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**Effect of the extrusion process on phytochemical, antioxidant, and cooking properties of gluten-free pasta made from broken rice and nopal****Efecto del proceso de extrusión sobre las propiedades fitoquímicas, antioxidantes y de cocción de pastas libres de gluten elaboradas a partir de arroz quebrado y nopal**

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**Abstract**

Gluten-free pasta (GFP) can be produced by combining broken rice flour with materials rich in bioactive compounds such as nopal flour (*Opuntia ficus-indica* L.). The objective of this work was to study the effect of extrusion temperature (ET: 88.5-125.5 °C), moisture content (MC: 21.27-34.73%), and nopal flour content (NFC: 1.91-22.09%) on the phytochemical, antioxidant and cooking properties of GFP. For the statistical analysis, the response surface methodology was used. The weight gain (WG) increased at high NFC. Also, the WG increased in two zones, one combining low MC and ET and another using high MC and ET. The cooking time decreased, and the total phenolic compounds content and antioxidant capacity (inhibition of oxidation of LDL) increased at high MC and NFC. The optimal processing conditions were ET = 118 °C, MC = 31.7% and NFC = 18.0%. The optimal pasta (OP) showed elevated sensory acceptability, similar to a commercial product. Likewise, the OP had a higher dietary fiber content than commercial pasta and control pasta. The results indicate that it is possible to obtain GFP using broken rice and nopal with acceptable phytochemical, antioxidant, and cooking properties whose consumption could eventually have health benefits.

**Keywords:** gluten-free pasta, extrusion, broken rice, nopal, bioactive compounds.

**Resumen**

Las pastas libres de gluten (PLG) pueden ser producidas combinando harina de arroz quebrado con materiales ricos en compuestos bioactivos tales como la harina de nopal (*Opuntia ficus-indica* L.). El objetivo del presente trabajo fue estudiar el efecto de temperatura de extrusión (TE: 88.5-125.5 °C), contenido de humedad (CH: 21.27-34.73%) y contenido de harina de nopal (CHN: 1.91-22.09%) sobre propiedades fitoquímicas, antioxidantes y de cocción de PLG. Para el análisis estadístico fue utilizada la metodología de superficie de respuesta. La ganancia de peso (GP) aumentó a altos CHN. Asimismo, una alta GP fue presentada en 2 zonas, una combinando altos TE y CH y otra utilizando bajos CH y TE. El menor tiempo de cocción, mayor contenido de compuestos fenólicos totales, y mayor capacidad antioxidante (inhibición de oxidación de LDL) fueron presentados a altos CH y CHN. Las condiciones óptimas de procesamiento fueron TE = 118 °C, CH = 31.7% y CHN = 18.0%. La pasta óptima (PO) mostró adecuada aceptabilidad sensorial similar a un producto comercial. Asimismo, la PO presentó mayor contenido de fibra dietaria que una pasta comercial y pasta control. Los resultados obtenidos indican que es posible obtener PLG utilizando arroz quebrado y nopal con aceptables propiedades fitoquímicas, antioxidantes y de cocción, cuyo consumo podría tener eventualmente beneficios en la salud.

**Palabras clave:** pasta libre de gluten, extrusión, arroz quebrado, nopal, compuestos bioactivos.

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## 1 Introduction

There are different disorders related to gluten intake, mainly celiac disease. It is estimated that around 10% of the world population presents a condition that shares gluten intake as an etiological factor, and the only treatment currently is performing a gluten-free diet for life (Ontiveros *et al.*, 2021). Celiac disease is determined by chronic inflammation and atrophy of the small intestine mucosa, leading to malabsorption syndrome with gastrointestinal and extra-gastrointestinal symptoms, affecting nutritional status (Murillo-Saviano *et al.*, 2019). Due to busy lifestyles, there is a high consumption of fast food, highlighting pasta. This food has gained popularity in the diet due to its long shelf life, easy preparation, low cost, and good palatability (Melini *et al.*, 2020). It is produced mainly using wheat semolina as the main ingredient, which is a source of gluten (Webb, 2019). Environmentally sustainable food production considers waste as by-products, which can be transformed to provide valuable compounds (antioxidants, fiber, etc.) and used as new products or raw materials in the food industry (Carciochi *et al.*, 2017; Hernández-Neri *et al.*, 2023) with economic value. Broken rice is a by-product of the rice milling industry. It is a high source of carbohydrates, low in fat, and gluten-free. Therefore, broken rice is an excellent alternative to produce gluten-free products. In addition, it has many advantages, such as a mild, colorless flavor, high digestibility, and low sodium level (Marcoa and Rosell, 2008). On the other hand, in México, the nopal is a traditional food with elevated consumption in the population (Espinosa-Solares and Domínguez-Puerto, 2023), and an important source of phytochemical compounds, antioxidant capacity, and dietary fiber, mainly soluble (Torres-Ponce *et al.*, 2015). Adding nopal flour to produce gluten-free pasta can increase its dietary fiber content and phytochemical compounds, predominantly phenolic compounds. Nopal has nutraceutical potential, as it has been shown to help reduce the risk of various diseases (Reyes *et al.*, 2023). Different technologies can be used to produce pasta, highlighting extrusion (Oliveira *et al.*, 2017). Extrusion is a versatile technology that combines high temperatures and pressures for a short time. This technology is characterized by causing changes in the properties of food that result in a better nutritional quality based on higher digestibility, a lower level of antinutritional factors, and an increase in the fractions of dietary fiber (Kour *et al.*, 2022). These changes depend on different variables of the extrusion process used during the production of foods, such as moisture content, temperature, screw speed, and the properties of the used raw materials. To obtain better extrusion processing conditions, the optimization method can be used through the response

surface methodology (RSM) (Yolmeh and Jafari, 2017). Likewise, another advantage that characterizes the extrusion process is that it can retain significant levels of bioactive compounds, such as phenolic compounds. These compounds are found in foods in free and bound form, and their presence has been associated with increased antioxidant capacity with potential benefits for human health (Zeng *et al.*, 2016). However, no scientific information has been found on combining raw materials such as broken rice and nopal flour, to produce gluten-free pasta by extrusion. Therefore, this work aimed to evaluate the phytochemical, antioxidant, and cooking properties of pasta made from broken rice and nopal.

## 2 Materials and methods

### 2.1 Raw materials

Broken rice (Grupo Ansera, Culiacan, Mexico) and dehydrated nopal flour (Viva Verde, Hermosillo, Mexico) were used for pasta production. The broken rice grains were ground in a Pulvex mill (Model 200, Mexico City, Mexico). Both materials were sieved to obtain products with a particle size  $\leq 420 \mu\text{m}$ .

### 2.2 Production of pasta by the extrusion process

The gluten-free pasta (GFP) was made from broken rice flour and dehydrated nopal flour. The concentration of nopal flour varied from 1.91% to 22.09%, and the moisture content was 21.27% to 34.73%, according to the experimental design (Table 1). The mixtures were fed using a constant mass flow rate of  $57 \pm 2 \text{ g/min}$ , into a twin-screw extruder (Shandong Light brand, model LT32L, China), with a compression ratio of 2:1. The screw speed was constant (115 rpm) and was used a circular die of 2 mm diameter. The temperature of the feeding and exit zones were kept constant at 80 °C and 70° C, respectively. At the same time, the temperature of the mixing/cooking zone varied according to the experimental design from 88.5 °C to 125.5 °C. The obtained pasta was dried in a room with ventilation and controlled temperature ( $\sim 25 \pm 2 \text{ °C}$ / 24 h) until reaching 8-10% moisture levels. After that, The GFP was stored in sealed black plastic bags and at room temperature of  $22 \pm 4 \text{ °C}$  until analysis.

### 2.3 Proximal analysis

The proximal composition of the raw materials (broken rice and dehydrated nopal flour), GFP obtained under optimal extrusion conditions (OP),

Table 1. Experimental design for the extrusion study.

Assay	Coded levels			Actual levels		
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	ET (°C)	MC (%)	NFC (%)
1	-1	-1	-1	96	24	6
2	1	-1	-1	118	24	6
3	-1	1	-1	96	32	6
4	1	1	-1	118	32	6
5	-1	-1	1	96	24	18
6	1	-1	1	118	24	18
7	-1	1	1	96	32	18
8	1	1	1	118	32	18
9	-1.682	0	0	88.5	28	12
10	1.682	0	0	125.5	28	12
11	0	-1.682	0	107	21.27	12
12	0	1.682	0	107	34.73	12
13	0	0	-1.682	107	28	1.91
14	0	0	1.682	107	28	22.09
15	0	0	0	107	28	12
16	0	0	0	107	28	12
17	0	0	0	107	28	12
18	0	0	0	107	28	12
19	0	0	0	107	28	12
20	0	0	0	107	28	12

ET = extrusion temperature; MC = moisture content; NFC = nopal flour content.

GFP without nopal (control product (CP)), commercial rice pasta (CRP), and commercial wheat semolina pasta (CWP) were analyzed according to the methodology reported by AOAC (2012) for protein (960.52), fat (920.39), ash (923.03), moisture (925.10) and crude fiber (962.09). Furthermore, the carbohydrate content was obtained by difference. Three measurements were made for each response.

#### 2.4 Cooking properties (cooking time (CT) and weight gain (WG))

For this determination, ≈50 g of product was cut into 5 cm pieces for each cooking property. The cooking time (CT) was evaluated as the time required for the dried central core to disappear when gently squeezed between two glass plates (D'Egidio *et al.*, 1990). Also, the percentage of weight gain (WG) was determined following the methodology described by AACC 16-50 (2000). In both determinations, three measurements were made for each treatment, and the WG (%) was calculated by weighing the pasta before and after cooking using the equation (1).

$$\text{Weight gain(\%)} = [\text{weight of cooked pasta (g)} - \text{weight of uncooked pasta (g)}] / \text{weight of uncooked pasta (g)} \times 100 \quad (1)$$

#### 2.5 Total phenolic compounds (TPC)

Free and bound phenolic compounds were extracted using the procedure described by Adom and Liu

(2002), with some modifications. The free phenolic compounds were extracted from one gram of sample by shaking 10 mL of 80% (v/v) ethanol for 10 min. Subsequently, a centrifugation process (Eppendorf 5804R, Hamburg, Germany) at 3000 xg for 10 min and concentration with a Heidolph rotary evaporator (model LABOROTA 4011, Germany) at 45 °C using low-pressure was performed. The extraction was repeated four times. The concentrate was dried using an oven and reconstituted using 50% methanol to determine free phenolic compounds. Likewise, the residue was stored to obtain the extracts of bound phenolics. This material was digested with 10 mL of NaOH 2M for 30 min in a water bath (Shel Lab, Model WS27), subsequently neutralized with 2 mL of concentrated HCl, and shaken for 2 min using a Thermolyne vortex equipment, model MG3215, USA. After that, hexane was added, and centrifugation was carried out (Eppendorf 5804R, Hamburg, Germany) at 3000 xg, eliminating the supernatant. Ethyl acetate was added, and a new centrifugation was performed at 3000 xg. The ethyl acetate was subsequently recovered and stored in a tube. The ethyl acetate was evaporated to dryness, and the extracted compounds were reconstituted using 50% methanol and stored at a temperature of 4 °C to determine the bound phenolic compounds. These extracts were used to measure the TPC and antioxidant capacity. The TPC content was obtained from the sum of free and bound phenolic compounds. For the determination of TPC content, the Folin-Ciocalteu spectrophotometric method was used with a spectrophotometer (Model

10, UV GENESYS, Series AQ7-2H7G229001, USA) by measuring the absorbance at 760 nm (Heimler *et al.*, 2006). Four replicates were performed for each treatment of the experimental design, and the TPC values were reported in mg gallic acid equivalents per gram of sample on a dry basis (mg GAE/g d.b.).

## 2.6 Antioxidant capacity-inhibition of oxidation of low-density lipoproteins (LDL)

This determination was performed using the methodology reported by Rocha-Guzman *et al.* (2012) using blood plasma donated by the EMED clinical analysis laboratory. The LDL was precipitated by using a precipitating reagent (HDL cholesterol SPINREACT, Girona, Spain), and then was added to a reaction mixture composed of the sample extracts, phosphate-buffered saline (PBS), and a copper sulfate ( $\text{CuSO}_4$ ) solution. The control consisted of adding PBS instead of sample extracts. The reaction mixture was incubated (3 h/37 °C), and then trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were added and heated to 95 °C/20 min. Subsequently, the mixture was cooled in water (3-5 °C) for 5 min. After that, n-butanol was added, and the mixture was centrifuged for phase separation. The upper phase was taken, and the absorbance was read at 532 nm (Loy *et al.*, 2002). The results were expressed as the percentage of LDL using the following formula:  $(1 - \text{absorbance of the mixture reaction/control absorbance}) \times 100$ .

## 2.7 Optimization

The optimization of the extrusion process was performed using the numerical method with the Design-Expert software (Stat-Ease, Inc., Minneapolis, MN, USA). The response variables used to optimize were WG, CT, TPC, and LDL (free fraction). The optimization aimed to find the best processing conditions to obtain the highest WG, TPC, LDL (free fraction), and the lowest CT values. The criteria for the optimization using the above responses were obtaining WG values  $\geq 200\%$  and  $\text{CT} \leq 11$  min (found in commercial pasta). Also, getting high TPC and LDL (free fraction) values due to the high levels of TPC and LDL in pasta can be related to potential beneficial effects on the consumer's health. Likewise, this information was used to evaluate the desirability function, which is one of the most widely used techniques for optimizing processes with multiple responses in the food industry, varying desirability from 0 (lower) to 1 (higher), according to Myers and Montgomery (1995). Two experimental tests were conducted with the optimal conditions, and WG, CT, TPC, and LDL (free fraction) responses were evaluated. The model was validated in triplicate,

comparing the predicted and experimental values.

## 2.8 Dietary fiber (DF)

The DF analyses were performed according to the AOAC (2012) methodology, with the method 985.29 and Prosky *et al.* (1988). This assay was carried out in the control pasta without nopal (CP), products obtained under optimal conditions (unprocessed and processed (OP)), and commercial rice pasta (CRP). The methodology is based on removing starch and protein using a TDF-100<sup>®</sup> enzyme kit (Sigma-Adrich, St. Louis, MO, USA). One gram of the sample was mixed with a phosphate buffer solution and gelatinized with heat stable  $\alpha$ -amylase. A digestion was performed with the enzymes protease and amyloglucosidase, carrying out different pH adjustments in the solution subjected to heating and cooling. The residue was filtered and washed with water, being saved for later use. Also, the residue was rewashed using ethanol and acetone, dried, and weighed to obtain the insoluble dietary fiber (IDF). Four volumes of ethanol were added to the combined filtrate and water washings previously saved to precipitate the soluble dietary fiber (SDF). The precipitate was filtered (Martin-Cabrejas *et al.*, 1995), washed using ethanol and acetone, dried, and weighed. The residual protein and ash were determined, and the IDF and SDF values were reported as percentages.

## 2.9 Sensory analysis

The sensory acceptability was evaluated in the pasta obtained under optimal conditions (OP), a control pasta without nopal (CP), and a commercial pasta (COP). The evaluation was performed in different locations (schools, supermarkets, homes, and others) by 120 untrained panelists of both genders (60 men and 60 women) over 18 years of age who usually consume pasta. The general acceptance was assessed using a 9-point hedonic scale, where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely, using the methodology reported by Delgado-Nieblas *et al.* (2018).

## 2.10 Statistical analysis

A central composite rotatable experimental design with an  $\alpha$  value = 1.682 was used. The independent variables were the extrusion temperature (ET, °C), the moisture content (MC, %), and the nopal flour concentration (NFC, %), with five levels for each factor (Table 1). The effect of these study factors on different phytochemical, antioxidant, and cooking properties of gluten-free pasta made from broken rice and nopal was evaluated. For the multiple regression



(MSR) and prediction of the experimental behavior of the data, the quadratic models produced by the statistical program Design Expert (Stat-Ease, 2017) version 11.0 were used. The second-order polynomial is shown in equation (2):

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_1^2X_1^2 + b_2^2X_2^2 + b_3^2X_3^2 + b_1b_2X_1X_2 + b_1b_3X_1X_3 + b_2b_3X_2X_3 \quad (2)$$

Where  $Y_i$  is the generic response,  $X_1$  is the extrusion temperature,  $X_2$  is the moisture content of the samples,  $X_3$  is the nopal flour concentration, and  $b_0$ ,  $b_1$ ,  $b_2$ ,  $b_3$ ,  $b_1^2$ ,  $b_2^2$ ,  $b_3^2$ ,  $b_1b_2$ ,  $b_1b_3$  and  $b_2b_3$  are the regression coefficients. The Pearson correlations were performed using the statistical software Statistica 7.0 (Statsoft, 2004). Whereas the means comparisons were made with the LSD test (Fisher,  $p \leq 0.05$ ) using the statistical software Design Expert (Stat-Ease, 2017) version 11.0.

### 3 Results and discussion

#### 3.1 Proximal analysis

The proximal composition of the broken rice, nopal flour, and pasta obtained under optimal processing conditions is shown in Table 2. The values in broken rice flour were similar to those reported by Ahmed *et al.* (2016), presenting high carbohydrate content and low lipid levels. Also, the values obtained for nopal flour are close to what was reported by Diego-Zarate *et al.* (2021), highlighting its high crude fiber and ash content. These high levels in the raw material nopal flour could have increased the values of crude fiber and ash for the optimal pasta (OP), compared to CP and the commercial pasta CRP and CWP. The elevated ash content indicates high levels of minerals

in foods (Delgado-Nieblas *et al.*, 2021). Likewise, the OP values are slightly lower (except ash) than those presented by Trivedi (2018) in a gluten-free pasta based on rice flour with spinach flour. The difference in the proximal analysis could be due to the different process conditions used to obtain the pasta and the various raw materials used for its production.

#### 3.2 Regression coefficients and statistical analysis

The regression coefficients and analysis of variance for the responses analyzed in the gluten-free pasta (GFP) are shown in Table 3. The dependent variables were fitted to a second-order model. All the evaluated responses showed a significant model ( $p \leq 0.001$ ), with  $R_{adj}^2$  values  $\geq 0.90$ , except BLDL, coefficients of variation between 3.05 and 10.72, and none of the variables of response showed a lack of fit ( $p > 0.05$ ). The statistical analysis (Table 3) indicated that all the factors of the study presented a significant effect ( $p \leq 0.05$ ), in their linear terms, on all the responses analyzed, except the extrusion temperature (ET) factor on the FLDL and BLDL responses, and the MC factor on the BLDL response. Also, the ET factor presented a significant effect ( $p \leq 0.05$ ) in the quadratic term on the WG and FLDL responses, the MC factor showed a significant impact ( $p \leq 0.05$ ) in the quadratic term on the WG, CT, and FLDL responses, and the NFC factor presented a significant effect ( $p \leq 0.05$ ) in the quadratic term on the WG and CT responses. At the same time, the ET\*MC interaction showed a significant impact ( $p \leq 0.05$ ) on the WG, CT, and FLDL responses. Likewise, the ET\*NFC interaction was significant ( $p \leq 0.05$ ) on the TPC and FLDL responses. Also, the MC\*NFC interaction presented a significant impact ( $p \leq 0.05$ ) on the CT and TPC responses.

Table 2. Chemical composition (% d. b.) and energy value (kcal/100 g) of the raw materials broken rice flour, nopal flour, control pasta (CP), optimal pasta (OP), commercial rice pasta (CRP), and commercial wheat semolina pasta (CWP).

Sample	Dry weight	Ash	Protein	Fat	Crude fiber	Carbohydrates	Energy value
<b>Raw materials</b>							
Broken rice flour	90.85 $\pm$ 0.02	0.61 $\pm$ 0.01	7.2 $\pm$ 0.01	0.44 $\pm$ 0.02	2.37 $\pm$ 0.17	89.38 $\pm$ 0.23	390.33 $\pm$ 0.03
Nopal flour	93.94 $\pm$ 0.05	23.61 $\pm$ 0.02	11.74 $\pm$ 1.05	1.65 $\pm$ 0.04	18.23 $\pm$ 0.32	44.77 $\pm$ 0.04	240.89 $\pm$ 0.02
<b>Pasta</b>							
CP	89.01 $\pm$ 0.02 <sup>d</sup>	0.61 $\pm$ 0.03 <sup>d</sup>	2.45 $\pm$ 0.4 <sup>c</sup>	0.02 $\pm$ 0.02 <sup>c</sup>	2.46 $\pm$ 0.04 <sup>c</sup>	94.46 $\pm$ 0.02 <sup>b</sup>	387.82 $\pm$ 0.01
OP	93.01 $\pm$ 0.01 <sup>a</sup>	4.77 $\pm$ 0.01 <sup>a</sup>	1.91 $\pm$ 0.01 <sup>d</sup>	0.81 $\pm$ 0.01 <sup>a</sup>	4.38 $\pm$ 0.01 <sup>a</sup>	88.13 $\pm$ 0.01 <sup>c</sup>	367.45 $\pm$ 0.01
CRP	91.92 $\pm$ 0.10 <sup>b</sup>	0.73 $\pm$ 0.01 <sup>c</sup>	3.00 $\pm$ 0.01 <sup>b</sup>	0.01 $\pm$ 0.01 <sup>c</sup>	1.10 $\pm$ 0.01 <sup>d</sup>	95.16 $\pm$ 0.03 <sup>a</sup>	392.73 $\pm$ 0.02
CWP	89.90 $\pm$ 0.02 <sup>c</sup>	0.91 $\pm$ 0.03 <sup>b</sup>	10.14 $\pm$ 0.01 <sup>a</sup>	0.19 $\pm$ 0.02 <sup>b</sup>	3.08 $\pm$ 0.01 <sup>b</sup>	85.68 $\pm$ 0.03 <sup>d</sup>	384.99 $\pm$ 0.02

Data are presented as mean  $\pm$  standard deviation. The data value of each parameter with different superscript letters in the rows is significantly different (LSD,  $p \leq 0.05$ ). d. b.= dry basis.

Table 3. Regression coefficients of the models, significance levels, analysis of variance, and predicted/experimental values for the gluten-free pasta made from broken rice and nopal.

	WG	CT	TPC	FLDL	BLDL
<b>Intercept</b>	214.74	9.19	1.12	67.37	43.59
<b>Linear</b>					
ET	2.46 (0.01)	-0.51 (0.021)	0.07 (< 0.001)	-0.65 (0.237)	6.77 (0.33)
MC	-2.54 (0.009)	-1.89 (< 0.001)	0.17 (< 0.001)	3.86 (< 0.001)	-0.23 (0.54)
NFC	-2.88 (0.005)	-1.29 (< 0.001)	0.39 (< 0.001)	8.98 (< 0.001)	2.2 (< 0.001)
<b>Quadratic</b>					
ET	4.84 (< 0.001)	0.25 (0.201)	–	-2.36 (0.009)	–
MC	6.43 (< 0.001)	1.14 (< 0.001)	–	-3.12 (< 0.001)	–
NFC	-2.93 (0.004)	0.51 (0.018)	–	-0.67 (0.214)	–
<b>Interactions</b>					
ET*MC	6.44 (< 0.001)	0.62 (0.03)	0.02 (0.07)	1.59 (0.042)	-0.03 (0.98)
ET*NFC	1.22 (0.075)	0.12 (0.624)	0.09 (< 0.001)	2.39 (0.005)	-0.01 (0.98)
MC*NFC	-1.35 (0.055)	0.87 (0.005)	0.16 (< 0.001)	-0.53 (0.451)	0.59 (0.24)
R <sup>2</sup>	0.90	0.91	0.98	0.95	0.74
CV (%)	10.72	6.68	5.78	3.05	3.17
p of F (model)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Lack of fit	0.52	0.09	0.06	0.09	0.074
<b>Optimization</b>					
Predicted values	231.79	8.92	1.62	79.96	–
Experimental values	234 ± 2.8	9.5 ± 0.3	1.67 ± 0.06	75.12 ± 4.3	–

ET = extrusion temperature; MC = moisture content; NFC = nopal flour content; WG = weight gain (%); CT = cooking time (min); TPC = total phenolic compounds (mg GAE/g d. b.); FLDL = free low-density lipoprotein inhibition (%); BLDL = bound low-density lipoprotein inhibition (%); Numbers within brackets indicate significance levels, dashes indicate terms of model non-used.

### 3.3 Weight gain (WG)

The percentage of weight gain in pasta is related to the water absorption capacity of the starch. The behavior of pasta after cooking is one of the most important quality parameters for consumers, including weight gain in conjunction with cooking time. To be classified as good quality, pasta must present a weight gain above 200% (Pagnussatt *et al.*, 2014). The effect of ET and NFC on the WG of pasta at an MC = 28% is shown in Figure 1a. The highest WG values (> 220%) were obtained at low and high ET combined with high NFC (> 15%). This behavior can be attributed to the fact that there is a higher proportion of soluble dietary fiber at high NFC, which is related to the water retention capacity within the pasta structure. Albuja-Vaca *et al.* (2020) reported a similar behavior in a gluten-free pasta based on rice flour with the addition

of lupine flour, reporting values of WG from 158.35 to 285.47%, related to the high-water absorption capacity of lupine flour. On the other hand, the effect of the interaction ET and MC (NFC = 12%) on the WG of pasta is shown in Figure 1b. It can be observed that the highest values of WG were obtained at low MC (< 25%) combined with low ET (< 107 °C). At low MC and ET, there could have been an elevated modification of the structural components of the pasta, mainly starch granules, due to less fluidity and an increase in friction and viscosity inside the extruder (Hernández-Medina *et al.*, 2008). Also, at high MC (> 32%) combined with high ET (> 117 °C) the levels of gelatinization could have increased. The above could have caused increased water absorption in the pasta, favoring an increase in its weight and volume. The water absorption of the pasta depends on the weakness

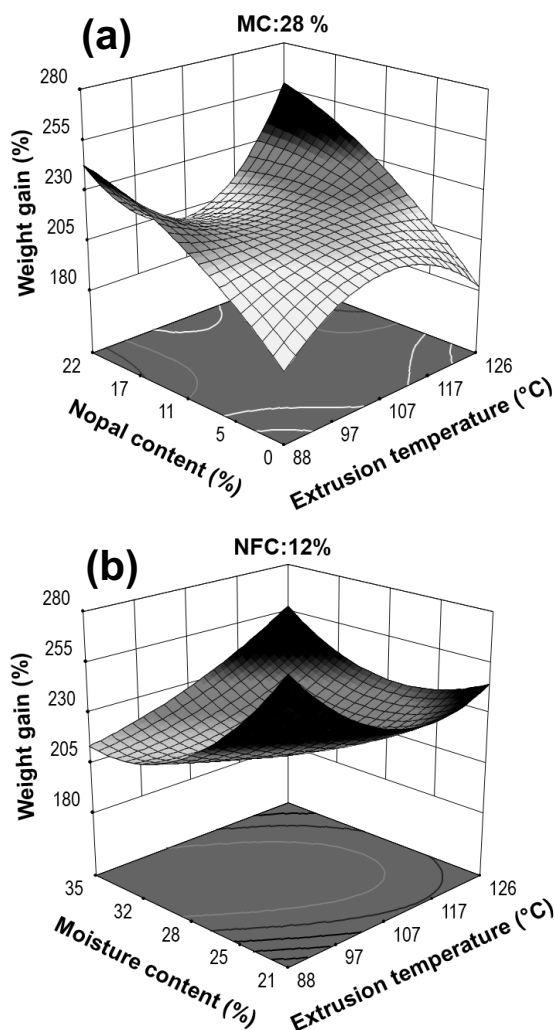


Figure 1. Effect of the extrusion temperature and the nopal content (a), and effect of the extrusion temperature and the moisture content (b) on the weight gain (%) of gluten-free pasta.

of the starch granules and is related to the amount of damaged starch. Faheid *et al.* (2022) obtained values of WG in a range of 159-190% in a gluten-free pasta made from rice flour, white corn flour, potato starch, soy protein isolate, and *psyllium* husk. The previous range of values is lower than that obtained in the present study.

### 3.4 Cooking time (CT)

One of the parameters contributing the greatest to consumers' high demand for pasta is its quick cooking. The cooking time for the pasta is the required time to obtain a complete gelatinization of the starch (Sissons *et al.*, 2022). The effect of MC and NFC on the CT at a constant ET = 107 °C is shown in Figure 2a. It can be observed that the lowest CT values (< 12 min) were obtained at high MC (> 28%) and high NFC (> 17%). This behavior can be attributed to the fact that as MC and NFC increase, there is a decrease in the

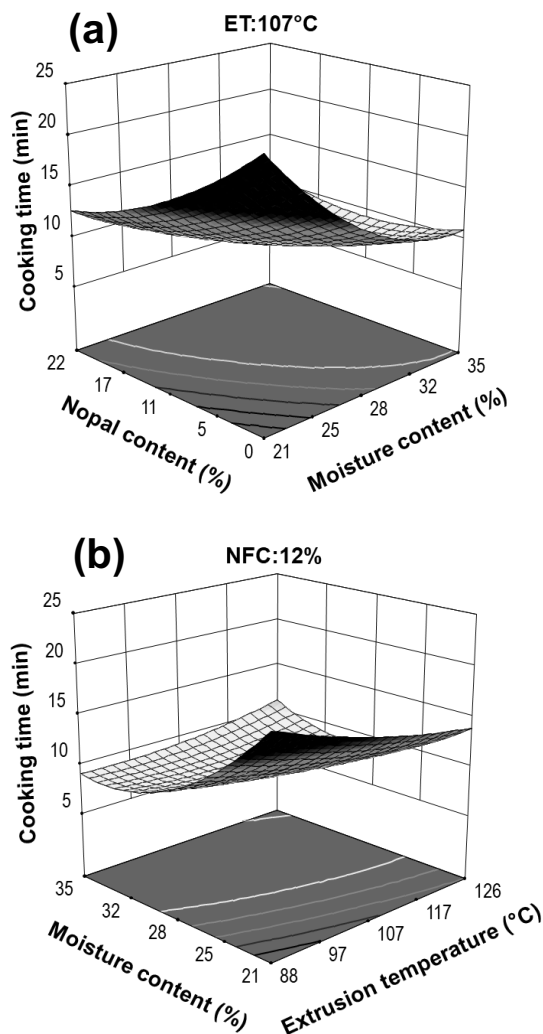


Figure 2. Effect of the moisture content and the nopal content (a), and effect of the extrusion temperature and the moisture content (b) on the cooking time (min) of gluten-free pasta made from broken rice and nopal.

expansion index (EI) of the pasta. A reduction in the EI increases the diffusion of water through the pasta matrix, reducing the time that the water and heat need to reach the center of the pasta during the cooking process (Granito *et al.*, 2014). The CT influences the texture and flavor of pasta; when it is not well cooked, its surface is hard, and its taste is characteristic of the flour used as raw material. Whereas, if the cooking time is longer than required, the pasta disintegrates, presenting a very soft and sticky texture and a color change, which consumers consider unpleasant (Samaan *et al.*, 2006). Also, the effect of the interaction between ET and MC (NFC= 12%) on the CT is shown in Figure 2b. It can be seen that the lowest CT values were obtained at low ET (< 107 °C) and high MC (> 28%). This behavior could be because as the moisture increased, the gelatinization process was more efficient due to the more significant interaction of water with amylose



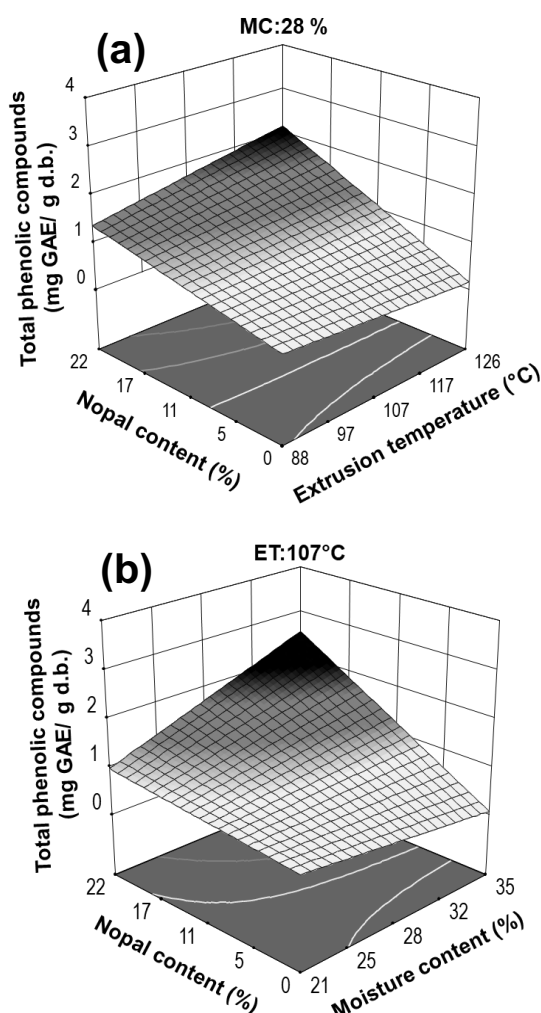


Figure 3. Effect of the extrusion temperature and the nopal content (a), and effect of the moisture content and nopal content (b) on the total phenolic compounds content (mg GAE/g d. b.) of gluten-free pasta.

and amylopectin chains. The amylose and amylopectin molecules in rice-based pasta play a key role in creating a starch network, which explains the integrity of the pasta texture after cooking (Marti *et al.*, 2010). Manthey *et al.* (2004) evaluated different levels of hydration of the raw materials (29, 30, 31, and 32%) on the cooking quality of pasta made with semolina and buckwheat (30%), finding cooking times from 9.3 to 10.8 min. Likewise, various authors have evaluated the cooking time in gluten-free pasta, reporting values of 8.5 min (Sereewat *et al.*, 2015) and  $13.8 \pm 0.1$  min (Criollo-Feijoo *et al.*, 2018), which are similar to those found in the present investigation work.

### 3.5 Total phenolic compounds (TPC)

Figure 3a shows the effect of the extrusion temperature (ET) and nopal flour concentration (NFC) on the total phenolic compounds (TPC) content of gluten-free pasta at a constant moisture content (MC = 28%). It can be seen that the highest TPC values ( $> 1.81$  mg

GAE/g db) were found by combining high ET ( $> 107$  °C) with high NFC ( $> 17\%$ ). The TPC presented a high positive Pearson correlation with the NFC factor ( $r = 0.86$ ,  $p \leq 0.001$ ). This behavior could be due to the high TPC value of the raw material nopal flour (12.33 mg GAE/g d.b.), which was higher than the TPC value obtained in the raw material broken rice flour ( $0.98 \pm 0.42$  mg GAE/g d.b.). According to Guevara-Figueroa *et al.* (2020), 4-Hydroxy benzoic acid, ferulic acid, and salicylic acid are the main phenolic acids in nopal. Also, iso-quercitrin, isorhamnetin 3-O-glucoside, nicotiflorin, rutin, and narcissin are the predominant flavonoids in this food. These authors mentioned that consuming these phenolic compounds has potential benefits for health. Also, Pang *et al.* (2018) reported that the phenolic compounds mostly present in white rice are phenolic acids, mainly in bound form, and ferulic acid, *p*-coumaric acid, and isoferulic acid are the most abundant. Likewise, the high content of these compounds in foods is positively correlated with their antioxidant activity. In this work, during the extrusion process, high temperatures and shearing generate a loss of structures due to the breaking of the bonds, which could have caused the release of phenolic compounds, making them more available for extraction and quantification (Herrera-Cazares *et al.*, 2021). On the other hand, Figure 3b presented the effect of MC and NFC on the TPC of pasta at a constant ET = 107 °C. It can be observed that the highest TPC values were found by combining high MC ( $> 28\%$ ) with high NFC ( $> 17\%$ ). This behavior could be due to the high MC in the samples that caused a lubricating effect during the extrusion process, provoking less thermomechanical damage of TPC (Ozer *et al.*, 2006). Also, some compounds present in nopal flour (mostly mucilages) have a lubricating effect (Pai *et al.*, 2009), which could have increased the fluidity of the mixtures in the extruder barrel, generating less heat degradation of the phenolic compounds.

### 3.6 Antioxidant activity-inhibition of oxidation of low-density lipoproteins (LDL)

Cardiovascular diseases related to atherosclerosis and increased LDL cholesterol values are the leading cause of mortality worldwide. A diet rich in antioxidants could reduce the incidence of cardiovascular diseases (Quiñones *et al.*, 2012). Figure 4a shows the effect of ET and NFC on inhibiting low-density lipoprotein oxidation (FLDL) from the free extract in gluten-free pasta at a constant MC = 28%. The highest values of FLDL ( $> 80.46\%$ ) were found at intermediate/high ET ( $> 97$  °C) and high NFC ( $> 17\%$ ). The FLDL presented a high positive Pearson correlation with the NFC factor ( $r = 0.82$ ,  $p \leq 0.001$ ) and TPC ( $r = 0.86$ ,

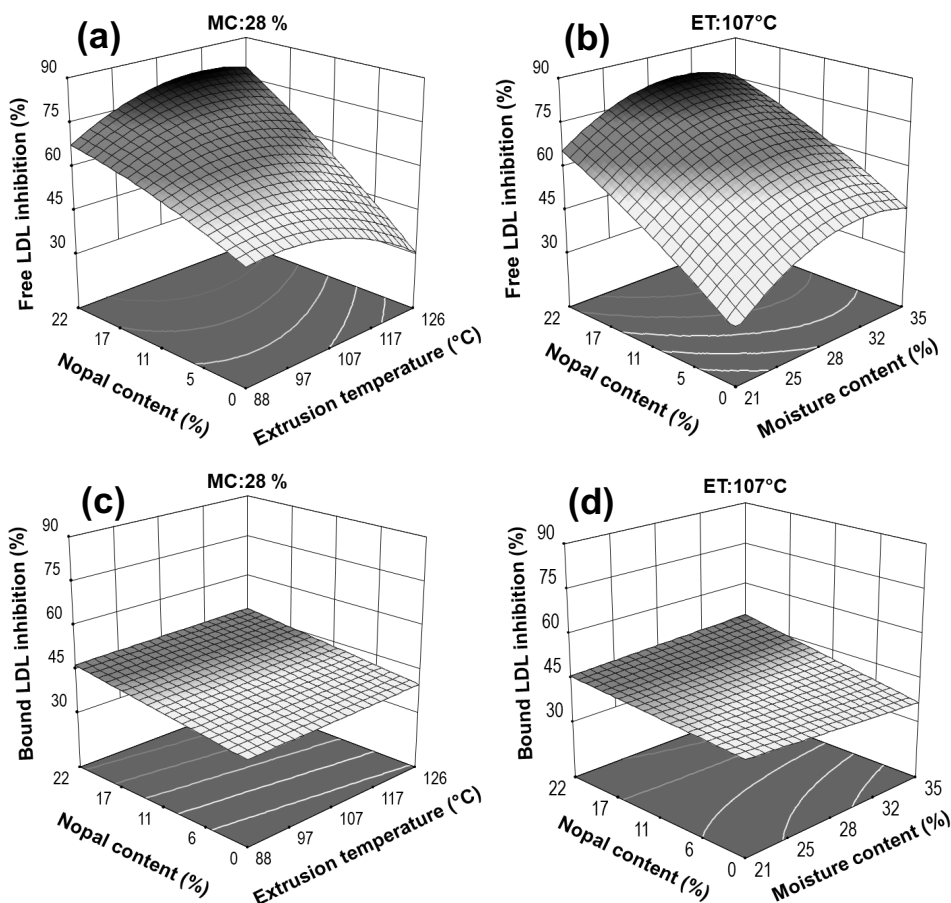


Figure 4. Effect of the extrusion temperature and the nopal content on the inhibition of oxidation of low-density lipoprotein from free extracts (a) and the inhibition of oxidation of low-density lipoprotein from bound extracts (c); effect of the moisture content and the nopal content on the inhibition of oxidation of low-density lipoprotein from free extracts (b) and the inhibition of oxidation of low-density lipoprotein from bound extracts (d).

$p \leq 0.001$ ). This behavior could be attributed to the phenolic compounds present in the nopal flour and its release by the ET, as well as the antioxidant activity of the raw materials, nopal flour ( $88.14 \pm 0.42\%$ ) and broken rice flour ( $40.26 \pm 0.13\%$ ). The phenolic compounds, including flavonoids and phenolic acids, have a high capacity to inhibit the oxidation of human low-density lipoproteins in vitro (Zunft *et al.*, 2001). The obtained results agree with what was reported by Rocha-Guzmán *et al.* (2012), who studied the LDL in extruded snacks produced from corn starch, yellow-corn flour, and pumpkin flour, where they found the best conditions at high ET and high levels of pumpkin flour. Figure 4b shows the effect of MC and NFC on the FLDL from the free extract in a gluten-free pasta at intermediate ET = 107 °C. The highest values were found at medium/high MC (> 25%) combined with high NFC (> 17%). Also, at high MC, the viscosity inside the extruder could have been reduced, presenting greater fluidity of the samples and decreasing the residence time, which caused less affection in the phenolic compounds and antioxidant capacity. Similar results were reported by Delgado-

Nieblas *et al.* (2017), presenting an inhibition value of  $67.13 \pm 6.71\%$  in Cehualca winter squash flours, which were subsequently used to produce extruded third-generation snack foods. On the other hand, Figure 4c shows the effect of ET and NFC (MC = 28%) on the BLDL (bound fraction) values. The highest values of BLDL (> 48%) occurred at high ET (> 117 °C) combined with high NFC (> 17%). This behavior might be because the ET could have reduced the viscosity and residence time of the materials in the extruder (Singha *et al.*, 2018), and the high polyphenols content provided by the nopal flour, being capable of providing health benefits (Moussaoui *et al.*, 2022). Likewise, Figure 4d shows the effect of MC and NFC (ET= 107 °C) on the BLDL values from bound extracts, presenting the highest values (> 48%) at high MC (> 28%) combined with high NFC (> 17%). This behavior could be due to the protective effect caused by the high levels of MC during the extrusion process of samples and the phenolic compounds provided by the nopal flour. This behavior agrees with what was reported by Álvarez-Parrilla *et al.* (2012), who studied the LDL in extracts of serrano pepper and

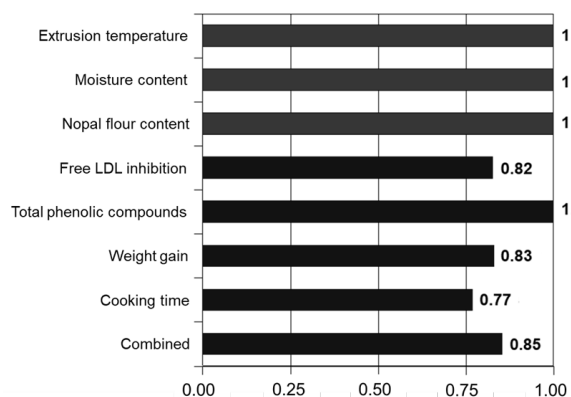


Figure 5. Individual and global desirability for the evaluated responses during the extrusion process optimization.

jalapeño pepper, noting that the phenolic acids and flavonoids present in the extracts can act as natural antioxidants against the oxidation of LDL in humans. Also, Thilakarathna *et al.* (2013), studied extracts of apple peel rich in phenolic compounds (quercetin), reporting that consuming these extracts reduced the plasmatic concentrations of oxidized LDL in obese people who presented metabolic syndrome.

### 3.7 Optimization

An optimization process was carried out to find the conditions of ET (°C), MC (%), and NFC (%) to obtain the gluten-free pasta with the best phytochemical, antioxidant, and cooking properties. The criteria used for the optimization were maximizing the values of WG, TPC, and FLDL and minimizing the CT values. A global desirability value of 0.85 was obtained (Figure 5). According to Fabila-Carrera (1998), a desirability value of 0.6 is considered acceptable. The previous global desirability value corresponds to the conditions: ET= 118 °C, MC = 31.7%, and NFC = 18%, with values predicted by the mathematic models for WG= 231.79%, CT= 8.92 min, TPC=1.62 mg GAE/g d.b, and FLDL= 79.96% (Table 3). Two experimental tests were carried out to obtain the gluten-free pasta with the addition of nopal flour using the optimal conditions (OP). WG, CT, TPC, and FLDL were evaluated in the optimal pasta (OP), obtaining experimental values of  $234 \pm 2.8\%$ ,  $9.5 \pm 0.3$  min,  $1.67 \pm 0.06$  mg GAE/g d.b., and  $75.12 \pm 4.3\%$ , respectively. These values were compared with the predicted values of the mathematic models during the validation. The values predicted by the models and the experimental values obtained in the validation were similar. Also, the WG value =  $234 \pm 2.8\%$  obtained was higher ( $p \leq 0.05$ ) concerning commercial pasta produced from rice (CRP,  $220.0 \pm 0.01\%$ ) and commercial pasta made from wheat (CWP,  $180 \pm 0.4\%$ ). Whereas the experimental value of CT ( $9.50 \pm 0.3$  min) was lower ( $p \leq 0.05$ ) than that obtained in CRP ( $11.0 \pm 0.2$  min) and CWP ( $12.0$

$\pm 0.1$  min). Also, the TPC value of  $1.67 \pm 0.06$  mg GAE/g d. b. was higher ( $p \leq 0.05$ ) than that found in the CRP ( $0.4$  mg GAE/g d. b.) and CWP ( $0.6$  mg GAE/g d. b.). Whereas the FLDL value of  $75.12 \pm 4.3\%$  was higher ( $p \leq 0.05$ ) than that found in CRP ( $34.06 \pm 2.1\%$ ) and CWP ( $36.89 \pm 0.5\%$ ). Therefore, the mathematical models used in each response are valid to find the optimal processing conditions for gluten-free pasta with appropriate phytochemical, antioxidant, and cooking properties.

### 3.8 Dietary fiber (DF)

The DF is an essential component of the diet for optimal health, and its consumption has been related to preventing non-communicable diseases such as diabetes, cardiovascular diseases, and gastrointestinal disorders (Almeida-Alvarado *et al.*, 2014). The DF values of the products obtained in the different processing stages (optimal unprocessed raw mix, optimally processed product (OP)) for getting gluten-free pasta are shown in Table 4. The OP presented the highest value ( $7.24 \pm 0.11\%$ ) of insoluble dietary fiber (IDF) concerning the control (CP) and commercial rice pasta (CRP). Also, there was a significant increase ( $p \leq 0.05$ ) of 14.92% due to the extrusion process concerning the optimal unprocessed raw mix. This behavior could be because the shear causes mechanical stress during the extrusion process, breaking the fibrous matrix's glycosidic bonds, thus increasing the fiber content (Bender *et al.*, 2019). Likewise, the OP presented the highest value of SDF concerning the control pasta and the commercial pasta. Also, there was a significant increase ( $p \leq 0.05$ ) of 53.51% concerning the optimal unprocessed raw mix. This increase could be because, during the extrusion process, high temperatures completely disorganize the original structure of materials, and fiber solubilization can occur depending on the severity of the process, increasing at high ET and low MC. Mechanical stress during the process can cause the degradation of the glycosidic bonds of the polysaccharides, leading to the release of oligosaccharides and, consequently, the increase of the soluble fraction (Vitaglione *et al.*, 2008). Likewise, the OP presented the highest total dietary fiber content (TDF.  $12.26 \pm 0.40\%$ ) concerning the CP and CRP. This could be due to the fiber content in the raw materials; broken rice flour presented an IDF value of  $1.33 \pm 0.12\%$ , a SDF value of  $0.93 \pm 0.07\%$ , and a TDF value of  $2.26 \pm 0.19\%$ . Also, nopal flour showed values of  $18.97 \pm 0.03\%$ ,  $14.28 \pm 0.12\%$ , and  $33.25 \pm 0.15\%$  for IDF, SDF, and TDF, respectively. The SDF value of nopal flour is similar to the value reported by Sáenz *et al.* (2010) in nopal flour, whereas the IDF value was lower. The lower value could be due to the nopal plant growth conditions, age, or maturation stage (Hernández-Urbiola *et al.*, 2010). Also, an increase of 28.10% was presented concerning the optimal unprocessed raw mix.

Table 4. Dietary fiber content of the control pasta (CP) and optimal pasta (OP) in the processing stages (unprocessed mixture and extruded product), and dietary fiber content of commercial rice pasta.

Product	Processing stages	dietary fiber (% , d.b)		
		Insoluble dietary fiber	Soluble dietary fiber	Total dietary fiber
Control pasta	Unprocessed mixture	1.43 ± 0.12a	0.98 ± 0.20b	2.41 ± 0.32b
	Extruded product	1.78 ± 0.34a	1.49 ± 0.13a	3.27 ± 0.47a
Optimal pasta	Unprocessed mixture	6.30 ± 0.20b	3.27 ± 0.16b	9.57 ± 0.36b
	Extruded product	7.24 ± 0.11a	5.02 ± 0.29a	12.26 ± 0.40a
Commercial rice pasta		0.68 ± 0.09	0.36 ± 0.18	1.04 ± 0.27

Data are presented as mean ± standard deviation. The dietary fiber values with different superscript letters between unprocessed mixture and extruded product treatments differ significantly ( $p \leq 0.05$ ). d. b.= dry basis.

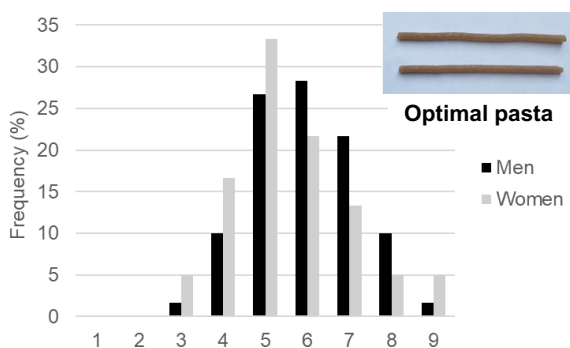


Figure 6. Frequency analysis in the sensory study (general acceptability) (9-point hedonic scale, where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike. 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely).

This behavior agrees with that reported by Stojceska *et al.* (2010), who showed that the extrusion technology could potentially increase the levels of total dietary fiber in gluten-free foods (cereals, fruits, and vegetables). In the present work, the optimal pasta can be classified as “high in fiber”, since its dietary fiber content is higher than 6% (Dello Ruso *et al.*, 2021).

### 3.9 Sensory analysis (SA)

The results for the general acceptability of the gluten-free pasta obtained under optimal processing conditions (ET= 118 °C, MC= 31.7%, and NFC= 18.0%) are shown in Figure 6. The sensory analysis results showed that approximately 85% of the panelists indicated a degree of acceptance  $\geq 5$  of the hedonic scale, of which 44% are men and 41% are women. The mean comparison test showed no statistically significant difference ( $p > 0.05$ ) in the general acceptability of gluten-free pasta between men and women. Regarding the general acceptability of the control pasta (CP) and the commercial rice pasta

(CRP), the results of the sensory analysis showed that approximately 83.2% and 86% of the panelists indicated a degree of acceptance  $>5$  of the HS, respectively. The general acceptability of the OP, CP, and CRP was similar.

## Conclusions

The mathematical models used for the statistical analysis of the different variables of response showed  $R^2_{adjusted}$  values  $\geq 0.90$  (except BLDL),  $p$  of F (model)  $\leq 0.001$ , without presenting a lack of fit ( $p > 0.05$ ). The optimal conditions of the extrusion process obtained with the numerical method were ET= 118 °C, MC= 31.7%, and NFC= 18.0%. The experimental values obtained in validating the optimal processing conditions were similar to the predicted values by the mathematical models in the studied responses. The gluten-free pasta obtained under optimal conditions showed better cooking, phytochemical, and antioxidant properties than commercial pasta. In addition, they showed an important content of TPC, antioxidant activity, and dietary fiber, contributed mainly by the nopal flour. The results obtained in the present study suggest that adding nopal flour was an effective strategy for getting gluten-free pasta based on broken rice. The results of the present study indicate that it is possible to obtain gluten-free pasta based on broken rice by adding nopal flour with excellent properties and sensory acceptability. The consumption of these foods could eventually positively affect consumers' health.

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