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Original Article

Hypoglycemic activity of food pasta based on amaranth/blue maize obtained by extrusion-cooking process

Actividad hipoglucemiante de pasta alimenticia elaborada a base de amaranto/maíz azul obtenida mediante el proceso de extrusión-cocción

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ABSTRACT
Extrusion has been used to develop functional foods. This study aimed to evaluate the hypoglycemic activity (HGA), antioxidant capacity (AoxC), and nutritional properties of food pasta based on amaranth/blue maize, obtained through the extrusion-cooking process. To obtain food pasta, the conditions were Extrusion Temperature = 82 °C / Humidity Content = 33 % and 10 % Semolina was added. A simulated gastrointestinal digestion was performed, and the HGA, AoxC, and nutritional properties were evaluated. Amaranth/blue maize flour (ABMF) showed 10.04 and 45.72 % inhibition of α-amylase (AphA) and α-glucosidase (AphG), respectively, while extruded pasta with semolina (EPS) showed a 41.31 and 42.94 % inhibition of AphA and AphG, respectively. EPS after hydrolysis showed good inhibition value for AphA and AphG in both fractions, *in* and *out*. The *out* fraction showed the highest percentage of inhibition for AphG and AphA (40.44 and 22.51 %, respectively). The ABMF and EPS had 18,844 and 12,652 μmol TE/100 g sample (dw) of AoxC, respectively. Most of the amino acids achieve the FAO recommendations. Protein digestibility *in vitro* showed an increment in EPS (76.61 %) with respect to ABMF (71.09 %). Additionally, C-PER had a positive effect after the extrusion-cooking process (3.05 vs. 2.94 for EPS and ABMF, respectively). Extrusion is a good technique for developing functional foods with potential biological activity.
Keywords: Functional foods, functional properties, biological activity.

RESUMEN
La extrusión ha sido utilizada para desarrollar alimentos funcionales. El objetivo de este trabajo fue evaluar actividad hipoglucemiante (HGA), capacidad antioxidante (AoxC) y propiedades nutricionales de pasta alimenticia a base de amaranto-maíz azul obtenida por extrusión-cocción. Para obtener la pasta se utilizaron condiciones de extrusión: Temperatura de Extrusión = 82 °C / Contenido de Humedad = 33 % y se añadió 10 % de Semolina. Se evaluaron HGA, AoxC y propiedades nutricionales después de la digestión gastrointestinal simulada. La harina de amaranto-maíz azul (ABMF) mostró 10.04 y 45.72 % de inhibición de α-amilasa (AphA) y α-glucosidasa (AphG), respectivamente. Mientras que la pasta extrudida añadida con semolina (EPS) mostró 41.3 y 42.94 % de inhibición de AphA y AphG, respectivamente. La EPS mostró buenos valores de inhibición de AphA y de AphG en ambas fracciones, *in* y *out*. La fracción Out presentó el porcentaje más alto para AphG y AphA (40.44 y 22.51 %, respectivamente). La ABMF y la EPS tuvieron 18,844 y 12,652 μmol ET/100 g de muestra (bs) de AoxC, respectivamente. La mayoría de los aminoácidos alcanzaron las recomendaciones de la FAO. La *DPIV* incrementó en EPS (76,61 % vs 71,09 % en ABMF). La extrusión incrementó C-PER (3.05 vs 2.94 EPS y ABMF, respectivamente). La extrusión es una buena técnica para desarrollar alimentos funcionales con potencial biológico.
Palabras clave: Alimentos funcionales, propiedades funcionales, actividad biológica.

INTRODUCTION
Non-communicable diseases (NCDs) such as type 2 diabetes, which is characterized by a metabolic disorder of high blood glucose levels (Chávez *et al*., 2018), are the leading cause of death around the world. Diet, which is modifiable through lifestyle changes, is a risk factor for developing NCDs (Phillips *et al*., 2019). Therefore, consumers are interested in the impact of food on health. Additionally, food scientists are interested in developing foods with potential health benefits that can reduce the risk of developing NCDs, acting as a nearly nutritional treatment or preventive measure (Orlien and Bolumar, 2019). These kinds of foods are produced using food-processing methods that aim to create high-nutritional-value foods with a desired texture and taste (Orlien and Bolumar,

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2019), utilizing grains like blue maize and amaranth. Blue maize is a pigmented cereal well known for having bioactive compounds with beneficial health effects (Bello-Pérez *et al*., 2015), and amaranth is a pseudocereal, with good nutritional quality due to its amino acid profile (rich in essential amino acids), making amaranth a source to improve protein quality (Montoya-Rodríguez *et al*., 2015; Orona-Tamayo and Paredes-López, 2017; Atukuri *et al*., 2019). Also, amaranth can be used to create new products using various food processing techniques (Coelho *et al*., 2018). Both grains, despite not being conventional ingredients, can be used to produce food pasta with potential health benefits (Camelo-Méndez *et al*., 2018), utilizing extrusion-cooking process (high temperature-short time) that is employed to obtain food with high nutritional value (Ruiz-Gutiérrez *et al*., 2017).

Pasta is highly consumed around the world, being a low-cost product with easy preparation, sensory attributes, nutritional quality, and a longer shelf life than other bakery products (Nilusha *et al*., 2019). Pasta can be enhanced with the addition of non-traditional ingredients, which can bring additional benefits to pasta by increasing bioactive compounds and providing health benefits, such as preventing non-communicable diseases (Camelo-Méndez *et al*., 2018). This study aimed to evaluate the hypoglycemic activity, antioxidant capacity, and nutritional properties of food pasta based on amaranth/blue maize, obtained through the extrusion-cooking process.

MATERIAL AND METHODS

Proximate Composition

The methods reported by AOAC (1999) were used to evaluate proximate composition. All determinations were made in triplicate.

RSM Experimental Design and Statistical Analysis for Extrusion Process

Response surface methodology (RSM) was used to get the best conditions of process variables to produce extruded amaranth/blue maize pasta with high antioxidant capacity (AoxC) and good technofunctional properties such as solid loss by cooking (SLC). Data from preliminary trials (not shown) were considered to select the number and range of process variables in the experimental design. The process variables used in this study were extrusion temperature (X1 = ET, 70°C – 140 °C) and humidity content (X2 = HC, 20 - 40%). On the other hand, the chosen dependent response variables were AoxC and SLC. Table 1 shows a Central Composite Rotatable Design (CCRD) that includes 13 experiments formed by 5 central points and 8 ($\lambda = 1.414$) axial points as well as the results for response variables. Individual experiments were carried out in random order and the quadratic polynomial regression model was the one used for predicting (*Y*) response variables. Models of the following form were developed to describe the two response (*Y*) surfaces:

$$Y = \beta 0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad \textbf{(1)}$$

Table 1. Combination of extrusion process variables used to produce food pasta based on amaranth/blue maize and experimental results for response variables (AoxC, SLC).
Tabla 1. Combinación de variables del proceso de extrusión utilizadas para producir una pasta alimenticia a base de amaranto/maíz azul y los resultados experimentales de las variables de respuesta (AoxC y PSC).

Treatment[a]	Process Variables[b]		Response Varibales[c]	
	ET	HC	AoxC	SLC
1	80.25	22.93	12753.05	30.37
2	80.25	37.07	13942.25	28.27
3	129.75	22.93	9898.02	30.62
4	129.75	37.07	11354.31	32.87
5	105.00	20.00	9639.73	32.43
6	105.00	40.00	10297.49	30.00
7	70.00	30.00	14643.51	28.54
8	140.00	30.00	12558.45	32.39
9	105.00	30.00	7494.46	27.33
10	105.00	30.00	7446.63	28.62
11	105.00	30.00	7317.23	28.49
12	105.00	30.00	7050.40	27.66
13	105.00	30.00	10096.27	25.08

[a]Does not correspond to order of processing; [b]ET= Extrusion temperature (°C); HC = Humidity content (%); [c]AoxC = Antioxidant Capacity (µmol TE/100 g sample, dw); SLC = Solid loss by cooking (%).

where *Y* is the value of the considered experimental predicted response variable (AoxC, or SLC), X_1 and X_2 are the values of ET and HC, respectively, $\beta 0$ is the constant value, β_1 and β_2 are linear coefficients, β_{12} is the interaction coefficient, β_{11} and β_{22} are quadratic coefficients. Applying the stepwise regression procedure, non-significant terms ($p \leq 0.05$) were deleted from the second order polynomial, and a new polynomial was recalculated to obtain a predictive model for each variable (Myers, 1971). All statistical analyses were performed using Stat-Ease Design Expert Software Ver. 7.0.0. (2005).

Optimization of Extrusion Process

Usually, response variables describing the quality characteristics and performance measures of the systems are to be optimized (maximized and minimized). Sometimes, when one response is maximized, the other one is minimized, which further complicates the situation (Jahani *et al*., 2008). To solve this problem, methods like the conventional graphical method (GPM) and desirability function are used. GPM superimposes the contour diagrams (figure 1) of the different response variables, while desirability combines all the responses into one measurement. Figures 1B and 1D present the effect of extrusion temperature (ET) and humidity content (HC) on antioxidant capacity (AoxC) and solid loss by cooking (SLC) of extruded food pasta, respectively. Superposition of these contour plots was carried out to obtain a new contour plot (figure 2) which was utilized for observation and selection of the best combination of extrusion-cooking process variables for producing optimized food pasta based

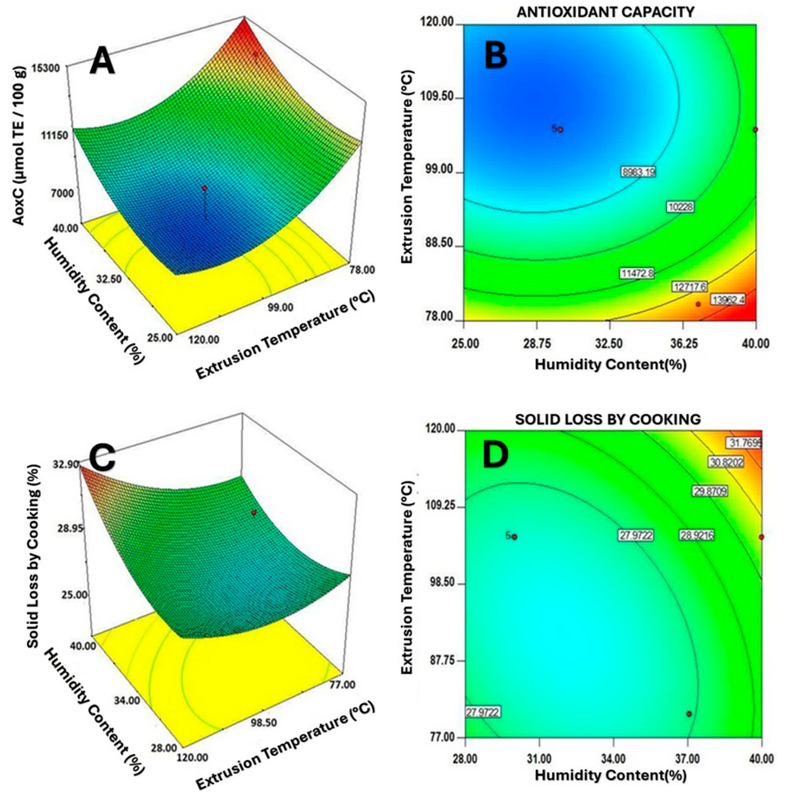

Figure 1. Response surface and contour plots for the effect of extrusion temperature (°C) and humidity content (%) on antioxidant capacity (A, B) and solid loss by cooking (C, D) of food pasta based on amaranth and blue maize.

Figura 1. Superficie de respuesta y gráficas de contorno para el efecto de la temperatura de extrusión (°C) y contenido de humedad (%) sobre la capacidad antioxidante (A, B) y pérdida de sólidos por cocción (C, D) de pasta alimenticia a base de amaranto y maíz azul.

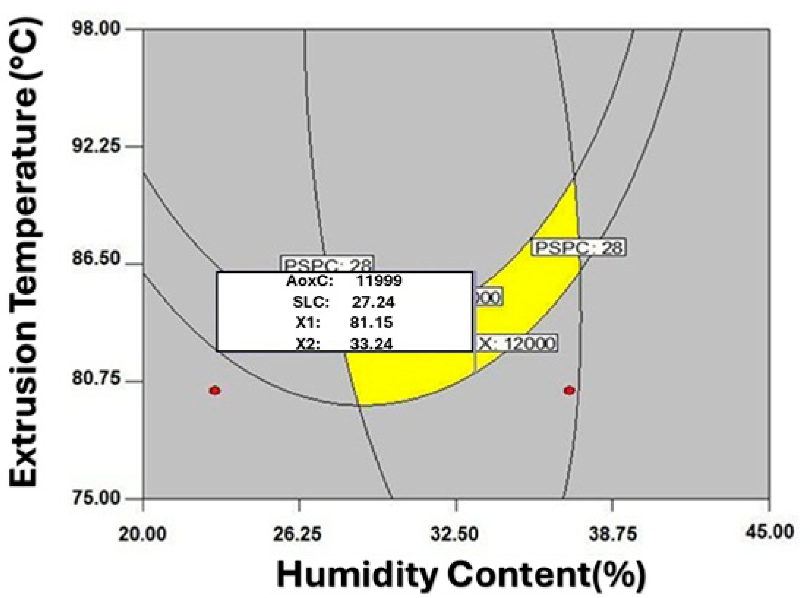

Figure 2. Region of the best combination of process variables (extrusion temperature / humidity content) for producing food pasta based on amaranth and blue maize with high antioxidant capacity and low solid loss by cooking.

Figura 2. Región de la mejor combinación de las variables de proceso (temperatura de extrusión / contenido de humedad) para producir pasta alimenticia a base de amaranto y maíz azul con alta capacidad antioxidante y baja perdida de sólidos por cocción.

on amaranth and blue maize with high AoxC and low SLC. Central point of the optimization region in figure 2 corresponds to a combination process variable of ET = 82 °C, SLC = 33 %. The predictive values as well as the validation of the optimization model area shown in Table 2.

De la Vara Salazar and Domínguez-Domínguez (2011) described the desirability method, and it was applied to find the best combination of extrusion process variables [extrusion temperature (ET)/ humidity content (HC)] that will result in an extruded amaranth/blue maize pasta with optimum values for the two dependent variables (AoxC, SLC). Both fitted models can be evaluated at any point $X = (X_1, X_2)$ of the experimental zone and as a result two values were predicted for each model, namely $\hat{Y}_1(X)$ and $\hat{Y}_2(X)$. Then each $\hat{Y}_i(X)$ is transformed into a value $d_i(X)$, which falls in the range (0, 1) and measures the desirability degree of the response in reference to the optimum value intended to be reached. In this study, AoxC response variables need to be as high as possible, while SLC needs to be low as possible. The transformation is:

$$d_i(x) = \begin{cases} 0 & if \hat{Y}(x) \leq Y_{i_*} \\ \dfrac{\hat{Y}_i(x) - Y_{i_*}}{Y^*_i - Y_{i_*}} & if Y_{i_*} \leq \hat{Y}(x) \leq Y^*_i \\ 1 & if \hat{Y}(x) \geq Y^*_i \end{cases} \quad (2)$$

where: $di(X)$ = Value of the desirability of the ith response variable, $\hat{Y}_i(X)$ = Estimated response variable, Y^*_i = Maximum acceptable value of the *i*th response variable, Y_{i*} = Minimum acceptable value of the *i*th response variable. Once the two individual desirabilities were calculated, the global desirability was obtained by the two response variables, using the mathematical function of transformation $D = (d_1 d_2)1/2$, where the ideal optimum value is $D = 1$; an acceptable value for D can be between 0.6 and 0.8 ($0.6 < D < 0.8$). This acceptable value was found by using Stat-Ease Design Expert Software Ver. 7.0.0. (2005). The AoxC and SLC predictive models (Table 3) were used to obtain individual desirabilities, which were employed to calculate a global desirability (*D*) (figure 3) for observation and selection of superior (optimum) combination of ET and HC for producing optimized extruded amaranth/blue maize pasta with high antioxidant capacity (AoxC) and good technofunctional properties such as solid loss by cooking (SLC).

Table 2. Results of the model validation.
Tabla 2. Resultados de la validación del modelo

METHOD	RESPONSE	95% CI low	PREDICTIVE	95% CI above
NUMERIC (*DESARABILITY*)	AoxC	9211.75	12000	14788.28
	SLC	24.03	27.24	30.46
GRAPHIC	AoxC	9211.75	11998.50	14788.28
	SLC	24.03	27.24	30.46
*EXPERIMENTAL**	*AoxC*	*14,453.75 µmol ET / 100 g*		
	SLC	*24.10%*		

*Model validation using the combination of ET and HC obtained after optimization of extrusion process to get food pasta based on amaranth and blue maize.

Preparation of Extruded Amaranth/Blue Maize Pasta

The extruded amaranth/blue maize pasta was obtained according to Montoya-Rodríguez *et al.* (2014) with some modifications. The amaranth and blue maize grains (500 g lots) were mixed with monoglyceride (1.5 % by 100 g and 10 % of semolina). The extrusion conditions used were obtained optimizing the extrusion process (figure 2): extrusion temperature (ET), 82 °C; humidity content (HC), 33 %; screw speed (SS), 50 rpm; and the feed rate was 30 rpm.

Technofunctional pasta properties: solid loss by cooking (SLC), cooking time, water solubility (WSI) index, water absorbance index (WAI), total color difference (ΔE), and pH

SLC was determined following the AACC (1995) Pasta and Noodle Cooking Quality – Firmness method. The optimum cooking time (OCT) was determined by cooking 25 g of pasta in 300 mL of distilled, boiling water. OCT was evaluated every 30 seconds and was reached when the white center in the core of the pasta disappeared. OCT represents the time required for the dried central core to disappear when gently compressed between two glass plates (D'Egidio *et al*., 1990); the experiments were performed in triplicate. The WSI, WAI, and ΔE were evaluated according to Milán-Noris *et al*. (2019) using these formulas:

$$WSI = (supernatant\ weight\ /\ sample\ weight) * 100$$

$$WAI = (sediment\ weight\ /\ sample\ weight) * 100$$

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

pH was evaluated according to method 02-52 (AOAC, 1999).

***In vitro* simulated gastrointestinal digestion of EPS**

The pasta was *in vitro* simulated digested, representing the physiological conditions of the mouth, stomach, and small intestine (Milán-Noris *et al*., 2018). Briefly, a pasta sample (3 g) was suspended in 100 mL of phosphate buffer (pH 6.9) containing 10 U/mL of α-amylase from porcine pancreas (EC 3.2.1.1), and incubated for 5 min (37 °C, 200 rpm). For gastric digestion, pH was lowered to 2 using 1 M HCl. Subsequently, 5 mL of pepsin from porcine gastric mucous (EC 3.4.32.1) solution (2250 U/mL in 0.01 M HCl) were added, and the reaction mixture was incubated for one hour (37 ºC, 200 rpm). For the

Table 3. Regression coefficients and analyses of variance of the second order polynomial models showing the relationship among response variables (Yk) and process variables (X).
Tabla 3. Coeficientes de regresión y análisis de varianza de modelo polinomial de segundo orden mostrando la relación entre las variables de respuesta (Yk) y las variables del proceso (X).

Coefficients	Antioxidant Capacity (Y_{CA})		Solid Loss by Cooking (Y_{PSC})	
	Coded	Uncoded	Coded	Uncoded
Intercept				
β	7881.00	83724.38	27.44	101.79
Linear				
β1	446.96^{NS}	-1290.05^{NS}	-0.41^{NS}	-2.89^{NS}
β2	-1048.96*	-1051.72*	1.29*	-0.63*
Quadratic				
β11	1094.33*	21.88*	1.81**	0.04**
β22	2910.52**	4.75**	1.44*	0.0023*
Interaction				
β12	67.77^{NS}	0.382^{NS}	1.09^{NS}	6.21^{NS}
R^2	0.907		0.912	
P≤	0.0017		0.0014	

^{NS}: Nonsignificant, *Significant: p≤0.05; **Significant: p≤0.01.

Predictive models for the AoxC to food pasta were the following: coded *AoxC* $= +7881.00 + 446.96 *A - 1048.96 *B + 66.77*A*B + 1094.33 *A^2 + 2910.52 *B^2$; *uncoded*: $AoxC= +83724.38 - 1290.05 *\% HC - 1051.72 *TE + 0.382 *\% HC*TE + 21.88 *\% HC^2 + 4.75*TE^2$. $R^2 = 0.9070$. Predictive models for the SLC for food pasta were the following: $SLC = +27.44 - 0.41*A + 1.29 *B + 1.09 *A*B + 1.81 *A^2 + 1.44 *B^2$; *uncoded*: $SLC = +101.79 - 2.89 *\% HC - 0.63 *TE + 0.0062 *\%HC*TE + 0.04 *\%HC^2 + 0.0023 *TE^2$.

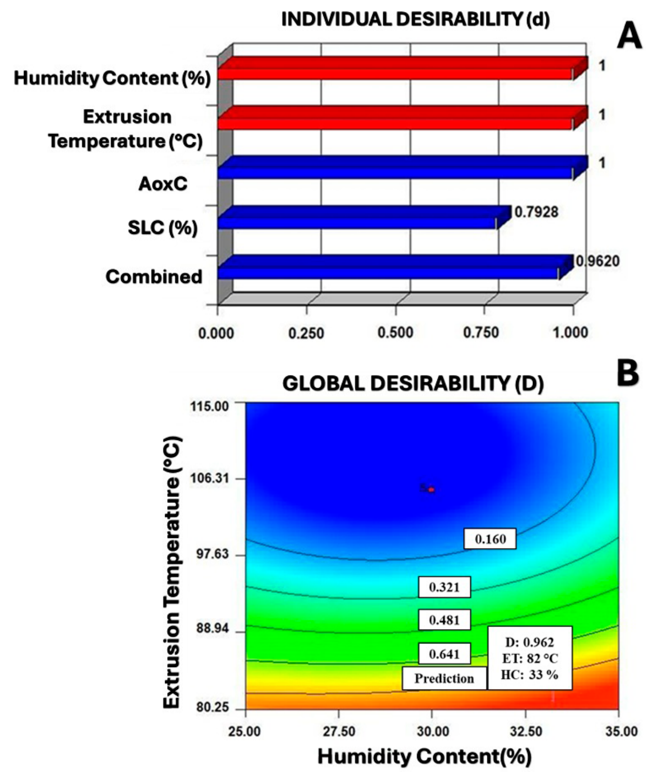

Figure 3. (A) Individual desirability (di) for response variables (antioxidant capacity/solid loss by cooking) and (B) global desirability (D = 0.962) for the optimized extruded food pasta based on amaranth and blue maize.
Figura 3. (A) Deseabilidades individuales (di) para las variables de respuesta (capacidad antioxidante / pérdida de sólidos por cocción) y (B) deseabilidad global (D = 0.962) para la optimización de la pasta extrudida a base amaranto y maíz azul.

intestinal digestion, the gastric digestion (adjusted to pH 6.5) and 5 mL of duodenal juice (200 mg of bile salts with 1980 U/mL of pancreatin from porcine pancreas) were added and incubated for two h (37 ºC, 200 rpm). After gastrointestinal digestion, the sample digests were heated (75 °C, 20 min) to inactivate the enzyme. Then, the samples were centrifuged (4 ºC, 20,000 xg) for 20 min. Fractions *In* and *Out* were collected; the *In* fraction (pellet) is non-absorbable, reaching the colon, whereas *Out* (supernatant) represents the absorbable fraction. The digested samples were freeze-dried and stored at -20 °C until analysis.

Hypoglycemic activity

AphA inhibition was determined using an α-amylase solution at 2 units / mL in 0.1 M sodium phosphate buffer (pH 6.9). Briefly, 50 µL of the extract were mixed with 100 µL of starch and 100 µL of a sodic potassic tartrate solution, and then 800 µL of phosphate buffer were added. From this solution, 200 µL were placed in a 96-well plate, and the absorbance was measured at 540 nm. The data were reported as a percentage of α-amylase inhibition. A color control for each sample and a curve of acarbose were used as a control. The results were expressed in µg equivalents of acarbose (AE) / 100 g of sample (µg AE / 100 g). AphG inhibition was carried out according to López-Angulo *et al*. (2014) with modifications. In a 96-well plate, 100 µL of a 0.5 units/mL α-glucosidase solution in 0.1 M sodium phosphate buffer (pH 6.9) were added, and the mixture incubated for 10 min. Aliquots of 50 µL of p-nitrophenyl-α-DD-glucopyranoside at 5 mM were added to the sample, and it was incubated (25 °C, 5 min). Samples without a substrate were used as a color corrector, and the absorbance was measured at 405 nm. The results were expressed as a percentage of inhibition relative to the positive control (100 % inhibition).

Antioxidant capacity (AoxC), total phenolic content (TPC), and flavonoids

The methodology previously reported by Dewanto *et al*. (2002) (minor modifications) was used to extract free phytochemicals. Bound phytochemicals were extracted according to the methods described by Adom and Liu (2002) and Adom *et al*. (2005). The oxygen radical absorbance capacity (ORAC) assay was used to determine the free and bound antioxidant capacities of the extracts, which were evaluated against Trolox as a standard, using fluorescein as a probe, as described by Ou *et al*. (2002). The absorbance of excitation and emission were set at 485 and 538 nm, respectively. The results of AoxC were expressed as micromoles of Trolox equivalents (TE) / 100

g. TPCs were determined according to Singleton *et al*. (1999) as was described by Gámez-Valdez *et al*. (2021). The results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry weight basis (DW). Flavonoid determination was carried out according to Liu *et al*. (2007), and the results were expressed as milligrams of catechin equivalents (CE) per 100 g of dry weight basis (DW).

Aminoacid analysis

Protein samples (2mg) were hydrolyzed in 6N HCl (4 mL) (110 °C, 24 h) in tubes sealed under nitrogen. Tryptophan was analyzed by HPLC after basic hydrolysis (Yust *et al*., 2004). Amino acids were determined after derivatization with diethyl ethoxymethylenemalonate by HPLC (Alaiz *et al*., 1992), using D, L-α-aminobutyric acid as an internal standard and a 300mm X 3.9mm i.d. reversed-phase column (Novapack C18, 4lm; Waters, Milford, MA, USA).

Protein digestibility *in vitro* (PDIV), C-PER, PDCAAS, and pBV

Protein digestibility *in vitro* was determined according to Tinus *et al*. (2012), following the method described by Hsu *et al*. (1977). 62.5 mg of protein flour were weighed and rehydrated in 10 mL of distilled water at 37 °C (1 h) with constant shaking (85 rpm). pH of the multienzymatic solution (prepared at the moment) was adjusted to 8.0 and kept at 37 °C until analysis. After rehydration, 1 mL of the multienzymatic solution was added. The initial and final pH were measured after 10 minutes of shaking. The pH change after 10 min was used to calculate the percentage of PDIV:

$$\%PDIV = 65.66 + 18.10\ (\Delta pH10min)$$

C-PER was calculated according to Satterlee *et al*. (1982), following the method by AOAC (1999). C-PER was evaluated using a regression equation obtained from the amino acid profile and PDIV of the sample and protein reference, as well as the relation of standard values of essential amino acids of FAO (2013). PDCAAAS and pBV were determined according to Milán-Noris *et al*. (2019).

Statistical Analysis

A one-way ANOVA was performed, and mean comparisons were evaluated using the GraphPad Prism 9 program with the Tukey test. All means were considered statistically different at $p < 0.05$. All the analyses were carried out in at least three independent replicates.

RESULTS AND DISCUSSION

Technofunctional properties and proximate composition of food pasta

Proximate composition and technofunctional properties of ABMF and EPS are shown in Table 4, and figure 4 shows the EPS obtained using a 50:50 mix of each grain (Montoya-Rodríguez *et al*., 2015a). WAI was higher in commercial pasta (CPW) (5.04 gel /sample g vs 3.58 and 3.26 gel / sample g, for EPS and ABMF, respectively). Extrusion increases WAI, as proteins are denatured during the extrusion-cooking process, starch is gelatinized, and fiber changes (Al-Shamsi *et al*., 2018; Cuj-Laines *et al*., 2018). Protein and aminoacid content are determinant to have differences in WAI (Peter and Gandhi, 2017). The extrusion-cooking process affects WSI, as extrusion degrades starch and fiber, producing soluble polysaccharides (Rashid *et al*., 2015). CPW had the highest WSI value, indicating that the starch damage was greater than that to EPS (Cuj-Laines *et al*., 2018). The oil absorption index (OAI) is a parameter that indicates the strength to retain flavor, and the extrusion-cooking process did not affect this value, as shown in Table 4 (Gupta *et al*., 2019). Protein content is one of the factors affecting OAI (Al-Shamsi *et al*., 2018). According to Joubert *et al*. (2018), pasta quality is determined by color, which is influenced by raw materials and extrusion-cooking process conditions, which was evident in the obtained pasta (brown) (figure 4), being different from CPW (Table 4). The latest could impact acceptability; however, it is important to mention that it is an integral pasta based on non-conventional ingredients.

Other parameters of pasta quality include optimal cooking time (OCT) and solid loss by cooking (SLC). OCT is important to know the exact time when pasta is well-cooked, since undercooked pasta can impact the flavor and acceptability. Some reports suggest that the use of non-conventional

Table 4. Technofunctional properties and proximate composition of amaranth/blue maize flour (ABMF), extruded pasta added with semolina (EPS) and wheat commercial pasta (CPW)[1].

Tabla 4. Propiedades tecnofuncionales y composición proximal de la harina de amaranto/maíz azul (HAMA), pasta extrudida con semolina (PES) y pasta comercial de trigo (PCT)[1].

PROPERTIES	ABMF	EPS	CPW[2]
Technofunctional properties			
WAI[5]	3.26±0.02^c	3.58±0.08^b	5.04±0.01^a
WSI[6]	10.67±0.46^c	16.50±1.05^b	22.40±0.40^a
pH	6.57±0.01^a	6.57±0.07^a	6.51±0.00^a
Optimal cooking time (mm:ss)		14±0.50^a	11.50±0.50^b
Solid losing by cooking (%)		19.06±1.50^b	9.80±0.03^c
Proximate composition (%)			
Proteins	11.26±0.43^a	11.09±0.61^a	6.0
Lipids	6.82±0.44^a	7.43±0.18^b	1.0
Ashes	3.07±0.29^a	2.54±0.14^b	
Carbohydrates	79.98±0.58^a	78.76±0.32^b	31.0

[1]The value corresponds to means ± SD of three replicates; different letter at the same row, are statiscally different ($p < 0.05$).[2]La Moderna® brand.

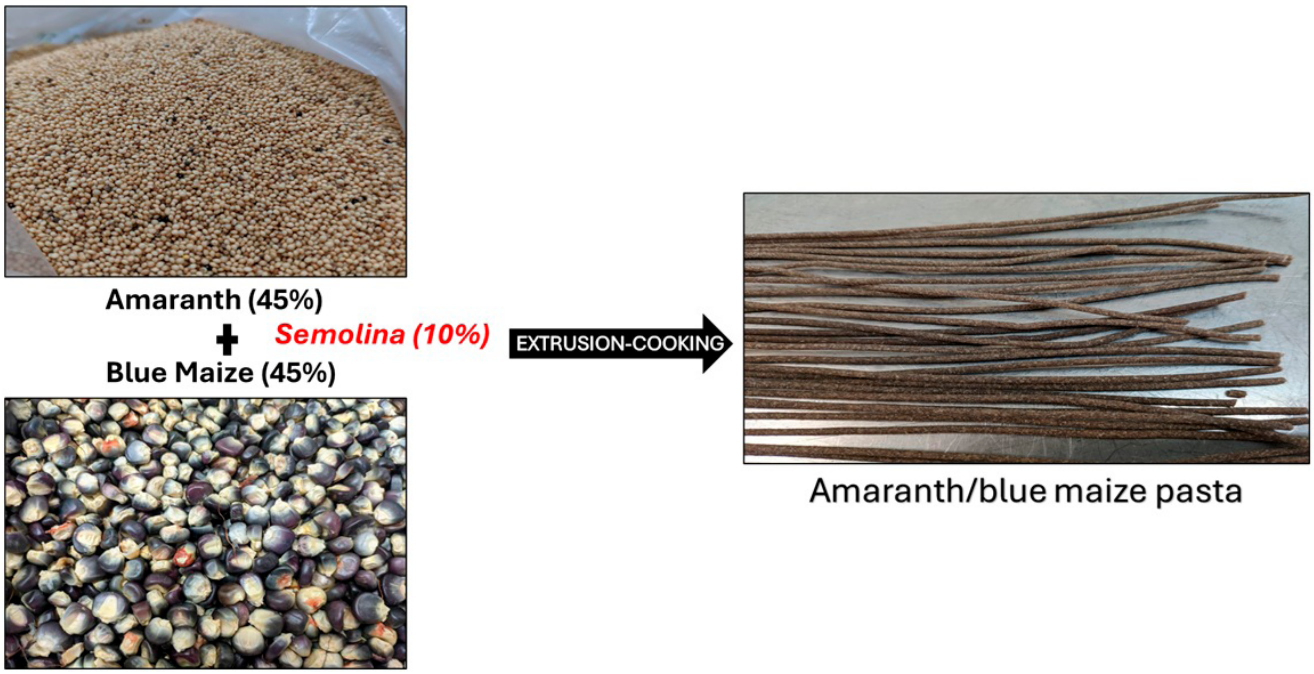

Figure 4. Food pasta based on amaranth/blue maize obtained by extrusion-cooking process.
Figura 4. Pasta alimenticia elaborada a base de amaranto/maíz azul obtenida mediante el proceso de extrusión.

ingredients could affect the elaboration of pasta due to the OCT decreases resulting from their addition (Cárdenas-Hernández *et al*., 2016). However, as shown in Table 4, the OCT was not affected despite the use of non-conventional ingredients. Palavecino *et al*. (2020) mention that this phenomenon could be explained by the starch gelatinization in these materials (no conventional), which have higher temperatures than those found in wheat. The SLC of CPW had the lowest value (9.80 %); whereas, EPS had 19.06 % of SLC, meaning that during the pasta cooking, this one could lose more components in the cooking water produced by using non-conventional material (Cárdenas-Hernández *et al*., 2016; Nilusha *et al*., 2019). Granito *et al*. (2009) replaced wheat semolina with 10 % legume, and the SLC obtained was 20.1 % (higher than this study), despite using conventional materials. Proximate composition of the EPS and ABMF showed good proteins (11.09 vs 11.26 %), lipids (7.43 vs 6.82 %), ashes (2.54 vs 3.07 %), and carbohydrate (78.76 vs 79.98 %) content. The protein content is higher in ABMF and EPS compared to maize but lower compared to amaranth. This can be explained since the protein content of the raw materials differs. Extrusion does not affect protein content (Astorga-Gaxiola *et al*., 2025); however, lipid content, which is helpful to protect other molecules during extrusion (Offiah *et al*., 2019), was higher (Table 4) in EPS due to the use of monoglyceride in pasta elaboration, despite some studies mentioning that they are affected negatively (Wang *et al*., 2022).

Extrusion-cooking effect on nutritional properties

Table 5 presents the results of the amino acid profile, chemical score, amino acid limiting, C-PER, pBV, and DPIV. The ABMF reached almost all the recommendations of amino acid content according to FAO for children (3 years and older) (Sá *et al*., 2023), except for Trp, which was the limiting AA, even though some reports mention that amaranth reaches the recommendations by FAO (Montoya-Rodríguez *et al*., 2015a). This could be explained due to the mixing with maize, since Trp is the limiting AA in maize (Montoya-Rodríguez *et al*., 2015b). Zhang *et al*. (2024) reported that extrusion cooking (130-135 °C) increased the amino acid digestibility of cereals like barley, wheat and rice. In this study, the amino acid profile was not affected, and this was probably due to the addition of monoglyceride during the extrusion-cooking process and the addition of semolina, which gave protection to temperature damage, which was different from Dias-Paes and Maga (2004) (130 °C vs 82 °C), where they reported that extrusion cooking affected the amino acid profile of maize. C-PER was improved after the extrusion-cooking process (Table 5); however, some reports suggest that extrusion conditions can either improve or decrease C-PER (Espinoza-Moreno *et al*., 2021). PDIV was higher in EPS with respect to ABMF, which could be explained by the extrusion-cooking process, as it produced protein denaturation, making proteins more accessible (Patil *et al*., 2016; Atukuri *et al*., 2019; Gámez-Valdez *et al*., 2020). Biological predicted value (pBV), which is a parameter that defines the protein anabolized in the body, was higher in EPS than in ABMF, and this is related to the results for PDIV (Milán-Noris *et al*., 2019). PCDAAS is the principal parameter to determine the quality of food protein. Its calculation is based on the limiting AAS according to the FAO recommendations for 2–5-year-old infants and the PDIV. The PDCAAS value of 1 is the highest, providing 100 % of the

Table 5. Chemical quality according to FAO and aminoacid profile, C-PER, *DPIV* and pBV of amaranth blue/maize flour (ABMF), extruded pasta with semolina (EPS) and wheat commercial pasta (CPW)[1].
Tabla 5. Calificación química de acuerdo con la FAO, perfil de aminoácidos, C-PER, DPIV y vBP de harina de amaranto/maíz azul extrudido (HAMA), pasta extrudida con semolina (PES) y pasta comercial de trigo (PCT)[1].

PROPERTIES	ABMF	EPS	FAO[2]
AA [3](g/100g protein)			
His	2.65	2.65	1.9
Ile	3.87	3.87	2.8
Leu	9.00	9.00	6.6
Lys	4.26	4.26	5.8
Met + Cys	2.62	3.31	2.5
Phe + Tyr	5.24	5.24	6.3
Thr	3.79	3.79	3.4
Trp	0.78	0.78	1.1
Val	4.99	4.99	3.5
Total	37.25	37.93	33.9
AAS[4] Chemical Score (%)	70.95	70.95	
AA limiting	Trp	Trp	
PDCAAS[5]	0.50	0.53	
C-PER[6]	2.94	3.05	
pBV[7]	33.94	40.55	
PDIV (%)	71.09±0.51[b]	76.61±0.64[a]	

[1]The value corresponds to means ± SD of three replicates. [2]FAO: Food and Agriculture Organization of the United Nations. [3]AA: aminoacids. [4]AAS: aminoacid score. [5]PDCAAS: corrected aminoacid score by protein digestibility. [6]C-PER: Calculated protein efficiency relation. [7]pBV: predicted biological value

essential amino acids recommendations (Azizi *et al*., 2025). The values of pBV in ABMF and EPS were lower (Table 5) than the minimum recommended value (0.6) (Milán-Noris *et al*., 2019), which could be attributed to the low chemical score in both samples.

Extrusion-cooking and simulated gastrointestinal digestion effect on nutraceutical properties.

Table 6 presents the results of AoxC, total phenolic content, and flavonoids of ABMF, EPS (digested *In* and *Out*), and CPW. Extrusion produced a reduction in AoxC ($p < 0.05$), and this could be explained by the high temperature used; however, EPS had a retention of 67 %, which is a better result than that reported by Milán-Carrillo *et al*. (2012) and Gámez-Valdez *et al*. (2020) for amaranth and blue maize, respectively. EPS had better AoxC ($p < 0.05$) than CPW (Table 6), making non-conventional ingredients a viable alternative for producing pasta. EPS was digested, and its protein profile analyzed (figure 5). The digested fractions (*In* and *Out*) showed good values for AoxC, with the *In* fraction having the highest value [5853 vs 4.60 micromoles of trolox equivalents/mg of soluble protein (Table 7)]. Since *In* is the non-absorbable fraction (reaching the colon), the results found are favorable because biocompunds will be available to have a beneficial effect against ROS

Table 6. Effect of extrusion, simulated digestion and semolina addition in AoxC, total phenolic content and flavonoids of amaranth blue/maize flour (ABMF), extruded pasta with semolina (EPS) and wheat commercial pasta (CPW)[1].
Tabla 6. Efecto de la extrusión, digestión simulada y adición de semolina en la AoxC, contenido de fenólicos totales y flavonoides de harina de amaranto/maíz azul extrudido (HAMA), pasta extrudida con semolina (PES, digeridos *In* y *Out*) y pasta comercial de trigo (PCT)[1].

	FREE	BOUND	TOTAL
Antioxidant Capacity (µmol TE / 100 g)			
ABMF	11704±469.20[a]	7141±306.20[b]	18844±573.30[a]
EPS	3169±73.85[c]	9483±396.90[a]	12652±454.60[b]
In[8] (µmol TE / g soluble protein)			5853±146.80[a]
Out[8](µmol TE / g soluble protein)			4.60±0.41[b]
CPW	5910±449.70[b]	3709±116.00[c]	9618±558.00[c]
Total Phenolic Content[6] (mg EGA / 100 g)			
ABMF	66.74±3.73[b]	259.7±2.20[a]	326.40±5.94[a]
EPS	62.73±1.25[b]	224.9±2.31[b]	287.60±3.39[b]
CPW	75.16±2.36[a]	176.40±7.89[c]	251.50±6.32[c]
Flavonoids[7] (mg catechin /100 g)			
ABMF	4.22±0.36[a]	4.83±0.16[b]	9.05±0.51[a]
EPS	0.55±.0.09[b]	6.65±0.53[a]	7.20±0.51[b]
CPW	0.87±0.12[b]	5.33±0.32[b]	6.20±0.43[b]

[1]The value corresponds to means ± SD of three replicates; different letter at the same measurement between samples, are statiscally different ($p < 0.05$).

(Milán-Noris *et al*., 2019). TPC and flavonoids were affected by the extrusion-cooking process, with CPW having the lowest value ($p < 0.05$). The latest could be explained due to the combination of humidity and temperature and considering that phenolic compounds are thermolabile (De la Rosa *et al*., 2019). Patil *et al*. (2016) reported similar flavonoid retention in extruded sorghum, whereas Gamez-Valdez *et al*. (2021) reported similar TPC retention (80.5 %).

Effect of extrusion-cooking and simulated gastrointestinal digestion on hypoglycemic activity

The results of AphA and AphG inhibition are shown in Table 8. The inhibition of AphA ranged from 8.28 to 41.31 %, whereas the inhibition of AphG ranged from 6.28 to 45.72 %. CPW had the lowest value ($p < 0.05$) for AphA, and the *In* fraction had the lowest value for AphG inhibition ($p < 0.05$). EPS had the highest value of AphA inhibition (41.31 %), and ABMF had the highest value of AphG inhibition (45.72 %); however, there was no difference between ABMF, EPS, and Out fraction from EPS ($p < 0.05$) (Table 4). Conforti *et al*. (2005) and Nkobole *et al*. (2021) reported similar results for AphA inhibition in amaranth varieties. pH changes affected inhibition of the AphA and AphG (Gutiérrez-Grijalva *et al*., 2018). These results suggest that EPS could retard the absorption of complex carbohydrates, preventing hyperglycemia (Kehinde and Sharma, 2020).

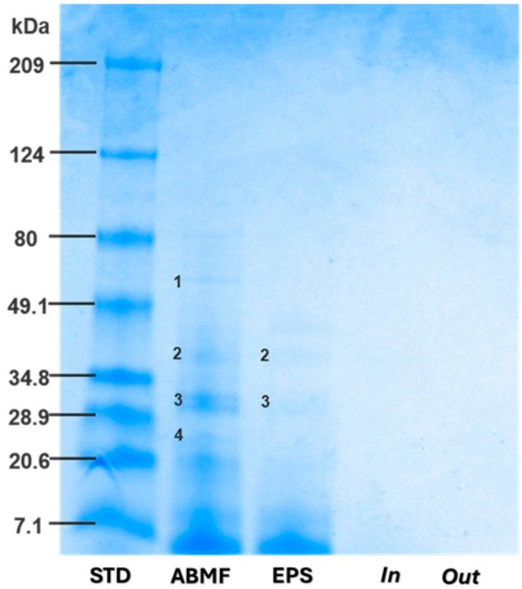

Figure 5. Protein profile (SDS-PAGE) of amaranth/blue maize flour (ABMF) extruded pasta with semolina (EPS) and the *In* and *Out* fractions obtained after EPS digestion.
Figura 5. Perfil de proteínas (SDS-PAGE) de la harina de amaranto/maíz azul (HAMA), pasta extrudida con semolina (PES) y las fracciones *In* y *Out* obtenidas después de la digestión de la PES.

Table 7. Effect of extrusion, simulated digestion and semolina addition in SP and AoxC of amaranth blue/maize flour (ABMF), extruded pasta with semolina (EPS) and wheat commercial pasta (CPW)[1].
Tabla 7. Efecto de la extrusión y digestión *in vitro* sobre el contenido de proteína soluble (SP) y capacidad antioxidante ORAC de harina de amaranto y maíz azul (HAMA), pasta extrudida con semolina (PES) y pasta comercial de trigo (PCT)[1].

Sample		SP	AoxC
		(mg SP/mL)	(µmol trolox eq/100g bs)
ABMF		4.83±0.42[a]	
EPS		2.65±0.23[c]	
	In	3.41±0.20[b]	5853±146.80[a]
	Out	0.53±0.02[e]	4.6±0.41[b]
CPW		1.98±0.42[d]	

[1]The value corresponds to means ± SD of three replicates; different letter at the same measurement between samples, are statiscally different ($p < 0.05$).

Table 8. Hypoglycemic activity from amaranth blue/maize flour (ABMF), extruded pasta with semolina (EPS), fractions *In* and *Out* (EPS digested) and commercial pasta of wheat (CPW)[1] and fractions *In* (colon available) and *Out* (basolateral fraction) of EPS digested[1].
Tabla 8. Actividad hipoglucemiante de la de harina de amaranto/maíz azul extrudido (HAMA), pasta extrudida con semolina (PES, digeridos *In* y *Out*) y pasta comercial de trigo (PCT)[1].

Sample	%AphA Inhibition	%AphG Inhibition
ABMF[2]	10.04±0.29[d]	45.72±0.64[a]
EPS[3]	41.31±1.05[a]	42.94±1.40[a]
Fraction *In*[5]	16.66±1.02[c]	6.28±2.44[c]
Fraction *Out*[6]	22.51±1.68[b]	40.44±8.33[a]
CPW[4]	8.28±0.29[d]	26.58±0.85[b]

[1]The value corresponds to means ± SD of three replicates; different letter at the same column, are statistically different ($p < 0.05$). %AphA Inhibition: inhibition percentage of α-amylase. %AphG Inhibition: inhibition percentage of α-glucosidase

CONCLUSIONS

A food pasta based on amaranth/blue maize with the addition of semolina was obtained through an extrusion-cooking process, which increased nutritional properties such as C-PER and PDIV. Additionally, the AA profile met almost all the necessities recommended by the FAO with exception of Trp. Nutraceutical properties, such as AoxC, TPC, and flavonoids, exhibited good retention values in EPS, making pasta a good source of antioxidants. EPS showed good hypoglycemic activity, exhibiting a high percentage of inhibition for AphA and AphG, making EPS a functional food with potential hypoglycemic properties. More studies are needed to elucidate this potential effect. The use of non-conventional ingredients, such as amaranth and maize, is a good alternative for producing functional food pasta.

CONFLICTS OF INTEREST

The authors declare they have no conflict of interest.

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