screw speed was fixed at 130 rpm. The extrudates obtained at 155 and 170 °C with 15.5% moisture had the best physical characteristics and were chosen for comparative analysis of nutritional changes between the unprocessed "hard-to-cook" bean/quality protein maize flour blend and the resulting extrudates. *In vitro* protein digestibility was higher in the extrudates (80%) than in the flour blend (76%). *In vitro* starch digestibility was higher at 155 °C (89%) and 170 °C (92%) than in the flour blend (12%). Processing conditions decreased dietary fibre content by 38% at 155 °C and 44% at 170 °C.

Keywords: Extrusion; QPM; Hard-to-cook beans; Nutritional changes

1. Introduction

Bean seeds undergo physical, biological and chemical changes during storage. Physical factors such as seed moisture content, temperature, seed condition and available oxygen in storage have a decisive effect on degradation because they influence seed quality (Rodríguez, 1992). Beans stored under high humidity (>75%) and high temperature conditions (30–40 °C) experience serious losses in quality characteristics, particularly increased cooking time due to hardening (Kigel, 1999). Some mechanisms proposed to explain hardening include conversion of lipids to oxygenated polymers, formation of insoluble pectates, lignification, protein denaturalization and hydrolysis; all occur mainly in the cotyledon. Hardening leads to what is called the hard-to-cook phenomenon. This involves changes in cell adherence that inhibit cell separation during cooking, which affects cooked seed texture, and limits protein availability due to denaturalization and hydrolysis, lowering seed nutritional contribution (Garcia, Filisetti, Udaeta, & Lajolo, 1998).

A number of alternative technologies have been proposed for use of hard-to-cook beans, such as dry and wet fractionating, soaking in saline solutions, alkaline thermal treatment and extrusion. Of particular interest is extrusion, since it is already widely used to incorporate hard-to-cook seeds into cereals which are then used to produce precooked flours, infant food and expanded snacks. These extruded products have advantages in terms of their sensory characteristics (texture, flavour, smell and colour) and nutritional properties (increased protein content and balanced amino acid profile).

A food extruder is a high temperature, short processing time bioreactor that can transform a variety of ingredients into intermediate or finished products such as precooked flours, expanded snacks, breakfast cereals, pastas and texturized protein (González, Torres, & Degreef, 2002). During extrusion,

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denaturalization causes the breaking of the hydrogen bridges and disulphur bonds responsible for the secondary and tertiary structures in proteins. This probably increases exposure of sites susceptible to enzymatic activity and thus improves *in vitro* digestibility (Camire, 2002). The thermal aspect of extrusion also improves digestibility by efficiently eliminating or reducing the content of antinutritional factors such as phytic acid, tannins and polyphenols (these form insoluble complexes with proteins and reduce susceptibility to proteolytic activity), as well as trypsin inhibitors and chemotrypsin (Alonso, Aguirre, & Marzo, 1998).

Increased susceptibility of starch to enzymes after extrusion is mainly caused by starch gelatinization, although inactivation of enzymatic inhibitors also influences digestibility (Dahlin & Lorenz, 1993). Higher *in vitro* starch digestibility is attributed to inactivation of α-amylase inhibitors (phytic acid, tannins and polyphenols), which increases at higher temperatures (Alonso, Aguirre, & Marzo, 2000).

Extrusion also changes the content, composition and physiological effects of dietary fibre. Fibre content can be lowered due to degradation of dietary fibre into lower molecular weight fragments, while its composition can change in response to modification of starch, which forms fractions resistant to enzyme attack that act *in vivo* as dietary fibre. Finally, macromolecular degradation of fibre by extrusion increases its solubility and changes its physiological effects (Lue, Hsieh, & Huff, 1991).

In Mexico, most diets are based on the combination of maize and a legume, mainly beans. Maize has a high carbohydrate content, but a very low protein content and low levels of lysine and tryptophan. These deficiencies have been addressed through development of maize hybrids (known as quality protein maize – QPM) in which lysine and tryptophan levels are twice that of normal corn. Mixing of QPM and hard-to-cook beans can be used to balance the amino acid profile of the resulting product without notably affecting sensory acceptance. The present study objective was to determine the most appropriate extrusion conditions for a QPM and hard-to-cook bean blend for use in producing a snack-type expanded product with adequate physical and nutritional characteristics.

2. Material and methods

2.1. Materials

White dent quality protein maize (S00TLWQ-TO) was provided by the Yucatan Scientific Research Centre (Centro de Investigaciones Científicas de Yucatán – CICY), and *Phaseolus vulgaris* seed was obtained from the 2004 harvest in the state of Yucatan, Mexico. Physical damage, cooking time and grain texture were evaluated following the methods dictated in applicable Mexican regulations (NMX, 2002a, 2002b). All chemical reagents were analytical grade (Sigma Co., St. Louis, MO, USA).

2.2. Flour preparation

Selected grains were milled before extrusion. The grains were processed in a Buhler–Miag roller mill, with a progressive, successive reduction in distance between rollers from 2 to 1 mm and from 0.5 to 0.25 mm. After each milling the flours were sifted through 1.168, 0.833, 0.351 and 0.208 mm screen. The 0.351- and 0.208-mm flours were used in the extrusion process. Using a pneumatic separator, particles >1.168 and >0.833 mm were divided into hull and germ, which were discarded, and flour. The flour was then milled again, and sifted through 1.168, 0.833, 0.351 and 0.208 mm screen to separate the 0.351- and 0.208-mm fractions, which were added to the 0.351/0.208 mm flour from the first milling.

2.3. Blend preparation

Flour moisture content was determined to establish the amount of water to be added to adjust moisture content to required levels. The maize and bean flours were blended at a 60/40 (w/w) ratio in quantities sufficient to produce 500 g of blended flour for each treatment. Once equipment (Brabender P600, Germany) functioning was stabilized, the blend was processed continuously.

2.4. Extrusion and physical evaluation

The flour blend (FB) was extruded using a Brabender 20 DN monoscrew extruder with the following specifications: pressure and temperature sensor; two heating zones; screw with 4:1 compression ratio; and a 3.5-mm diameter × 20-mm long (3.5 × 20) die. A 3^2 model was used to evaluate extrusion conditions. The evaluated factors and levels were temperature (155, 170 and 185 °C) and moisture content (15.5, 17.5 and 19.5 g/100 g), with a feed rate of 200 g/min at a fixed screw speed of 130 rpm. After extrusion, the products were placed on trays to cool for 10 min. Product moisture content was conditioned for 24 h at 60 °C until attaining 6 g/100 g, and the products were stored in polyethylene bags until analysis. Physical evaluation of the samples included expansion index (EI), density, resistance to compression (RC) and specific mechanical energy (SME).

The expansion index (EI) was measured as described by Gujska and Khan (1990), i.e. by dividing extrudate diameter by die orifice diameter.

Density was determined following Wang, Klopfenstein, and Ponte (1993). Extrudate diameter (d), length (l) and weight (Pm) were measured and then density calculated as:

$$\text{Density} = \frac{\text{Pm}}{\pi(d/2)^2 l}$$

Resistance to compression (RC) was determined according to Park, Rhee, and Rhee (1993) with an Instron model 4411 universal machine, at a compression speed of 10 mm/min, using an 8-mm diameter probe and a 5000 N load cell.

The torque and mass output values were used to determine the specific mechanical energy consumption (SME) (González et al., 2002), using the following formula: SME (J/g) $= C \times T \times N \times \text{QA}^{-1}$, where k is: 61.6×10^{-3}; T is torque in Brabender units (BU); N is screw rpm and QA (g/min) is

the mass output (OC), referred to feeding moisture level. The value of C takes into account unit conversion and constants as follows: $2\pi \times 9.81 \times 10^{-3}$ mm/s^2 and QA (g/min) = OC (100 − EM)/100 − PM where EM was extrudate moisture and PM was established processing moisture.

Optimum extrusion conditions were selected from among the 11 tested extrudate treatments (Table 1) based on product physical characteristics, and the two optimum treatments (155 and 170 °C, 15.5 g/100 g moisture content, 130 rpm screw speed) further analyzed in comparison to the FB.

2.5. 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 nitrogen-free extract (NFE).

2.6. In vitro protein digestibility

This was determined following Hsu, Vavak, Satterlee, and Miller (1977), using 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 (III grade Sigma P-7500 with 50–100 units/g powder) per mL. 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 = pH of protein suspension immediately after digestion with multi-enzymatic solution for 10 min.

2.7. Amino acid analysis

The amino acid profiles of the products were determined according to Alaiz, Navarro, Vioque, and Vioque (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) consisted of a model 600E multi-solvent delivery system, a Wisp Model 712 automatic injector and a model 484 UV–vis detector. Samples with 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 constantly determined using a binary gradient system. The solvents used were (A) 25 mmol/L sodium acetate containing 0.02% sodium azide (pH 6.0), and (B) acetonitrile. Solvent was 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 was determined by high-performance liquid chromatography (HPLC) with spectrophotometric detection at 280 nm (Yust et al., 2004). Samples (10 mg) were dissolved in 3 mL of 4 mol equi/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 equi/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) prior to injection. Standard tryptophan solutions were prepared by dilution of a stock solution (0.51 mg tryptophan/mL, 4 mol equi/L sodium hydroxide) to 3 mL with 4 mol equi/L sodium hydroxide, followed by incubation as described above. 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.

2.8. Available lysine

Available Lys content was determined with the method of Hurrel, Lerman, and Carpenter (1979). Briefly, 0.5 g of sample was weighed into two tubes (A, B), 1 mL of 2-propanol was added to each tube and the tubes agitated vigorously for 5 min. After agitation, 4 mL sodium acetate (5%) were added to tube A, and 4 mL sodium acetate plus 0.3 mL of propionic anhydride added to tube B. The tubes were shaken vigorously for 15 min, 40 mL of colouring solution added to each, and then shaken again for 60 min. Levels of excess colouring concentration were determined at 475 nm using a spectrophotometer (Thermospectronic Genesis 10uv, Madison, WI, USA). Lysine levels were determined by the B–A difference. In both tubes, the millimoles of bound colouring = (40 mL/1000 mL) (100/weight of sample) (0.146), where 0.146 = lysine conversion factor.

Table 1
Expansion index (EI), density, resistance to compression (RC) and specific mechanical energy (SME) of extrudates

Sample	Temperature (°C)	Moisture content (g/100 g)	EI	Density (kg/m^3)	RC (N)	SME (J/g)
1	155	15.5	2.10^{a}	307.61^{abc}	53.7^{a}	564^{a}
2	155	17.5	2.03^{abc}	381.20^{cd}	52.8^{a}	513^{b}
3	155	19.5	1.94^{bc}	436.23^{d}	52.9^{a}	433^{c}
4	170	15.5	2.06^{ab}	237.17^{a}	51.9^{a}	502^{b}
5	170	17.5	1.90^{cd}	271.71^{abc}	54.7^{a}	440^{c}
6	170	17.5	1.85^{cd}	308.64^{abc}	53.5^{a}	452^{c}
7	170	17.5	1.82^{cd}	301.34^{abc}	55.1^{a}	443^{c}
8	170	19.5	1.68^{de}	360.22^{bcd}	53.9^{a}	344^{d}
9	185	15.5	1.66^{de}	261.02^{ab}	53.4^{a}	454^{c}
10	185	17.5	1.54^{ef}	339.78^{abcd}	53.4^{a}	378^{d}
11	185	19.5	1.40^{f}	348.98^{abcd}	54.9^{a}	300^{e}

$^{a-f}$Different superscripts in the same column indicate statistical difference ($P < 0.05$).

2.9. Protein efficiency ratio (cPER)

This parameter was calculated following the applicable AOAC (1997) method, employing the *in vitro* digestibility value and the amount (g) of amino acid (AA)/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 cannot surpass 50% of the total of their respective combinations.

2.10. Total starch (TS)

This was quantified by adapting the methodology of Tovar, Björck, and Asp (1990) using 4 mol/L KOH to guarantee starch solubilization. Hydrolysis was done using thermostable α-amylase (Sigma TO-3306) and amyloglucosidase (BioChemika 10115 70 solid units/mg). Reactivate glucose oxidase/peroxidase (GOD–PAP) (DiaSys, RE. 10250021) was used for colorimetric determination of glucose, and concentration was determined to 500 nm using a spectrophotometer (Thermospectronic Genesis 10UV, Madison, WI, USA). Calculation of sample glucose concentration was done with the equation: Glucose [mg/dL] = (EA − BA/SA − BA) (SC), followed by % Starch = (mg of glucose × 2 × 0.9/sample weight) (100), where, EA = Extrudate absorbance, BA = Blank absorbance, SA = Standard absorbance, SC = Standard concentration = 100 mg/dL, 2 = dilution factor and 0.95 = glucose to glean transformation factor.

2.11. Available starch (AS)

This was quantified in the same way as TS, adapting the methodology of Holm, Björck, Drews, and Asp (1986), with the exception that 4 mol/L KOH was not added.

2.12. Resistant starch (RS)

This parameter was calculated by the difference between TS and AS: RS = TS − AS.

2.13. In vitro starch digestibility

In vitro hydrolysis was determined using the method of Holm et al. (1986), which is based on the reducing power of the maltose released by the action of pancreatic amylase through 3,5-dinitrosalicylic acid (DNS). A standard maltose curve was generated and the calculations done as follows: % Hydrolysis in time 0: % Hydrolysis = [(mg maltose to 0 min − mg maltose 0 m) × 0.95 × 100]1.82^{-1}, where 0 m is a blank mixture of 0.3 mL H_2O, 0.5 mL standard, 1 mL DNS and 0.2 mL of sample. % Hydrolysis 5–60 min: % Hydrolysis = [mg maltose−(mg maltose to 0 min − 1.0)] × 0.95 × 100]1.78^{-1}, where, 1.0 is subtracted because 1.0 mg maltose was added to the 0 min and 0 m samples to ensure their detection in the spectrophotometer; 0.95 = glucose to glean transformation factor; and 5–60 = minutes hydrolysis times.

2.14. Total dietary fibre (TDF)

This parameter was determined with the gravimetric enzymatic method proposed by Prosky, Asp, Schweitzer, Debris, and Furda (1989). Briefly, 1 g of sample was weighed into each of four flasks, and 50 mL of 0.05 mol equi/L phosphate buffer at pH 6 added to each. The flasks were then placed in a Galena bath at 100 °C, 0.1 mL thermostable α-amylase enzyme (Sigma A-3306) added to each and then they were agitated at 60 rpm for 15 min. After cooling, pH was adjusted to 7.5. The flasks were returned to the bath at 60 °C, 0.1 mL protease (Sigma P-3910) added to each and then they were agitated at 60 rpm for 30 min. After cooling, pH was adjusted to 4.0. The flasks were again placed in the bath at 60 °C, 0.3 mL amyloglucosidase (Sigma A-9913) added and then they were agitated for 30 min. Finally, 95 g/kg ethanol, preheated to 60 °C, was added at a 1:4 (v/v) ratio. In a vacuum, flask content was filtered into crucibles containing celite (Sigma C-8656). The residue remaining 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. Crucible content was dried at 105 °C. Protein ($N \times 6.25$) was determined for the residue in two crucibles and the residue in the remaining two was burned at 550 °C for 4 h.

$$\mathrm{TDF(g/kg)} = \frac{[\text{Residue weight(g)} - \text{protein(g)} - \text{ash(g)} - \text{Blank}] \times 1000}{\text{Sample weight(g)}}$$

2.15. Insoluble dietary fibre (IDF)

This was determined following the method of Prosky et al. (1989), which is similar to that for TDF, except that addition of 95 g/kg alcohol at 1:4 is omitted.

2.16. Soluble dietary fibre (SDF)

Calculated by the difference between TDF and IDF: SDF = TDF − IDF.

2.17. Statistical analysis

Statistical treatment of the extrusion process results was done by multi-variate analysis following Johnson and Wichern (1992). An analysis of variance (ANOVA) and a means comparison were applied to establish differences using the LSD method, regression analysis and surface response, according to Montgomery (2004). Nutritional change data for starch, protein and fibre were processed using central tendency (mean) and dispersion (standard deviation) measurements. All analyses were done with the Statgraphics 5.0 program.

3. Results and discussion

3.1. Extrusion process and physical evaluation

The multi-variate analysis results indicated that both temperature and moisture significantly ($P < 0.05$) affected expansion index (EI), density, and specific mechanical energy (SME), but not resistance to compression (RC). 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 allow description of the IE, density and SME as a function of the significant factors (i.e. temperature and moisture). The mathematical models indicate that these behaviours are represented by Eqs. (1)–(3).

3.2. Expansion index

Temperature (X_1) and moisture (X_2) significantly ($P > 0.05$) affected extrudate EI. Both factors negatively affected variable response (Eq. (1)) in that the EI increased as both factors decreased. Maximum expansion was produced with extrusion at 155 °C and 15.5 g/100 g moisture content (E155) (Table 1). This can be explained by the fact that when materials are forced through an extruder die their water content vaporizes, and the simultaneous vapour flash-off expands their starch content, producing a porous, sponge-like structure in the extrudate. Extrudate degree of expansion is closely linked to the size, number and distribution of air cells within the material. Higher material moisture content creates a lubricating effect in the extrudation barrel which lowers friction and increases vaporization of superheated water as the extrudate exits the die, both of which can interfere with product expansion (Kokini, Lai, & Chedit, 1992; Pérez, Cruz, Chel, & Betancur, 2006).

$$\mathrm{EI} = 1.86 - 0.26(X_1) - 0.16(X_2) \qquad r^2 = 0.98 \tag{1}$$

3.3. Density

Temperature and moisture significantly ($P < 0.05$) affected density, although the effect was negative for temperature and positive for moisture (Eq. (2)). This coincides with Balandrán-Quintana, Barbosa-Cánovas, Zazueta-Morales, Anzaldúa-Morales, and Quintero-Ramos (1998), who reported a 20% reduction in density in bean extrudates when moisture was decreased from 25 to 20 g/100 g. Compared to products made only with maize, those including legume flour have higher protein content, which can also influence density since friction and shear during extrusion cause extensive interlacing between proteins and lead to their texturization: high protein content extrudates are denser and more rigid.

$$\mathrm{Density} = 299.55 - 29.21(X_1) + 56.605(X_2) \qquad r^2 = 0.95 \tag{2}$$

3.4. Resistance to compression

The RC values were statistically similar ($P > 0.05$), with values ranging from 54.9 to 51.9 N (Table 1). This physical parameter was influenced more by raw material characteristics and composition than by process temperature and moisture, meaning the normal relationship of higher RC as the EI increases (Díaz, 2003) was not observed here. This is a function of hardness, expressed as the maximum break force by compression, which reflects alveolar wall resistance and the number of alveoli per unit of height. These characteristics vary greatly depending on material composition and degree of protein denaturalization. The latter leads to greater interaction with other components and, even when extrudate protein content remains unchanged, can produce more rigid and denser products that are more resistant to breaking.

3.5. Specific mechanical energy

Both temperature (X_1) and moisture (X_2) had a significant ($P < 0.05$) and negative influence on extrudate SME (Eq. (3)). The SME values tended to decrease as temperature and moisture content increased, with the lowest SME values observed at the highest levels for both factors (185 °C and 19.5 g/100 g moisture). Higher temperature facilitated the transformation from solid flow to viscoelastic flow, and higher moisture produced a lubricating effect, resulting in less energy use. Starch gelatinization is positively influenced by SME during extrusion. The higher the SME the higher the degree of gelatinization since mechanical energy favours gelatinization by promoting rupture of intermolecular hydrogen bonds (Gropper, Moraru, & Kokini, 2002). Degree of gelatinization, in turn, is directly related to product expansion (Díaz, 2003), which agrees with the present results in that the extrudates with the highest SME had the highest EI.

$$\mathrm{SME} = 442.0 - 63.0(X_1) - 73.8(X_2) \qquad r^2 = 0.99 \tag{3}$$

Of the tested extrusion conditions those at 155 °C (E155) or 170 °C (E170) and 15.5 g/100 g moisture produced optimum physical characteristics (Table 1): the highest expansion index values (2.1 at 155 °C and 2.06 at 170 °C), lowest densities (307.61 kg/m^3 at 155 °C and 237.17 kg/m^3 at 170 °C) and highest SME values (564 J/g at 155 °C and 502 J/g at 170 °C). These products were selected to evaluate extrudate nutritional characteristics.

3.6. Nutritional evaluation

3.6.1. Chemical composition

The proximate composition of the flour blend (FB) and extrudates (Table 2) showed that moisture content after extrusion dropped to 8.9% in the E155 and 8.6% in the E170. This is to be expected since a portion of the water vaporizes during final extrudate expansion. Protein content was similar between treatments with 15.7% in the FB, 15.5% in the E155 and 15.4% in the E170. Although extrusion does not change

Table 2
Proximate composition of raw flour blend (FB) and extrudates (155 and 170 °C) (% db)

Component	FB	E155	E170
Moisture	(11.52 ± 0.01)	(8.99 ± 0.03)	(8.61 ± 0.05)
Protein	15.69 ± 0.03	15.45 ± 0.04	15.37 ± 0.02
Fat	2.62 ± 0.02	2.53 ± 0.03	2.37 ± 0.01
Crude fibre	2.45 ± 0.01	2.61 ± 0.06	2.68 ± 0.04
Ash	2.29 ± 0.09	2.27 ± 0.02	2.37 ± 0.06
NFE	77.07 ± 0.01	77.02 ± 0.06	77.12 ± 0.03

protein content, the high temperature, pressure and mechanical force of the process change protein physical and chemical properties.

Asp and Björck (1989) stated that high temperature (>200 °C) and screw speed (>300 rpm) during extrusion can cause lipids degradation. Also, fatty acids in the material can form complexes with amylose, making it more difficult to extract crude fats for quantification. The temperatures (155–185 °C) and screw speed (130 rpm) used in the present study, however, were far below these levels and probably had no effect on these parameters. Indeed, no differences were observed in crude fat content between the FB and the extrudates (E155 and E170).

Neither were differences noted in crude fibre content between the FB and extrudates (E155 and E170). Rabe (1999) mentioned, however, that insoluble and soluble fibres are redistributed after extrusion, producing thermomechanical transformations that will not appear in a proximal determination of crude fibre due to the technique's low sensitivity.

Mineral components resist the high temperatures, pressures and mechanical forces of extrusion. In an evaluation of extrusion of QPM at 130, 150 and 170 °C, at a screw speed of 300 rpm and 14% moisture content, Díaz (2003) reported no differences between flour and extrudate ash content. This agrees with the present results in which ash content in the FB was unmodified in the E155 and E170 (Table 2).

3.7. In vitro protein digestibility

The digestibility value for the E155 was 80% and that for the E170 was 79% (Table 3). These are similar to the 83% for *P. vulgaris* L. extrudates (160 °C, 22 g/100 g moisture) reported by Balandrán-Quintana et al. (1998), and the 82% for a 50/50 (w/w) maize/Lima bean flour blend reported by Pérez et al. (2006). The increased digestibility observed here in both extrudates (155 and 170 °C) may be due to two phenomena caused by extrusion: (1) protein denaturalization, which may increase exposure of sites susceptible to enzymatic activity (Camire, 2002); and (2) inactivation of trypsin and chemotrypsin inhibitors, leading to improved digestibility (Alonso et al., 1998).

Table 3
Protein nutritional parameters of raw flour blend (FB) and extrudates (155 and 170 °C)

Component	FB	E155	E170
In vitro protein digestibility (%)	76.05 ± 0.05	80.12 ± 0.03	79.31 ± 0.01
Available lysine (g/16 g N)	5.18 ± 0.02	4.33 ± 0.07	3.84 ± 0.05
cPER	1.62 ± 0.01	1.55 ± 0.03	0.94 ± 0.04

3.8. Amino acid profile

The essential amino acids content of the FB and the E155 exceeded that of a reference protein of the FAO/WHO (1991), except in Trp, which was 18% below the reference level (Table 4). Both extrudates had amino acids contents below that of the FB, although the E170 exhibited the greatest decrease, with a 63.37% sulphur amino acid level versus the reference (Asp & Björck, 1989). This is similar to the 10% decrease in sulphur amino acids reported by Díaz (2003) for QPM extrudates (130 °C, 20% moisture, 300 rpm screw speed). In an evaluation of Cys, Trp, Met, Arg and Lys losses during extrusion of maize flour at variable temperatures (150–180 °C), moistures (13–17%) and velocities (57–81 rpm), Ilo and Berghofer (2003) reported amino acid losses of 10, 15, 17, 19 and 43%, respectively, for extrudates produced at 180 °C, 13% moisture and 57 rpm. They concluded that temperature, moisture content and material residence time in extruder (screw speed) all affect amino acids loss. In the two extrudates analyzed here, screw speed was fixed at 130 rpm and moisture content was constant at 15.5%, meaning thermomechanical treatment intensity determined the observed decreases in both types of amino acids, particularly in the E170.

3.9. Available lysine

Available Lys content decreased 17.3% in the E155 and 26.9% in the E170 (Table 3). Ilo and Berghofer (2003) reported a 43% Lys decrease in maize extrudates. As was the case with the other amino acids, the decrease in Lys in the present study resulted from the thermomechanical treatment, and was, therefore, more pronounced (26.9%) at 170 °C. The high temperatures (155 and 170 °C) and moisture content (15.5 g/100 g) used here may have favoured Maillard reactions. In this sense, Lys is known to be more susceptible to reductions in physiological availability because its ε position amino group makes it more reactive and tends to interact with the reducing sugars produced by the process, a reaction that is further favoured by the mechanical action (Camire, 2002). The presence of Maillard reactions in the extrudates was corroborated by their colour, which varied from light yellow to dark brown due to the melanoidins generated by this type of reactions.

3.10. Calculated protein efficiency (cPER)

The extrusion process lowered cPER by 4% in the E155 and by 42% in the E170 compared to the FB (Table 3). In a study of extrudates (190 °C, 18.5 g/100 g moisture, 100 rpm) of hard-to-cook bean and rice blends (25/75 (w/w)), Steel, Sgarbieri, and Jackix (1995) reported decreases of 8% in cPER compared to the raw material. Based on the observed reduction in essential amino acids, they concluded that this reduction in protein nutritional value was caused by the thermal treatment. This is

Table 4
Amino acid content (g/100 g protein) of raw flour blend (FB) and extrudates (155 and 170 °C)

Amino acid	FB	E155	E170	FAO/WHO
Isoleucine	3.67 ± 0.01	3.56 ± 0.01	3.36 ± 0.02	2.8
Leucine	9.12 ± 0.01	9.02 ± 0.03	8.88 ± 0.02	6.6
Lysine	5.99 ± 0.04	5.85 ± 0.01	5.56 ± 0.02	5.8
Methionine + Cysteine	3.0	2.7	1.9	2.5
Phenylalanine + Tyrosine	8.3	8.2	8.0	6.3
Threonine	4.88 ± 0.02	4.81 ± 0.04	5.03 ± 0.01	3.4
Tryptophan	0.91 ± 0.05	0.91 ± 0.02	0.80 ± 0.03	1.1
Valine	5.27 ± 0.06	5.51 ± 0.01	4.92 ± 0.03	3.5
Histidin	3.36 ± 0.01	3.24 ± 0.02	3.13 ± 0.01	1.9

similar to the present results, particularly in the observed decreases in sulphur amino acids and Lys.

3.11. Total starch (TS)

Total starch values were similar in the FB and the E155 (46.2%) and E170 (48.9%) (Table 5). These levels are lower than the 62.9% reported by Pérez et al. (2006) for a 50/50 QPM/Lima bean blend. Molecular degradation in the starch occurred as a function of temperature, moisture and screw speed since increases in these parameters would produce severe degradation in the starch and produce lower weight molecules. The studied extrusion conditions (150 and 170 °C, 15.5 g/100 g moisture, 130 rpm) were not extreme enough to cause this degradation, which explains the similar starch contents in the FB and extrudates (E155 and E170).

3.12. Available starch (AS)

Available starch increased from 84% of total starch in the FB to 94.7% in the E155 and 95.9% in the E170 (Table 5). These values are similar to the 97.4% reported by Betancur (2001) for maize starch, and higher than the 80.7% reported by Pérez et al. (2006) for an extrudated QPM/Lima bean blend (50/50 (w/w)). Starch loses its crystalline structure due to cooking during extrusion, leaving its molecules available for hydrolysis (Steel et al., 1995). This would explain the increased AS values in the two extrusion treatments studied here, which both had significantly higher AS than the FB.

3.13. Resistant starch (RS)

The FB had a RS content of 15.9% of total starch, higher than the 10.8% RA reported by Pérez et al. (2006) for an unprocessed QPM/Lima bean blend (50/50 (w/w)). This value decreased to 5.3% in the E155 and to 4.3% in the E170, which are higher than the 2.6% reported by Pérez et al. (2006) for the same blend as above. The higher RA in the FB in the present study was probably due to the 40% proportion of HCB in the blend. Bean starch granules are more resistant to hydrolysis by digestive enzymes because of their C-type diffraction pattern (a combination of A and B) structural characteristics. This pattern is a mixture of rhomboid and hexagonal crystals, the latter of which are indigestible by enzymes due to their compact supramolecular packing (Steel et al., 1995). Depending on process conditions, resistant starch can form during extrusion; at low moisture contents (15–20%) molecules are less mobile, favouring the formation of hydrogen bridges between contiguous chains. In addition, screw speed during processing is important because it determines material residence time, with longer residence times favouring RA formation. High temperatures (180–200 °C) can also increase RA formation (Cabrejas, Esteban, Perez, Maina, & Waldron, 1997). In the present study, however, RA content decreased with extrusion, probably because the conditions (150 and 170 °C, 15.5% moisture, and 130 rpm) did not meet the moisture content, residence time and temperature requirements for greater production of RA. They did cause cooking of the material, however, which agrees with the observed increases in extrudate AS (E155 and E170).

Table 5
Nutritional changes in starch and fibre of raw flour blend (FB) and extrudates (155 and 170 °C)

Component	FB	E155	E170
Total starch (%)	49.08 ± 0.03	48.75 ± 0.04	48.94 ± 0.09
Available starch (%)	41.29 ± 0.07	46.19 ± 0.04	46.88 ± 0.01
Resistant starch (%)	7.79 ± 0.01	2.57 ± 0.02	2.06 ± 0.09
In vitro starch digestibility (%)	12.03 ± 0.02	89.45 ± 0.05	91.89 ± 0.08
Total dietary fibre (%)	27.50 ± 0.05	17.03 ± 0.09	15.03 ± 0.06
Insoluble dietary fibre (%)	23.08 ± 0.01	13.64 ± 0.02	11.57 ± 0.03
Soluble dietary fibre (%)	4.42 ± 0.02	3.39 ± 0.04	3.46 ± 0.02

3.14. In vitro starch digestibility

As expected, the starch in the FB was not gelatinized, meaning it was practically indigestible by α-amylase action. The resulting hydrolysis level of 12% (Table 5) was similar to the 10% reported by Betancur (2001) for maize, Lima and velvet bean starches. This low digestibility is caused by the crystalline structure of starch, which protects the glucoside bonds and limits enzyme hydrolytic action. Once gelatinized, however, this crystalline structure is lost, leaving the molecules open for hydrolysis, which breaks the glucoside bonds and, therefore, increases digestibility. This is what occurred in the present study as degree of hydrolysis increased to 89% in the E155 and to 92% in the E170. These values are similar to the 92% for maize starch and greater than the 84% for Lima bean reported by Betancur (2001).

3.15. Total dietary fibre (TDF)

The TDF content in the FB was higher (Table 5) than the 14.46% reported by Pérez et al. (2006) for a QPM/Lima bean blend (50/50 (w/w)). Extrudate TDF was similar to the 17.2% reported by Cabrejas et al. (1997) for hard-to-cook bean extrudates (140 °C, 25 g/100 g moisture, and 130 rpm). Process conditions modified TDF content in the extrudates by 38% (E155) and 44% (E170) versus the FB. This modification probably occurred through degradation into low molecular weight fragments since extrusion causes considerable solubilization of dietary fibre components, particularly pectic polymers and cellulose. Cabrejas et al. (1997) stated that temperature is what causes this degradation, which coincides with the present results in that the greatest decrease in TDF content was observed in the E170.

3.16. Insoluble dietary fibre (IDF)

Insoluble dietary fibre in the FB accounted for 84% of TDF (Table 5), a percentage similar to the 80% reported for hard-to-cook bean (*P. vulgaris* L.) flour (Cabrejas, Jaime, Karanja, & Downie, 1999). The IDF accounted for 80% of TDF in the E155 and 78% in the E170, values similar to those reported for extrudates from HCB flours (83 and 79%; 160 °C, 25 g/100 g moisture, and 130 rpm) (Cabrejas et al., 1999). Compared to the FB, IDF in the E155 decreased by 26%, and that in the E170 by 34%. This reduction was caused by the increased solubilization of insoluble fibre components, particularly arabinose and uronic acid, as well as degradation of pectic polymers and cellulose (Cabrejas et al., 1999).

3.17. Soluble dietary fibre (SDF)

Soluble dietary fibre in the FB accounted for 16% of TDF (Table 5), which is lower than the 20% reported for SDF in HCB (*P. vulgaris* L.) flour (Cabrejas et al., 1999). The SDF fraction accounted for 20.6% of TDF in E155 and 22.7% in E170, which are higher than SDF values reported for HCB flours extrudated at 140 °C (17% SDF) or 160 °C (21% SDF) (25 g/100 g moisture, 130 rpm) (Cabrejas et al., 1999). Increased SDF can be caused by release of the soluble fraction from hemicellulose as a result of heating, which coincides with the highest SDF being observed in E170.

4. Conclusions

Extrudates with adequate physical characteristics were produced from a blend of quality protein maize and hard-to-cook bean (60/40 (w/w)) flours. Of the tested extrusion conditions, those at 155 or 170 °C, 15.5 g/100 g moisture and 130 rpm screw speed produced optimum physical characteristics: the highest expansion index values (2.1 at 155 °C and 2.06 at 170 °C); lowest densities (307.61 kg/m^3 at 155 °C and 237.17 kg/m^3 at 170 °C); and highest SME values (564 J/g at 155 °C and 502 J/g at 170 °C). Combination of the legume with the maize provided the blend with a protein content of almost 15%, producing extrudates with good nutritional quality. *In vitro* protein digestibility increased after extrusion, although the extrudates' amino acid content decreased versus the flour blend, lowering cPER values, especially in the extrudate produced at 170 °C. Total starch content in the flour blend and extrudates was unmodified. Both available starch content and *in vitro* starch digestibility increased due to extrusion. Total and insoluble dietary fibre decreased in the extrudates compared to the flour blend, but soluble dietary fibre increased. Using a blend of flours from QPM and a legume of no commercial value and low nutritional value, extrusion at 155 °C, 15.5 g/100 g moisture content and 130 rpm screw speed produced an extrudate with adequate physical and nutritional characteristics.

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