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ORIGINAL ARTICLE

Physico-chemical, nutritional and infrared spectroscopy evaluation of an optimized soybean/corn flour extrudate

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Abstract A central composite design using RMS (Response Surface Methodology) successfully described the effect of independent variables (feed moisture, die temperature and soybean proportion) on the specific parameters of product quality as expansion index (EI), water absorption index (WAI), water solubility index (WSI) and total color difference (ΔE) studied. The regression model indicated that EI, WAI, WSI and ΔE were significant ($p<0.05$) with coefficients of determination (R^2) of 0.7371, 0.7588, 0.7622, 0.8150, respectively. The optimized processing conditions were obtained with 25.8 % feed moisture, 160 °C die temperature and 58 %/42 % soybean/corn proportion. It was not found statistically changes in amino acid profile due to extrusion process. The electrophoretic profile of extruded soybean/corn mix presented low intensity molecular weight bands, compared to the unprocessed sample. The generation of low molecular weight polypeptides was associated to an increased in In vitro protein digestibility (IVPD) of the extrudate. The FTIR spectra of the soybean/corn mix before and after extrusion showed that the α-helix structure remained unchanged after extrusion. However, the band associated with β-sheet structure showed to be split into two bands at 1624 and 1640 cm^{-1} . The changes in the β-sheet structures may be also associated to the increased in IVPD in the extruded sample.

Keywords Protein changes · Extrusion cooking of soybean/corn · Response surface methodology · Amino acid profile and electrophoresis · In vitro protein digestibility · Infra-red spectroscopy (FTIR)

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Introduction

Extrusion is the process by which starchy and/or proteinaceous materials are plasticized and cooked in a tube by a combination of moisture, pressure, temperature and mechanical shear. Extrusion cooking is an important processing technique in the food industry, which has advantage over other conventional cooking/processing, due to its capacity for developing a variety of products with distinct characteristics. Some main advantages of extrusion process are related to product texture, including expansion, crispiness and general mouthfeel; versatility; high productivity; low operating costs; energy efficiency and shorter cooking times. Under certain process conditions such as, high temperature, moisture, pressure, and mechanical force, it is possible to obtain a series of products such as

breakfast cereals, snacks, precooked meals, etc. (Brennan et al. 2011, 2013). Chemical transformation of macromolecular components occurs during extrusion processing which modify the functional and digestive characteristics of the extrudates (Singh et al. 2007; Jyothi et al. 2009). Therefore, extrusion is considered an important processing technique for the preparation of nutritious foods with improved functional properties (Hernandez-Diaz, et al. 2007).

The physico-chemical changes than can occur in biopolymers during extrusion cooking include: binding, cleavage, loss of native conformation, fragment recombination and thermal degradation (Joy-Steel et al. 2012). Because proteins are biopolymers with a great number of chemical groups, they are more reactive (Mitchel and Areas 1992) than carbohydrates and undergo many changes during the extrusion process, with protein denaturation being the most important (Camire et al. 1990). Denaturation of proteins during the extrusion process results in increased digestibility, reduction of protein solubility, and inactivates antinutritional factors (e.g., antitrypsin factor and lectins) (Areas 1992).

Soybean is a rich source of protein with high nutritional value and excellent physicochemical properties. Additionally, soybean's biologically active proteins and peptides produce beneficial effects on health, such as aid in hormone balance, interference of cell cycle in obesity and cancer processes (Wang & Gonzales, 2005; Mora-Escobedo et al. 2009; Gonzalez et al. 2011). The limitations of some essential amino acids as cysteine, methionine, and tryptophan, of soybean can affect their nutritional value. However, the combination of soybean with cereals such as corn allows for a better balance of essential amino acids in the mix. Corn is one of the cereals commonly used in extrusion; starch present in this cereal is of main importance for the production of highly acceptable extruded snack foods (Rampersad, Badrie, and Comissiong 2003). A combination of legumes and cereals may improve the physical, chemical and nutritional quality of an extruded product. Novotni et al. (2009), reported in breads made from a mixture of extruded soybean meal and maize a high in vitro protein digestibility and a significantly improved amino acid composition and lysine score. Abd et al. (2003), studied the nutritional value of several legume cultivars and reported an increase in vitro protein digestibility and a decrease in antinutritional factors in the samples processed by extursion. Also, Nwabueze (2007) reported similar results in extruded African breadfruit-corn and defatted soybean mix. Taking into account functional and nutraceutical properties of soybean and corn, the aim of this study was to evaluate physicochemical, nutritional and infrared spectroscopy changes of an optimized soybean/corn flour extrudate.

Material and methods

Raw material preparation

Soybean (*Glycine max L.*) seeds and white corn (*Zea mays*) were purchased from a local market in Mexico City. A total of 20 kg of both soybean and corn, were ground into flour in a mill 2HP Engine 16 in (Raymond, USA). The flours were sieved to pass through a 150 μm screen. Soybean and corn flours were mixed at various weight ratios, and the total moisture contents of the blends adjusted to the desired values, prior to processing. All the formulations were kept in polyethylene bags and stored at room temperature 24 h before extrusion processing. Three different runs were performed with the optimum conditions given by the response surface methodology.

Extrusion conditions

Extrusion cooking was performed in a single screw extruder (CICATA-IPN, Mexico City, Mexico). The length the screw was 950 mm and the screw compression ratio ~~of~~ was 2.5:1. The extruder barrels were 60 mm in diameter and contained three controlled heating and cooling zones. Three thermocouples were used to monitor the barrel temperatures and one extra was inserted in the die plate, to determine the die temperature. The feed was introduced into the extruder by a feeder run at 16 rpm, controlled with a Baldor Electric Co. Cat. GPP 7454 MDF (FT Smith, AR, USA). The die was equipped with a circular die insert 2.5 mm in diameter. The extruder's screw was run at a constant speed of 150 rpm. The extrusion process variables were: feed moisture (18–26 %), die temperature (110–180 °C), and soybean proportion (proportion of soybean in the mix=0–100 %).

Expansion index (EI)

EI was determined according to the method reported by Jin et al. (1994). It was used the following formula:

$$\mathbf{EI} = \textit{Sample diameter}/\textit{diameter of the extruder die}.$$

The values were average of ten measurements.

Water absorption index (WAI) and Water solubility index (WSI)

WAI and WSI were determined as described by Gonzalez-Soto et al. (2007) with minor modifications. The

determinations were made in triplicate. The WAI were analyzed using the following formula:

$$\mathbf{WAI} = (\text{weight of wet sediment/weight of dry sample}) \times 100$$

And the WSI using the following formula:

$$\mathbf{WSI} = (\textit{weight of dissolved solids in supernatant/ weight of dry sample solids in the original sample}) \times 100$$

Total colour difference (ΔE)

The colour was analysed in a Sphere Spectrophotometer (Colour Mate HDS Milton Roy, USA). The evaluation of colour was determined using as standar samples unprocess soybean/corn mix (20.3–79.7 %, 50–50 %, 79.73–20.27 %, 0–100 % and 100–0 %). The CIELab values L, a* and b* were measured for triplicate and the numerical total colour difference (ΔE) was calculated as follows (Alarcon-Valdez et al. 2005).

$$\Delta\mathbf{E} = \left(\Delta L^2 + \Delta a^2 + \Delta b^2\right)^{1/2}$$

Where:

ΔL $L_{std} - L_{sample}$
Δa $a_{std} - a_{sample}$
Δb $b_{std} - b_{sample}$

Experimental design

The experimental design was based on a central composite design of three independent variables and five levels that generated a total of 20 runs, which included 6 replicates at the central point. The independent variables were feed moisture content (X_1), die temperature (X_2), and the proportions of soybean (X_3) in the mix. The code levels for independent variables are shown in Table 1. The response variables were expansion index (EI), water absorption index (WAI), water solubility index (WSI), and total colour difference (ΔE). Response surface methodology (RSM) of experimental data was used for the generation of response surface plots, in order to see clearly the influence of the independents variables in the responses. Design Expert version 7.0 (Statease Inc., Minneapolis, MN, USA) was used for all determinations.

Table 1 Code levels for the independent variables.

Variables	Levels				
	−1.682	−1	0	1	1.682
X_1=Feed moisture (%)	18	19.62	22.0	24.38	26
X_2=Die temperature (°C)	110	124.2	145.0	165.8	180
X_3=Soybean proportion (%)	0	20.3	50.0	79.73	100

Amino acid profile

The amino acid profile was analysed according to the methodology of Bidlingmeyer et al. (1984) with slight modifications. This analysis was made for extrusion obtained with optimal conditions of extrusion and unprocessed soybean/corn mix. A 10 mg sample was dissolved in water or 0.1 M hydro-chloric acid. A volume corresponding to 0.1 to 5.0 μg was pipetted into a 50 × 6 mm tube, and placed in a vacuum vial. The vial was then attached to a Workstation manifold, and the solvent removed under vacuum. After drying, the vacuum was released and 200 μl of 6 N HCl (constant boiling) containing 1 % (v/v) phenol was pipetted into the bottom of the vacuum vial. The vacuum vial was then reattached to the manifold, evacuated and sealed under vacuum (130–260 Pa). Samples were hydrolysed in a Workstation at 110 °C for 24 h in a nitrogen atmosphere. Amino acid standard (Pierce H, Waters Assoc. Milford; MA, USA) were run under the same conditions. Standards of free amino acids, or individual samples, containing up to 25 nmol of each amino acid were placed in the 50×6 mm tubes and dried under vacuum. Free amino acids and hydrolyzed samples were dried down again after adding 10–20 μl of ethanol-water- triethylamine (TEA) (2:2:1) to each tube. The derivatization reagent was made fresh daily and consisted of ethanol-TEA-water-PITC (7: l: l: l). PTC amino acids were formed by adding 20 μl of reagent to the dried samples and sealing them in the vacuum vials for 20 min at room temperature. The reagents were then removed under vacuum using the Workstation. The samples and standards were dissolved in sample buffer (sodium acetate and TEA pH 6.1) and were then injected into HPLC (Agilent Technologies 1200).

The column used in this experiment was Waters 3.9× 15 mm C18. The amino acids were detected at the wavelength of 254 nm. The mobile phases were composed of sodium acetate 0.14 M with 0.5 ml/l of TEA adjusted to pH 6.1 with glacial acetic acid (solvent A) and 60 % acetonitrile in water (solvent B). The injection volume was 4 μl and the gradient of mobile phases were as follow: Initial, A:B=96:4, 0–10 min; A:B=56:46, 10–10.5 min; A:B=0:100, 10.5–12.50 min; A:B=96:4, 12.5–17 min; A:B=96:4; total HPLC run time was 17 min, the flow rate was 1 ml/min.

In vitro protein digestibility (IVPD)

The analysis of in vitro protein digestibility was made following the methodology proposed by Hsu et al. (1977). A multi-enzyme system consisting of porcine pancreatic trypsin type IX, bovine pancreatic chymotrypsin type II and porcine intestinal peptidase grade III (Sigma Chemical Co., St Louis, MO, USA) was selected. 50 ml were prepared of each sample solution at a concentration of 6.25 mg protein/ml adjusted the pH to 8.0 with NaOH 0.1 N while stirring in a 37 °C water bath. The multi-enzyme solution [(8 mg trypsin+15.5 mg chymotrypsin+6.5 mg peptidase)/5 ml distilled water] was maintained in an ice bath and adjusted to pH 8.0. Five milliliters of the multi-enzyme solution were then added to each protein suspension with stirred at 37 °C. The pH drop was recorded automatically over a 10 min period using a recording pH meter. IVPD was calculated applying the equation reported by Hsu et al. (1977): PD=210.46−18.10X, value X=pH of protein suspension after 10 min of digestion with the multi-enzyme solution. The values were average of three measurements.

Electrophoresis

This analysis was made for raw material (soybean and corn), unprocessed soybean/corn mix and extruded obtained with optimal conditions of extrusion. The samples were analyzed by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). Was conducted in a Minin-Protean 3 device (Bio-Rad Laboratories, Richmond, CA) using a 13 % polyacrylamide slab gel under reducing conditions at 110 V. Eighteen micrograms of protein were loaded in each lane, according to the procedure reported by Mora-Escobedo et al. (2009). A prestained SDS-PAGE broad range standard (Bio Rad) was used as molecular weight marker.

Infrared sectroscopy

Fourier transform infrared spectroscopy (FT-IR) spectra were recorded using a FTIR-Bruker Equinox 55 for mid and near IR regions, with a spectral range of 25–30 cm^{-1}. The samples were mixed with KBr and compressed on tablets. They were measured in a range of frequency from 4000 to 400 cm^{-1} according to the report by Flores-Morales et al. (2012).

Statistical analysis

Data analysis for each response variable was analyzed using multiple regression procedures with Design Expert version 7.0 (Statease Inc., Minneapolis, MN, USA). In order to determine the effect of each independent variable in the responses, the experimental data was fitted to the selected models and regression coefficients obtained, the model proposed for each response (Y) was:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

Where Y=the response, X_1=feed moisture (%), X_2=die temperature (°C), X_3=proportion soybean (%), b_0=intercepts, b_1, b_2, b_3 are linear, b_{11}, b_{22}, b_{33}, = are quadratic and b_{12}, b_{13} and b_{23} are interaction regression coefficient terms, respectively. Also, the coefficients of determination (R^2) were calculated. For each response, a surface plot was generated from the regression equations by holding the variable with the least effect on the response equal to a constant value and changing the other two variables. A numerical multi-response optimization was used to determine the optimal processing conditions to further evaluate the physical (EI, WAI, WSI and ΔE), chemical (protein and amino acid profiles) and nutritional (IVPD) properties of the extrudate. Data were expressed as mean±standard deviation (SD) of triplicate determinations. Analysis of variance (ANOVA) and Least Significant Difference (LSD) tests were used to determine the significance differences of their means. Statgraphics Plus software version 5.1 (USA) was used for these determinations.

Results and discussion

Expansion index (EI)

The EI of an extrudate is one of the most important properties related to the crispiness and crunchiness of the finish product (Berrios et al. 2004; Patil et al. 2007).

The regression model for the response variables as a function of the variables analyzed can be described by the following equation in terms of coded values:

$$\mathbf{EI} = -68.86755 + 6.33587X_1 + 0.37227X_2 + 0.35887X_3 - 0.031996X_1X_2 - 0.033481X_1X_3 - 0.14809X_1^2 - 1.12365E{-}004X_2^2 + 7.48998E{-}004X_1^2X_2 + 7.73797E{-}004X_1^2X_3 \quad (1)$$

According to the regression coefficients and their p-values (Table 2), the terms that made significant contribution to the EI were a lineal effect of soybean proportion (X_3), a quadratic effect of die temperature (X_2^2), and the interactive quadratic effect of feed moisture and soybean proportion (X_1^2,X_3). The

Table 2 Significance of *p*-values for quality parameters of the extrudate

Variable	Expansion index *p*-value	Water absorption index	Water solubility index	Total colour difference
X_1	0.1876	0.1045	0.0819	
X_2	0.2981	0.0587	0.0099 *	<0.0001 *
X_3	0.0151*	0.6569	0.0323 *	<0.0001 *
X_1X_2	0.1128	0.03*	0.1393	
X_1X_3	0.174	0.2474	0.021 *	
X_2X_3		0.106	0.4112	
X_1^2	0.8298	0.1517	0.335	
X_2^2	0.0372 *	0.0103 *	0.6062	
X_3^2		0.1924	0.7244	
$X_1^2X_2$	0.0646			
$X_1^2X_3$	0.0119 *			

*Significant at $P<0.05$

regression model indicated that EI was significant ($p<0.05$) with coefficient of determination $R^2=0.7371$. The R^2 indicated that 74 % of the variability in EI could be explained by the model. Additionally, the ANOVA (Table 3) showed that the lack of fit was not significant ($p>0.05$) which further indicated the model could be used for predicting purposes within the experimental range of data analyzed in the study.

Interpretation of regression equation can be improved by response surface plot presenting constant values of dependent variables as a function of two independent variables studied over their range of variation. In Fig. 1a, the effect of different die temperatures and soybean proportion on the EI of the extrudate is shown. It was observed that the intermediate values of die temperature of 140–160 °C, had the highest effect on EI. On the other hand, the proportion of soybean showed to be inversely proportional to EI. This is, as the proportion of soybean increased the EI decreased. The decrease on EI with an increase in die temperature can be attributed to large dextrinization of the starch and weakening of protein structure, which may have caused the collapse of the bubbles in the molten extrudate, resulting in a reduced expansion of the final product (Kokini et al. 1992; Mendonça et al. 2000; Meng et al. 2010). On other hand, at the lowest die temperature the starch made have not been completely gelatinized, which resulted in a lower EI (Chinnaswamy and Hanna 1988; Case et al. 1992). The relationship between the amount of starch in the product and process in temperature is considered the critical factor in the expansion. Upon, heating, the starch in the presence of water swelled due incorporation of water into the granule. As heating progresses, the starch granules absorbed more water, lost its crystalline structure and the starch gelatinized, which favored the expansion of the product. It is recognised that the addition of food ingredients such as lipids alter the physical and chemical properties of the extrudate. The soybean is a food associated with high lipid content (Gonzalez et al. 2011). Extrusion cooking of lipid-containing foods results in the formation of amylose-lipid complexes (Mercier et al. 1980; Colonna and Mercier 1983). Complexes of amylose-lipid in the granular starch particles play a key role in restriction of water penetration into the particles, which decrease the degree of starch gelatinization (Lin et al. 1997) and expansion of the extrudates (Chinnaswamy and Hanna 1988; Lue and Huff 1991).

Water absorption index (WAI)

The predicted model for WAI can be described by the following ecuation :

$$\begin{aligned}\mathbf{WAI} = {} & +47.11830-2.05544X_1-0.30458X_2-0.011752X_3 \\ & + 6.28820E{-}003X_1X_2 \\ & + 2.13900E{-}003X_1X_3-3.53553E{-}004X_2X_3 \\ & + 0.025169X_1^2 + 6.67511E{-}004X_2^2 \\ & + 1.45081E{-}004X_3^2 \end{aligned} \quad (2)$$

Based on the stadistical analysis of the data, the following independent variables significanly ($p<0.05$) afected WAI: the interaction feed moisture and die temperature (X_1X_2) and quadratic effect of die temperature (X_2^2) (Table 2). The ANOVA (Table 3) showed that the model of WAI was significant ($p<0.05$) with a coefficient of determination of $R^2=0.7588$, which indicated that 76 % of the changes on WAI could be explained by the model. Also, the ANOVA indicated no lack of fit for this model. Therefore, the model could be considered significant and predictive within experimental range of data analyzed in the study. In the response surface graph (Fig. 1b), it was observed that the maximum value of WAI was obteined at the highest die temperature and feed moisture content. This may be do to rupture of intermolecular hydrogen links and molecular disorder in the starch granule, resulting in greater

Table 3 Analysis of variance for different models

Response	df.	Source	Sum of squares	Mean squares	*F*-value	*p*-value
Expansion index	9	Model	0.17	0.019	3.12	0.0456*
	10	Residual	0.060	5.975×10^{-3}		
	5	Lack of fit	0.036	7.121×10^{-3}	1.47	0.3403
	5	Pure error	0.024	4.830×10^{-3}		
	19	Total	0.23			
Water absorption index	9	Model	3.81	0.42	3.49	0.0321*
	10	Residual	1.21	0.12		
	5	Lack of fit	0.96	0.19	3.89	0.0811
	5	Pure error	0.25	0.05		
	19	Total	5.03			
Water solubility index	9	Model	267.71	29.75	3.56	0.0302*
	10	Residual	83.52	8.35		
	5	Lack of fit	70.82	14.16	5.58	0.0413*
	5	Pure error	12.70	2.54		
	19	Total	351.23			
Total colour difference	2	Model	751.16	375.58	37.45	< 0.0001*
	17	Residual	170.51	10.03		
	12	Lack of fit	152.51	12.71	3.53	0.0866
	5	Pure error	17.99	3.6		
	19	Total	921.67			

*Significant at $P<0.05$, df: degrees of freedom

water penetration into the matrix and enhanced starch gelatinization, leading to an increased in WAI. Rayas-Duarte et al. (1998) and Yağcı and Göğüş (2008) have previously reported that water absorption has been generally attributed to the dispersion of starch in excess water which increased by the degree of starch damage do to gelatinization.

Water solubility index (WSI)

The model for WSI as a function of the variables analyzed is shown below:

$$\begin{aligned}\mathbf{WSI} = {} & -215.57140 + 12.14661X_1 + 1.04918X_2 \\ & + 0.76122X_3 - 0.033158X_1X_2 - 0.039545X_1X_3 \\ & + 1.41623E{-}003X_2X_3 - 0.13631X_2{}^2 - 9.35424E \\ & -004X_2{}^2 - 3.12358E{-}004X_2{}^2 \end{aligned} \quad (3)$$

According with the regression coefficients and *p*-values (Table 2), the independent variables that had significant effect ($p<0.05$) on WSI were the linear effect of die temperature (X_2) and proportion of soybean (X_3), and the interaction of feed moisture and soybean proportion (X_1X_3). The ANOVA for WSI showed *p*-values of 0.0302 and 0.0413 for the quadratic model and the lack of fit, respectively (Table 3). Therefore, both the model and the lack of fit were considered significant ($p<0.05$) with a coefficient of determination $R^2=0.7622$.

The effect of die temperature and soybean proportion on the WSI of the extrudate is presented in the Fig. 1c. A steady increased in WSI was observed as the extruder die temperature and soybean proportion decresed. The WSI is related to the quantity of soluble molecules and to dextrinization of starch components (Mercier et al. 1998). As the proportion of soybean in the mix decreased the amount of corn increased, and so the starch content. On the other hand, at low die temperatures the severity of the process on the material under extrusion increased do to high shear in the barrel. The combination of these two factors may have caused fragmentation and partial depolymerization of the starch, resulting in the observed increased on the WSI. Therefore, WSI could be use as indicator of the molecular degradation and evaluation of the degree of starch conversion during extrusion cooking (Colonna et al. 1989; Ding et al. 2005). At the highest die temperature and soybean proportion (maximun protein content in the mix) an opposite effect was observed on the WSI, which may be due to loss of protein solubility by denaturation and possible cros-linking between proteins and starch components. Protein denaturation provoke structural changes, which enable hydrophilic groups such as –OH, $-NH_2$, –COOH and –SH to form cross links with starch (Goel et al. 1999).

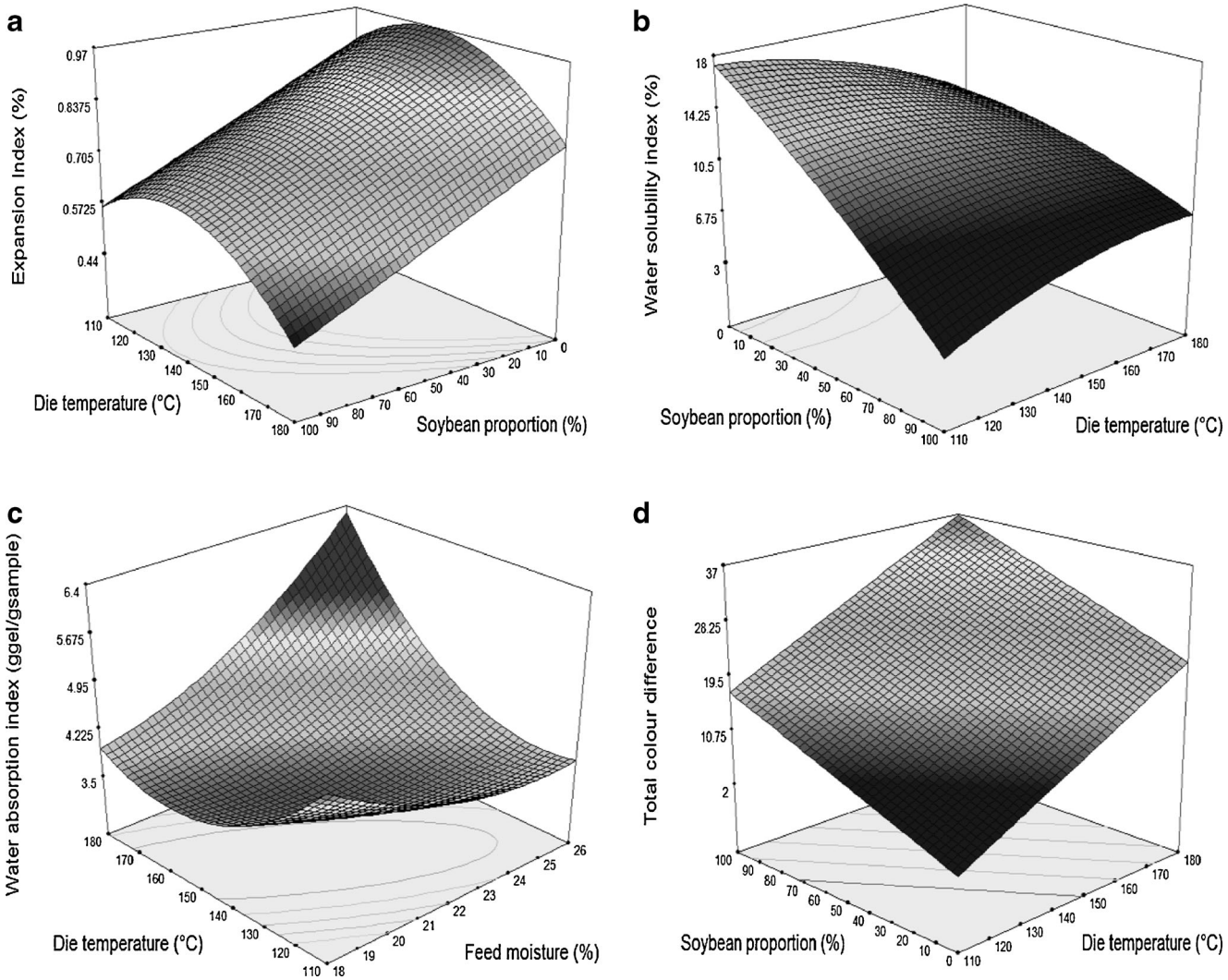

Fig. 1 **a**. Response surface plot for EI as a function of die temperature and soybean proportion. **b**. Response surface plot for WAI as a function of die temperature and feed moisture. **c**. Response surface plot for WSI as a function of soybean proportion and die temperature **d**. Response surface plot for total color difference as a function of soybean proportion and die temperature

Total colour difference (ΔE)

The evaluation of ΔE as an indicator of effect of processing was reported considering the unextruded mixtures of soybean/corn as references.

The effect of the independent variables analyzed by regression analysis allowed to observe the influence of these variables on ΔE. The predicted regression model can be described by the following equation:

$$\Delta \mathbf{E} = -29.23802 + 0.28446X_2 + 0.15026X_3 \quad (4)$$

A significant ($p<0.05$) linear relationship was observed for the terms die temperature (X_2) and soybean proportion (X_3) (Table 2). Additionally, the ANOVA model for ΔE indicated that it was significant ($p<0.05$) with no lack-of-fit (Table 3). Therefore, the model was considered predicted with a coefficient of determination $R^2=0.8150$.

A better observation of the effect of the independent variables on ΔE was obtained by using response surface graphs (Fig. 1d). An increased on die temperature and soybean proportion clearly increased the values of ΔE. It is known that reaction between protein and sugars under high temperature favour the browning, non-enzymatic Maillard reaction (Mercier et al. 1998; Berrios et al. 2010). The high barrel temperature and mechanical shear conditions during extrusion processing have a direct effect on denaturation of proteins, gelatinization and dextrinization of starch, which favour the Maillard reaction (Mercier et al. 1998; Robin 2001). Moreover, Noguchi and Cheftel (1983) reported that the brown color of a protein- enriched product increasing significantly with increasing the barrel temperature from 170 to 210 °C. Therefore, we concluded that the observed increasing values

of ΔE, which indicated a darkening of the extrudate, was mainly do to the Maillard reaction.

Optimum conditions

Base on the numeral multi-response optimization used in this study, the processing conditions obtained for developing an optimized extrudate from a mixture of soybean and corn were 25.8 % feed moisture, 160 °C die temperature, and 58 % of soybean in the mix. The values for the physico-chemical characteristics of the extrudate obtained with these conditions are presented in Table 4.

Amino acid profile

Table 5 present the amino acid profile obtained in the unprocessed soybean/corn mix and the developed extruded product. In general, it was observed that the amino acid profile in the final extrudate compare satisfactorily with the requirement for essential amino acid reported by a Report of a Joint WHO/FAO/UNU Expert Consultation, 2007 (World Health Organization/Food and Agriculture Organization of the United Nations/United Nations University) for human adults. Asparagine and glutamic acid increased significantly ($p>0.05$) from the 11.45, 14.30 % to 13.53, 16.40 %, respectively. Similarly, the sulfur amino acids methionine and cysteine also increased due to processing. Anuonye et al. (2010), reported an increase in cysteine, when an acha/soybean blend was extruded, which goes along with the results obtained in the present study. Lysine is considered one of the most unstable amino acid under high temperature and shear extrusion conditions (Dias and Maga 2004) with losses in the range of 12–49 % (Ilo et al. 1996). A decreased in lysine as well as a decrease in the non-essential amino acids, arginine and phenylalanine, were obtained in the extruded product. Based on our knowledge, reduction of arginine and phenylalanine during extrusion has not been previously reported. However, reduction of lysine as a result of extrusion cooking is well known (Dias and Maga 2004; Ilo et al. 1996; Ruiz-Ruiz et al. 2008). Moreover, reduction of lysine due to the Maillard reaction and the formation of bonds between lysine and glucose (CH-N type bond, instead of CO-NH) have been also reported (Hood-Niefer and Tyler 2010).

Electrophoresis

The electrophoretic profile of corn, soybean, unprocessed soybean/corn flours, and extruded product are presented in Fig. 2a and b. Figure 2a, line 2, shows the different subunits present in the corn flour sample. Globulin, glutelin and zein subunits were observed with molecular weights within the range of 66 and 116 kDa, 25 to 50 kDa, and 22–24 kDa, respectively. Similar electrophoretic profile of corn proteins was previously reported by Sadeghi and Shawrang (2006). Soybean displayed the characteristic 7S and 11S fractions (line 3). The molecular weight distribution, within the different protein subunits, matched well with those presented by Wang and Gonzalez de Mejia, (2005) and Bittencourt et al. (2007). The molecular weight bands below 21 kDa represented the Bowman Birk trypsin inhibitors (Kato and Matsuda 1997).

The effect of extrusion cooking on the electrophoretic profile of soybean/corn flour is presented in Fig. 2b. It was observed that extrusion caused drastic molecular weight redistribution, within the different protein subunits of the soybean/corn flour mix, compared to the unprocessed sample. Most of the molecular weight fractions present in the unprocessed sample were absent in the extruded product.

The extent of macromolecular degradation during extrusion processing is a function of barrel temperature, feed moisture, screw speed, shear stress and residence time. According to Diosady et al. (1985) and Meuser et al. (1987), mechanical effects, such as shear, are more significant for macromolecular depolymerization than thermal effects. Depolymerization of protein during extrusion could generate very low molecular weight polypeptides, which may have eluted off the SDS-PAGE gel during electrophoresis. This in part may explain the absence of certain molecular weight bands in the extruded sample. Additionally, Li and Lee (1996) reported that low intensity molecular weight bands in an electrophoresis gel could be due to the formation of larger aggregates of polymerized proteins, making those proteins too large to enter the gel.

In vitro protein digestibility (IVDP)

The IVPD of the soybean/corn mix was significantly increased ($p<0.05$) from 76 to 84 %, which represented a 10.50 % increment in digestibility at consequence of extrusion cooking (Table 6). During high temperature extrusion cooking, structural changes occur in the proteins that affect noncovalent interactions responsible for the stabilization of the structure, so polypeptides and open internal peptide bonds are exposed and can be more easily hydrolyzed by digestive enzymes (Dahlin and Lorenz 1993; Allan and Booth 2004; Ruiz-Ruiz et al. 2008). Also, heating inactivated enzymes inhibitors and aids protein digestibility (Lorenz et al. 1980). These indicated factors support the beneficial effect of extrusion cooking on protein digestibility, since the anti-nutritional factors (lectin and protease Kunitz inhibitors) in the soybean/corn mix that have antagonistic effect on protein digestibility, are thermo-labile. Therefore, inactivation of the anti-nutritional factors during extrusion cooking favored the digestibility of the extrudate. Additionally, the extruded mix presented and improved protein digestibility compared to the corresponding raw materials (corn and soybean), which show the beneficial effect of extrusion cooking on the IVPD.

Table 4 Optimum values obtained for the evaluated physico-chemical characteristics of the extrudate

Expansion index	Water absorption index ggel/gsample	Water solubility index Percent	Total colour difference
0.64±0.05	4.14±0.39	22.50±0.90	25.89±0.10

Mean value of triplicates ± standard deviation

Moreover, extrusion processing exert mechanical and thermal effects that cause depolymerization of proteins undergoing extrusion with the generation of low molecular weight polypeptides that can improve the digestibility of the protein (Diosady et al. 1985; Meuser et al. 1987). These reports correlate well with results of this study, which presented low intensity molecular weight bands on the electrophoretic profile of extruded soybean/corn mix, compared to the unprocessed sample mix (Fig. 2b). The IVPD of the raw materials and unprocessed soybean/corn mix were not significantly different ($p>0.05$).

Infrared spectroscopy

The FTIR absorption spectra of the unprocessed and extruded soybean/corn mix are presented in Fig. 3a. The decrease in intensity of the broad band located between the frequency range of 3300 to 3400 cm^{-1}, corresponds to the stretching vibration (O–H) of water molecules in the soybean/corn mix and is indicative of the dehydration resulted at a consequence of extrusion cooking. The subsequent four peaks, arising from carbohydrates at 2955 and 2872 cm^{-1}, are associated with asymmetric and symmetric stretching modes of CH_3, respectively. On the other hand, the peaks at 2926 and 2855 cm^{-1} are associated with asymmetric and symmetric stretching modes of CH_2.

In the fingerprint region (800–1800 cm^{-1}) the peak at 1745 cm^{-1} appears due to the stretching vibration of carboxylic group (C=O) in lipids. The next band, which is centered near 1652 cm^{-1}, is associated to an amide I functional group from proteins. Such the components of amide I arise from the C=C stretching and N–H bending modes and also predominantly from the C=O stretching mode. The band at 1545 cm^{-1}, which corresponds to amide II of protein, shows C–N stretching vibrations with smaller contributions from the C–O in-plane bend and the C–C and N–C stretching vibrations. As for the amide I vibration, the amide II vibration is only marginally affected by side-chain vibrations. But, the correlation between secondary structure and frequency is less straight forward than for the amide I vibration and N–H bending. The frequency of the vibration at 1457 cm^{-1} corresponds to COOH group. Whereas, the peak at 1378 cm^{-1} corresponds to the COO^- due to the loss of the hydrogen ion.

Table 5 Amino acid content in unprocessed soybean/corn mix and extruded soybean/corn mix

Amino acids	Unprocessed soybean/corn mix g/100 g of protein	Extruded soybean/corn mix	Amino acid requirements for adults*
Asparagine	11.45±0.48[b]	13.53±0.10[a]	
Glutamic acid	14.30±0.54[b]	16.40±1.13[a]	
Serine	5.73±0.05[a]	5.56±0.20[a]	
Glycine	4.89±0.13[a]	4.76±0.35[a]	
Histidine	2.42±0.04[a]	2.27±0.12[a]	1.0
Arginine	5.57±0.35[a]	4.30±0.18[b]	
Threonine	3.86±0.03[a]	3.61±0.24[a]	1.5
Alanine	5.10±0.19[a]	4.81±0.12[a]	
Proline	9.10±0.45[a]	8.71±0.67[a]	
Tyrosine	4.28±0.35[a]	4.36±0.33[a]	
Valine	5.39±0.15[a]	5.22±0.12[a]	2.6
Methionine	1.38±0.04[b]	1.55±0.01[a]	1.5
Cysteine	0.74±0.04[b]	0.84±0.02[a]	
Isoleucine	5.40±0.13[a]	5.24±0.10[a]	2.0
Leucine	7.78±0.01[a]	7.76±0.27[a]	3.9
Phenylalanine	5.94±0.10[a]	5.67±0.09[b]	2.5
Lysine	7.51±0.23[a]	5.41±0.30[b]	3.0

Results are expressed in dry basis (DB) as the mean value of triplicates±standard deviation, analysis was performed between columns. Samples with the same letter do not show significant differences ($p<0.05$).* Report of a Joint WHO/FAO/UNU Expert Consultation (2007)

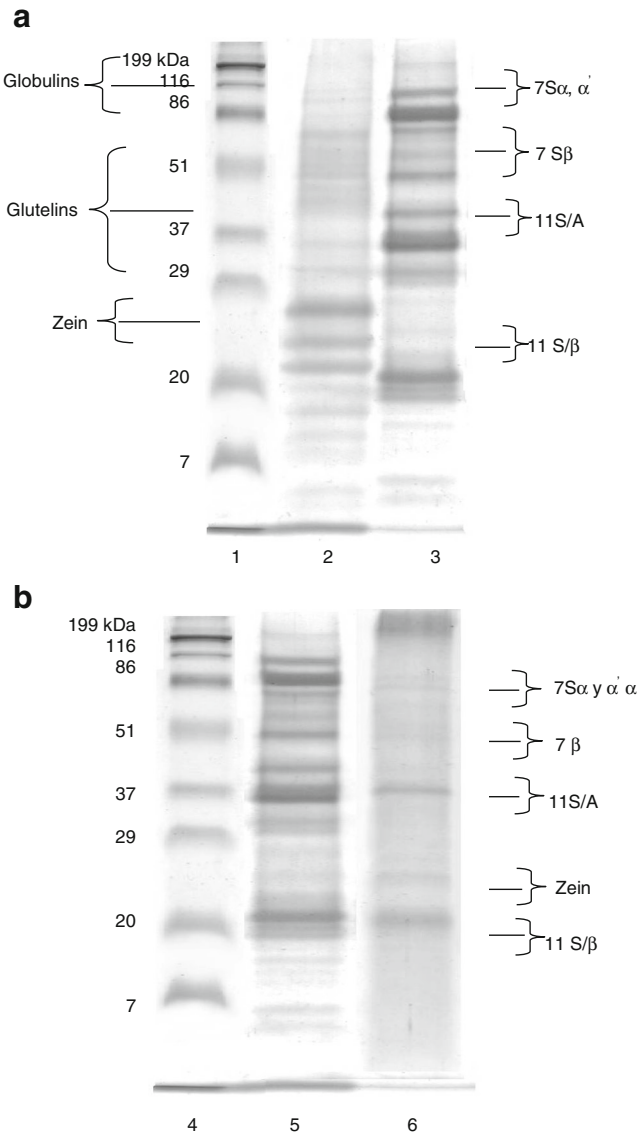

Fig. 2 **a**. SDS-PAGE of corn and soybean flours. Line 1, molecular weight distribution of standard molecular weight markers. Line 2; corn flour. Line 3; soybean flour. **b**. Line 4, Molecular weight distribution of standard molecular weight markers. Line 5; unprocessed soybean/corn mix. Line 6, extruded soybean/corn

Table 6 Comparative in vitro protein digestibility of the samples

Sample	Percent
Casein*	92.3±1.53[a]
Corn	76.9±2.01[c]
Soybean	75.3±0.63[c]
Unprocessed soybean/corn mix	75.9±1.33[c]
Extruded soybean/corn	83.9±2.36[b]

Results are expressed in dry basis (DB) as the mean value of triplicates± standard deviation. Samples with the same letter do not show significant differences ($p<0.05$)

*Protein of reference

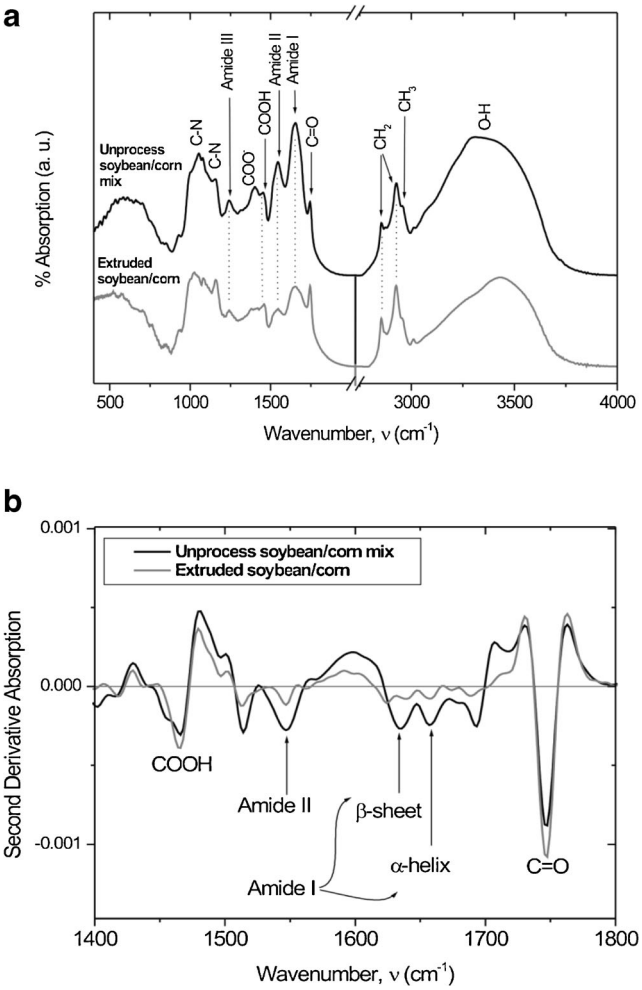

Fig. 3 **a**. FTIR absorption spectra of unprocessed soybean/corn and extruded soybean/corn, **b**. Second derivative FTIR spectra of unprocessed soybean/corn and extruded soybean/corn

The amide III vibration is observed at approximately 1244 cm^{-1}, which is characterized by the C–N stretching vibration with small contributions from the C–O in plane bending and the C–C stretching vibration. In polypeptides, the composition of this mode is more complex as it is dependent on a side-chain structure, and because N–H bending contributes to several modes in the 1400 to 1200 cm^{-1} region. Additionally, signals were observed at 1158, 1085 and 1024 cm^{-1}, which are attributed to C–N bond. Moreover, a band associated to N–H bending mode of the amide V group from proteins was observed at 871 cm^{-1}. The amide V mode is primarily an NH out-of-plane (NH ob) motion with some CN torsion (CN t) (Banker 1992). This amide V mode was mainly an NH ob mode in the work of Miyazawa (1967).

To evaluate the effect of extrusion cooking on the nutritional value of soybean/corn mix, it was important to put emphasis on the proteins regions. According to Fig. 3a, the intensity of the absorption of the Amide I band (centered at 1652 cm^{-1}) decreased nearly by half due to extrusion cooking.

This result agrees well, with the result obtained by SDS-PAGE (Fig. 2b) where it was observed a fading of molecular weight bands from the electrophoretic protein profile of the extruded soybean/corn mix, which could be attributed to the increased IVDP determined on this sample.

The characteristic vibration frequencies of the Amide I band (1652 cm^{-1}) arise from the overlapping secondary structural components of the polypeptide chains in the proteins from the soybean/corn mix. To observe more detailed spectral features of the Amide I band, the second derivative of the FTIR spectra of the soybean/corn mix before and after extrusion was calculated and the results are shown in Fig. 3b. A splitting of the amide I band in two more specific bands was observed: one at 1656 cm^{-1}, which is associated to the α-helix of the secondary structure of proteins; another one at 1636 cm^{-1}, which is attributed to β-sheet structures. Similar bands were observed for amide I in heated soybean protein isolate (Maltais et al. 2008). The α-helix structure remained unchanged after extrusion. However, the band associated with β-sheet structure (1636 cm^{-1}) showed to be split into two bands at 1624 and 1640 cm^{-1}, respectively. This structural change observed in the β-sheet structures may be also associated to the increased in IVPD in the extruded sample. Dahlin and Lorenz 1993; Allan and Booth 2004; Ruiz-Ruiz et al. 2008 reported that during high temperature extrusion processing, changes occur in the secondary, tertiary and quaternary protein structure, so polypeptides and open internal peptide bonds are exposed and can be more easily hydrolyzed by digestive enzyme. This finding correlates well with the results of the present study.

Conclusions

A central composite design using RMS successfully described the effect of independent variables (feed moisture, die temperature and soybean proportion) on the specific parameters of product quality (EI, WAI, WSI, ΔE) studied. The optimized processing conditions were obtained with 25.8 % feed moisture, 160 °C die temperature and 58 %/42 % soybean/corn proportion. The amino acid profile in the final extrudate compared satisfactorily with the requirement for essential amino reported by FAO/WHO/UNU, for human adults. The reduction of the amino acid lysine in the extruded product was attributed to the Maillard reactions. The electrophoretic profile of extruded soybean/corn mix presented low intensity molecular weight bands, compared to the unprocessed sample. The generation of low molecular weight polypeptides was associated to an increased in IVPD of the extrudate. The FTIR spectra of the soybean/corn mix before and after extrusion was determined. The α-helix structure remained unchanged after extrusion. However, the band associated with β-sheet structure showed to be split into two bands. The observed changes in the β-sheet structures may be also associated to the increased in IVPD in the extruded sample. Further studies are needed to conform these observations.

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