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EFFECT OF EXTRUSION COOKING ON THE FUNCTIONAL PROPERTIES AND STARCH COMPONENTS OF LENTIL/BANANA BLENDS: RESPONSE SURFACE ANALYSIS

EFECTO DE LA EXTRUSIÓN EN LAS PROPIEDADES FUNCIONALES Y COMPONENTES DEL ALMIDÓN DE MEZCLAS LENTEJA/PLÁTANO: ANÁLISIS DE SUPERFICIE DE RESPUESTA

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Abstract

Banana and lentil flour blends were processed in a single screw extruder modifying the flour properties of the blend (20.5-79.5%), at selected range of die temperature (145-175 °C) and the feeding moisture content (20-24%).Functional characteristics evaluated in the extrudates were water absorption index (WAI), water solubility index (WSI), bulk density (BD), paste viscosity properties, microstructure and resistant starch content. The concentration of lentil/banana blends and temperature were the most important variables affecting dependent variables WAI, WSI, BD and viscosity properties. The results of this study indicated that extrusion cooking induced desirable functional characteristics to lentil/banana blends by increasing their resistant starch content.

Keywords: extrusion, lentil flour, banana flour, functional properties.

Resumen

Se procesaron harinas de lenteja y plátano en un extrusor de tornillo simple, modificando la proporción de harina (20.5-79.5%), la temperatura de dado (145-175 °C) y la humedad de alimentación (20-24%). Las propiedades funcionales evaluadas en los extrudidos fueron índice de absorción de agua (IAA) y de solubilidad (ISA), densidad aparente (DA), perfil de viscosidad, cambios microestructurales y contenido de almidón resistente. La proporción de las harinas de lenteja/plátano fueron las variables independientes que más afectaron a las variables dependientes IAA, ISA, DA y viscosidad. Los resultados de este estudio muestran que la mezcla de lenteja/plátano, después de la cocción por extrusión provee características funcionales deseables, con un alto contenido de almidón resistente.

Palabras clave: extrusión, harina de lenteja, harina de plátano, propiedades funcionales.

1 Introduction

Extrusion cooking technology is a continuous, high-temperature, short-time process with high capability of production at low cost. Extrusion converts raw materials into shelf-stable finished food products with enhanced textural attributes and flavor. Extrusion has been used to develop various types of snack foods, mainly from corn meal, rice, wheat or potato flour, in many shapes and variety of textures. Extrusion cooking modifies the digestible characteristic and functional properties, such as paste viscosity, water absorption and water solubility indexes, expansion index and bulk density of protein and starch molecules

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(Ali *et al*., 1996; Hernandez-Diaz, *et al*., 2007).

Published studies have focused on the use of extrusion technology to process cereals, vegetables, fruits and legumes to improve their nutritional profile (Liu *et al*., 2000; Berrios, 2006). Lentil flour has potential for traditional and newer product developments with health benefits since it contains about 25% protein, 56% carbohydrate and 1% fat. Lentils are considered as one of the best and low-cost sources of vegetable proteins and are also a good source of B-complex vitamins, such as folate, thiamine, niacin and riboflavin (Fikry *et al*., 1980; Adsule *et al*., 1989). They have appropriate balance of minerals, a high amount of dietary fiber and complex carbohydrates involved in low glycemic index (Shams *et al*., 2008). Lentils have been associated with cholesterol and lipid lowering effects in humans, along with a reduction of the incidence of colon cancer and type-2 diabetes (Leterme, 2002; Shams *et al*., 2008). On the other hand, the consumption of banana is mainly in its ripe state. In Mexico, the postharvest handling of banana is relatively poor and, as a consequence, large quantities of this fruit are lost during commercialization. Unripe banana has been investigated and the studies indicated that these fruits had 17.5% of resistant starch (RS) and 14.5% of dietary fiber. Based on this finding, the consumption of unripe bananas could improve the colon health (Faisant *et al*., 1995; Juarez-Garcia *et al*., 2006). For this reason the flour obtained from this fruit could be considered a functional ingredient for the food industry.

The objective of this study was to investigate the effect of extrusion processing on the functional properties and starch components of blend of lentil and banana flours.

2 Methodologies

2.1 *Raw materials*

Commercial hard green (unripe) pre-climacteric bananas (*Musa paradisiaca* L.) were purchased from a local market in Cuautla, Morelos, Mexico. Fruits were peeled and cut into 5 mm slices and immediately rinsed in citric acid solution (0.3% w/v). The slices were dried at 50°C in a convection oven. The lentils (*Lens culinaris*) were purchased from "Local Productores Unidos de Huaniqueo", (Huaniqueo, Michoacán, México). Lentils and dried banana slices were ground using a commercial grinder (Mapisa Internacional S.A. de C.V., D.F., México) to pass through a US 40 sieve and stored at 25°C in sealed plastic containers until further analyses.

2.2 *Chemical analysis*

Moisture, ash, protein and fat analyses of raw materials were carried out according to the procedures of AOAC (1990). All the determinations were performed in triplicate.

2.3 *Samples conditioning*

Banana and lentil flours were mixed (Maren type 10, Effort, Copenhagen, Denmark) and spray-atomized with water, to reach the various moisture contents indicated in the central composite experimental design (Table 2). The samples were stored in plastic bags at 4°C for subsequent processing.

2.4 *Extrusion*

Materials were processed in a single-screw extruder (CICATA - IPN, México, D.F.), 60 mm diameter and 950 mm length screw. The barrels contained three controlled heating and cooling zones. The diameter of the hole in the die was 12.5 mm. Three thermocouples were used to monitor the barrel temperatures and one extra thermocouple was inserted in the die plate. The materials were fed to the extruder at 25 Hz, controlled with a Baldor Electric Co. Cat. GPP 7454 MDF (FT Smith, AR, USA). Extrusion was carried out at different temperatures, moisture contents and flour blend proportions, according to the experimental design shown in Table 2. The resulted extruded products were stored in polyethylene bags until further analysis.

2.5 *Experimental design and statistical analysis*

To analyze the influence of independent variables on functional characteristics of lentil and banana blends, response surface methodology (RSM) was used. A central-composite rotable design (Montgomery, 2004; Velázquez-Trujillo *et al*., 2010) was selected with three variables: ratio of lentil to banana flour (20.5-79.5%), feed moisture content (20-24%) and die temperature (145-175°C). Six repetitions were made at the central point conditions. Treatments were performed randomly and results were analyzed using the statistical program Design expert ® version 8.0.4.

(Minneapolis, MN, USA). The significance of the models was tested by variance analysis (test F) and the determination coefficient R^2, and values and significance of the independent variables were also calculated, considering, $\alpha = 0.05$.

2.6 Characterization of the extruded samples

The extruded samples were milled to pass through a 60 mesh sieve and stored at room temperature in plastic bags. In all analytical determinations, the average of three measurements was reported.

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

The WAI and WSI of the extruded materials were determined using the procedure reported by Anderson (1982).

2.6.2. Bulk density (BD)

The BD was determined by the method proposed by Hsieh *et al*. (1990).

2.6.3. Viscosity profile: Maximum viscosity (MV) and Final Viscosity (FV)

The viscosity profile was determined according to the method used by Guerra-DellaValle *et al*. (2009). Tests were carried out in a Rapid Visco Analyser (RVA-4D; Newport Scientific Pty, Warriewood, Australia). The sample preparation was done, using a total test sample of 28 g, taking into account the initial moisture of each extruded sample. The samples were placed in a canister and the suspension was heated from 50 to 90°C in 5 min and held at 90°C for another 5 min, then cooled down to 50°C for an extra 6 min so that the total time of analysis was 16 min at 160 rpm. Relative viscosity was reported in centipoises (cPs) and the following parameters were obtained from the curve: MV (maximum viscosity obtained during the heating) and FV (final viscosity at the end of the test).

2.6.4. Total and resistant starch

Total starch (TS) was determined by the method of Goñi *et al*. (1997); 50 mg of sample were dispersed in 2 M KOH (30 min); then samples were incubated with amyloglucosidase (Boehringer, No. 102857, 60°C, 45 min, pH 4.75), and glucose was determined using the glucose oxidase assay GOD-POD. TS was calculated as released glucose (mg) × 0.9.

Resistant starch (RS) was evaluated by the method of Goñi *et al*. (1996). Protein and digestible starch were removed with pepsin (P-7012, 2500- 3500 units/mg protein, Sigma Chemical Co., St. Louis, MO) by incubation at 40 °C, pH 1.5 for1 h and with α-amylase (A-3176, 10-30 units/mg solid, Sigma Chemical Co.) incubating at 37 °C, pH 6.9 for 16 h. The residue was treated with 2 M KOH and then incubated with amyloglucosidase (A-7255, 5000 units/g solid, Sigma Chemical Co.) at 60°C and pH 4.75, for 45 min. Glucose was determined using glucose oxidase/peroxidase assay (SERA-PAK ® Plus, Bayer de México, S.A. de C.V., Edo. de México). RS was calculated as mg glucose × 0.9.

2.6.5. X-ray diffraction

Samples, before the analysis were stored in a sealed container at a relative humidity of 82% for obtaining a constant moisture content; then, they were analyzed between $2\theta = 10^o$ and $2\theta = 50^o$ with a step size $2\theta = 0.02^o$ in an X-ray diffractometer (Philips PW 1710, The Netherlands) using Cu $K\alpha$ radiation ($\lambda = 1.543$), 50 kV and 30 mA. The diffractometer was equipped with a 18 divergence slit and a 0.1 mm receiving slit (González-Soto *et al*., 2007). Starch crystallinity determination with X-ray difraction is generally carried out following different methods (Rindlav-Westling *et al*., 2002). These methods calculate crystallinity by subtracting the amorphous contribution from the measured spectra. A sample containing amorphous maize starch was prepared and its diffraction spectrum used as the amorphous contribution in the measured spectra. Crystallinity was calculated by subtracting the amorphous contribution in each spectrum according to Equation (1) (Rodríguez, 1995):

$$\%C = At/Ap - N \quad N = 149.6cps * deg \tag{1}$$

Where:

$\% C$ = Percent crystallinity

Ap = Area of crystalline peaks (from the difference between the area under the curve and of the amorphous halo).

At = Total area under the curve

N = Instrument noise

3 Results and discussion

3.1 Chemical composition of raw materials

The raw materials had very similar mineral composition, whereas moisture, fat and protein content were higher in lentil flour (Table 1). Lentil protein, lipids and ash content were similar to values reported by Cai *et al*. (2001). As expected, the highest resistant starch content was found in banana flour.

3.2 Water absorption index

The Water Absorption Index (WAI) measures the amount of water absorbed by starch and can be used as an index of gelatinization (Anderson *et al*., 1969). The gelatinization is the conversion of raw starch into a cooked and digestible material by the application of water and heat. Also, gelatinization is one of the important effects that extrusion has on the starch component of foods.

Table 3, shows the regression coefficients for a second-order model for WAI. The coefficients that had a significant effect ($P < 0.05$) on WAI, were the linear term M (flour moisture content) and T (die temperature), the quadratic effect of T and the interaction of M and T.

The response surface graph (Fig. 1) shows that the maximum WAI value was found at the highest moisture content. Similar effect of moisture content on WAI has been reported for rice and pea grit (Singh *et al*., 2007). At high moisture content, the viscosity of the starch would be low, allowing for extensive internal mixing and uniform heating which, in turn, would account for enhanced starch gelatinization (Lawton *et al*., 1972); it also may lead to increased water absorption. Lower WAI values were observed at lower moisture content and high lentil flour concentration in the blend. Lentils are reach source of protein. So, as the lentil flour increase in the blend, the protein content increased in the extrudates. The WAI depends on availability of hydrophilic groups which bind water molecules and on the gel forming capacity of macromolecules. Although legume proteins have hydrophilic groups, the protein denaturation during extrusion cooking leads to loss of hydration capacity of proteins. The lower hydration capacity is favored by the formation of inter- and intra-molecular protein bonds with amylose and amylopectin (Fernandez-Gutierrez *et al*., 2004).

3.3 Water solubility index

The Water Solubility Index (WSI) is related to the quantity of water soluble molecules, and is associated to dextrinization. In other words, WSI can be used as an indicator of the degradation of molecular compounds, and measures the starch degradation resulted from extrusion cooking (Colonna, *et al*., 1989; Ding *et al*., 2005). Recently, WSI was used as an indicator for evaluating the degree of cooking in bean extrudates (Drago *et al*., 2007).

The model equation predicting the response is given in the Table 3, the determination coefficient for this model was $R^2 = 0.8472$ and $P = 0.0044$. The terms that showed significant effect on WSI ($P < 0.05$) were: T, C, T^2 and MT. This surface response model is shown in Fig. 2. The lower WSI values were found at moisture 22%, temperature 150 C and 50% of banana flour and 50% lentil flour, whereas the maximum values of WSI were obtained in the extrudates processed at moisture 20%, 180 C and highest concentrations of banana flour in the blends.

The blends with highest levels of lentil concentration (consequently higher protein content) processed at high or low moisture content and low temperature, presented low WSI values (Fig. 2).

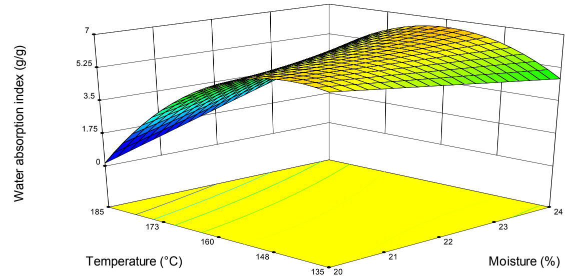

Fig. 1. Effect of extrusion variables on water absorption index: extruder temperature vs. moisture content (lentil/ banana flour concentration: 50-50).

Table 1. Chemical composition of banana and lentil flours (%)

Component	Lentil flour	Banana flour
Moisture	9.39 ± 0.09	5.77 ± 0.13
Fat[a]	0.97 ± 0.01	0.15 ± 0.04
Protein[a,b]	24.59 ± 0.62	2.44 ± 0.09
Ash[a]	2.88 ± 0.06	2.42 ± 0.09
Total starch[a]	39.90 ± 0.52	74.9 ± 0.54
Resistant starch[a]	4.77 ± 0.11	24.6 ± 0.39

[a]Mean of three replicates ± standard deviation, dry basis.
[b]N × 6.25.

Table 2. Extrusion conditions with actual variable levels for experimental design

Run	Actual values		
	Moisture (%)	Temperature (°C)	Lentil flour (%)
1	20.00	145.00	20.50
2	24.00	145.00	20.50
3	20.00	175.00	20.50
4	24.00	175.00	20.50
5	20.00	145.00	79.50
6	24.00	145.00	79.50
7	20.00	175.00	79.50
8	24.00	175.00	79.50
9	18.64	160.00	50.00
10	25.36	160.00	50.00
11	22.00	134.77	50.00
12	22.00	185.23	50.00
13	22.00	160.00	0.39
14	22.00	160.00	99.61
15	22.00	160.00	50.00
16	22.00	160.00	50.00
17	22.00	160.00	50.00
18	22.00	160.00	50.00
19	22.00	160.00	50.00
20	22.00	160.00	50.00

Table 3. Regression coefficients for second-order model for water absorption index (WAI), water solubility index (WSI), bulk density (BD), maximum viscosity (MV), final viscosity (FV), total starch (TS) and resistant starch (RS) of extrudates

Variable	Water absorption index (g/g)	Water solubility index (%)	Bulk density (g/cm^3)	Maximum viscosity (cPs)	Final viscosity (cPs)	Total Starch (%)	Resistant starch (%)
Intercept	35.1718	204.0040	7.6575	25438.0500	10990.7430	92.4101	8.0389
Moisture content (M)	*-4.4197	2.2916^{ns}	*-0.3295	-1317.6780^{ns}	-475.0094^{ns}	5.5690^{ns}	*-0.9363
Die temperature (T)	*0.3567	*-2.9187	*-0.0404	*-103.1469	*-62.9949	-0.8288^{ns}	0.0425^{ns}
Lentil content (C)	-0.3053^{ns}	*0.4894	*-0.0210	*-69.2584	*-21.3979	*-0.4747	*0.0097
M^2	ns	ns	*0.0080	ns	*5.4135	ns	ns
T^2	*-0.0034	*0.0214	*0.0001	ns	*0.0852	ns	*-0.0005
C^2	ns	ns	ns	ns	ns	ns	ns
M×T	*0.0273	*-0.1548	ns	*3.8583	*81.3750	ns	*0.0063
M×C	ns	ns	*0.0010	ns	ns	ns	ns
T×C	ns	ns	ns	*0.2576	*0.0661	ns	ns
R^2	0.7832	0.8472	0.8367	0.8348	0.8314	0.9137	0.8399
P	0.0084	0.0044	0.0021	0.0007	0.0024	< 0.0001	0.0006

^{ns}Non-significant. * Significant $p < 0.05$

This result indicated that protein present in the blends lost solubility by denaturation, when it was subjected to high extrusion temperature. Also due to loss of protein solubility by denaturation, structural changes may have occurred which allowed hydrophilic groups such as -OH, $-NH_2$, -COOH, and -SH to form cross-links with starch (Ramírez-Ortiz *et al.*, 2008).

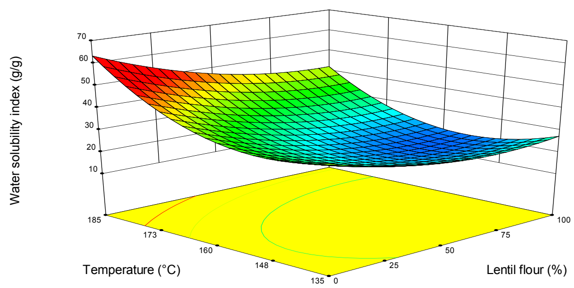

Fig. 2. Effect of extrusion variables on water solubility index: extruder temperature vs. lentil flour (moisture content: 22%).

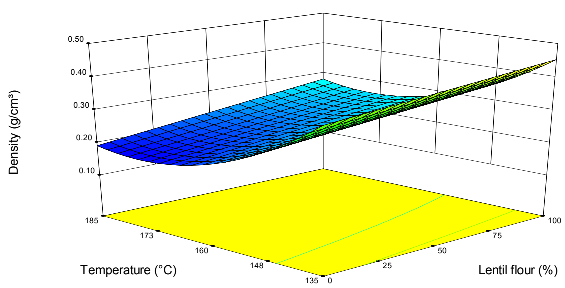

Fig. 3. Effect of extrusion variables on bulk density: extruder temperature vs. lentil flour (moisture content: 22%).

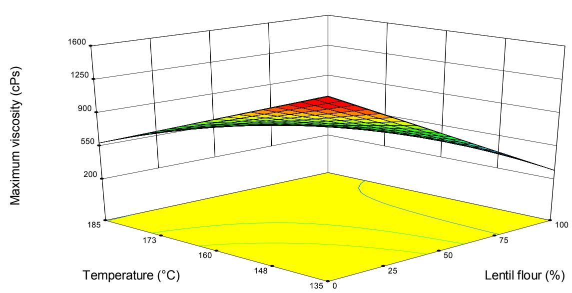

Fig. 4. Effect of extrusion variables on maximum viscosity: extruder temperature vs. lentil flour (moisture content: 22%).

3.4 Bulk density

Bulk density is a very important parameter in the production of expanded and formed food products, as the bulk density considers expansion in all directions (Wang and Ballington, 2007). The regression equation for BD is presented in the Table 3. The extrusion variables that had a significant effect on the BD, were M, T and C, M^2, T and MC. The BD of the extrudates was between 0.17 and 0.54 g/cm^3, and the effect of extrusion variables on BD are reported in Fig. 3. The response surface graph obtained, corresponding to the model showed that the lowest BD values were obtained when the blend with highest level of banana flour was extruded at higher temperatures (Fig. 3). The BD values decreased when the extrusion temperature increased due to higher starch gelatinization. This agreed with the report of Mercier and Feillet (1975) and Case *et al.* (1992) who indicated that as gelatinization increases, the volume of extruded products increases and bulk density decreases.

The highest value of BD was obtained at high levels of lentil flour in the blend processed at high moisture content. The increase of protein content on the extrudates can also influence density, since friction and shear during extrusion cause extensive interlacing between proteins and lead to their texturization and because high protein content promote denser and rigid extrudates products (Ruiz-Ruiz *et al.*, 2008).

3.5 Maximum viscosity

RVA is used extensively to characterize the pasting properties of starch in grains and processed foods (Bryant *et al.*, 2001; Kadan *et al.*, 2003). In extruded products, the viscosity determined by RVA reflects the changes in pasting properties that result from starch structural changes during cooking and therefore gives an indirect evaluation of the extent of starch conversion. The model prediction for maximum viscosity (MV) is presented in Table 3, the determination coefficient for this model was R^2 = 0.8348 and P = 0.0007, the terms T, C and the interaction of the terms MT and TC had a significant effect ($P < 0.05$) on MV.

The highest values of viscosity in the extrudates were associated with a high proportion of ungelatinized starch, whereas the lowest values of viscosity might reflect greater degradation and gelatinization of starch that is attributed to depolymerization and molecular entanglement resulting from the processing conditions (Hagenimana, *et al.*, 2006). The response surface graph obtained for MV is shown in Fig. 4. The highest values of viscosity on the extrudates were determinated in the blends with highest level of banana flour, processes at low moisture content and low temperature. Under those conditions starch is not complete gelatinized, since gelatinization process is carry out in excess of water, while fusion of starch granules is produce at low water content but high temperature. The lowest values for MV were determined at low moisture content and high temperature. At high temperature the extruded mass became plastic and less viscous allowing the molecules to become more susceptible to compression during extrusion. Thus, greater thermal and mechanical action was produced, resulting in degradation of the starch granules and, consequently, lower viscosity value was obtained. Carvalho *et al*., (2002), has previously reported that high barrel temperatures and low feed moisture resulted in increased mechanical effort during the extrusion process of third-generation snacks, resulting in great starch degradation and low viscosity values. The lower viscosity values of the extruded materials compared with those of the untreated samples are probably a consequence of the denaturation of the protein, as well as the starch-protein interactions that produce structures with lower capacity for interaction with water and consequently low viscosity. The MV results for the extruded materials is attributed to the modification of the conformation of the proteins; many of the covalent bonds that stabilize the secondary structure are destroyed and new bonds can be form between the subunits (Lampart-Sczapa, *et al*., 2006) enabling the formation of starch-lipid and starch-protein complex, as well as retrograded amylose (Guha *et al*., 1998).

3.6 Final viscosity

The final viscosity (FV) is a measure of starch reassociation, which in extruded products, depends on modifications that occur in the structure of granules and molecules, and could give an indirect indication of how much RS can be formed via retrogradation of starch. The retrogradation has been reported to be of considerable practical importance since it affects textural changes in starchy foods. Amylose leaching, friction between swollen granules, granule swelling, and competition between leached amylose and remaining ungelatinized granules for free water have been reported to affect hot paste viscosity (Liu, *et al*., 1997).

The quadratic model fitted to the experimental results for FV that showed significant ($P < 0.05$) effect were the linear terms T and C, the quadratic terms M and T and the interactions MT and TC. Figure 5 illustrates the response surface graph obtained for the FV. An increase in FV was determinate for the extruded products with the highest proportion of banana flour in the blend and extruded at low temperature. This result could be associated with the increase of the starch content in the mixture and the extent reassociation tendency of the starch. However the FV decreased when the blend with high concentration of lentil flour and low moisture content was extruded at high temperature; this is probably due to the fact that during cooling the starch and the protein produce a weaker and less stable gel.

3.7 Total and resistant starch

Total starch value in the extruded products showed an increase from 34% to 65%, which was related to the level of the raw flours used in the blend. The regression equation for the relationship between resistant starch (RS) and the independent variables of lentil-banana flour concentration, moisture content and temperature obtained are presented in Table 3. The coefficient of determination (R^2) was 0.8399 and it was significant ($P < 0.05$).

The linear effects of moisture and lentil flour concentration as well the quadratic effect of temperature and the interaction between moisture content and die temperature significantly influenced the resistant starch content in the products. Figure 6 illustrates the response surface graph obtained for the RS that ranged between 1.3% to 2.4%. The higher RS content was determined in the extrudates processed at 22-24% feed moisture, 160-175ºC and highest proportion of banana flour in the blend. Some authors reported that amylose content of 37% in banana starch (Yoshimoto *et al*., 2000; Tester, *et al*., 2004) and that extrudates with highest proportion of banana flour in the extruded blend showed the highest RS concentration.

3.8 X-ray diffraction

X-ray diffraction was used to study the changes in the crystalline structure of the extruded products at the molecular level. The banana flour studied showed a relative crystallinity of 2.45% and C-type X-ray pattern with main reflections at 2θ values of 17°, 18° and 23° which agreed with González-Soto *et al*. (2007) report. The diffraction spectra of the lentil flour showed peaks at 2θ values of 15°, 17.2° and 23.2°. This pattern closely matches the reported values for B-type legumes starches and the relative crystallinity of 1.30% determined in the flour 1.30% (Hoover and Ratnayake, 2002; Kaur *et al*., 2004).

During the extrusion process the organized crystalline structure of the raw materials are partially or totally destroyed, depending on

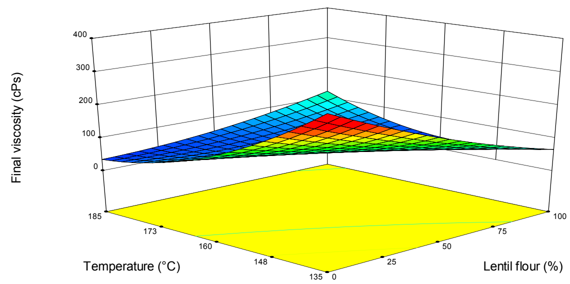

Fig. 5. Effect of extrusion variables on final viscosity: extruder temperature vs. lentil flour (moisture content: 22%).

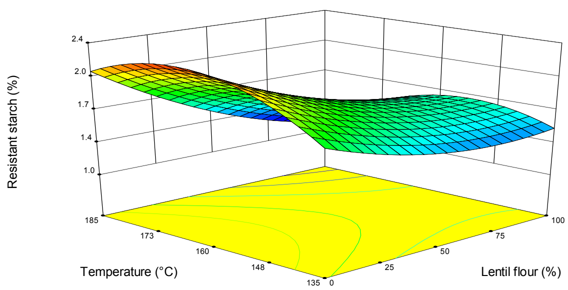

Fig. 6. Effect of extrusion variables on resistant starch: extruder temperature vs. lentil flour (moisture content: 22%).

the extrusion conditions principally due to the barrel temperature and feed moisture. These changes in the structure are also related with the starch source and other components present in the flour, such as proteins and fibers (McPherson, *et al*., 2000). Some degree of crystallinity in the extruded products was detected, partially organized in B-type crystalline structures. Those structures are very stable and known to be very resistant to α-amylase hydrolysis. These results showed the influence of the unripe banana flour, due to the main component of unripe banana which is starch as Guerra-Della *et al*. (2009) reported. These changes were also reflected in functional properties of the extrudate as BD and Viscosity and RS content.

Conclusions

Lentil and banana flours had several desirable attributes as functional ingredients to produce healthy new food products. The extrusion process induced additional modifications such as changes in WSI, BD, viscosity values and resistant starch content. The concentration of lentil/banana blends and temperature were the most important variables affecting the dependent variables (WSI, BD, MV and FV). All the dependent variables could be controlled by appropriated processing conditions. Mixtures with the highest concentration of banana flour and processed at 155 °C produced products with highest resistant starch content.

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Nomenclature

M	moisture content of lentil/banana flours
T	die temperature °C
C	lentil flour content
RSM	response surface methodology
R^2	determination coefficient
WAI	water absorption index, g/g
WSI	water solubility index, %
BD	bulk density, g/cm^3
MV	maximum viscosity, cPs
FV	final viscosity, cPs
TS	total starch, %
RS	resistant starch, %
$\%C$	percent crystallinity
Ap	area crystalline peaks
At	total area under the curve
N	instrument noise

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