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Assessment on the extrusion parameters, physical and functional properties of a Blue Corn/Red Chief Lentil extrudates added with starch rich amylose and dietary fiber

Evaluación de los parámetros de extrusión, propiedades físicas y funcionales de extruidos de maíz azul y lentejas rojas adicionadas con almidón rico en amilosa y fibra dietética

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Abstract

The aim of this work was to evaluate the effect of adding commercial un-modified food starch, based on high amylose corn (HylonV®), and soluble prebiotic dietary fiber (FiberSol®-2) in extrudates samples of a mixing base of Blue Corn (BC) and Red Chief Lentil (RL). Extruded samples with varying formulation were produced in a co-rotating twin-screw extruder and physical and functional properties of the expanded extrudates and extrusion parameters were evaluated. In addition, an acceptability test was performed with untrained judges. PCA was employed to analyze correlation amongst variables. The results obtained showed that the first component from PCA was more related to starch content effect per se and the second component to the effect of the FiberSol®-2 content in the food matrix during the extrusion process. In conclusion, based on the desirability function, the optimal formulation for extrusion cooking was 69.93 g/100 g wb BC:RL-mixture, 20.08 g/100 g wb HylonV® and 10.0 g/100 g wb FiberSol®-2 with adequate physical and functional properties of extrudates. The acceptability test showed that untrained judges preferred more expanded extrudates with higher FiberSol®-2 and BC:RL-mixture content and lower HylonV® content.

Keywords: extrusion cooking; mixture design; physical properties; functional properties; principal component analysis.

Resumen

El objetivo fue evaluar el efecto de la adición de almidón comercial no modificado, a base de maíz de alta amilosa (HylonV®), y fibra dietética prebiótica soluble (FiberSol®-2) en extruidos con una base de mezcla de Maíz Azul (BC) y Lenteja Roja (RL). Las muestras extruidas con diferentes formulaciones se elaboraron en un extrusor de doble husillo co-rotativo y se evaluaron las propiedades físicas y funcionales de los extruidos expandidos y los parámetros de extrusión. Además, fue realizada una prueba de aceptabilidad con jueces no entrenados. Se empleó PCA para analizar la correlación entre las variables. Los resultados mostraron que el primer componente estaba relacionado con el efecto del contenido de almidón per se, y el segundo componente al efecto del contenido de FiberSol®-2 en la matriz alimenticia durante el proceso de extrusión. En conclusión, con base en la función de deseabilidad, la formulación óptima fue 69.93 $g/100g_{bs}$ mezcla BC:RL, 20.08 $g/100g_{bs}$ HylonV® y 10.0 $g/100g_{bs}$ FiberSol®-2 con propiedades físicas y funcionales adecuadas de los extruidos. La prueba de aceptabilidad mostró que los jueces no entrenados prefirieron extruidos más expandidos, con mayor contenido de FiberSol®-2 y mezcla BC:RL, y menor contenido de HylonV®.

Palabras clave: cocción por extrusión; diseño de mezclas; propiedades físicas; análisis de componentes principales.

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1 Introduction

Nowadays, there has been a trend among consumers to purchase low-calorie foods that have health benefits (Altan *et al.,* 2008; Aguilera & Lillford, 2008). Because of this trend, many research work studies have been incorporated novel ingredients that are potentially beneficial to health into new food products (López-Fernández *et al.*, 2021; Flores-Silva *et al.,* 2021). It has been reported that the addition of legumes to cereal-based products allows for an increase in both the protein content and quality (da Silva *et al.*, 2014). On the other hand, the extrusion process, and in particular of raw materials from food sources, deals with the process of mixing, cooking and transformation of the flours, which with the help of the shear energy exerted by a screw (of different configurations), and together with the supply of an additional heat flow in the compartment containing it, the food material is heated to its melting point, or plasticization point. This process occurs under controlled conditions to form a new material or extruded product, with physicochemical, functional, and sensory properties, very different from the raw materials used (Fellows, 2000). So, extruded legumes have been observed to have good expansion, and are therefore considered highly feasible for the development of high nutritional value, low calorie snacks (da Silva *et al.*, 2014). Extrusion is a widely used technology in chemical and food industry, ranging from processing in a 3D Printing (Wang *et al.,* 2019) to the use by-products from the food industry to obtain functional foods (Beltrán-Medina *et al.,* 2020; Medina-Rendon *et al.,* 2021). Extrusion cooking offers an excellent alternative of processing, extruders are very versatile equipment as they can process a wide variety of powdered ingredients including wholemeal flours and are very effective in the production of low-fat foods (Frame, 1994; Guy *et al.,* 2001).

Different cereals have been used to make expanded extrudates, such as white corn, which has a considerable amount of starch, allowing for the production of ready-to-eat expanded foods. A healthy alternative is the use of blue corn, which has a similar composition to white corn with the benefit of containing phenolic compounds and anthocyanins. The consumption of blue corn has been linked to the prevention of degenerative and chronic diseases, such as cataracts, cancer, and cardiovascular diseases (Camacho-Hernández *et al.,* 2014; Sánchez-Nuño *et al.,* 2024). Pulses (such as red chief lentil) are a good source of dietary fiber, protein, starch, minerals, folate and isoflavones, and are low in fat (they do not contain cholesterol) and sodium. Their components have been associated with beneficial health effects, such as hypocholesterolemic effects (proteins), prevention of some cancers and osteoporosis (isoflavones) (Nayak *et al.,* 2011; Madar & Stark 2002). Lentil polyphenols were reported to have antioxidant, antidiabetic, anticancer and cardioprotective activities. The phenolic compounds in lentils (flavanols) are good enzyme inhibitors of α-glucosidase and lipase, enzymes related to the digestion of glucose and lipids, respectively (Mustafa *et al.,* 2022). The most abundant amino acids in lentils are glutamic acid, aspartic acid, arginine, leucine, and lysine (which is in adequate levels). Lysine is the main limiting amino acid in cereals, so lentils are ideal complementary foods to cereals, which are relatively good sources of the sulfur-containing essential amino acids but low in lysine (Dhull *et al.,* 2023). Extrusion generally does not affect the protein content of extrudates, although a small reduction in certain essential amino acids has been reported due to their interaction with sugars during Maillard reactions. However, it is important to note that adding pulses to extrudates, increases the protein and essential amino acids content compared to cereal-based extrudates such as corn. It has also been shown that the extrusion process increases in vitro protein digestibility when comparing extrudates with raw doughs (García-Cordero *et al.,* 2024).

HylonV® (HyV) food starch is unmodified, based on high amylose corn and contains approximately 55% amylose. As dietary fiber source is use FiberSol®-2 (FS), that is a water-soluble, non-viscous and highly digestion-resistant maltodextrin, with a meal stimulated production of satiety hormones (Ye *et al.,* 2015). However, the incorporation of new ingredients in extrusion-cooking causes wide ranging changes of the morphological and molecular structure of starch granules because of mechanical and thermal energy dissipation (Brümmer *et al.,* 2002; Masatcioglu *et al.,* 2017). As far as these changes are related to the variables (parameters) of the extrusion cooking process it is necessary to differentiate between the effect of the specific mechanical energy input (SME) and that of the product temperature (PDT) on the extruded starch (Brümmer *et al.,* 2002).

Food formulation is a complicated process that requires sophistication, dedication, and a thorough background, both theoretical and practical in formulation chemistry, and could culminates in the introduction of a new product (Stauffer, 2004). The novelty of this work was to formulate an extruded food from a novel ingredient (blue corn) enriched with protein (Red Chief Lentil), also added with ingredients such as commercial corn starch (HyV) and dietary fiber (FS), where, the advantages they offer are taken, primary for its composition and standardized characteristics and secondly, for the benefits that they offer to the finished product (HyV is a biopolymer that provides characteristics of a resistant starch, and FS that allows the study of the addition of dietary fiber). Therefore, the objectives of this work were to develop an extruded food product through an extreme vertices mixture design by adding Blue Corn and Red Chief Lentil (70:30), commercial corn starch (HyV) and dietary fiber (FS), and to evaluate the effect of the extrudates samples on the operation parameters [specific mechanical energy (SME), product temperature (PDT) and die pressure (DP)] and the physical [porosity (X), sectional expansion index (SEI), bulk density (BD), brittleness (B) and hardness (H)], and functional [water solubility index (WSI), water absorption index (WAI), cold viscosity (CV), pasting temperature (PT), raw peak viscosity (RPV), final viscosity (FV), antioxidant activity (AA), total phenolic content (TPC) and *in vitro* protein digestibility (IVPD)] properties of the food product.

2 Materials and methods

2.1 Materials

Sunny state blue corn (BC) and decorticated red chief lentils (RL) (Lens culinaris Medik) were acquired from a local distributor (California, USA) and kept at room temperature before milling process was carried out. Lentil and corn were ground to a coarse flour and then ground to a fine flour (< 1 mm) in a pin-mill, Alpine 160Z (Hosokawa Alpine AG, Augsburg, Germany). FS was purchased from Archer Daniels Midland/Matsutani LLC (Decatur, IL, USA) and HyV (55 g/100 g wb amylose) from National Starch and Chemical Company (Bridge-water, NJ, USA).

2.2 Blend preparation

Formulations were blended 10 minutes in a mixer, Hobart V-1401 (The Hobart Mfg. Co., Troy, OH, USA), set to the lowest speed, to obtain a uniform batch. Formulations were prepared using a Blue Corn and Red Lentil blend base (BC:RL-mixture, 70:30 p/p wb), following the extreme vertices mixture design, and stored in 4-gallon HDPE airtight buckets until extrusion.

2.3 Extruder and processing conditions

A twin-screw extruder, EVOL HT32-H (Clextral Inc., Tampa, FL, USA) with co-rotating and closely intermeshing screws was used. The D/L ratio was 24 and the extruder was provided with six-barrel sections, BS (each 128 mm long). The process conditions were set as: a screw speed of 500 rpm; a temperature profile BS1 to BS6 of 5, 80, 100, 100, 120, 140 ± 1 °C; a die temperature of 140 ± 1 °C.

Formulations were fed at a rate of 12.41 kg/h (wb), using a gravimetric loss-in-weight feeder, LWFD5-20 (K-Tron Corp., Pitman, NJ, USA). Water was supplied to the extruder to achieve a final moisture content of 20 g/100 g wb, using a triplex variable stroke piston pump, VE-P33 (Bran and Luebbe, Wheeling, IL, USA). The exit diameter of the two circular dies was 3.0 mm each. The pressure in the die was monitored during the extrusion runs, using a PT412-5M pressure transducer (Dynisco Instruments, Sharon, MA, USA). Extruder parameter data were collected at 1 s intervals by Intouch software (FITSYS PLUS ver. 1.23) when operating conditions (torque and pressure) reached steady state (Morales *et al*., 2015).

2.4 Extrusion parameters

Specific mechanical energy (SME), product temperature (PDT) and die pressure (DP) were obtained directly from the extruder software.

2.5 Physical properties

2.5.1 Sectional expansion index

Sectional expansion index (SEI) of the extrudates was evaluated according to the method described by Patil *et al.* (2007). The diameters of 30 samples were measured with a digital caliper, with measurements taken at five randomly chosen locations on the extrudates, each about 4 cm long. The sectional expansion index was calculated as the ratio of the average sectional area of the expanded extrudates to the area of the extruder die (3.0 mm).

2.5.2 Texture

A TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA) was used to measure the peak force as an indication of hardness (H), using 3-point bend test with a pasta blade. The test speed and the distance between two supports were 2 mm/s and 22 mm, respectively. Texture Exponent 32 software (version 5.0) was employed to record and analyze the curve. Brittleness (B) was assessed as the distance (mm) at which a product breaks and was measured from the force–distance curve (Altan *et al.,* 2008). Thirty measurements were performed on each formulation.

2.5.3 Bulk density, true density and porosity

Bulk density (BD) of each formulation was determined based on a method explained by Hwang & Yakawa (1980). The volumetric displacement method used glass beads with a diameter range of 1.00 to 1.18 mm as a displacement medium. Values were the average of three measurements of five grams each. Real density was obtained with a pycnometer, AccuPyc II 1340 (Micromeritics Instrument Corp., USA) and helium was used as displacement gas. Five values of true density for samples with higher and lower expansion were measured. All the measurements were taken place after leaving the sample for at least 48 h at 25 °C on desiccators. Porosity (X) was calculated according to the equation (1):

$$X = \frac{(\rho t - \rho b)}{\rho b} \tag{1}$$

where: ρt = true density; ρb = bulk density (BD).

2.6 Functional properties

2.6.1 Water absorption index and water solubility index

Water Absorption Index (WAI) and Water Solubility Index (WSI) were analyzed as techno-functional properties. Data is expressed in percentage of change from the original value. Values could be positive if they increased and negative if they decreased.

The methodology described by Anderson et al. (1970) was followed to determine WAI and WSI. One gram of ground sample was added to 10 mL of distilled water in a centrifuge tube and shaken vigorously until completely dispersed on a vortex mixer. Then, it was placed on a rotary shaker at 25°C for 30 minutes. The suspension was centrifugated at 6000 g. WAI was expressed as the weight of the hydrated sample per gram of original sample. Determinations were performed in triplicate.

2.6.2 Pasting properties

The Rapid Visco Analyzer, RVA-4500 (Newport Scientific, Warriewood, Australia) was used to evaluate the pasting properties. 3 g milled sample and 25 mL HPLC grade water were mixed in an RVA-canister prior to analysis. Measurements were performed in triplicate at a constant shear rate of 160 rpm. The temperature program started at 298.15 K and the samples were held for 2 min, then heated to 368.15 K at 14 K/min, held for 3 min, and finally cooled at 17.5 K/min and held at 298.15 K for 1 min. Parameters recorded were cold viscosity (CV), raw peak viscosity (RPV), pasting temperature (PT) and final viscosity (FV) (Crosbie & Ross, 2007).

2.6.3 Antioxidant Activity (AA) and Total Phenolic Content (TPC)

Anthocyanins and soluble phenolic compounds in ground materials were extracted and quantified according to the following protocol: (a) one gram of each sample was mixed with 20 mL of acidic methanol (HCl):water solution (50:50 v/v, pH 2) per triplicate; (b) samples were kept for 1 h at room temperature with constant horizontal shaking in capped centrifuge tubes; (c) samples were centrifuged at 3000 g, 15 min at 4ºC; (d) pellets obtained before were extracted again with 20 mL acetone:water 70:30 v/v; (e) samples were kept for 1 hour at room temperature with constant horizontal shaking; (f) samples were centrifuged at 3000 g, 15 min at 4ºC; and (g) both supernatants of each sample were put together and used for AA by DPPH (Swain & Hillis, 1959) and TPC determination (Brand-Williams *et al.,* 1995).

2.7 Acceptability test

The acceptability test was divided in two steps. Twenty-two people from the western regional research center (Albany, CA) volunteered as untrained judge for the first step. Untrained judges were asked to try separately two codified sets of 6 extruded samples to rate texture acceptability from strongly dislike (2) to strongly like (10) in five levels, where the first and second sets included formulations from 1 to 6, and from 7 to 11 plus formulation 2, respectively. Both sets results were analyzed together by Fisher's least-significant difference (LSD) test and the top five liked formulations were identified. For the second step forty-two volunteers were requested to rank the top five formulations from strongly dislike (1) to strongly like (5). Fisher's least-significant difference (LSD) test was employed for analyzing data (Meilgaard *et al.,* 1999). Three-digit random numbers were used to code the samples and were served in the order of the formulation number. Water and unsalted crackers were provided to judges to cleanse their palates between samples and expectoration cups if they did not wish to swallow the samples.

2.8 Protein digestibility

In vitro protein digestibility (IVPD) was evaluated according to the method of Hsu & Vavak (1977), in samples selected by untrained judges in the acceptability test plus 100 g/100 g wb BC:RL-mixture (as a control), using a multienzyme system (trypsin, chymotrypsin and peptidase). The pH drop after 10 min incubation period was recorded and the percent protein digestibility (Y) was calculated from the equation (2):

$$Y = 210.469 - 18.10X \tag{2}$$

where (X) is the pH change after 10 minutes.

2.9 Experimental design and statistical

For the correlation between extrusion parameters, physical and functional properties, principal components analysis (PCA) was employed (Statgraphics Centurion XV).

Extrusion formulations were established and analyzed with an extreme vertices mixture design

Table 1. Three component extreme vertices mixture design.

Formulation	BC:RL-mixture[1,3]	HylonV®[3]	FiberSol®-2[3]
1	100	0	0
2	90	0	10
3	60	40	0
4	50	40	10
5	95	0	5
6	80	20	0
7	55	40	5
8	70	20	10
9	75	20	5
10	82.5	10	7.5
11	62.5	30	7.5
12[2]	55	40	5
13[2]	62.5	30	7.5

[1] BC:RL-blend: Blue Corn:Red Chief Lentil (70:30). [2] Repeats. [3] g/100 g wb.

(Table 1) with the following variables and levels: BC:RL-mixture (100-50 g/100 g wb), HyV (0-40 g/100 g wb) and FS (0-10 g/100 g wb) (Altan *et al.,* 2008; Caltinoglu *et al.,* 2014). For design and analysis of the extreme vertices mixture, the STATGRAPHICS Centurion XV package was used. Statistically significant differences between values were determined with a p-value < 0.05. A simultaneous optimization was performed to find the best combination of components using the desirability function method, considering the response variables whose models meet the traditional assumptions and that the coefficient of determination (R^2_{adj}) of each model was at least 70%. (Gutiérrez & de la Vara, 2012). The results were expressed as mean values ± standard errors of three separate determinations.

3 Results and discussion

The experimental design formulations for the extrusion process allowed us to obtain extrudates based on blue corn, red chief lentil, HyV starch and FS. Various physical properties were studied on the extruded products; Table 2 summarized them together with extrusion parameters and their standard deviations.

Data for pasting properties due to the extrusion process, antioxidant activity and total phenolic content are shown in Table 3.

3.1 *ANOVA for mixture design*

Analysis of variance for extrusion parameters, physical and functional properties showed that all but SEI (p=0.07), B (p=0.069), WAI (p=0.28), CV (p=0.137), RPV (p=0.141) and FV (p=0.105), were significantly affected (p<0.05) by BC:RL-mixture, HyV and FS content. Models fitting data were lineal for H (p=0.0224), DP (p=0.0071), AA (p=0.0000) and TPC (p=0.0000); quadratic for BD (p=0.0292) and X (p=0.0376); and cubic for PDT (p=0.0390), SME (p=0.0072), WSI (p=0.0468) and PT (p=0.0036).

Table 2. Measured physical properties of the extruded products from de mixture design and extrusion parameters.

Formulation	SEI	BD (g/cm^3)	H (N)	B (mm)	X	PDT (ºC)	SME (kJ/kg)	DP (kPa)
1	3.57±0.01	0.50±0.00	5.89±1.03	0.14±0.02	0.66±0.00	171.84±8.45	566.50±17.20	7427±187
2	3.68±0.01	0.44±0.00	5.29±1.05	0.16±0.02	0.69±0.00	174.76±8.79	504.65±13.98	6170±107
3	5.89±0.02	0.33±0.00	6.09±1.01	0.20±0.02	0.78±0.00	182.81±8.31	856.06±18.20	7391±283
4	2.88±0.02	0.56±0.04	3.36±0.73	0.11±0.02	0.62±0.04	184.36±8.42	584.68±17.57	7173±172
5	3.62±0.03	0.50±0.01	4.28±0.81	0.14±0.02	0.66±0.01	180.48±10.83	531.93±19.72	7059±236
6	3.27±0.01	0.52±0.01	5.52±0.99	0.14±0.02	0.65±0.01	180.21±4.00	642.16±35.44	8101±339
7	3.28±0.01	0.52±0.02	3.29±0.62	0.11±0.02	0.65±0.02	173.25±8.96	637.52±21.72	8429±157
8	3.58±0.02	0.53±0.00	3.78±0.55	0.11±0.02	0.64±0.00	183.77±3.54	548.56±21.98	6626±100
9	3.48±0.01	0.59±0.02	4.67±0.98	0.14±0.02	0.60±0.02	177.34±6.87	575.65±8.36	7017±154
10	3.74±0.01	0.48±0.01	4.03±0.77	0.13±0.02	0.67±0.01	174.18±8.24	469.82±11.55	6718±118
11	3.26±0.01	0.52±0.03	4.51±1.12	0.13±0.02	0.65±0.02	180.06±9.12	655.05±23.12	7459±195
12	3.23±0.01	0.45±0.05	3.87±0.72	0.14±0.02	0.70±0.05	172.62±6.94	665.32±12.47	8271±119
13	3.12±0.02	0.63±0.02	4.79±0.96	0.14±0.02	0.57±0.02	177.71±7.33	621.53±15.7	6844±159

SEI: Sectional expansion index; BD: Bulk density; H: Hardness; B: Brittleness; X: Porosity; PDT: Product temperature; SME: Specific mechanical energy; DP: Die pressure.

Table 3. Results of functional properties.

Formulation	WSI[1,4] [%]	WAI[1,5] [g gel/g sample]	CV[1,6] [cP]	PT[1,7] [ºC]	RPV[1,8] [cP]	FV[1,9] [cP]	Antioxidant activity[2] Extrudates	Antioxidant activity[2] Raw formulation	Total phenolic content[3] Extrudates	Total phenolic content[3] Raw formulation
1	15.94±0.13	3.38±0.04	139.00±4.58	60.30±0.00	1211.00±32.14	2744.33±24.75	4.33±0.14	4.43±0.28	1.29±0.08	1.28±0.09
2	20.55±0.05	3.30±0.06	75.50±3.44	61.75±0.14	849.00±9.90	2268.00±22.63	4.20±0.32	4.00±0.26	1.29±0.07	1.15±0.09
3	15.14±0.03	3.68±0.00	190.50±2.12	60.20±0.07	544.50±2.12	1082.50±2.12	3.11±0.03	3.27±0.21	0.85±0.06	0.83±0.06
4	18.88±0.01	3.17±0.03	33.00±1.73	62.18±1.48	360.33±2.52	717.00±7.21	3.07±0.30	2.84±0.18	0.81±0.09	0.70±0.06
5	17.70±0.04	3.15±0.01	76.33±5.51	60.30±0.09	1035.00±8.19	2493.67±9.50	4.37±0.48	4.21±0.27	1.26±0.12	1.22±0.09
6	13.91±0.02	3.65±0.06	91.50±3.54	60.40±0.07	855.00±11.31	2039.00±11.31	3.96±0.21	3.85±0.24	1.07±0.06	1.05±0.08
7	16.07±0.14	3.36±0.00	44.50±0.71	60.45±0.07	626.00±1.41	1451.50±9.19	3.35±0.06	3.05±0.20	0.95±0.07	0.76±0.06
8	20.26±0.11	3.12±0.15	54.33±1.15	61.05±0.77	616.33±2.08	1472.00±26.85	3.96±0.15	3.42±0.22	1.10±0.07	0.92±0.07
9	16.97±0.06	3.61±0.01	88.50±2.12	60.30±0.14	701.00±1.41	1613.00±4.24	3.74±0.12	3.63±0.23	1.07±0.02	0.99±0.07
10	19.61±0.03	3.27±0.01	87.33±0.58	60.28±0.06	791.67±40.20	1820.33±13.87	4.04±0.15	3.82±0.24	1.15±0.09	1.07±0.08
11	18.16±0.04	3.42±0.01	54.50±0.71	60.28±0.11	481.00±2.83	1005.00±0.00	3.13±0.26	3.24±0.21	0.91±0.07	0.84±0.06
12	15.06±0.03	3.45±0.00	40.50±0.71	60.30±0.07	485.50±0.71	966.00±1.41	3.31±0.24	3.05±0.20	0.87±0.06	0.76±0.06
13	18.13±0.04	3.44±0.04	54.33±1.53	60.60±0.35	540.00±5.00	1239.33±25.58	3.66±0.27	3.24±0.21	0.99±0.08	0.84±0.06

[1]Mean ±SD; [2]mg TROLOX/g sample wb; [3]mg Gallic Acid/g sample wb;[4]Water Solubility Index (WSI);[5]Water Absorption Index (WAI); [6]ColdViscosity (CV); [7]Pasting Temperature (PT);[8]Raw Peak Viscosity (RPV); [9]Final Viscosity (FV)

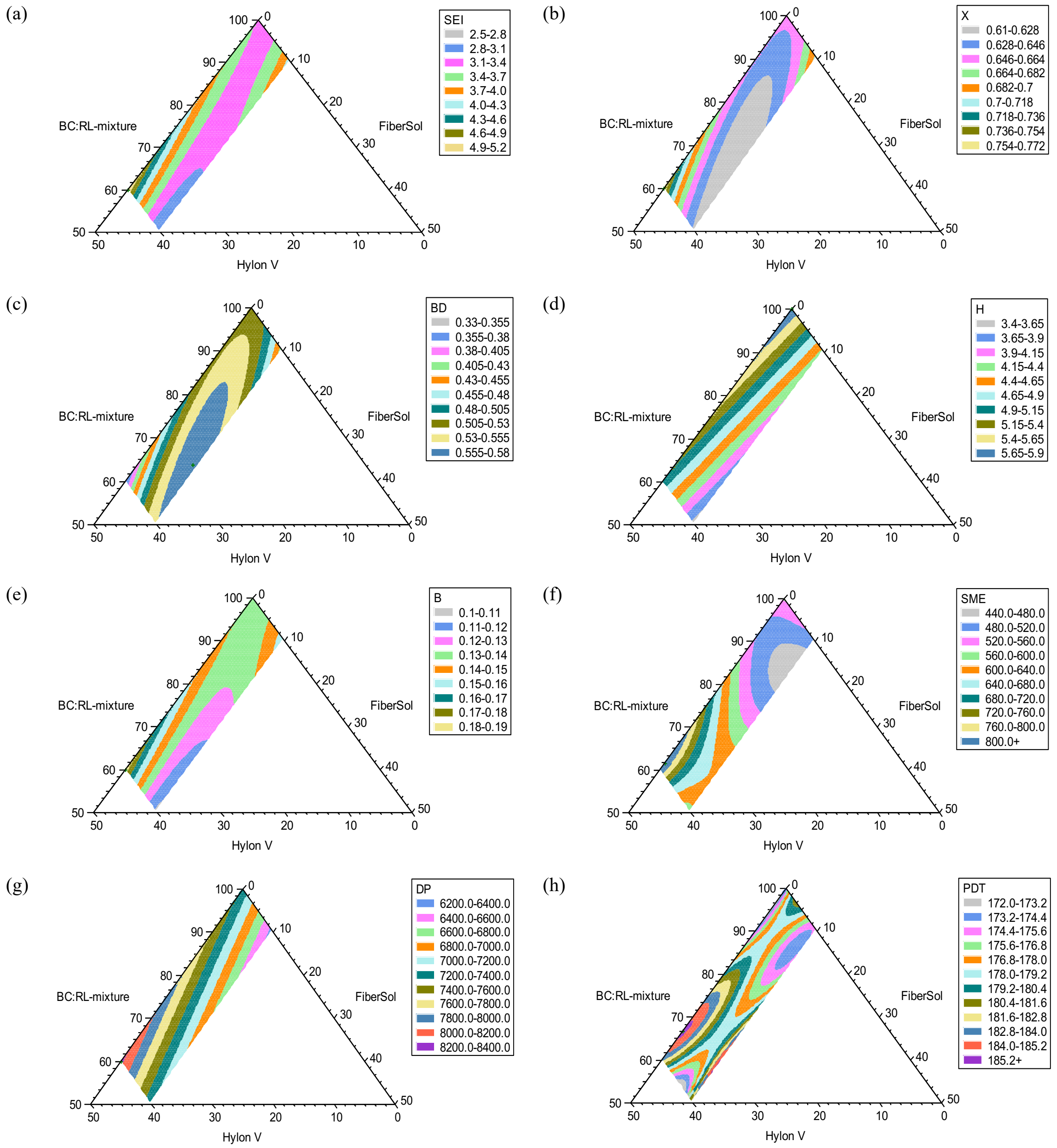

Figure 1. Mixture contour plots for: (a) Sectional expansion index [SEI]; (b) Porosity [X]; (c) Bulk density [BD]; (d) Hardness [H]; (e) Brittleness [B]; (f) Specific mechanical energy [SME]; (g) Die pressure [DP]; and (h) Product temperature [PDT]. BC:RL-mixture, HylonV® and FiberSol®-2 expressed in g/100 g wb.

3.2 Physical properties

The SEI, BD, and X values of expanded extrudates prepared from different formulations containing varying BC:RL-mixture, FS and HyV contents are shown in Table 2. The SEI of extrudates was in the range of 3.12 and 5.89, similarly to another study (Meng *et al.,* 2010), indicating that BC:RL-based extruded products can be obtained with a desirable expansion. Greater expansions were observed in extrudates made from mixtures without FS, being the 40 g/100 g wb HyV mixture the one with the greatest SEI (Figure 1a). This could be due to the fact that by increasing the starch content (HyV) and reducing the protein content (BC:RL-mixture) and without the addition of fiber (FS), the starch will have a greater amount of available water, increasing its gelation capacity and therefore its expansion (García-Cordero *et al.,* 2024). The X values ranged from 0.57 to 0.78, alike to Yağci & Göğüş (2008). A similar behavior to SEI´s was depicted for X, having the maximum value at 40 g/100 g wb HyV and the greatest values when FS content was zero (Figure 1b). BD maximum value (0.63 g/cm^3) was obtained when extrudates were made from mixtures with the highest FS content (SEI, 3.12; X, 0.57). What is in accordance with different studies (Altan *et al.,* 2008; Yağci & Göğüş, 2008; Vanier *et al.,* 2016), they found expansion positively correlated to porosity and both negatively related to BD. Figure 1c shows that as higher the FS content was, higher the BD, this behavior was countered by increasing HyV concentration. Maskan & Altan (2012) explained that the presence of fiber particles tended to break the cell walls before the gas bubbles had expanded to their maximum potential. Thus, the increase in bulk density may be due to the increase in fiber content of the feed material.

3.2.1 Textural properties

The H measurements that were taken for the expanded extrudates ranged from 3.29 to 6.09 N are shown in Table 2 and represented graphically in Figure 1d. Where can be seen that increasing HyV content while keeping FS constant produced a decrease in H values due to higher expansion. Additionally, H was inversely related to FS content for both cases, keeping either BC:RL-mixture content constant or HyV content constant, thus, lower H values were got at maximum FS content (10 g/100 g wb). What would be also related to the matrix disruption (Altan *et al.,* 2008).

As it was reported, physical properties are related to the amount and the type of starch added to the formulation (Vanier *et al.,* 2016). FS particles compete with starch molecules for water, what would prevent mixture matrix to melt, thus producing softer extrudates (Maskan & Altan, 2012). B represented the distance to break the extrudates, with the shortest distance being the most brittle product (Altan *et al*., 2008). Measurements for extrudates ranged from 0.11 to 0.20 mm. It is noticeable that, B values got reduced when FS increased, either keeping constant HyV or BC:RL-mixture content (Figure 1e). B values are in line with H behavior in this work, hence, FS content interfering effect on the matrix from fiber molecules would produce less expanded and more brittle extrudates.

3.2.2 Extrusion parameters

Table 2 depicts behavior for DP, SME, and PDT. Maximum value for SEM was obtained when FS and HyV contents were 0 and 40 g/100 g wb, respectively (Figure 1f), what matches with maximum values found for SEI and X. Higher starch concentrations would increase matrix viscosity while extruding, increasing this way SME values due to stronger gels. Meng *et al.* (2010) explained that higher SME values produced larger expansions due to the starch disruption generated through shear stress, inducing gelatinization and consequently expansion. Therefore, higher viscous dissipation was got, increasing PDT over DP value (Table 2). The highest DP values were obtained when HyV content was maximum (Figure 1g), thus, its behavior would be due to the stronger gel formed and to the water overheating inside the bubbles in the matrix before exiting. High HyV and low FS contents would increase PDT (Figure 1h) due to viscous dissipation.

3.3 Functional properties

3.3.1 AA and TPC

Data are presented in Table 3. Values for extrudates ranged from 3.07 to 4.37 mg TROLOX/g sample wb and from 0.81 to 1.29 mg Gallic Acid/g sample wb, AA and TPC, respectively. These values are like the ones reported by Mora-Rochin *et al.* (2010) for blue corn extrudates. There was not statistically significant difference (NSSD) between extruded and raw formulations for each sample, neither for AA nor for TPC. This might indicate either that the conditions were soft enough to maintain AA or a protective effect from the matrix occurred, preventing phenolic compounds from reacting and losing their structure and/or antioxidant capacity. Thus, phenolic compounds retention with AA after extrusion could be an important finding because contrary to this study, Camacho-Hernandez *et al.* (2014) and Brennan *et al.* (2011) reported anthocyanin content reduction after extrusion. Also, Castro-Montoya *et al.* (2024) reported that a high moisture content or some compound present in the food matrix could cause a lubricating effect during the extrusion process, causing less thermomechanical damage to the phenolic compounds, avoiding their losses during the extrusion process.

The optimization of the formulation using the desirability function (Gutiérrez & de la Vara, 2012) in the indicated region, maximizing PDT (R^2_{adj}=87.75%), SME (R^2_{adj}=96.13%), AA (R^2_{adj}=86.75%) and TPC (R^2_{adj}=94.23%), and minimizing WSI (R^2_{adj}=86.08%) and PT (R^2_{adj}=97.56%), showed that the optimal values for the studied components were 69.93 g/100 g wb BC:RL-mixture, 20.08 g/100 g wb HyV and 10.0 g/100 g wb FS. The overall desirability was 0.5953. The predicted response values with the desirability function were 183.134°C (PDT), 553.253 kJ/kg (SME), 14.158% (WSI), 60.449°C (PT), 3.7353 mg TROLOX/g sample wb (AA) and 1.0645 mg GA/g sample wb (TPC).

3.4 Correlation amongst formulations, extrusion parameters, physical and functional properties

Twenty-three pairs of variables were found to have a strong correlation ($p<0.05$) in Spearman's rank correlation analysis. Therefore, a principal component analysis (PCA) was performed to better understand the phenomena occurring during extrusion cooking process and the relationship amongst formulation, physical and functional properties. Five components were necessary to explain 89.054% of the variability in the original data. First component explains 33.959% of the variability of the data, the second 25.029%, the third 13.165%, the fourth 10.134% and the fifth 6.767%. Figure 2 depicts the correlation amongst independent variables, extrusion parameters, physical and functional properties of products. It is noteworthy that the first component can be observed by looking at the horizontal axis where the behavior of the variables is mainly explained by the HyV/BC:RL-mixture ratio. This was more related to starch content effect per se. A higher starch concentration would increase matrix viscosity, inducing higher SME values and therefore higher PDT due to viscous dissipation. DP resulted to be strongly correlated to HyV, this correlation might be due to a strongest gel structure formed at higher starch contents, being capable to retain vapor before explosion (Korkerd *et al.,* 2016; Yu *et al.*, 2012). Thus, at constant moisture and extrusion conditions, response variables were mainly controlled by the starch concentration in the matrix. WAI is related to the amount of water absorbed by starch granules after swelling in excess water, being an index of degree of gelatinization (Rodriguez-Miranda *et al.,* 2012). BC:RL-mixture was directly related to WAI, a higher starch concentration might increase matrix gel strength and viscosity. The use of starch as a stabilizer and thickener has been widely studied (Moscicki, 2011; Manrique-Quevedo *et al.,* 2007; Saha & Bhattacharya, 2010). WAI was positively correlated to RPV. Blanche & Sun (2004) explained that RPV is a measure of the degree of starch transformation (cooking) and Braşoveanu & Nemţanu (2020) stated that RPV indicate the capacity and ability of water absorption of granules. Thus, higher RPV and WAI values indicate cooked extrudates. PT provides an indication of the minimum temperature required to cook the starch (Wang *et al.,* 2014) and was positively correlated to HyV content. After heating starch above the gelatinization temperature, the process of viscosity development in starch pasting occurs (Braşoveanu & Nemţanu, 2020). Furthermore, less cooked extrudates agreed with higher BC:RL-mixture content, AA and TPC, showing a positive strong correlation amongst them, indicating that AA and TPC on extrudates were directly related to initial formulation. Hu & Xu (2011) and Sarawong *et al.* (2014) also reported correlation coefficients between AA (determined by DPPH assay) and TPC higher than 0.90 for soluble compounds.

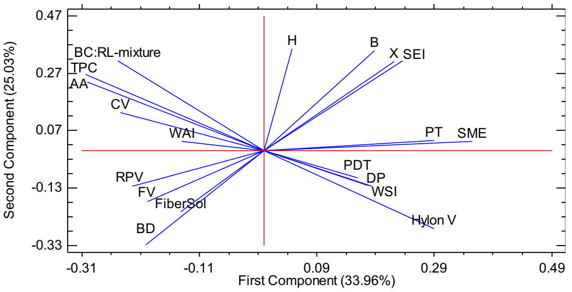

Figure 2. Principal component analysis: loading plot of first two principal components describing the variation and correlation amongst the different ingredients, extrudates properties and system parameters. Two close lines indicate two properties that are highly correlated.

The second component explains the variables behavior which could be observed along the vertical axis and are mainly explained by the FS effect (Figure 2). FS content was positively related to BD and negatively related to H, B, X and SEI. This could be due to a dilution effect, because an increase in FS content in raw mixtures will produce less starch availability and would also interfere in gelatinized matrix formation during extrusion process. Thus, inducing production of denser, less expanded, softer, and more brittle extrudates. Yağci & Göğüş (2008) explained that after certain concentration fiber molecules disrupt the continuous structure of the melt in extruder, preventing elastic deformation during extrusion. The appearance of FV variability in the middle way of both components indicates the positive effect of BC:RL-mixture and FS on it. Lower fiber contents would let the matrix to strengthen, increasing viscosity, shearing stress, starch granules disruption, expansion (SEI) and finally break down starch molecules (CV) and starch dextrinization (WSI) if shearing is high enough. This could explain the negative correlation of FS with SEI, WSI and CV. Similarly to Wani & Kumar (2016), Stojceska *et al.* (2009) and Yağci & Göğüş (2008) findings. BC:RL-mixture appears in the middle way of both components, indicating that a higher BC:RL-mixture concentration would increase H due to an increase of the fiber and protein content in the mixture. Some authors found that denser and less expanded extrudates can be obtained increasing protein and fiber content in raw mixtures (Korkerd *et al.*, 2016; Yu *et al.*, 2012).

3.5 Acceptability test

The acceptability test was carried out to select the samples that would be evaluated in the protein digestibility test, using the formulations with the greatest acceptance by untrained judges. The first panel consisted mainly of males, being mostly Asian and Caucasian between 30 and 59 years old. Besides, the second panel was formed by the same number of men as women, mostly Asian and Caucasian between 18 and 39 years old. The mean scores of both texture evaluations are shown in Figure 3, it is noticeable that in general, untrained judges preferred more expanded extrudates, as can be seen in Table 2 (SEI). Analysis of the surface representing the effect of formulation on texture acceptability behavior showed that untrained judges preferred extrudates with lower content of HyV, higher content of FS and BC:RL-mixture. It has been described that in terms of overall acceptability, formulations with moderate proportions (15 to 35%) of pulses have been better rated by the panelists (García-Cordero *et al.,* 2024), which coincides with this work, since the RL content in formulation 2 was 27%, in 3 it was 18% and in 10 it was 24.75%.

3.6 Protein digestibility

Table 4 shows that IVPD of extrudates increased significantly by extrusion process. The IVPD results agree with those of Rathod & Annapure (2016) on pulses, where the IVPD greatly varied from raw samples. A more significant improvement of IVPD by extrusion cooking was produced in extrudates with less amount of HyV, what might indicate a protective effect from starch over the protein structure. Rathod & Annapure (2016) found that the increase in digestibility was greater in starch than in protein produced by the extrusion processing.

As Stauffer (2004) states, successful food formulation culminates in the introduction of a new product that is tasty, nutritious, economical, convenient, and safe and that consumers react positively to by increasing their purchases of the item. Thus, this study allows us to direct efforts to the development of a functional food, which has the advantage of being ready to eat. The study of the effect of changes in composition by monitoring simple parameters to measure, allows explaining how it affects the structure of the product and could finish in a future in a new healthy product.

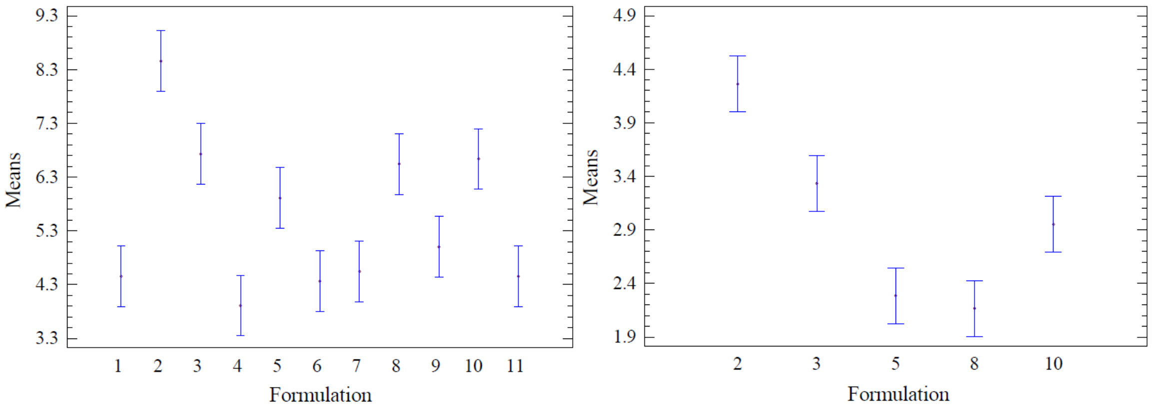

Figure 3. Formulation means' graphs; 95% Fisher's LSD.

Table 4. Protein digestibility.

Formulation	BC:RL- mixture^{2,3}	HylonV®^{3}	FS^{3,5}	Protein digestibility^{4}	
				Extrudates^{1}	Raw formulations^{1}
1	100	0	0	83.56±0.18	78.13±0.00
2	90	0	10	84.23±0.69	78.66±0.51
3	60	40	0	82.93±0.13	78.76±0.13
10	82.5	10	7.5	83.56±0.18	78.31±0.26

^{1}Mean±SD; ^{2}BC:RL-mixture: BlueCorn:RedChiefLentil (70:30); ^{3}g/100 g wb; ^{4}%; ^{5}FS: FiberSol®-2.

Conclusions

It is concluded that directly expanded extrudates can be obtained from a mixture of blue corn and red chief lentil, without any additives or extra ingredient. However, addition of HylonV® increased extrudates expansion indirectly. FiberSol®-2 had a great effect on extrudates physical properties, decreasing sectional expansion index, porosity and hardness. The optimal formulation for extrusion cooking of extrudates with adequate physical properties was 69.93 g/100 g wb BC:RL-mixture, 20.08 g/100 g wb HylonV® and 10.0 g/100 g wb FiberSol®-2.

During extrusion-cooking, new structures of products are formed due to starch, protein and fiber damage. WAI and pasting properties of extruded products were influenced by the ingredient's concentration. It was found a strong correlation amongst antioxidant activity and total phenolic content for soluble compounds, moreover, not statistically significant difference was found amongst extruded and raw formulations. The analysis of acceptability showed that untrained judges preferred more expanded extrudates that contained FiberSol®-2 and lower HylonV® content. Amongst the samples selected by untrained judges, the lowest protein digestibility was obtained in the formulation with the highest HylonV® content.

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