

Influence of extrusion on the bioactive compounds and the antioxidant capacity of the bean/corn mixtures

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Abstract

The aim of this investigation was to examine the influence of extrusion on the bioactive compounds and the antioxidant capacity of bean/corn mixtures. Whole bean flour and nixtamalized corn were mixed in a 60:40 proportion and extrusion was performed in different moisture (14.5%, 15.4%, 17.1% and 18.0%) and temperature (150°C, 160°C, 170°C, 180°C and 190°C) conditions in order to find the optimal extrusion conditions. According to their functional properties and antioxidant status, the mixtures 142°C/16.3% H, 170°C/16.3% H and 198°C/16.3% H were defined as optimal, moderate and bad, respectively. Total polyphenols and flavonoids in the mixture of 142°C/16.3% H (15.09 ± 1.7 mg gallic acid equivalent [GAE]/g dry weight [DW] and 1.57 ± 0.2 mg catechin equivalent [CE]/g DW) were significantly higher ($P < 0.05$) than in the sample 170°C/16.3% H (9.42 ± 1.1 mg GAE/g DW and 1.4 ± 0.1 mg CE/g DW) and the mixture 198°C/16.3% H (6.46 ± 0.8 mg GAE/g DW and 0.78 ± 0.1 mg CE/g DW). The antioxidant activity (37.02 ± 3.8 and 25.01 ± 2.5 μ M Trolox equivalent [TE]/g DW) of mixture 142°C/16.3% H, determined by the cupric reducing antioxidant capacity with Trolox equivalent antioxidant capacity and β -carotene–linoleic acid (β -carotene, % of inhibition) assays, was significantly higher ($P < 0.05$) than in 170°C/16.3% H (25.69 ± 2.8 and 17.02 ± 1.8 μ M TE/g DW) and in mixture 198°C/16.3% H (13.93 ± 1.5 and 8.94 ± 0.9 μ M TE/g DW), respectively. The free polyphenols, flavonoids and the antioxidant activities showed lower results than the hydrolyzed ones. The correlation coefficients between polyphenols, flavonoids, and cupric reducing antioxidant capacity capacities were between 0.93 and 0.99.

In cereal proteins extracted and separated by electrophoresis, some differences were found in the sodium dodecyl sulfate–protein bands in the region from 36 to 45 kDa for 142°C/16.3% H, in comparison with other samples. Therefore, there is a need to find such conditions for the extrusion procedures that would take into consideration the contents of the bioactive compounds and the antioxidant capacity in the end product.

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Introduction

Extrusion is a widespread procedure, the aim of which is to improve the quality of the end products: confectionary products, baby foods, snacks, ready-to-eat breakfast cereals and pat foods (Akdogan 1999). In this investigation was used a bean/nixtamalized corn mixture. Nixtamalization is a traditional alkali treatment, whereas corn is precooked with CaOH_2 and conditioned for 6–18 h. After nixtamalization, the corn is wet milled. During nixtamalization, starch is partially gelatinized and protein is partially denatured. Nixtamalized corn flour has better nutritional properties than untreated corn flour (Fernández-Muñoz et al. 2002). Therefore, nixtamalized corn was used in the studied mixtures.

Some investigators have studied the effects of extrusion conditions on the properties of flour (Ding et al. 2005, 2006). These authors show the effect of extrusion conditions, including the feed rate (20–32%), feed moisture content (14–22%), screw speed (180–320 rpm), and barrel temperature (100–140°C), on the physicochemical properties (density, expansion, water absorption index [WAI]), and water solubility index (WSI) and sensory characteristics (hardness and crispness) of an expanded rice snack. An increasing feed rate results in extrudates with a higher expansion, lower WSI, and higher hardness. Increasing feed moisture content results in extrudates with a higher density, lower expansion, higher WAI, lower WSI, higher hardness and lower crispness. A higher barrel temperature increased the extrudate expansion but reduced density, increased the WSI and crispness of extrudate. Screw speed had no significant effect on the physicochemical properties and sensory characteristics of the extrudates.

However, we found only one published paper concerning the effect of extrusion on the bioactive compounds and the antioxidant capacity. Therefore, the aim of this investigation was not only to determine the effect of extrusion parameters on the functional properties of bean/nixtamalized corn mixture. It was very interesting to know how the extrusion conditions influence the bioactive compounds including proteins and the antioxidant capacity of the studied beans/corn mixtures. To achieve this aim, the contents of polyphenols, flavonoids and the antioxidant capacity of the total extracts of all samples were determined and the electrophoresis technique was applied.

It was shown that the content of bioactive compounds in natural products does not necessarily indicate their antioxidant capacity (Lotito and Frei 2004). It was suggested that the synergetic effect, which could exist between individual bioactive compounds means that the antioxidant potential may be greater than their sum (Lotito and Frei 2004). Therefore, the total antioxidant capacity was also evaluated. There are many methods for total antioxidant determination, and every one has its limitations (Yu et al. 2002). Some of these antioxidant assays give different antioxidant activity trends (Ou et al. 2002). Therefore, in order to receive reliable results, two other complemented assays for the determination of the total antioxidant capacity were used:

1. Cupric reducing antioxidant capacity (CUPRAC) (Apak et al. 2004).
2. β -Carotene–linoleic acid assay (β -carotene) (Ferreira et al. 2006).

The protein profile was also determined.

Materials and methods

Chemicals

Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid), butylated hydroxyanisole, Folin–Ciocalteu reagent, $\text{CuCl}_2 \times 2\text{H}_2\text{O}$, neocuproine (2,9-dimethyl-1,10-phenanthroline), β -carotene, sodium dodecyl sulfate, β -mercaptoethanol, acrylamide, polyacrylamide, Coomassie Brilliant Blue R and molecular weight marker (14–205 kDa) were obtained from Sigma Chemical Co. (St Louis, MO, USA). All reagents were of analytical grade. Deionized and distilled water were used throughout.

Samples and mixture preparation

Whole bean flour (*Phaseolus vulgaris* L.) cultivar Pinto Villa from the high lands of Durango, Mexico was used. Beans were grown in spring 2006. The beans were milled in a commercial mill (MLI 204; Buehler AG, CH-9240 Uzwil, Switzerland). Corn (*Zea mays* L.) from the cultivar CAFIME was grown in 2005. The nixtamalized corn was milled in a nixtamal stone mill (Villamex, Guadalajara, Mexico). Bean and nixtamalized corn (N-corn) flours were mixed in a proportion of 60:40, respectively. Samples of mixed flour were conditioned to 14.5%, 15.4%, 17.1% or 18.0% moisture for 12 h in closed plastic containers.

Extrusion procedures

Extrusion was done with a single-screw extruder (CINVESTAV, Queretaro, Mexico) with a compression of 3:1, a screw diameter of 19 mm, a length–diameter ratio of a 20:1 and a dice diameter of 3.0 mm. A constant screw speed of 90 rpm (60 Hz) and a constant feeding speed of 28 rpm were used. The temperature in the third zone of the extruding zone was varied (150°C, 160°C, 170°C, 180°C and 190°C). To determine the moisture and temperature for extrusion, an experimental central rotary design of second order was used. Samples were identified as follows: 1 = 142°C/16.3% H; 2 = 190°C/18.0% H; 3 = 190°C/14.5% H; 4 = 170°C/16.3% H; 5 = 170°C/18.7% H; 6 = 170°C/16.3% H; 7 = 150°C/14.5% H; 8 = 170°C/13.8% H; 9 = 170°C/16.3% H; 10 = 198°C/16.3% H; 11 = 170°C/16.3% H; 12 = 150°C/18.0% H and 13 = 170°C/16.3% H.

The expansion index and hardness were measured as previously described by Gujska and Khan (1990).

Determination of the contents of bioactive compounds

A 50 mg aliquot of lyophilized sample was accurately weighed in a screw-capped tube. The total phenols were extracted with 5 ml of 1.2 M HCl in 50% methanol/water (TP). The samples were vortexed for 1 min and heated at 90°C for 3 h with vortexing every 30 min. The samples were cooled, diluted to 10 ml with methanol and

centrifuged for 5 min at $4,000 \times g$ with a benchtop centrifuge to remove solids (Vinson et al. 2001).

Determination of the contents of polyphenols and flavonoids and the electrophoresis were done as we previously described (Gorinstein et al. 2007).

Determination of the antioxidant capacity

The antioxidant capacity of the total extracts of all studied samples was determined by the following assays.

1. CUPRAC was performed according to Apak et al. (2004). This assay is based on utilizing the copper (II)–neocuproine reagent as the chromogenic oxidizing agent. To the mixture of 1 ml copper (II), neocuproine, and NH_4Ac buffer solution, antioxidant sample (or standard) solution (x ml) and H_2O [$(1.1 - x)$ ml] were added to make the final volume of 4.1 ml. The absorbance at 450 nm was recorded against a reagent blank.
2. The β -carotene–linoleic acid assay was performed according to Ferreira et al. (2006). A stock solution of β -carotene and linoleic acid was prepared by dissolving 0.5 mg β -carotene in 1 ml chloroform and adding 25 μl linoleic acid together with 200 mg Tween 40. The chloroform was evaporated. One hundred milliliters of aerated water were added to the residue. To 2.5 ml of this mixture, 300 μl each extract were added. The samples were incubated in boiling water for 120 min together with two blanks, one containing the antioxidant butylated hydroxyanisole and the other one without antioxidant. The absorbance was measured at 470 nm.

Protein extraction and electrophoresis

Total proteins from defatted lyophilized cereal samples of 60 mg each were extracted with 1 ml sample buffer (0.0625 M Tris–HCl, pH 6.25) containing 2% sodium dodecyl sulfate, 10% glycerol, 5% mercaptoethanol and 0.001% bromophenol blue. The extracts were allowed to stand overnight at room temperature. Samples were boiled for 5 min, and then centrifuged at $18,000 \times g$ for 15 min at 15°C .

A Hoeffer SE-600 apparatus (Hoeffer Pharmacia Biotech Inc., San Francisco, CA, USA) was used for sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The resolving gel was 12.7% total acrylamide and 1.3% cross-linker, and the stacking gel was 6% total acrylamide and 1.7% cross-linker (Laemmli 1970, Gorinstein et al. 2007). The gel size was $140 \times 160 \times 1.5 \text{ mm}^3$. Supernatants (20 μl) were loaded onto gel. The run was carried out at constant current of 25 mA per gel. Gels were stained with 0.25% Coomassie Brilliant Blue G-250 in methanol/water/glacial acetic solution (5:5:1 v/v) and destained in 1% solution of Brij 35. The following molecular weight markers (Sigma Chemical Co.) were used: myosin (205 kDa), β -galactosidase (116 kDa), phosphorylase b (97 kDa), bovine albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3 phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20 kDa), and α -lactalbumin (14 kDa).

Statistical analysis

The values of the investigation of the bioactive compounds and antioxidant capacity are presented as means \pm standard deviations of five measurements. Differences between groups were tested by two-way analysis of variance. In the assessment of the antioxidant potential, the Spearman correlation coefficient (R) was used. Linear regressions were also calculated. $P < 0.05$ was considered significant.

Results

The characteristics of all 13 studied samples are summarized in Table I. Based on physicochemical properties, relatively low moisture and temperature of extrusion samples 1 (142°C/16.3% H), 6 (170°C/16.3% H) and 10 (198°C/16.3% H) were assessed as optimal, moderate and bad, respectively. The results of the determination of the contents of polyphenols, flavonoids and the level of the antioxidant capacity of all studied samples after the extrusion procedures are summarized in Table II and Figure 1. As can be seen, in samples 1 and 4 (extrusion moisture 16.3% and extrusion temperature 142°C and 170°C, respectively) were found the highest contents of polyphenols and flavonoids and the highest antioxidant capacity as determined by all used tests. Therefore, correlation between the best quality of the mixture after extrusion procedures (sample 1: extrusion moisture 16.3% and extrusion temperature 142°C) and the mixtures with highest contents of the polyphenols and flavonoids and the highest antioxidant capacity were found ($R = 0.9971$ for CUPRAC and $R = 0.9293$ for β -carotene assay; Figure 2).

The investigated samples 1–13, sample 14 (bean flour) and sample 15 (corn flour) were compared with sample 16 (the non-treated mixture of bean and corn of 60:40%). Small changes were detected only in the region of 45–50 kDa. The changes

Table I. Characteristics of the 13 samples studied.

Sample	Bean/corn composition (%)	Extrusion moisture (%)	Extrusion temperature (°C)	Expansion index	Hardness
1	60/40	16.3	142	1.7 ^{ab}	4.5 ^{ab}
2	60/40	18	190	1.6 ^b	2.2 ^a
3	60/40	14.5	190	1.8 ^a	2.9 ^a
4	60/40	16.3	170	1.6 ^b	4.8 ^{ab}
5	60/40	18.7	170	1.7 ^{ab}	4.1 ^{ab}
6	60/40	16.3	170	1.6 ^{ab}	1.7 ^a
7	60/40	14.5	150	1.7 ^{ab}	4.8 ^{ab}
8	60/40	13.8	170	1.6 ^b	7.3 ^b
9	60/40	16.3	170	1.8 ^{ab}	3.2 ^a
10	60/40	16.3	198	1.6 ^{ab}	3.1 ^a
11	60/40	16.3	170	1.8 ^{ab}	5.5 ^{ab}
12	60/40	18	150	1.7 ^{ab}	6.2 ^{ab}
13	60/40	16.3	170	1.6 ^{ab}	3.1 ^{ab}

Data are means \pm standard deviation of five measurements. Means in columns without lowercase superscript letters in common differ significantly ($P < 0.05$). Samples: 1 = 142°C/16.3% H; 2 = 190°C/18.0% H; 3 = 190°C/14.5% H; 4 = 170°C/16.3% H; 5 = 170°C/18.7% H; 6 = 170°C/16.3% H; 7 = 150°C/14.5% H; 8 = 170°C/13.8% H; 9 = 170°C/16.3% H; 10 = 198°C/16.3% H; 11 = 170°C/16.3% H; 12 = 150°C/18.0% H; and 13 = 170°C/16.3% H.

Table II. Bioactive compounds and antioxidant activities of investigated samples.

Sample	CUPRAC (μM Trolox equivalent/g)	β -carotene (%)	Flavonoids (mg Catechin equivalent/g)	Polyphenols (mg Gallic acid equivalent/g)
1	37.02 ± 3.7^b	25.01 ± 2.5^b	1.57 ± 0.16^a	15.09 ± 1.51^b
2	20.55 ± 2.1^b	17.98 ± 1.8^b	1.31 ± 0.14^b	10.19 ± 1.11^b
3	30.02 ± 3.1^b	33.00 ± 3.3^b	2.09 ± 0.22^a	17.40 ± 1.75^c
4	32.57 ± 3.3^a	34.20 ± 3.4^a	2.89 ± 0.28^a	16.27 ± 1.65^b
5	9.55 ± 1.1^a	10.32 ± 1.1^a	1.89 ± 0.18^c	7.09 ± 0.74^a
6	25.69 ± 2.6^b	17.02 ± 1.6^b	1.57 ± 0.16^c	9.42 ± 0.97^c
7	19.18 ± 1.8^b	13.10 ± 1.4^b	1.25 ± 0.12^c	7.75 ± 0.77^c
8	16.61 ± 1.7^a	15.00 ± 1.6^a	1.15 ± 0.11^b	8.71 ± 0.87^b
9	16.07 ± 1.5^a	14.00 ± 1.5^a	1.39 ± 0.13^a	8.41 ± 0.85^a
10	13.93 ± 1.4^a	8.94 ± 1.1^a	0.78 ± 0.04^c	6.46 ± 0.65^c
11	24.96 ± 2.5^b	16.70 ± 1.5^b	1.66 ± 9.3^d	9.74 ± 0.98^d
12	28.22 ± 2.9^b	20.30 ± 2.4^b	1.77 ± 9.6^d	11.31 ± 1.15^c
13	32.52 ± 3.3^b	11.95 ± 1.2^b	1.89 ± 8.8^b	11.95 ± 1.22^b

Data are means \pm standard deviations of five measurements. Means in columns without lowercase superscript letters in common differ significantly ($P < 0.05$). Samples: 1 = 142°C/16.3% H; 2 = 190°C/18.0% H; 3 = 190°C/14.5% H; 4 = 170°C/16.3% H; 5 = 170°C/18.7% H; 6 = 170°C/16.3% H; 7 = 150°C/14.5% H; 8 = 170°C/13.8% H; 9 = 170°C/16.3% H; 10 = 198°C/16.3% H; 11 = 170°C/16.3% H; 12 = 150°C/18.0% H; and 13 = 170°C/16.3% H.

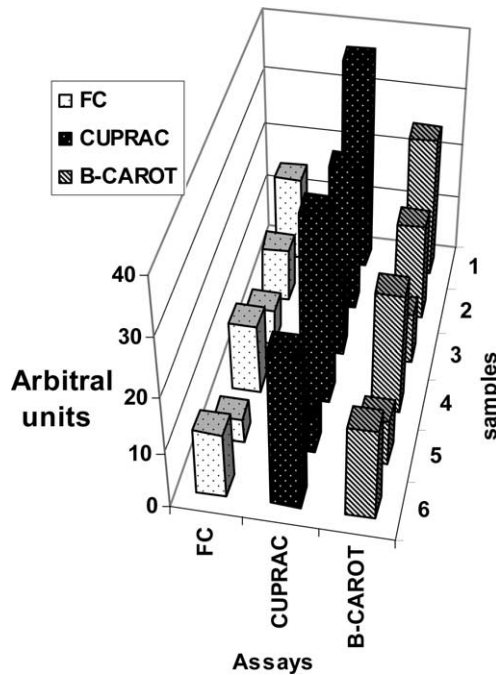


Figure 1. Polyphenol and antioxidant activities of investigated samples. FC, polyphenols; B-CAROT, β -carotene-linoleic acid assay. Samples: 1 = 142°C/16.3% H; 2 = 142°C/16.3% H; 3 = 198°C/16.3% H; 4 = bean flower; 5 = corn flower; 6 = bean/N-corn of 60%/40%.

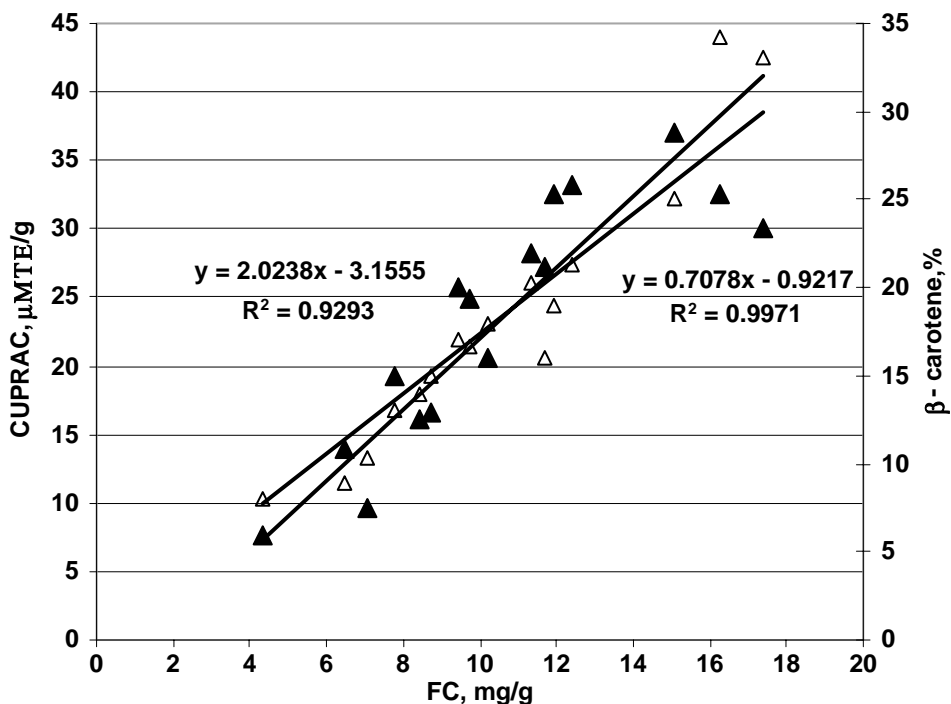


Figure 2. Correlation coefficients between (▲) polyphenols (FC) (mg GAE/g DW, x) and the antioxidant capacity by CUPRAC of mixture extract (μM Trolox equivalents/g, y_1), and (△) polyphenols (mg GAE/g DW, x) and the antioxidant capacity by β -carotene of mixture extract (% inhibition, y_2). β -carotene, β -carotene-linoleic acid assay; DW, dry weight.

in the proteins of the studied samples were minimal (Figure 3), which is important for the overall value of proteins after extrusion (Arija et al. 2006; Perez-Navarrete et al. 2006).

Discussion

Our results of the study of the extrusion samples corresponded with the data of Farouk et al. (2000), who investigated extruded wheat flour at 100–120°C with 5% d-glucose or mixtures of 5% d-glucose and 0.5% or 2.0% l-alanine, l-leucine, l-lysine, l-threonine or l-cysteine. They found that extent of browning was only moderate, and yellow and red pigments were produced. The odor intensity increased with the addition of either glucose or a mixture of glucose and amino acids.

Osman et al. (2000) suggested that extrusion conditions can be optimized to influence the physicochemical structures in the extrudate matrix so that oil absorption can be minimized. The extrusion was at $192 \pm 1^\circ\text{C}$ for 10–40 sec to complete expansion, but the extruded product was produced using a co-rotating twin-screw extruder, dehydrated to a uniform moisture content. Manthey et al. (2004) investigated the effect of hydration level on processing properties of buckwheat bran flour and of the drying temperature on the physical and cooking quality of spaghetti. Specific mechanical energy transferred to the dough during extrusion decreased 69% for semolina and 79% for semolina containing 30% w/w buckwheat bran flour, as the

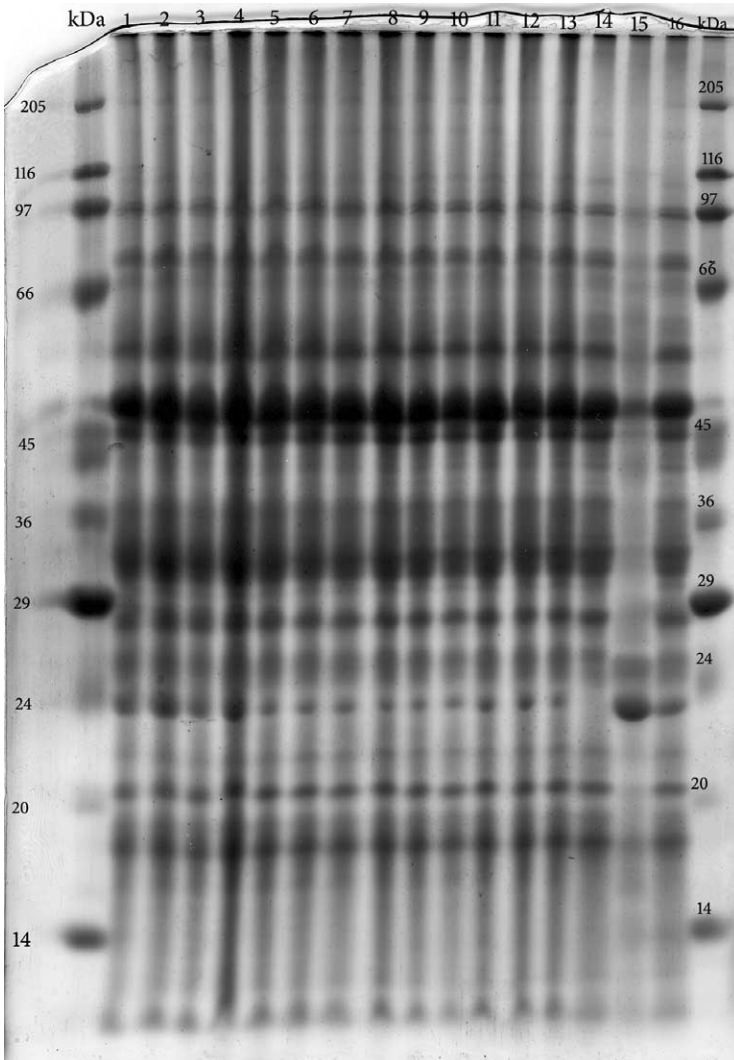


Figure 3. Comparison of the band intensity of proteins extracted from cereal samples and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Molecular markers: myosin (205 kDa), β -galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3 phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20 kDa), and α -lactalbumin (14 kDa). Examined samples: samples 1–13, 13 mixed flour samples of bean/corn in the ratio 60%/40% extruded at different temperatures and moisture contents; sample 14, bean flour; sample 15, nixtamalized corn flour; and sample 16, control sample (non-extruded) with bean/corn in the ratio 60%/40%. Samples identified as follows: 1 = 142°C/16.3% H; 2 = 190°C/18.0% H; 3 = 190°C/14.5% H; 4 = 170°C/16.3% H; 5 = 170°C/18.7% H; 6 = 170°C/16.3% H; 7 = 150°C/14.5% H; 8 = 170°C/13.8% H; 9 = 170°C/16.3% H; 10 = 198°C/16.3% H; 11 = 170°C/16.3% H; 12 = 150°C/18.0% H and 13 = 170°C/16.3% H.

hydration level increased absorption 29–32%. The hydration level before extrusion did not affect the cooking loss of spaghetti made from semolina. However, the cooking loss was greater from spaghetti made with semolina buckwheat bran flour, which was hydrated to 32% compared with 29–31% for absorption. In our research the highest

percentage of moisture was about 18%, and Kim et al. (2006) extruded pastry wheat flour under various conditions of feed moisture (20%, 40%, and 60%) and screw speed (150 rpm, 200 rpm, and 250 rpm), at a constant barrel temperature profile (40°C, 60°C, 80°C, 100°C, and 120°C, feed port to exit die). The results obtained by these authors indicate that feed moisture and storage time were both important factors for the formation of resistant starch formation from pastry wheat flour during extrusion. Chanvrier et al. (2007) investigated the rheological properties of wheat flour. During extrusion (with 28% moisture content, wet basis) the rheological properties are influenced by the molecular changes of its components. There was no simple relationship between the wheat-flour characteristics and their rheological properties. It was observed that the shear viscosity of the blends under controlled conditions (35% moisture content, and 140°C temperature) have to be modified by both gluten and amylose contents. Our results differ with the experimental conditions, such as moisture, temperature and the composed cereals in the blends.

We found that during the extrusion procedure proteins have been slightly changed, which corresponds with the obtained protein profile of other work (Arija et al. 2006; Perez-Navarrete et al. 2006). Also, Chanvrier et al. (2007) discussed the changes undergone by wheat gluten to determine the levels of unextractable polymeric proteins, to follow the polymerization of protein under processing. This study indicated that, in low hydrated products in the molten state, shear viscosity is affected by the structure of the blends and by the molecular changes occurring during processing. All of above cited investigations (Farouk et al. 2000; Osman et al. 2000; Manthey et al. 2004; Arija et al. 2006; Kim et al. 2006; Perez-Navarrete et al. 2006; Chanvrier et al. 2007) are dealing with different aspects of the influence of extrusion procedure on the end products. However, only one study has been published concerning the influence of extrusion procedures on the contents of the bioactive compounds and antioxidant capacity of the end products (Korus et al. 2006). These data are very important, because many authors recommend consumption of food only with high contents of bioactive compounds, proteins and high antioxidant capacity (Paganga et al. 1999; Proteggente et al. 2002; Sun et al. 2002; Haruenkit et al. 2007). The results of this investigation show that, after the extrusion procedures, the contents of polyphenols and flavonoids remained high— 15.09 ± 1.51 mg gallic acid equivalents (GAE)/g, 17.40 ± 1.75 mg GAE/g and 16.27 ± 1.65 mg GAE/g in samples 1, 3 and 4, respectively. Also the antioxidant capacity of the studied samples as determined by the used tests remained high particularly in the same samples 1, 3 and 4 (from 37.02 to 32.57 μ M Trolox equivalents/g). We found a good correlation between the results of the extrusion procedures (extrusion moisture, extrusion temperature) and the bioactive compounds and the antioxidant capacity. So, the best conditions of the extrusion procedures was registered in sample 1 with the highest contents of polyphenols and flavonoids and the highest antioxidant capacity, hardness and expansion index. Also, other workers investigated the influence of extrusion on polyphenol content and antioxidant activity (Korus et al. 2006): determining the influence of extrusion parameters on the polyphenol content and composition and antioxidant activity in common beans of two cultivars (Augusta, Nigeria). These authors found that the total polyphenol contents varied from 777 to 996 mg/100 g dry matter. In this research the total polyphenols varied from 434 to 1,740 mg/100 g dry matter. The contents of most polyphenol compounds were decreased after extrusion compared with raw seeds. The lowest losses of polyphenols were observed in

extrudates obtained at 20% initial moisture content and a temperature of 120°C. The largest decrease in antioxidant activity evaluated in the β -carotene/linoleic acid system was observed in extrudates obtained at 20% moisture and 180°C temperature. Also the cited authors found changes in the contents of polyphenols and the antioxidant activity connected to the extrusion conditions. The increase in the content of polyphenols in sample 1 (15.09 mg/g) in comparison with the raw mixture (11.67 mg/g) was about 23%; CUPRAC values have increased by 27% and of β -carotene by 36%. Therefore, future investigators have to find such conditions of the extrusion procedures, which would take into consideration the bioactive compounds and the antioxidant capacity of the end product (Zasytkin and Lee 1998; Linn et al. 2002).

Conclusion

The best extrusion procedure was achieved using an extrusion moisture of 16.3% and an extrusion temperature of 142°C, respectively, with the highest contents of polyphenols and flavonoids and antioxidant capacity. The protein composition during the extrusion did not change drastically; small differences were detected in the range of 45–50 kDa.

There was correlation between the best extrusion procedure and the contents of the bioactive compounds and the antioxidant capacity in the end product.

Future investigators have to determine conditions for the extrusion procedures that would take into consideration the contents of the bioactive compounds and the antioxidant capacity in the end product.

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