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DEVELOPMENT OF EXTRUDED READY-TO-EAT SNACKS USING PUMPKIN SEED (*Cucurbita pepo*) AND NIXTAMALIZED MAIZE (*Zea mays*) FLOUR BLENDS

DESARROLLO DE BOTANAS EXTRUDIDA LISTA PARA COMER USANDO MEZCLAS DE HARINAS DE SEMILLA DE CALABAZA (*Cucurbita pepo*) Y MAÍZ NIXTAMALIZADO (*Zea mays*)

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Abstract

Extruded ready-to-eat snacks were prepared from flour blends made with pumpkin seed (PSF) and nixtamalized maize (NMF) using a single-screw extruder with a compression screw ratio of 3:1. A central composite rotatable design was used to investigate the effects of the PSF proportion in formulations (0 - 30 g/100 g), feed moisture content (14 - 20 g/100 g) and extrusion temperatures (120 - 180 °C) on the physical properties like expansion index (EI), bulk density (BD), water absorption index (WAI), water solubility index (WSI), hardness (H), pH and total color difference (ΔE). The results indicated that EI, BD and ΔE were significantly affected by increasing the proportion of PSF and BD, whereas increasing ΔE resulted in an opposite effect on EI (P <0.05). Temperature significantly negatively affected (P <0.05) EI and H, while increased moisture content only caused a significant increase (P <0.05) in WAI. Optimal conditions were at an extrusion temperature of 120 °C, feed moisture content of 20 g/100 g and PSF content of 10.36 g/100 g and protein content =11.74 g/100 g. The ready-to-eat snack developed in this research could be considered as a functional food with nutritional and health benefits.

Keywords: *Cucurbita pepo*, extrusion process, maize flour, ready-to-eat snacks.

Resumen

Se prepararon botanas extrudidas listas para comer a partir de mezclas de harinas de semilla de calabaza (HSC) y maíz nixtamalizado (MN) usando un extrusor de un solo tornillo con una relación de tornillo de compresión de 3:1. Se utilizó un diseño central compuesto rotable para investigar los efectos de la proporción HSC en las formulaciones (0 - 30 g / 100 g), contenido de humedad de alimentación (14 a 20 g /100 g) y temperaturas de extrusión (120-180 ° C) sobre las propiedades físicas como índice de expansión (IE), densidad aparente (DA), índice de absorción de agua (IAA), índice de solubilidad en agua (ISA), dureza (D), pH y la diferencia de color total de (ΔE). Los resultados indicaron que el IE, DA y ΔE se vieron afectados de manera significativa por el aumento de la proporción de HSC y DA, mientras que el aumento de ΔE produjo un efecto contrario a la IE (P <0.05). La temperatura afecto significativamente negativamente (P <0.05) el IE y D, mientras que el aumento de contenido de humedad sólo causó un aumento significativo (P <0.05) en el IAA. Las condiciones óptimas fueron a una temperatura de extrusión de 120 ° C, contenido de humedad de alimentación de 20 g /100 g y HSC de 10.36 g /100 g y contenido de proteína = 11.74 g /100 g. La botana listos para comer desarrollada en esta investigación podría ser considerada como un alimento funcional con beneficios nutricionales y de salud.

Palabras clave: *Cucurbita pepo*, proceso de extrusión, harina de maíz, botana lista para comer.

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1 Introduction

Pumpkin (*Cucurbita pepo*) is a vegetable that is native to the Western Hemisphere, with different varieties found in North America, Continental Europe, Australia, New Zealand and India. Pumpkin has also been cultivated in Tropical Asia in countries such as Indonesia, Malaysia and Philippines. After roasting and salting, its seeds are often eaten as a snack in Arab countries, as well as in other countries including Mexico. Pumpkin seeds are excellent sources of protein (25.4 - 35.4%) and oil (41.5 - 49.1%) (Norfezah *et al*. 2011; Rodríguez-Miranda *et al*. 2012). Seed kernels have been used as an additive in some food dishes, and there are several reports on the nutritional value of pumpkin seed kernel proteins and oils (Rodríguez-Miranda *et al*. 2014). Pumpkin seeds have also been reported to have a positive effect on human health, including anti-diabetic, anti-hypertensive, anti-tumor, anti-bacterial, anti-hypercholesterolemic and anti-inflammatory actions (Radočaj *et al*. 2012). Pumpkin seed are usually a by-product of pumpkin pulp processing at either the artisanal- or large-scale. It is estimated that seeds constitute 2.9% in weight of the fresh fruit, while in their dry form, they account for 32% of the weight (Sedano-Castro *et al*. 2005). These by-products could be used in the development of new food products such as snack foods. These products have become included in the feeding habits of the majority of the world's population because they provide convenient portions and alleviate short-term hunger (Rodríguez-Miranda *et al*. 2011). One of the most important technologies that has shown great potential for the development of new snack products is extrusion cooking, which is a high temperature short-duration process in which food materials are plasticized and cooked by a combination of temperature and mechanical shear, resulting in molecular transformations and chemical reactions. This technology uses a continuous process with high productivity and the significant retention of nutritional quality, natural color, and food flavor (Nayak *et al*. 2011). Extrusion has been used to develop various types of snack foods, mainly from corn meal, rice, wheat flour or potato flour, in many shapes and a variety of textures. The application of extrusion to legume flours is a relatively new area of investigation, with the exception of soy beans (Rodríguez-Miranda *et al*. 2011; Ramos-Diaz *et al*. 2013). Extruded products are commonly obtained from starchy materials, especially cereal grains such as corn, sorghum and rice (Escalante-Aburto *et al*. 2013). Maize is an important part of the diet in Central American countries. It is consumed daily in different forms, one of them being tortilla. The nixtamalization process (alkaline heat treatment) is used to prepare maize (Zambrano-Zaragoza *et al*. 2013; Estrada-Girón *et al*. 2014). This thermal-alkaline process improves physicochemical, functional and nutritional properties. For example, nixtamalization has been reported to increase the protein quality, calcium content and niacin bioavailability, while reducing the aflatoxin levels of maize grains (Sefa-Dedeh *et al*. 2004).

Extruded snacks based on maize have shown good physical, functional, nutritional and sensory characteristics by varying certain process conditions such as temperature, moisture level, screw speed, residence time and calcium hydroxide concentration (Rodríguez-Miranda *et al*. 2011; Zazueta-Morales *et al*. 2002). However, no information has been reported yet regarding the combined use of pumpkin seed flour with nixtamalized maize to produce new composite extruded snacks. Therefore, the aim of this work was to investigate the processability of flour mixtures made from pumpkin seed and nixtamalized maize flour to produce ready-to-eat snack foods in a single-screw extruder. Additionally, we evaluated the effect of extrusion temperature, moisture content and proportion of pumpkin seed flour on a set of physical and functional properties of extruded snacks.

2 Materials and methods

2.1 Raw materials

Pumpkins (*Cucurbita pepo*) were acquired from a local market in the community of Durango, Durango State, Mexico. Fruits were collected during the 2014 spring-summer agricultural cycle. Ripe pumpkins were cut, and the seeds removed. Yellow maize (*Zea mays* L.) grains of the criollo variety were obtained from a local market in Durango, Durango (Mexico) and stored in 5 kg bags at room temperature (25 ± 1 °C) until use.

2.1.1 Preparation of pumpkin seed flour

Pumpkin seed flour (PSF) was obtained from cleaned dehulled seeds with no physical damage. Seeds were dried in a convection air drier (ED 115 Binder Oven, Im Mittleren Ösch 578532 Tuttlingen, Germany) at 60 ± 2 ºC for 24 h and ground in a domestic blender (Oster 465) until the particles were passed through a No. 30 mesh screen (0.59 mm, USA standard test sieve ASTM E-11 Specification W.S. Tyler, USA). The powdered seeds were placed in sealed polyethylene bags and stored at 4 ± 0.5 ºC until use.

2.1.2 Preparation of nixtamalized maize flour

Nixtamalized maize flour (NMF) was prepared according to the procedure reported by Rodríguez-Miranda *et al*. (2011) and the NMX-F-046-S-1980 method from Mexican Official Norm. Maize portions of 100 g were soaked and cooked in an alkaline solution (1.08 g $Ca(OH)_2$/100 mL of water) in 0.33 g of maize grain/mL of lime water at 85 ± 2 °C for 45 min. After a resting period of 8 h, the cooking solution, or "nejayote", was eliminated, and the cooked maize grains were rinsed four times with purified water. The resulting grains were dried at 55 °C for 25 h, milled using a Cross Beater Mill (Glen Mills Inc., New Jersey, USA) and then sieved at 0.59 mm. The nixtamalized flour was stored in sealed glass containers at room temperature (25 ± 1 °C) until use.

2.2 Chemical composition of the flours and extruded blend

The approximate compositions of the flours and extruded blend (extruded products were evaluated only to determine the optimal conditions) were determined in triplicate following standard AOAC (2005) methods: moisture (925.10), ash (942.05), protein (960.52) and fat (948.22). Crude fiber was determined by acid-alkaline digestion (Tejeda, 1992) and the difference in carbohydrate levels.

2.3 Physicochemical properties of the flour

The pH of flours was recorded using a calibrated pH meter (Ultrabasic Denver UB-10, Denver Instrument Company 6542 Fig Street Arvada, Colorado 80004, USA). One gram of each flour sample was mixed with 10 mL of distilled water at 25 ± 1 °C. The pH electrode was immersed into the dispersion, shaken and allowed to equilibrate until a stable reading was observed. Flour color was determined using a Hunter Lab colorimeter Model 45/0L (Hunter Associates Lab., Ind., USA). Clarity (L^*), red/green chromaticity (a^*) and yellow/blue chromaticity (b^*) were measured, and the total color difference (ΔE) was calculated from these values.

2.4 Extrusion

A laboratory scale single-screw extruder (Model E 19/25 D, Instruments Inc. South Hackensack, NJ, USA) was used with the following characteristics: four heating zones, screw compression ratio 3:1, longitude/diameter relation (L/D) 20:1, 3 mm internal diameter of the exit die. Before extrusion, a mixture of the formulations was performed as well as the adjustment of the feed moisture content of 14 to 20 g/100 g (w. b.) according to the experimental design (Table 1). The extruded products were dried at 45 ºC for 20 h until reaching 6 g moisture/100 g content, and they were milled and stored at room temperature (25 ºC) in polyurethane sealed bags for subsequent analysis.

2.5 Characterization of extruded products

2.5.1 Expansion index (EI) and bulk density (BD)

The EI was measured according to the Gujska and Khan (1990) method: dividing the diameter of the extrudate by the diameter of the exit die. The BD was determined according to the technique reported by Wang *et al*. (1993). We measured the diameter (d) and the longitude (l) of 10 randomly selected samples. Later, each extrudate was weighed (W) to ultimately determine the density using Eq. 1. The results were expressed in kg/m^3.

$$\text{Bulk density} = \frac{W}{\pi(d/2)^2 l} \quad (1)$$

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

The WAI and WSI were determined as outlined by Anderson *et al*. (1969). One gram of ground product was sieved at 0.420 mm and dispersed in 10 mL of water at room temperature (25 ± 1 °C). The resulting suspension was gently stirred for 30 min, and then the samples were centrifuged at 1006 x g for 15 min (Universal Compact Centrifuge HERMLE 211 Labortechnik GmbH Mod Z 200A, Germany). The supernatant was decanted into a tarred aluminum pan. The WAI was calculated as the weight of sediment or gel obtained after removal of the supernatant per unit weight of the original dry solids. The WSI was the weight of dry solids in the supernatant expressed as a percentage of the original weight of the dry sample.

Table 1. Coded levels of variables the extrusion process.

Variables	Coded	Levels				
		- α	-1	0	+1	+ α
Temperature (ºC)	X_1	120	132.16	150	167.84	180
Feed moisture content (g/100 g)	X_2	14	15.22	17	18.78	20
Pumpkin seed flour proportion (g/100 g)	X_3	0	6.08	15	23.92	30

α=1.682

Table 2. Independent variables and experimental design levels expressed in coded and real units for extrusion.

Trial no.	Independent variables					
	Coded			*Actual*		
	X_1	X_2	X_3	Temperature (ºC)	Feed moisture content (g/100 g)	Pumpkin seed flour proportion (g/100 g)
1	-1	-1	-1	132.16	15.22	6.08
2	1	-1	-1	167.84	15.22	6.08
3	-1	1	-1	132.16	18.78	6.08
4	1	1	-1	167.84	18.78	6.08
5	-1	-1	1	132.16	15.22	23.92
6	1	-1	1	167.84	15.22	23.92
7	-1	1	1	132.16	18.78	23.92
8	1	1	1	167.84	18.78	23.92
9	-1.682	0	0	120	17	15
10	1.682	0	0	180	17	15
11	0	-1.682	0	150	14	15
12	0	1.682	0	150	20	15
13	0	0	-1.682	150	17	0
14	0	0	1.682	150	17	30
15	0	0	0	150	17	15
16	0	0	0	150	17	15
17	0	0	0	150	17	15
18	0	0	0	150	17	15
19	0	0	0	150	17	15
20	0	0	0	150	17	15

Table 3. Proximate chemical composition for pumpkin seed flour (PSF), nixtamalized maize flour (NMF) and extruded blend (optimal conditions).

Component (g/100 g)	PSF	NMF	Extruded blend (PSF – NMF)[2]
Moisture	7.18 ± 0.04[a]	6.56 ± 0.18[b]	11.89 ± 0.17[c]
Protein (N x 6.25)	26.39± 0.04[a]	10.83 ± 0.03[b]	11.74 ± 0.34[c]
Fat	49.85 ± 0.39[a]	6.38 ± 0.17[b]	10.27 ± 0.19[c]
Crude fibre	1.13 ± 0.15[a]	1.64 ± 0.05[b]	1.50 ± 0.25[c]
Ash	5.56 ± 0.13[a]	1.72 ± 0.10[b]	2.00 ± 0.16[c]
Carbohydrates[1]	9.89 ± 0.17[a]	72.87 ± 0.14[b]	62.60 ± 0.21[c]

Means ± standard deviation. Different superscripts letter in the same row indicate significant difference ($P<0.05$).

[1]Obtained by difference.

[2]Extruded at 120 °C, feed moisture content 20 g/100 g and PSF content of 10.36 g/100 g.

2.5.3 Hardness (H)

The hardness of extrudates was determined using a Stable Micro system TA-XT2 texture analyzer (Stable Micro Systems, Ltd., Surrey, UK) fitted with a 2 mm cylinder probe in compression mode. The samples were punctured by the probe to a distance of 30 mm. Hardness in Newton (N) was determined by measuring the maximum force required to break the extruded samples. A force-time curve was recorded and the area under the force-time curve was measured. Twenty-one randomly collected samples of each extrudate were measured and averaged.

2.5.4. Color and pH

Color parameters were measured with a tristimulus colorimeter (MiniScan 45/0L, Hunter Lab, Hunter Associates Laboratory 11491, Sunset Hills Road Reston, Virginia 20190, USA). Clarity (L), red/green chromaticity (a^*) and yellow/blue chromaticity (b^*) were measured, and ΔE was calculated from these values. The pH of extrudates was measured by dispersing the extrudate milled in distilled water at 25 °C.

2.6 Experimental design and data analysis

A central composite rotatable experimental design was used with three independent variables (Table 1 and 2). The independent variables considered were the extrusion temperature (X_1), feed moisture content (X_2) and the pumpkin seed flour proportions (PSF - NMF) (X_3) (Table 4 and 5). The response variables were the EI, BD, WAI, WSI, H, pH and ΔE. The experimental data were analyzed according to the surface response methodology using the commercial statistical package, Design Expert (Design Expert 8.0.2, Stat-ease INC., Minneapolis, USA). The experimental data were adjusted to quadratic models (Eq. 2) and the regression coefficients were obtained. The statistical significance of the terms of the regression was examined by using a variance analysis (ANOVA) for each response.

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (2)$$

2.6.1 Process optimization

Numerical optimization was performed using the superposition methodology of response surfaces. The responses used were EI, BD, WAI, WSI, H, pH and ΔE. Desired goals were assigned for all the parameters to obtain the optimum numerical values for the responses. The levels of pumpkin seed flour proportion, feed moisture content and extrusion temperatures were maintained within the desired range. The response parameters EI and BD were maintained at maximum and minimum values, respectively. H (minimum), WAI (maximum), WSI, pH and ΔE were maintained within a set range.

3 Results and discussion

3.1 Chemical composition of the flours

The chemical composition of PSF and NMF flours is shown in Table 3, and both flours have statistically significant differences (P <0.05) in all components. The PSF had a higher content of protein, lipid and ash and lower crude fiber and carbohydrates values, while NMF had higher crude fiber and carbohydrates levels. The observed PSF protein content was within the range reported by others (25 to 38 g/100 g), while the lipid content was higher (22 to 49 g/100 g) (Rodríguez-Miranda *et al*. 2012; Rezig *et al*. 2012). These results are important because the mix between these two flours could increase the nutritional value of the resulting products. However, it should be noted that the high lipid content of the PSF flour could be an adverse factor in the processing of directly expanded extruded snacks (Ilo *et al*. 2000). However, from a nutritional standpoint, these lipids are a potential source of polyunsaturated fatty acids such as oleic and linoleic acid, which have been shown to prevent cardiovascular disease (Rezig *et al*. 2012). With respect to protein content, some authors have mentioned that pumpkin seeds have high amounts of essential amino acids, in addition to being a rich source of some micro-elements such as K, Cr, Na, Mg, Zn, Cu, Mo and Se (Yadav *et al*. 2010).

The carbohydrate content in NMF is within the previously reported range of 66.86 to 81.5 g/100 g (Rodríguez-Miranda *et al*. 2011; Gutiérrez-Dorado *et al*. 2008; Ayala-Rodríguez *et al*. 2009). Among carbohydrates, starch is the most important component in the extrusion process because it is responsible for the phenomenon of snack expansion due to its viscoelastic properties (Rodríguez-Miranda *et al*. 2014; Bisharat *et al*. 2013). The expansion of the snack obtained by the extrusion process mainly depends on the degree of gelatinization of starch, which can be up to 90 %, and this degree of gelatinization promotes molecular interaction among the ingredients of the formulation (Seth and Rajamanickam 2012; Umar *et al*. 2013).

3.2 *Physicochemical properties of the flours*

The pH values of PSF and NMF are shown in Table 4, and the results indicate statistically significant differences (P <0.05) between meals. The PSF had the highest pH value (6.91). Additionally, it was found that PSF differs (P <0.05) from NMF in the four color parameters (L*, a*, b* and ΔE) (Table 4). The brightness value (L*) was higher in NMF, indicating that it has a higher degree of whiteness than PSF. This difference is due to the pigments in PSF and the amount of starch in NMF. As for the red (+ a*) to green (-a*) tonality, the PSF value was a green tonality (-4.89), while NMF was located in the red tonality (0.27). Within the range of yellow (+ b*) to blue (-b*), yellow was more predominant, with PSF (24.47) having the most intense tone. Overall, there was a significant ΔE (P <0.05) between samples. The difference between the measured color parameters of the samples is mainly due to the green layer of the pumpkin seeds, which is located in the inner part of the seed coat and is composed of chlorophyll (Kreft *et al*. 2009).

3.3 *Effect of pumpkin seed flour proportion, extrusion temperature and feed moisture content on extrudates*

3.3.1 Regression coefficients

In Table 6, the regression coefficients for all responses are analyzed. The extrusion temperature had a significant effect (P <0.05) in the linear term for EI, H and ΔE also in its

Table 4. pH values and color parameters for pumpkin seed flour (PSF) and nixtamalized maize flour (NMF).

Parameter		PSF	NMF
pH		6.91 ± 0.01[a]	6.11 ± 0.04[b]
Color	*L**	55.34 ± 0.03[a]	88.92 ± 0.02[b]
	*a**	-4.89 ± 0.03[a]	0.27 ± 0.02[b]
	*b**	27.47 ± 0.12[a]	14.17 ± 0.02[b]
	ΔE	50.61 ± 0.05[a]	16.43 ± 0.02[b]

Means ± standard deviation. Different superscripts letter in the same row indicate significant difference (P<0.05). ΔE = Total color difference.

Table 5. Responses of dependent variables to the extrusion condition for physicochemical characteristics (EI; BD, WAI, WSI, H, pH and total color difference (ΔE))

Trial no.	Dependent variables						
	EI (--)	BD (kg/m^3)	WAI (g/g)	WSI (%)	H (N)	pH	ΔE
1	1.59	843.01	4.16	6.60	90.95	6.37	35.46
2	1.43	845.46	4.06	8.91	59.58	6.10	32.49
3	1.72	681.16	5.46	4.16	75.24	6.55	36.99
4	1.45	724.42	4.04	12.31	53.49	6.19	32.53
5	1.28	1015.67	3.69	10.42	67.75	6.74	44.14
6	1.33	888.28	3.73	12.68	57.66	6.46	40.34
7	1.47	851.29	4.28	9.91	79.84	6.61	43.43
8	1.30	912.89	4.49	7.74	68.34	6.49	40.41
9	1.61	854.04	3.99	8.54	95.60	6.28	38.65
10	1.43	626.02	4.45	6.19	60.16	6.34	36.83
11	1.35	875.70	3.87	9.73	74.07	6.43	40.11
12	1.39	935.23	4.29	6.51	56.93	6.41	39.28
13	1.83	564.11	4.56	8.12	65.01	6.25	32.46
14	1.35	923.23	4.19	10.41	70.96	6.54	45.17
15	1.45	828.51	4.25	11.49	69.17	6.42	38.71
16	1.42	892.40	4.17	11.35	76.57	6.42	38.07
17	1.43	845.80	4.42	11.09	77.92	6.45	38.12
18	1.49	812.77	3.95	11.26	71.52	6.64	38.62
19	1.47	893.29	4.43	11.69	83.14	6.42	37.76
20	1.44	916.80	4.21	11.48	80.96	6.31	38.35

EI = Expansion index; BD = Bulk density; WAI = Water absorption index; WSI = Water solubility index; H = Hardness.
The values are mean of three replicates.

quadratic term for EI, WSI, H and pH. On the other hand, feed moisture showed a significant effect (P <0.05) in the linear term for EI, whereas the concentration of PSF was significant (P <0.05) in the linear term for BD and ΔE and the quadratic term for EI and H. The interaction $X_{1,2}$ had a significant effect (P <0.05) on EI as well the interaction $X_{1,3}$. The quadratic regression model was fitted to the experimental data (P <0.05), except for WAI (P >0.05). In all the analyzed response variables, it was determined that the models had an $R^2 > 0.676$.

3.3.2 Expansion index (EI)

The EI in extruded snacks is an important parameter of quality, functionality and acceptability of the final product (Escalante-Aburto *et al.* 2013; Navarro-Cortez *et al.* 2016). In the extrusion process, air bubbles give a porous expanded structure to form extrudates (Navarro-Cortez *et al.* 2016). The porosity created during extrusion can be used to describe the expansion properties of the product (Rathod and Annapure 2016; Rodríguez-Miranda *et al.* 2014; Seth and Rajamanickam 2012). The range of EI found in this study was 1.28 to 1.83 (Table 5). Expansion decreased with increasing temperature, which could be attributed to increased starch degradation during the extrusion process that prevents bubble growth and weakens the structure (Bisharat *et al.* 2013). This behavior is consistent with that reported by other researchers (Escalante-Aburto *et al.* 2013; Bisharat *et al.* 2013). The increase in the proportion of PSF (0 - 30 g/100 g) showed lower values of EI. This could be due to different effects: 1) a dilution effect wherein as the amount of PSF is increased, the amount of NMF is decreased and hence the starch content is decreased, affecting the viscoelastic properties of the dough (Robin *et al.* 2012); 2) PSF has a high lipid content (Table 3), which, depending on its concentration, can act as a lubricant, reducing friction between the extruder barrel, screw, and the sample and reducing the degree of gelatinization and the melting of the starch granules, consequently affecting EI (Bisharat *et al.* 2013). Additionally, it is known that the degree of gelatinization of starch is affected by starch-lipid complexes due to a decrease in the rate of hydration of the granule (Hernández-Hernández *et al.* 2011; De Pilli *et al.* 2012). Norfezah *et al.* (2011) reported that EI decreased by increasing the concentration of pumpkin seed flour (*Cucurbita maxima*) in mixtures with corn. They also observed that the extrusion of vegetables restricts the expansion ratio of the extruded product, but the addition of starch could enhance the expansion of extruded foods for the development of an expanded product.

3.3.3 Bulk density (BD)

Determining the BD is commonly used to define the physical characteristics and quality of the extruded products (Escalante-Aburto *et al*. 2013). BD is inversely related to EI obtained during the extrusion process (Rodríguez-Miranda *et al*. 2011). In our study, BD ranged between 564.11 and 1015.67 (kg/m^3) (Table 5). The concomitant increase of BD with PSF could be due to the high lipid content (Table 3). Similarly, the protein content of PSF can affect the degree of starch gelatinization and therefore the rheological properties of the melted material melt during the extrusion process (Yağci and Göğüs 2008). This can also be attributed to the dilution effect of starch in NMF by increasing PSF. Some authors have shown that the expansion of extruded products depends on the degree of gelatinization of the starch and the amount of this product in the design and process conditions (Rodríguez-Miranda *et al*. 2011).

3.3.4 Water absorption index (WAI)

Water absorption capacity indicates structural changes of the native starches to be thermally processed. In this study, the WAI interval was determined to be 3.69 to 5.46 g/g (Table 5). The decrease in WAI with increased PSF content could be associated with the lubricating effect of the lipids present in the flour pumpkin seed during the extrusion process, whereas the increased temperature is associated with a decrease in viscosity, flowing material faster into the extruder and reducing the severity of the extrusion process (De Pilli *et al*. 2012).

Such behavior could occur because the water acts as a plasticizer during the extrusion process, lowering the viscosity of the dough and reducing the residence time and degradation of the starch granules by increasing the water absorption capacity (Hageniman *et al*. 2006). Ding *et al*. (2005) reported that increasing the moisture content of the food significantly increased the WAI values in a rice-based snack. This relationship has also been reported in other studies (Chakraborty *et al*. 2011; Seth *et al*. 2015).

3.3.5 Water solubility index (WSI)

WSI is used in the extrusion process as an indicator of the degradation of the molecular components such as starch, fiber and protein (Seth and Rajamanickam 2012; Suksomboon *et al*. 2011). The WSI interval was determined to be 4.16 to 12.68% (Table 5). Humidity and temperature are directly related to the decreased viscosity of the mixture, and the decreased retention time in the extruder barrel, which causes less degradation, mainly in starch (Chiu *et al*. 2013; Seth *et al*. 2015). On the other hand, the increase in solubility with increasing PSF could be related to the solubilization of proteins during extrusion due to an effect of mechanical cutting (Oikonomou and Krokida 2011; Ghumman *et al*. 2016).

3.3.6 Hardness (H)

The hardness of direct expanded products is an important factor for the industry and the consumer. If the product has a low hardness, it will break easily during packaging and distribution, resulting in economic losses for the snack processing industry. However, if the product has a high breaking strength, the consumer could find the product difficult to bite and chew (Zazueta-Morales *et al*. 2002; Paula and Conti-Silva 2014). Extrudate hardness is a subjective parameter that is associated with the expansion and the porous structure of the product. Therefore, the hardness of the extruded product is the maximum force required to break the extruded material (Meng *et al*. 2010). The hardness values of the extrudate products were 53.49 to 95.60 N (Table 5). This can be explained because the increased temperature in the barrel causes a decrease in the viscosity of the mass required to produce a low density product, resulting in the formation of small bubbles with thin walls, which results in the production of materials with lower values of H (Suksomboon *et al*. 2011). Some researchers (Seth *et al*. 2015) found similar effects of temperature on H. The decrease in hardness with increasing temperature could be due to the increased degradation of the starchy component that decreases the mass expansion at elevated temperatures. The increase in the moisture content decreased the H of the extruded products, which could be due to an increased expansion of the extrudate that results from increased starch gelatinization and a lower rate of degradation at higher moisture content.

Table 6. Values of the coefficients estimated by multiple linear regressions for physicochemical characteristics (EI; BD, WAI, WSI, H, pH and total color difference (ΔE))

Coefficients		Response						
		EI	BD	WAI	WSI	H	pH	ΔE
	Intercept	**1.450***	**863.217***	**4.236***	**11.361***	**76.578***	**6.375***	**38.291***
Linear	X_1	**-0.062***	-29.552	-0.036	0.482	**-9.835***	-0.055	**-1.268***
	X_2	0.026	-23.618	**0.246***	-0.725	-2.039	0.037	-0.034
	X_3	**-0.119***	**86.260***	-0.158	0.924	0.317	-0.023	**3.824***
Quadratic	X_1^2	0.015	-32.966	-0.002	**-1.207***	0.265	**-0.064***	-0.306
	X_2^2	**-0.039***	25.525	-0.052	-0.940	**-4.112***	0.045	0.386
	X_3^2	**0.039***	-31.678	0.051	-0.535	**-3.235***	**0.098***	0.075
Interaction	$X_1 X_2$	**-0.041***	28.724	-0.142	0.176	1.026	0.000	-0.090
	$X_1 X_3$	**0.038***	-13.939	0.222	-1.294	3.941	-0.027	0.075
	$X_2 X_3$	0.002	17.891	0.010	-0.801	**5.571***	0.059	-0.277
R^2		0.935	0.759	0.676	0.700	0.885	0.760	0.877
P of F (model)		0.000	0.032	0.102	0.076	0.001	0.030	0.001

*Bold numbers indicate significant parameter estimates (P<0.05). X_1 = Temperature (^{0}C); X_2 = Feed moisture content (g/100g); X_3 = Pumpkin seed flour proportions (g/100g); EI = Expansion index; BD = Bulk density; WAI = Water absorption index; WSI = Water solubility index; H = Hardness; R^2 = coefficient of determination.

The relationship between hardness and moisture content has been extensively studied (Seth *et al*. 2015; Chiu *et al*. 2013).

3.3.7 pH

Table 5 shows the results of the pH of snacks, and the range of the reported pH values is 6.10 to 6.64 (Table 5). This effect likely occurs due to the high concentration of free fatty acids present in PSF (Rodríguez-Miranda *et al*. 2012), which causes an increase in pH. A decrease in pH due to the temperature can result from the loss of fatty acids by percolation (De Pilli *et al*. 2012). Bowen *et al*. (2006) reported that the presence of free fatty acids will cause some changes in the pH of the system and may result in the starch hydrolysis. Sriburi and Hill, (2000), report an increase of pH in extruded products with initial values ranging from 1 to 6.5 increasing to 2.15, and 6.63 respectively.

3.3.8 Total color difference (ΔE)

Color parameters are important because they are used to assess changes in raw material and formulation due to boiling within the extruder barrel (Mjoun and Rosentrater 2011); therefore, color is important because it is the first attribute perceived by consumers, and it determines the acceptance or rejection of a product (Rampersad *et al*. 2003). Table 5 shows the results of the ΔE snacks. The ΔE interval was 32.46 to 44.14.

The increased ΔE could be due to the increased PSF concentration which causes a darker formulation color of the snacks (Table 4). To compare the values of different formulations, ΔE was observed at a temperature range of 120 to 180 °C. The extrusion process caused a significant decrease (P <0.05) in ΔE. It is known that redox reactions between sugars and proteins (amino acids) in foods at high temperatures can promote non-enzymatic browning (Maillard reaction), resulting in the darkening of the final product (Nayak *et al*. 2011). Therefore, the observed decrease in ΔE values can be attributed to the Maillard reaction as a result of the extrusion process. This effect is consistent with what has been reported by other researchers (Norfezah *et al*. 2011). Furthermore, pigment degradation due to extrusion temperatures could have generated products of the Maillard reaction that promoted changes in color values, as observed in the extruded and unprocessed (raw) products. Therefore, it can be concluded that changes in the color of the ready-to-eat snacks are due to increasing concentrations of PSF and the processing conditions.

3.4 Pearson correlation

The Pearson correlation analysis (Table 7) shows that the extrusion temperature is negatively correlated with H (r = -0.74, P <0.01) feed moisture content and is positively correlated with WAI (r = 0.55, P <0.05), while the concentration of PSF is negatively correlated with EI (r = -0.74, P <0.01), and positively correlated with BD (r = 0.67, P <0.01) and ΔE (r = 0.92, P <0.01). With respect to the correlations between responses, EI is negatively correlated with BD (r = -0.73, P <0.01) and ΔE (r = -0.57, P <0.01), and positively correlated with WAI (r = 0.56, P <0.05); BD is negatively correlated with WAI (r = -0.52, P <0.05) and positively correlated with ΔE (r = 0.65, P <0.01); WAI is negatively correlated with WSI (r = -0.62, P <0.01).

3.5 Optimization

A numerical optimization was performed to determine the best processing conditions to develop meal snacks with PSF and NMF. The best process conditions were at an extrusion temperature of 120 °C, feed moisture content of 20 g/100 g and PSF content of 10.36 g/100 g, with EI 1.7, BD 711.1 kg/m^3, H of 53.5 N and WAI of 5.4 g/g. We determined that a low temperature is required, representing an energy-saving means to obtain a ready-to-eat snack using PSF with acceptable physical characteristics.

3.6 Optimal chemical composition of extruded product

The approximate optimal composition profiles of the extruded product are shown in Table 3. The chemical composition was protein content of 11.74 g/100 g and lipid content 10.27 g/100 g (Table 3). The protein and lipid content was higher than that reported in other studies. In taro flour-maize flour and taro flour-nixtamalized maize flour blends, Rodríguez-Miranda *et al*. (2011) reported protein and lipid contents of 1 g/100 g and 0.49 g/100 g, respectively. For third generation snacks obtained from orange by-products, Tovar-Jiménez *et al*. (2015) observed a protein content of 5.11 g/100 g and a lipid content of 6.80 g/100 g. For extruded ready-to-eat snacks using corn, black gram, roots and tuber flour blends, Reddy *et al*. (2014) observed a protein content of 10.46-11.91 g/100 g and a lipid content of 5.14-10.89 g/100 g. From a nutritional standpoint, the high lipid content in the ready-to-eat snack is a potential source of polyunsaturated fatty acids such as oleic and linoleic acid, which have been shown to prevent cardiovascular disease (Rezig *et al*. 2012). With respect to protein content, some authors have mentioned that pumpkin seeds have high amounts of essential amino acids (Yadav *et al*. 2010). The ready-to-eat snack developed in this research could be considered as a functional food with nutritional and health benefits.

Table 7. Pearson linear correlation for extrudate properties

	T	FMC	PSF	EI	BD	WAI	WSI	H	pH	ΔE
T	1.00									
FMC	0.00	1.00								
PSF	0.00	0.00	1.00							
IE	-0.39	0.16	**-0.74****	1.00						
BD	-0.23	-0.18	**0.67****	**-0.73****	1.00					
WAI	-0.08	**0.55***	-0.36	**0.56***	**-0.52***	1.00				
WSI	0.17	-0.26	0.33	-0.44	0.38	**-0.62****	1.00			
H	**-0.74****	-0.15	0.02	0.31	0.21	0.10	-0.06	1.00		
pH	-0.30	0.21	-0.13	0.28	0.10	0.15	-0.17	0.08	1.00	
ΔE	-0.31	-0.01	**0.92****	**-0.57****	**0.65****	-0.25	0.18	0.19	0.01	1.00

*Bold numbers indicate significant parameter estimates (P<0.05). ** Bold numbers indicate significant parameter estimates (P<0.01).T = Temperature; FMC = Feed moisture content; PSF = Pumpkin seed flour proportions; EI = Expansion index; BD = Bulk density; WAI = Water absorption index; WSI = Water solubility index; H = Hardness.

Conclusions

The results of this study showed that extrusion cooking of enriched flour mixtures of pumpkin seed and cornmeal with a single screw extruder can be used effectively to produce ready-to-eat snacks with a high probability of acceptance by the consumer. Increasing pumpkin seed meal decreased (P <0.05) the rate of expansion, and increased bulk density and the total color difference. An increase in temperature caused a decrease (P <0.05) in the rate of expansion and the hardness of snacks, while the increase of feed moisture content alone caused a significant increase (P <0.05) in the rate of water absorption. The optimum process conditions were obtained at an extrusion temperature of 120 °C with a feed moisture content of 20 g/100 g, containing 10.36 g/100 g of flour pumpkin seed, for a ready-to-eat snack with a 1.7 expansion ratio, bulk density of 711.1 kg/m^3, hardness of 53.5 N, and a water absorption index of 5.4 g/g, protein content of 11.74 g/100 g and lipid content of 10.27 g/100 g, which is higher than that of other extruded snacks. These lipids are a potential source of polyunsaturated fatty acids such as oleic and linoleic acid, which have been shown to prevent cardiovascular disease. The ready-to-eat snack developed in this research could be considered as a functional food with nutritional and health benefits. The physical characteristics exhibited by the product indicate that the extruded product would have a high probability of acceptance if tested for consumer acceptability.

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