Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (Phaseolus vulgaris L.) flour: Effects of bean addition and extrusion cooking

Alex A. Anton*, R. Gary Fulcher, Susan D. Arntfield

Department of Food Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2

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ABSTRACT

Navy and red bean flours (BF) were added to corn starch at levels of 15%, 30%, and 45% and submitted to extrusion cooking to produce fortified puffed snacks. Process variables (screw speed, moisture, and temperature of the final zones) of a twin screw extruder were kept constant (150 rpm, 22% and 160 °C). Corn starch-bean extrudates were denser, less expanded, and harder. However starch fortified with 30% BF produced extrudates with percentage of deformation – an instrumental measurement of crispness - comparable to corn starch alone. At this level, crude protein was increased 12-fold, while total phenols, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and oxygen radical absorbance capacity (ORAC) in vitro antioxidant activities (AA) were also increased. Red bean fortification yielded extrudates with higher levels of phenols and both DPPH and ORAC AA compared to navy beans. In navy and red bean extrudates, total phenols, DPPH, and ORAC AA were reduced by 10%, 17%, and 10%, and by 70%, 62%, and 17% after extrusion, respectively. Phytic acid and trypsin inhibitors levels were reduced in nearly 50% and 100% in all bean extrudates compared to raw mixtures, indicating that these materials were safe for human consumption.

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1. Introduction

Extrusion cooking is an important processing technique in the food industry as it is considered to be an efficient manufacturing process. Food extruders provide thermo-mechanical and mechanical energy (shear) necessary to cause physico-chemical changes of raw materials with an intense mixing for dispersion and homogenization of ingredients (Anton & Luciano, 2007; Linko, Colonna, & Mercier, 1981; Wiedman & Strobel, 1987).

Extruded foods are composed mainly of cereals, starches, and/or vegetable proteins. The major role of these ingredients is to give structure, texture, mouth feel, bulk, and many other characteristics desired for specific finished products (Launay & Lisch, 1983; Tahnoven et al., 1998). Consumer acceptance of extruded foods is mainly due to the convenience, value, attractive appearance and texture found to be particular for these foods, especially when it concerns snack products (Anton & Luciano, 2007; Harper, 1981).

While corn starch provides all the features for production of highly acceptable extruded snack foods, its nutritional value is far from satisfying the needs of health-conscious consumers (Rampersad, Badrie, & Comissiong, 2003). Several attempts to improve the nutritional profile of extruded starch have been reported (Liu, Hsieh, Heymann, & Huff, 2000; Onwulata, Konstance, Smith, & Holsinger, 2001; Rampersad et al., 2003). Among other materials, incorporation of legume flours has been shown to cause a positive impact on levels of proteins and dietary fibre of corn starch-based extruded snacks (Berrios, 2006). On the other hand, addition of high-fibre, high-protein alternate ingredients to starch has been demonstrated to significantly affect the texture, expansion and overall acceptability of extruded snacks (Liu et al., 2000; Veronica, Olusola, & Adebowale, 2006). For the production of nutritious acceptable snacks, rates of starch fortification seem to vary according to the nature of each material. Legumes, for example, have been reported to cause good expansion and are regarded as highly feasible for the development of high-nutritional, low-calorie snacks (Berrios, 2006).

Taking into account the nutritional and economical aspects of common beans (Phaseolus vulgaris L.) (Anton, Ross, Beta, Fulcher, & Arntfield, 2008; Tharanathan & Mahadevamma, 2003), fortifying corn starch with flours and fractions of varied bean cultivars for the production of extruded snacks appears to be promising. High in fibre, protein, and low in fat, bean consumption has been inversely associated with reduced risk of coronary diseases and some types of cancer (Azevedo et al., 2003; Winham & Hutchins, 2007). In addition, there is solid scientific evidence that coloured dry beans possess strong in vitro antioxidant activity (Anton, Ross, Beta, et al., 2008; Beninger & Hosfield, 2003; Madhujith & Shahidi, 2005).
which may explain, in part, the protective benefits of bean consumption on development of degenerative diseases.

Antioxidants in beans are related to the presence of phenolic compounds that influence their seed coat colour (Beninger & Hosfield, 2003; Madhujith & Shahidi, 2005). In this regard, coloured dry beans such as red, pinto and black, are expected to possess stronger antioxidant activity than navy beans. Although little is known about the effect of extrusion cooking on phenolic composition and antioxidant activity of dry beans (Korus, Gumul, & Czechowska, 2007; Korus, Gumul, Folta, & Bartoń, 2007), thermal processing of beans has been reported to cause important changes on these parameters (Anton, Ross, Beta, et al., 2008; Rocha-Guzmán, González-Laredo, Ibarra-Pérez, Nava-Berümen, & Gallegos-Infante, 2007).

Additionally, extrusion cooking has been used to partially or totally inactivate several antinutritional compounds that limit the widespread use of beans as a primary staple food (Alonso, Aguirre, & Marzo, 2000; Shimelis & Rakshit, 2007). These compounds, such as phytic acid and trypsin inhibitors, might produce adverse effects for human and animal nutrition (Martín-Cabrejas et al., 2004). Extrusion has also been reported to be the most effective method for improving protein and starch digestibility of kidney beans extrudates (Alonso et al., 2000; Berrios, 2006). Consequently, fortification of corn starch with bean flour is believed to add value to dry beans as well as result in a product with high-nutritional appeal.

This work aimed to determine the technical feasibility of adding varied levels of navy and red bean flour (15%, 30%, and 45%) to corn starch for production of puffed snack foods through extrusion, as well as to examine the effect of extrusion cooking on levels of nutritional and antinutritional compounds of the various formulations. Parameters such as bulk density, expansion ratio, breaking strength and deformation were used to evaluate the physical properties of extrudates. They were aimed to reflect the technical feasibility of incorporating bean flours into corn-based extruded materials. Additionally, levels of protein, antioxidants, total phenolics, trypsin inhibitors, and phytic acid were measured to determine the nutritional impact of bean fortification and to assess the consequences of the thermal treatment on these parameters.

2. Materials and methods

2.1. Acquisition of samples and preparation of flours

Navy (variety GTS 531) and small red (variety AC Earlired) beans were obtained from the Agriculture and Agrifood Canada Research Station in Morden, MB, Canada. The cultivars were grown and harvested in 2006 and exposed to the same environmental conditions in order to avoid external variation. The weight of 100 seeds was determined gravimetrically and expressed as mean ± SD of three determinations. Crude protein (AOAC, 1990) content of bean samples were: 24.06% for navy, and 21.27% for small red beans.

Whole seeds were ground in a Jacobson pilot scale hammer mill (Model No. 120-B, Minneapolis, MN, USA) to pass a 500 μm sieve (35 mesh US Standard Sieve Series). Ground samples were added at different levels (15%, 30%, and 45%) to regular corn starch (9.8% moisture, 25% amylase and 75% amylopectin – Casco, Ethibioce, ON, Canada) and the composite flours were stored at 5 °C in opaque, closed containers for further use. Composite flours were made in triplicate for each level of substitution for each bean cultivar. The raw composite flours, as well as the extruded products, were analyzed for their moisture content by AOAC (1990) method 925.10.

2.2. Extrusion

A laboratory scale twin screw extruder (MPF 19:25, APV Baker Inc., Grand Rapids, MI, USA) under high shear and high temperature in the final zones was used. The barrel diameter was 19.0 mm and the screw configuration with a length to diameter (L/D) ratio of 25.0 was as follows: 8 D feed screws, 6×30° forward kneading paddles, 6D feed screws, 1×kneading paddle, 1D single lead screw, 2×60° forward kneading paddles, 2×60° reverse kneading paddles, 1D single lead screw, 3×60° forward kneading paddles, 1D single lead screw, 2×60° forward kneading paddles, 4×60° reverse kneading paddles, 3D single lead screws. Screw diameter was equal to 19.00 mm (1D) and one kneading paddle was equal to 1/4 D.

Composite flours were added to the feed hopper and deionized water was injected as the mixture reached the screw zone, allowing a fixed feed moisture of 22%. Based on preliminary experiments, the following conditions were kept constant: 150 rpm screw rotation, 1.8 kg/h feed rate, 4.5 mm die diameter. The barrel consisted of five independent zones, electrically heated and cooled by water. Barrel temperature zones profile was set to 30/80/120/160/160 °C. Extruded products were cooled for 30 min in room temperature and then placed in sealed plastic bags for 24 h in room temperature. Extrudates were analyzed for their physical properties 24 h after production.

2.3. Physical analysis

Expansion ratio was determined as the diameter of extrudates divided by the diameter of the die exit (4.5 mm) (Gujska & Khan, 1991). Diameters at three different locations along the 40 mm strand of an extrude were measured first and the expansion ratio was calculated by dividing the average diameter of the strand in mm by 4.5. The specific length of extrudates was evaluated as their straight length divided by the equivalent weight of each individual strand (Alvarez-Martinez, Konduy, & Harper, 1988). Density (ρ) was determined following the method of Wang, Klopfenstein, and Ponte (1993) by measuring the diameter (d), length (l) and weight (Pm) of each extrudate. It was calculated as

\[
\rho = \frac{P_m}{\pi(d/2)^2/l}
\]

Mechanical properties of extrudates were determined through a three point bending test using a Zwick 2005 materials testing machine (Zwick USA, Kennesaw, GA, USA) equipped with a 1 kN load cell and a Warner-Bratzler shear cell (1 mm thick blade). Tests were controlled and data were compiled using the software TextXpert II (Zwick GmbH, Ulm, Germany). The extrudates were analyzed at a cross head speed of 0.2 mm/s. Breaking strength index (BSI) was calculated using: BSI = peak breaking force (n)/extrudate cross-sectional area (mm²). dl (Fmax) was defined as deformation at maximum force, meaning how much the shear cell penetrated the sample until breaking. This information was used to calculate the % deformation, defined as dl (Fmax) × 100/extrudate diameter. For all physical analysis so far described, at least ten strands of each type of extrude were assayed for each test. Following the described measurements, extrudates were ground in a coffee grinder (Smart Grind, Black and Decker, Towson, MA, USA) so that the meal passed through a 500 μm sieve (35 mesh US Standard Sieve Series). The ground samples were stored at 5 °C for no more than 3 weeks in opaque, closed containers.

Colour measurements (CIE L*, a*, b* colour space) were performed on ground samples using a Minolta CM-3600d model spectrophotometer (Konica Minolta, Ramsey, NJ, USA). The colour of extrudates was expressed as the average of three L*, a*, and b* readings, where L* stands for brightness, +a* redness, −a* greenness, +b* yellowness.
yellowness, and \( b^* \) blueness. A white calibration plate was used to standardize the equipment prior to colour measurements. Chemical analyses were performed on ground samples only after they were warmed to room temperature.

2.4. Chemical analysis

Nitrogen content was determined by using the Kjeldahl method and was multiplied by a factor of 5.7 to estimate protein content (AOAC, 1990).

Total phenol content and antioxidant activity were determined in both raw and cooked mixtures with 3 or 4 replications depending on the method, as stated in the tables footnotes. For such determinations, 100 mg of finely ground sample was extracted in 2.5 mL of acetone/water (80:20, v/v) (Fisher, Ottawa, ON, Canada) for 2 h in a rotary shaker. After this period, the samples were centrifuged at 3000g in a table centrifuge (GLC-1, Sorval, Newton, CT, USA) for 10 min. Thereafter the supernatant was transferred to a 3 ml syringe (Fisher) and filtered through a 0.45 \( \mu \)m sterile PVDF filter unit (Fisher). The filtrate was collected for further analysis.

The total phenolic content was determined using the Folin–Ciocalteau method (Singleton & Rossi, 1965) as modified by Gao, Wang, Oomah, and Mazza (2002). An aliquot (0.2 mL) of extract was added to 1.5 mL of freshly diluted 10-fold Folin–Ciocalteau reagent (BDH, Toronto, ON, Canada). The mixture was allowed to sit for 5 min and then 1.5 mL of sodium carbonate solution (60 g/L) (Sigma, St. Louis, MO, USA) was added. Afterwards, the mixture was incubated for 90 min and the absorbance read at 725 nm. Acetone/water (80:20, v/v) was used as a blank and ferulic acid (Sigma) was used as the standard. The results were expressed in mg of ferulic acid equivalents per 100 g of sample. Linearity range of the calibration curve was 20–200 \( \mu \)g (\( r = 0.99 \)).

For measuring the antioxidant activity two methods were employed. Antioxidant activity was initially measured using a modified version of Chen and Ho (1995). For this assay, 200 \( \mu \)L of extract was reacted with 3.8 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Fisher) solution (6.34 \( \times 10^{-4} \) M in methanol). The decreasing absorbance was monitored at 517 nm (Ultrascop 200, Pharmacia Biotech Piscataway, NJ) in the dark at 30 min against a methanol blank. The control consisted of 200 \( \mu \)L of acetone/water (80:20, v/v) in 3.8 mL of DPPH solution. The results were obtained as a percent of discolouration according with the formula

\[
1 - \left( \frac{Absorbance_{sample}}{Absorbance_{control}} \right) \times 100
\]

Simultaneously to the samples, 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox) (Sigma) was used as a standard and the results were expressed as \( \mu \)mol of Trolox equivalents per 100 g of sample. The linear range of the calibration curve was 2.5 to 20 \( \mu \)mol (\( r = 0.99 \)).

The second method for determining antioxidant activity was the oxygen radical absorbance capacity (ORAC) assay according to the procedures described by Huang, Boxin, Hampsch-Woodill, Flanagan, and Prior (2002) as modified by Li, Wei, White, and Beta (2007). An FL \( \times 800 \) microplate fluorescence reader (Bio-Tek Instruments Inc., Winooski, VT) was used with fluorescence filters for an excitation wavelength of 485/20 nm and an emission wavelength of 528/20 nm. The plate reader was controlled by KC4 3.0 software (version 29). Samples, rutin control, and the Trolox standard were diluted manually. Three hundred microliters of buffer solution (blank), diluted sample, rutin control, or Trolox standard was transferred to a 96 well flat-bottom polystyrene microplate (Corning Incorporated, Corning, NY) by hand according to their designated positions. A full automation of plate-to-plate liquid transfer was programmed by using a Precision 2000 microplate pipetting system (Bio-Tek Instruments Inc., Winooski, VT). Trolox standard, diluted samples, and 20 \( \mu \)M rutin control from designated wells of the first 96-well microplate was transferred to designated wells of the second 96-well microplate. The latter was quickly covered with an adhesive sealing film, then shaken for 3 min at 37 °C in the incubator and incubated in the preheated (37 °C) FL \( \times 800 \) microplate reader for a total period of 20 min. The second 96-well microplate was transferred back to its original station in the Precision 2000 automated microplate pipetting system, followed by automatically transferring 60 \( \mu \)L of AAPH solution from the reagent holder to designated wells. Thus, the total volume for each well was 200 \( \mu \)L. The second 96-well microplate was quickly covered again with an adhesive sealing film and immediately transferred to the FL \( \times 800 \) microplate reader, and the fluorescence was measured every minute for 50 min at 37 °C. The peroxyl radical was generated by 2,2'-azobis(2-aminopropane) dihydrochloride (AAPH) during measurement, and fluorescein was used as the substrate.

Final ORAC values were calculated by using a regression equation between the Trolox concentration and the net area under the fluorescence decay curve. The area under curve (AUC) was calculated as

\[
AUC = 0.5 + f_1/f_0 + \cdots + f_i/f_0 + \cdots + f_n/f_0 + 0.5(f_0/f_0)
\]

where \( f_0 \) is the initial fluorescence reading at 0 min and \( f_i \) is the fluorescence reading at time \( i \) min. The net AUC was obtained by subtracting the AUC of the blank from that of the sample. ORAC values were expressed as Trolox equivalents according to the standard curve. Final results were expressed as mg TE per 100 g of sample.

Phytic acid levels were determined by the method of Latta and Eskin (1980). This analysis was done with a chromatographic column (0.7 cm × 15 cm) containing 0.5 g of an anion-exchange resin (100–200 mesh, chloride form; AG1-X8, Bio-Rad Co., Hercules, CA, USA). The Wade reagent (1 mL, 0.03% FeCl₃ - 6H₂O and 0.3% sulfosalicylic acid in distilled water) was added into the extract (3 mL), and the mixture vortexed for 30 s. The absorbance of the supernatant was measured at 500 nm with a UV–Vis spectrophotometer.

Trypsin inhibitor activity was measured following the procedure by Kakade, Rackis, McGhee, and Puski (1974), using \( N \)-benzoyl-\( \alpha \)-arginine-p-nitroanilide hydrochloride (BAPNA) (Sigma) as the substrate for trypsin. 500 mg of finely ground sample was extracted with 25 mL of 0.01 N sodium hydroxide for 3 h at room temperature in a rotatory shaker. Extracts were centrifuged at 17,500g (RC5C, Sorval, Newton, CT, USA) at 4 °C for 20 min, and the supernatants filtered through Whatman No. 1 filter paper. Thereafter extracts were diluted to 30% in distilled water so that 1.0 mL could inhibit 50% of trypsin activity in the conditions presented herein. Five portions of extracts (0, 0.6, 1.0, 1.4, and 1.8 mL) were pipetted into test tubes and the final volume was adjusted to 2 mL with distilled water. Trypsin solution (2 mL, 20 mg/L in 0.001 M HCl) was added and the tubes were placed in the water bath at 37 °C, followed by addition of 5 mL of \( N \)-benzoyl-\( \alpha \)-arginine-p-nitroanilide (BAPNA) solution (0.4 mg/mL in Tris-buffer 0.05 M, pH 8.2) previously warmed to 37 °C. After exactly 10 min the reaction was stopped by adding 1 mL of 30% acetic acid to each test tube. The absorbance was read at 410 nm and the reagent blank prepared by adding 1 mL of 30% acetic acid to a test tube containing trypsin and water (2 mL of each) before the BAPNA solution was added. One trypsin unit was arbitrarily defined as an increase of 0.01 absorbance unit at 410 nm per 10 mL of the reaction mixture under the conditions used herein.

2.5. Statistical analysis

All data were recorded as means ± SD and analyzed by GraphPad Instat for Windows (version 3). One-way analysis of variance
3. Results and discussion

3.1. Effect of bean flour addition on some physical properties of corn starch-based extrudates

The most appropriate feed moisture (22%) and temperature (160°C) for production of the most expanded extrudates observed for our Flours are in accordance with the findings of Balandrán-Quintana, Barbosa-Cánovas, Zazueta-Morales, Anzaldúa-Morales, and Quintero-Ramos (1998), whose work reported the best extrusion conditions for production of extruded whole pinto bean meal.

Table 1 summarizes the impact of added bean flour on some physical properties of corn starch extrudates. As expected, increasing levels of bean flour resulted in a significant decrease in expansion. By decreasing the amount of corn starch in the mixtures and increasing the concentration of protein and fibre through addition of bean flour, less expanded products were formed due to interactions between these components and the starch. This lower expansion can also be explained on the basis that fibre can rupture cell walls and prevent air bubbles from expanding to their maximum potential (Pérez-Navarrete, Gonzáles, Chel-Guerrero, & Betancur-Ancona, 2006). Such an argument may also support the fact that navy bean flour fortification produces slightly less expanded products than small red beans. This was probably due to the higher amount of fibre from the seed coats in the flour of navy beans, which is based on the weight of 100 seeds (navy: 15.17 ± 0.41 g; small red: 27.21 ± 1.83 g); when the mass of the seeds is less, the seed coat comprises a larger area relative to one whole seed. Similar behaviour has been reported for maize and Lima bean flour extrudates (Pérez-Navarrete et al., 2006).

Density of extrudates increased significantly with bean flour addition, except for fortification with 15% of small red bean flour, which did not significantly affect this parameter. There is solid evidence in the literature that as high-fibre, high-protein materials are added to starch-based extruded products, density is expected to increase (Omwulata et al., 2001; Veronica et al., 2006). In this study, our parameter was inversely correlated with expansion ratio \((r = -0.89, P < 0.01)\) based on the same rationale. Gujska and Khan (1991) suggested that the degree of expansion affects the density, fragility and overall texture of extruded products.

The specific length of extrudates correlates their length with their weight as an expression of axial expansion. Negative correlations between radial and axial expansion ratios have been reported (Alvarez-Martínez et al., 1988; Launay et al., 1983), which agrees with our findings \((r = -0.88, P < 0.01)\).

Table 2 shows the effect of bean cultivar on the properties herein studied. Cultivar effects were present at all levels of bean flour substitution for colour, phenolic content and antioxidant activity. Fortification with navy bean flour caused a slight impact on colour, while small red bean flour addition resulted in clearly seen colour changes. Nonetheless, as there is a market opportunity for introducing new food products with high-nutritional appeal, the health-conscious consumer is likely to accept different organoleptic features such a appearance, flavour, and texture (Anton, Luciano, & Maskus, 2008).
Physical properties of extrudates were negatively affected by added bean flours (Table 1). While breaking strength index increased with higher levels of bean flour substitution, instrumental parameters aimed to reflect the crispness of extrudates were decreased. The distance traveled by the shear cell until total breakage of extrudate, together with the corresponding percentage of deformation of each extrudate, were taken as a measure of crispness. A crispier product is reflected by longer distances traveled by the shear cell at maximum force (dl), higher percent deformation and a larger number of major peak forces during analysis of theuffed extrudates. Higher deformation as a result of many fracture events is regarded as a consequence of crispy extruded products (Roudaut, Dacremont, Pàmies, Colas, & Le Meste, 2002; Veronica et al., 2006) and was used to evaluate crispness in this study. Thus, crispness of bean products was significantly affected only in 45% substitutions, demonstrating that although bean addition produced harder and less puffed extrudates, 30% fortification resulted in extrudates with percentage of deformation comparable to corn starch alone (Table 1). This is logical by understanding the basic constitution of corn starch and beans, which serves as an argument for most of the physical properties herein discussed. It also agrees with the work of Areas (1992) in a sense that addition of protein to starch-rich flours produces the usual “protein-type” extrudates that are harder and less expanded. Density and breaking strength index were positively correlated ($r = 0.9$, $P < 0.01$).

3.2. Effect of bean flour addition on some nutritional properties of corn starch-based cooked extrudates

Bean flour addition, regardless of cultivar, produced a great impact on selected nutritional properties of corn starch-based extrudates (Table 3). As whole legume flour contains more proteins than cereal starch (Tharanathan & Mahadevamma, 2003), levels of crude protein increased as a function of increasing rate of bean fortification. It is noteworthy that although we did not characterize the amino acids present our materials, it can be assumed that the amino acid profile of extrudates containing bean flour has changed from almost non-existent (corn starch control) to a relevant source of lysine (Tharanathan & Mahadevamma, 2003). In bakery goods, addition of legume flour to cereal-based formulations has proven to positively impact their essential amino acid balance (Koehler, Chang, Scheier, & Burke, 1987; Shehata, Darwish, El-Nahry, & Razek, 1988; Tharanathan & Mahadevamma, 2003). Addition of high protein-high lysine material is known to positively affect the protein quality of cereal foods, since cereal grains are deficient in this amino acid (Pomeranz, 1970). Shehata et al. (1988) showed that addition of 10%, 15%, or 20% of broad bean flour to Egyptian wheat bread caused a significant ($P < 0.01$) increase on protein efficiency ratio (PER) in breads fortified with 10% of bean flour, however the authors suggested that higher levels of substitution could possibly result in significant increases in bread protein quality.

Total phenols and antioxidant activities determined in the cooked products showed significant variation with respect to bean flour concentration and bean cultivar. Bean flour addition had a positive impact on the levels of these phytochemicals. However, fortification with small red bean flours was to a great extend more effective in producing extrudates with higher nutritional functionality than navy bean flours. As discussed in ours (Anton, Ross, Beta, et al., 2008; Anton, Ross, Lukow, Fulcher, & Arnfield, 2008) and other authors works (Madhujiht & Shabidi, 2005), the colour of dry beans reflects the phytochemical profile of their seed coats, which is composed by compounds such as flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins) (Feenstra, 1960). Since the colour of the seed coats of the cultivars studied are cream white (navy) and dark red (small red), this may explain the differences observed. Recently, the presence of kaempferol, a natural flavonoid with strong antioxidant activity, has been identified in seed coat extracts of red and pinto beans (Hu et al., 2006). In view of the fact that the authors had not found kaempferol in the seed coat extracts of black and white beans, this may also contribute to justify our findings.

In all cooked extrudates, total phenol levels were significantly ($P < 0.001$) correlated to both DPPH ($r = 0.98$) and ORAC ($r = 0.98$) antioxidant activities. The method for determining antioxidant activity yielded expressively different antioxidant values. Results from the ORAC assay were much higher than the ones from the DPPH method, yet they were significantly correlated ($r = 0.98$, $P < 0.001$). Nonetheless, similar finding have been reported in the literature (Wang & Ballington, 2007; Xu, Yuan, & Chang, 2007), suggesting that although coloured beans may be an important source of antioxidants, the quantification method of such plays an important role on determining their antioxidative potential.

3.3. Effect of extrusion cooking on some selected nutritional and antinutritional properties of corn starch-based raw mixtures

As observed in Table 3, extrusion cooking resulted in a significant decrease in the antioxidative potential of corn starch-bean mixtures. Total phenols were reduced on average 10% in starch and navy bean extrudates, in comparison to the raw mixtures. More important reductions occurred in small red bean extrudates, which had their total phenols content decreased in approximately 70%. These observations can be extended to what was observed in...
the levels of both DPPH and ORAC antioxidant activities after processing of corn starch-bean mixtures, that is, they were both importantly reduced after extrusion. DPPH antioxidant activity decreased nearly 22% in starch and navy bean extrudates, while antioxidants reached 65% reduction in materials to which small red bean flour was added. ORAC values were also affected by extrusion, however to a lesser extent. Through this method, overall reduction of antioxidant activity was on the range of 1–37%, with no effect of bean cultivar detected through analysis of variance (ANOVA). As mentioned before, different methods for determining antioxidant activity are expected to give different outcomes. Xu and Chang (2008) reported that the ORAC is the only method so far that combines both inhibition time and degree of inhibition into a single quantity (Cao & Prior, 1999). The antioxidant reaction mechanism of ORAC is quite different than that of DPPH: ORAC reactions involve a hydrogen atom transfer mechanism, while DPPH mechanism involves a single electron transfer (Prior, Wu, & Schalich, 2005). In the ORAC, antioxidant activity provokes the inhibition of the free radical damage to the fluorescent compounds. The different values herein reported may be attributed to the capacity of each method of detecting the antioxidant activity of various compounds. Arnnao (2000) speculated that lower DPPH values may be attributed to the interference of other pigments that also absorb at the wavelength used in the DPPH method (515 nm), such as carotenoids and anthocyanins (Diamini, Taylor, & Rooney, 2007).

Nonetheless, our results are in accordance with previous works on polyphenols and antioxidant activities of raw and extruded common beans (Alonso et al., 2000; Korus, Gumul, & Czechowska, 2007; Korus, Gumul, Fonta, et al., 2007). Korus, Gumul, and Czechowska (2007a) reported that the effect of extrusion on the phenolic content of beans depended on the cultivar. In their study, one bean cultivar showed an increase of 14% in the amount of phenolics in extrudates compared to raw beans, while the other two were decreased by 19% and 21%. They also observed that extrusion at 180 °C and 20% moisture of the feed material resulted in the least active materials and decreased antioxidant activity in comparison to the raw flours. Additionally, it is suggested that extrusion may have also promoted the polymerization of phenolic acids and tannin (Remy, Fulcrand, Labarbe, Cheynier, & Moutounet, 2000), thus affecting the extractability of such compounds, and their related reduced antioxidant activities (Diamini et al., 2007).

On the other hand, antinutritional compounds such as phytic acid and trypsin inhibitors were also significantly reduced by extrusion cooking (Table 4). Phytic acid, which was significantly higher in mixtures containing navy beans compared to small red, had an overall reduction of nearly 44% after extrusion. We suggest that during cooking inositol hexaphosphate could have been hydrolyzed to lower molecular weight forms, which is in agreement with the work of Alonso et al. (2000), who reported a significant reduction in phytic acid content in beans submitted to extrusion cooking. Using high performance liquid chromatography (HPLC), these authors revealed that during extrusion, some molecules of inositol hexaphosphate were hydrolysed to penta-, tetra- and triphosphates. Similar reductions in phytic acid levels in whole beans processed under various conditions, including soaking, roasting, autoclaving, and pressure-cooking have been reported (Alonso et al., 2000; ElMaki et al., 2007). It appears that the mechanisms of reduction of phytic acid involved in the processing of whole seeds and flours of beans are quite different. In whole legume seeds it seems to concern the leaching of phytate that occurs during soaking and cooking (Estévez, Castillo, Figuerola, & Yánez, 1991), the phytase activity at a temperature of 40–55 °C that may degrade inositol hexaphosphate to the pentaphosphate or lower molecular weight forms (ElMaki et al., 2007), or the formation of insoluble complexes between phytate and other components during cooking, therefore reducing phytate availability (Kumar, Venkataraman, Jaya, & Krishnamurthy, 1978).

Trypsin inhibitors determined in the raw mixtures were significantly higher in navy beans composites than in small red, and increased as a function of increased level of bean fortification (Table 4). Since these inhibitors are thermolabile and their inhibitory activity can be reduced extensively by an appropriate thermal treatment (Alonso et al., 2000; Anton, Ross, Lukow, et al., 2008; Shimelis & Rakshit, 2007), low levels were expected in the bean extrudates. In fact, extrusion cooking in the conditions applied was able to reduce trypsin inhibitors by 100% in all samples. This is in agreement with the findings of Alonso et al. (2000) and Baladrán-Quintana et al. (1998), who observed that extrusion cooking was one of the best processing methods for improving the protein quality of legumes.

### Table 4

<table>
<thead>
<tr>
<th>(%)</th>
<th>Phytic acid (mg/10 g)</th>
<th>Trypsin inhibitors (TIU/100 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw Cooked</td>
<td>Raw Cooked</td>
</tr>
<tr>
<td>Starch</td>
<td>100</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>13.77 ± 0.83a</td>
<td>5.69 ± 0.47a</td>
</tr>
<tr>
<td>30</td>
<td>29.91 ± 1.52b</td>
<td>9.73 ± 0.46b</td>
</tr>
<tr>
<td>45</td>
<td>41.93 ± 1.89c</td>
<td>22.19 ± 1.11c</td>
</tr>
<tr>
<td>Navy</td>
<td>15</td>
<td>11.79 ± 0.9a</td>
</tr>
<tr>
<td>30</td>
<td>25.61 ± 2.07b</td>
<td>16.69 ± 0.62b</td>
</tr>
<tr>
<td>45</td>
<td>37.91 ± 0.66c</td>
<td>23.32 ± 1.37c</td>
</tr>
<tr>
<td>Small Red</td>
<td>15</td>
<td>ND</td>
</tr>
<tr>
<td>30</td>
<td>25.61 ± 2.07b</td>
<td>16.69 ± 0.62b</td>
</tr>
<tr>
<td>45</td>
<td>37.91 ± 0.66c</td>
<td>23.32 ± 1.37c</td>
</tr>
</tbody>
</table>

Table 4: Effect of extrusion cooking on some antinutritional properties of corn starch added of bean flours.

TIU, trypsin inhibitory units; ND, non detectable.

All the values are mean ± SD of four determinations adjusted to dry matter. Data followed by a different character in the same column, within the same bean flour, are significantly different (P < 0.05) using a Tukey test comparing all pairs of columns. All materials were significantly different than their respective raw flours for both phytic acid and trypsin inhibitors (P < 0.05).

### 4. Conclusions

The effect of bean cultivar was more relevant on the nutritional, rather than physical, properties of corn starch-based extrudates (Table 2). Physically, critical differences were observed mainly for colour, which reflects the differences observed in terms of total phenol content and antioxidant activities. Fortification of corn starch with small red bean flour yielded extrudates with higher nutritional functionality compared to navy bean flour substitution. The replacement of corn starch by bean flour, regardless of cultivar, indicates to be feasible at 30%.

Attempts to improve the nutritional and physical properties of bean extrudates by addition of different additives have been reported (Berrios, 2006; Berrios, De, Wood, Whitehand, & Pan, 2004; Martin-Cabrejas et al., 1999). Recently, Berrios et al. (2004) reported that by adding increasing levels of sodium bicarbonate to black bean flour they could produce more expanded bean extrudates. This observation was attributed to the release of CO2 from NaHCO3 facilitated through the heat and moisture provided by the extrusion process. Therefore, it is suggested that bean flour can be incorporated at higher levels in corn starch-based extruded snacks, without great impact on their physical properties, by elaborating processing and formulation with the use of adequate food additives.

At 30% substitution, crude protein was increased 12-fold, while the other chemical compounds studied were also significantly increased. In comparison to raw flours, extrusion significantly decreased concentration of phenolics, antioxidants, TI, and phytic acid.
Further sensory studies and biological trials must be carried out in order to evaluate the acceptability of bean extrudates by a consumer panel and to verify the impact of such foods on animal and human nutrition. Additionally, a modified manufacturing procedure and the application of food additives, such as sodium bicarbonate, may help improve the nutritional profile and the texture of extrudates added at higher levels of bean flour. Nonetheless, it appears that corn starch-bean extrudates have a strong potential to replace regular extruded snacks as a healthier option.

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References


