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Production of extruded amaranth flour as an ingredient for the preparation of gluten-free bread.

Elaboración de harina de amaranto extrudido como ingrediente para la elaboración de pan libre de gluten.

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ABSTRACT

Gluten-free bread (GFB) is primarily made from grains and starch, making it nutrient-deficient. The objective was to optimize the extrusion conditions [extrusion temperature (ET) and screw speed (SS)] and the inclusion level of extruded amaranth flour (EAF) to develop a mixture composed of rice flour-corn starch (RF/CS) and EAF suitable for producing GFB with better nutritional quality and adequate techno-functional/ sensory properties. A 3-factor, 9-response rotatable composite central design was used; 20 treatments. Response surface methodology was applied as an optimization technique. The optimal conditions found (ET = 89 °C, SS = 74 rpm, and EAF = 15 %) resulted in a high protein content bread (8.3 %), acceptable specific volume (2.3 mL/g), and sensory properties (crumb color = 74.9, crust color = 70.4, crumb texture = 69.7, crust texture = 68.6, bread softness = 71.7, bread flavor = 71.0, and overall bread acceptability = 71.9) evaluated between “I like it moderately” and “I like it a lot”. The results found show the impact of the optimized EAF as an ingredient for the production of nutritionally improved and sensorially acceptable GFB.

KEY WORDS: *Amarantus hypochondriacus*, extrusion, bread for celiacs, optimization, techno-functional properties, sensory evaluation

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RESUMEN

El pan sin gluten (GFB) se elabora principalmente a partir de cereales y almidón, lo que lo hace deficiente en nutrientes. El objetivo fue optimizar las condiciones de extrusión [temperatura de extrusión (ET) y velocidad del tornillo (SS)] y el nivel de inclusión de la harina de amaranto extrudido (EAF) para desarrollar una mezcla compuesta de harina de arroz-almidón de maíz (RF/CS) y EAF adecuada para elaborar GFB con mejor calidad nutricional y propiedades tecno-funcionales/ sensoriales adecuadas. Se utilizó un diseño central compuesto rotable de 3 factores y 9 respuestas; 20 tratamientos. Se aplicó metodología de superficie de respuesta como técnica de optimización. Las condiciones óptimas encontradas (ET = 89 °C y SS = 74 rpm y EAF = 15 %) dieron como resultado un pan con contenido de proteína alto (8.3 %), volumen específico aceptable (2.3 mL/g) y propiedades sensoriales (color de la miga = 74.9, color de la corteza = 70.4, textura de la miga = 69.7, textura de la corteza = 68.6, suavidad del pan = 71.7, sabor del pan = 71.0 y aceptabilidad global del pan = 71.9) evaluadas entre “me gusta moderadamente” y “me gusta mucho”. Los hallazgos de esta investigación resaltan el potencial de la EAF optimizada como componente para mejorar el valor nutricional y la aceptabilidad sensorial del GFB.

PALABRAS CLAVE: *Amarantus hypochondriacus*, extrusión, pan para celíacos, optimización, propiedades tecno-funcionales, evaluación sensorial

Introduction

Celiac disease (CD) affects around 1 % of the global population (Caio *et al*., 2019). Its prevalence has been reported to be higher among women and increases in individuals with affected first-degree relatives (Fasano & Catassi, 2012). The only effective treatment for CD is lifelong adherence to a strictly gluten-free diet (Caio *et al*., 2019). This means avoiding consuming any food or product containing wheat, barley, rye, and their derivatives. Gluten-free food production mainly relies on several starch types and flour from gluten-free cereals, such as corn and rice (Ziena *et al*., 2019).

Due to the absence of gluten, gluten-free bread (GFB) has significantly different sensory characteristics compared to wheat bread. GFB typically has low volume, a crumbly texture, a pale color, an unpleasant taste, and rapid hardening (Masure *et al*., 2016). Corn starch is employed

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to improve the GFB sensory properties due to gelatinization, gas retention, and lower CO_2 loss and crust collapse (Sciarini *et al*., 2016). However, adding starches to GFB reduces its nutritional value, including protein, fiber, and minerals. A solution to this issue is the incorporation of highly nutritious flours made from legumes and/or pseudocereals.

In this regard, several studies have explored the impact of including legumes and pseudocereals on the nutritional and sensory qualities of GFB (Rybicka *et al*., 2019; De Aguiar *et al*., 2022). Authors found that incorporating raw amaranth flour into bread improves its nutritional quality but decreases the sensory acceptability, mainly due to its taste. Amaranth flour is an excellent alternative because amaranth is a pseudocereal with high nutritional value, containing high levels of proteins, fats, fiber, and minerals (Soriano-García & Aguirre-Díaz, 2019). Amaranth proteins are relatively rich in lysine and tryptophan compared to FAO (2013) standards (Juan *et al*., 2007). To enhance the nutritional, techno-functional, and sensory characteristics of amaranth, it is subjected to heat treatment, such as extrusion cooking.

The extrusion process causes significant biochemical changes, including protein denaturation, starch gelatinization, lipid modifications, increased soluble dietary fiber, and the inactivation of microorganisms and enzymes. Among these changes, extrusion variables, particularly temperature, have a significant effect. Extrusion temperature has an integral impact on the final properties of extruded foods. It is crucial to carefully adjust this parameter to optimize the texture, flavor, nutritional value, and other product sensory properties (Moreno *et al*., 2018). The temperature is controlled based on specific ingredients and the desired characteristics of the final product, focusing on achieving the optimal balance between nutritional quality and sensory appeal. Additionally, controlling the extrusion process conditions is crucial, as the Maillard reaction can affect the nutritional value of proteins, depending on the type of material, composition, and process conditions (Singh *et al*., 2007). Therefore, careful control of extrusion conditions is essential to enhance the nutritional and sensory quality.

Several studies have used extrusion as a cooking method to improve the nutritional value of extruded flour. A study involved obtaining cooked corn tortillas fortified with 30 % extruded amaranth flour (Gámez-Valdez *et al*., 2021), showing higher protein content, dietary fiber, protein digestibility, and protein chemical quality compared to regular tortillas. Extruded amaranth flour addition did not significantly affect the sensory properties of the tortillas. Moreover, extrusion has been used to improve the specific volume and sensory properties of rice flour as an ingredient in the preparation of GFB (Clerici *et al*., 2009). However, there are no reports in the literature on the impact of the extrusion process in improving the nutritional, techno-functional, and sensory properties of amaranth as an ingredient in the production of GFB.

This work aimed to optimize the extrusion conditions and the inclusion level of amaranth flour to develop a mixture of rice flour, corn starch, and extruded amaranth flour suitable for producing gluten-free bread with improved nutritional value and physicochemical, techno-functional, and sensory properties.

Material and Methods

Elaboration of Extruded Amaranth Flour

Amaranth grains (*Amaranthus hypochondriacus*) of the Nutrisol variety (2021 harvest) were acquired from the DEMEGARCIA company in Atlixco, Puebla. The extruded amaranth flour (EAF) was obtained following the methodology described by Gámez-Valdez *et al*. (2021). Distilled water was added to the amaranth flour to get a moisture content of 28 %. Extrusion was carried out using a laboratory single-screw extruder, Model 20 DN (CW Brabender Instruments, Inc., NJ, USA), equipped with a 19 mm diameter screw, 20:1 length/diameter ratio, a nominal compression ratio of 1:1, and a 4 mm die. The extruder operating conditions were: Extrusion temperature (ET) = 70-170 °C and screw speed (SS) = 50-240 rpm. The feed rate was 70 g/min; the extruded amaranth was cooled down at 25 °C, ground (LM 3100, USA) (80-US mesh = 0.177 mm), and stored at 4 °C.

Preparation of Mixtures and Bread

For bread preparation, mixtures were made containing 75 % rice flour (RF), corn starch (CS) ranging from 0 % to 24.5 %, and extruded amaranth flour (EAF) ranging from 0.5 % to 25 %. For bread production, 420 g of the mixture was used, along with 5 g of yeast, 16 g of sugar, 5 g of salt, 3 g of baking powder, 5 g of xanthan gum, 410 g of milk, 16 g of oil, 7 g of vinegar, and 2 eggs (each ingredient was weighed separately). The ingredients were mixed for 8 minutes (at low speed) with a handheld mixer (Black & Decker®). The dough was then left to ferment (60 min / 25 °C) and baked at 180 °C for 40 minutes in a conventional stove oven (Koblenz®). Finally, the bread was allowed to cool at room temperature before the evaluation.

Evaluation of Response Variables

Protein Content and Specific Volume

The protein content was determined using the Micro Kjeldahl Method (Method 960.52; AOAC 2012), applying a conversion factor of 6.25 to the bread samples. The specific volume was determined by dividing the volume of the sample by its weight (cm^3/g), according to AACC Method 10-05 (1995).

Sensory Properties

To evaluate sensory perception, a panel of 50 untrained judges was selected, including both sexes, aged between 18 and 35 years, who were regular consumers of wheat bread. The evaluated attributes were: crumb color (CmC), crust color (CsC), crumb texture (CmT), crust texture (CsT), bread softness (BS), bread flavor (BF), and global acceptability of the bread (GAB). The evaluation was conducted using a LAM (labeled affective magnitude) scale, which

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is an 11-point hedonic scale ranging from -100 (indicating the greatest possible dislike) to +100 (indicating the greatest possible liking). The midpoint of the scale represents zero, indicating a neutral opinion (Pohjanheimo, 2010). These values were then transformed to a scale of 0 to 100, where 0 represents the greatest imaginable dislike, 100 represents the greatest imaginable liking, and 50 indicates a neutral opinion (neither like nor dislike) (Cardello & Schutz, 2004).

Experimental Design, Statistical Analysis, Optimization, and Validation

Experimental Design: A central composite rotatable design with three factors and five levels of variation was employed (2 factorial levels, 2 axial levels, and 1 central level) [extrusion temperature (ET = 70, 90, 120, 150, and 170 °C), screw speed (SS = 50, 88, 145, 201, and 240 rpm), and % inclusion of EAF (0.5, 5.5, 12.75, 20, and 25)]. From the combination of the factor levels, 15 different treatments were obtained (8 factorial treatments, 6 axial treatments, and 1 central treatment); the central treatment (ET = 120 °C, SS = 120 rpm, and % EAF = 12.75) was replicated 5 times to estimate the lack of fit in the model. In total, the experimental design consisted of 20 treatments (**Table 1**). The response variables were protein content (PC), specific volume (SV), crumb color (CmC), crust color (CsC), crumb texture (CmT), crust texture (CsT), bread softness (BS), bread flavor (BF), and global acceptability of the bread (GAB).

Statistical Analysis of Regression and Variance: To investigate the relationship between the factors and response variables (regression models), multiple linear regression using least squares from response surface methodology was employed. To verify the significance of the model parameters (regression coefficients, β), analysis of variance was applied with $\alpha = 0.05$, and the significance of the full regression model was also estimated using $\alpha = 0.05$. The goodness of fit of the regression models was assessed using statistical parameters including the coefficient of determination (R^2), adjusted coefficient of determination ($R^2_{adjusted}$), predicted coefficient of determination ($R^2_{predicted}$), coefficient of variation (CV), and lack of fit probability ($p_{lack\ of\ fit}$). The obtained prediction models were used to graphically represent the system, allowing the graphical analysis of the effect of process variables (factors) on the response variables.

Optimization: The numerical desirability method from the response surface methodology was used for optimization. The nine regression models obtained for the response variables were employed to estimate the theoretical values of the nine response variables at various randomly selected points within the experimental region. These predicted values were transformed into individual desirability values for each response variable [di(X)] using a transformation equation proposed by the response surface methodology, aimed at maximizing or minimizing each response variable. This desirability method involves transforming the predicted values from the mathematical models $\hat{Y}i$ (X) into an individual desirability value [di(X)], which ranges from 0 to 1 and measures the degree of desirability of the response relative to the optimal value sought (in this case, the maximum value for each response). The geometric mean of the individual desirabilities for each response variable was used to determine the global desirability with the mathematical function $D = (d^1 \times d^2 \times d^3 \times d^4 \times d^5 \times d^6 \times d^7 \times d^8 \times d^9)^{1/9}$, where the ideal optimal value is D = 1; however, a D-value between 0.6 and 0.8 is considered acceptable.

Validation: To validate the optimal conditions, five replicas of extruded amaranth flour were prepared under the best process conditions to subsequently produce gluten-free bread (GFB) in quintuplicate with the best inclusion percentage of EAF. Each of the response variables was experimentally evaluated in triplicate. Additionally, to validate the optimal conditions, a theoretical confidence interval for each response was constructed, based on the optimization zone and using a 95 % confidence level. For a model to pass the validation test, the average of the five replicas of each response must fall within the aforementioned theoretical range. The experimental design, statistical analysis, optimization, and validation were performed using Design Expert Software version 11 (Design Expert, 2018; Stat-Ease, Inc., 1300 Godward Street Northeast, Suite 6400, Minneapolis, MN 55413, USA).

Results and Discussion

Results of Response Variables for the 20 Treatments

The results for the response variables showed diverse behavior depending on the treatments (**Table 1**). The protein content (PC) values ranged from 6.7 % to 10.7 %. The highest PC value was observed in Treatment 14, which included the highest percentage of EAF in the GFB, while the lowest value was found in Treatment 9. De Aguiar *et al*. (2022) prepared bread with 60 % amaranth flour / 40 % rice flour, achieving good sensory acceptance with a protein content of 6.9 %. Overall, most of the PC values found in this study were higher; this difference could be associated with the employed amaranth variety, quantity, and type of ingredients used in bread preparation. One of the physical properties of bread that influences consumer acceptance is color. This parameter is affected by non-enzymatic browning reactions that occur between an amino group of proteins and a carbonyl group of simple sugars (Castro *et al*., 2017). This means that a higher protein content in bread favors the development of color, which can influence product preference.

Regarding the specific volume (SV), it ranged from 1.4 to 2.4 mL/g. The highest value was found in treatment 1, which had the lowest percentage of EAF in the GFB. On the other hand, the lowest value was found in treatment 14. The bread with the highest protein content had the lowest specific volume. The SV values in our bread were similar to those reported by Clerici *et al*. (2009), who prepared bread using 90 % raw rice flour and 10 % extruded rice flour with lactic acid, obtaining specific volumes in the range of 1.63 to 2.25 mL/g. This similarity could be due to the employment of rice flour in bread preparation. However, our results were slightly lower than those reported by De la Barca *et al*. (2010), who made GFB with an SV of 3.5 mL/g using 60-70 % popped amaranth flour and 30-40 % raw amaranth flour. This difference might be due to the increased amount of damaged starch in the amaranth associated with the popping and toasting process. Kohyama *et al*. (2022) reported that as the degree of toasting increases, so does the damaged starch, which absorbs more water compared to intact starch. This property affects the texture and moisture retention in baked products.

For CmC, values ranged from 57.93 to 76.60; for CsC, from 63.22 to 73.38; for CmT, from

57.41 to 71.15; for CsT, from 56.67 to 70.25; for BS, from 58.75 to 75.17; for BF, from 57.98 to 72.22; and for GAB, values ranged from 55.78 to 73.12. These values were found on the LAM scale between "like slightly" (55.62) and "like very much" (78.06). It is worth noting that the lowest scores for 4 of the 7 sensory properties (CsC, BS, BF, and GAB) were found in treatment 8, which had severe extrusion processing conditions [ET (149.7 °C), SS (201.5 rpm)] and a high percentage of EAF (20 %). In contrast, the highest scores for the sensory properties CmC, BS, and GAB were found in treatment 16, at the midpoint of the design. We also observed that the acceptance of CsC and CsT was favored by lower extrusion temperatures (treatment 9). In **Table 1**, it can be seen that for CmC, the lowest score was found in treatment 10 (ET = 170 °C) and the highest score in treatment 16 (ET = 120 °C). It is important to state that treatments 10 and 16 have the same SS and EAF values. Therefore, the difference between these two treatments is the temperature at which the amaranth flour was extruded, indicating that this parameter was relevant. Gómez *et al*. (2011) reported that crumb color is affected by the Maillard reaction (which starts around 120 °C) generated during thermal processing for bread making.

Prediction Models and Response Surface Graphs

Table 2 shows the results of the regression analysis; significant experimental mathematical models ($p < 0.0001$) were obtained for each response, with determination coefficients R^2 ranging from 0.8702 to 0.9221. The models showed no significant lack of fit ($p > 0.05$). Furthermore, the relative dispersion of the experimental points concerning the predicted values from the models was less than 10 %. These values indicate that the models were adequate and reproducible. The models included significant linear, quadratic, and interaction terms, and their relative impact on the response can be noted by comparing their coefficients. For model improvement, non-significant terms were removed, and only significant or necessary terms were retained. The analysis of the results focused on 5 out of the 9 response variables (PC, SV, CmC, CmT, and GAB) based on comments obtained in the sensory evaluation, which were directed towards observations concerning the bread crumb. However, CsC, CsT, BS, and BF were also considered to describe the behavior of GAB.

A significant quadratic model was obtained for the PC ($F = 18.06$, $p < 0.0001$). The EAF had a positive effect on the linear term ($F = 13.97$, $p < 0.0001$) and the quadratic term ($F = 7.87$, $p = 0.0149$). The linear term is 141 % greater than the quadratic term, meaning that the linear factor of EAF is twice as relevant for the PC as the quadratic one. This model explained 89.29 % of the total variability ($p < 0.0001$) of the PC values of the GFB. In the quadratic model ($F = 16.55$, $p < 0.0001$) obtained for the SV, the term with the highest coefficient was the SS ($F = 39.75$, $p < 0.0001$), with a negative effect on the response, meaning that a higher SS results in a lower SV. Additionally, the quadratic term of the ET ($F = 33.91$, $p < 0.0001$) had a significant effect on the SV; therefore, SV was favored at intermediate ET levels. For CmC, a quadratic model was obtained ($F = 20.21$, $p < 0.0001$). The negative linear term of ET had the greatest effect ($F = 33.96$, $p < 0.0001$) on the acceptance of CmC, meaning that high ET values decreased the acceptance towards CmC. The predictive model explained 90.32 % of the total variability ($p < 0.0001$) of the bread CmC values. For bread CmT property (evaluated sensorially), another quadratic model was obtained ($F = 14.57$, $p < 0.0001$). The model obtained for CmT was similar to that of CmC. However, in this model, the linear term of EAF had the greatest effect ($F = 34.30$, $p < 0.0001$), meaning that high EAF values decreased the acceptance by CmT. Additionally, the interaction effect of ET*EAF ($F = 19.17$, $p = 0.0006$) was of great importance, so the acceptance by CmT depended on the ET at which the EAF was extruded. For the GAB property, a quadratic model ($F = 14.57$, $p < 0.0001$) similar to that of CmC and CmT was obtained. In this model, the term with the greatest effect was the negative interaction of ET*EAF ($F = 22.47$, $p = 0.0004$), indicating that the effect on GAB of these two independent variables is conditioned by each other.

Once the mathematical models were obtained, response surface and contour plots were generated (**Figure 1**). In **Figure 1B**, it was observed that the highest PC values were found at high EAF contents and intermediate ET, which could be due to a higher presence of damaged starch, resulting in a higher amount of sugars available for yeast fermentation and therefore higher CO^2 production (Schober *et al*., 2005). As a result, the bread had fewer carbohydrates due to their release as CO^2 and more proteins due to a concentration effect. Similarly, it was observed on SV lower values at intermediate ET (**Figure 1E**), and higher PC values at the same ET.

On the other hand, the sensory quality of bread, including its brown color and characteristic flavor, is mainly due to the Maillard reaction, which occurs when the amino group of a protein, amino acid, or peptide reacts with reducing sugars such as glucose and maltose (Camire *et al*., 1990). This reaction, along with caramelization, generates a variety of compounds known as Maillard reaction products (MRP) (Capuano *et al*., 2008). MRP formation is a complex process influenced by factors such as time, temperature, pH, and the specific types of amino acids and sugars present (Liu *et al*., 2020). Lysine and tryptophan are mainly responsible for the intense browning observed in bread during this process (Bertrand *et al*., 2018). In our study, the acceptance scores for CmC [**Figure 1 (G-I)**] and CmT [**Figure 1 (J-L)**] were favored with low to intermediate ET and inclusion percentages below 15 % of EAF in the bread. The decrease in the acceptance of the bread's CmC with the inclusion of EAF at high temperatures could be because, under these conditions, Maillard reactions are promoted (Singh *et al*., 2007), which manifested as color change in the bread's CmC. Additionally, using a lysine-rich pseudocereal such as amaranth in bread formulation, could have contributed to higher browning. Lysine is one of the most reactive amino acids; therefore, with higher lysine content, the Maillard reaction becomes more prominent (Sahagún & Gómez, 2018). On the other hand, the decrease in CmT acceptance with the increase in amaranth flour proportion in the formulation above 15 % EAF was related to the proteins present in the flour, resulting in a gummy bread texture (De Aguiar *et al*., 2022). Finally, the results of the sensory evaluation of GAB were linked to the flavor and characteristics of the bread's crust.

In **Figure 1M**, it can be observed that GAB reaches its maximum values around 95 °C; at temperatures higher than 95 °C, GAB decreases with higher ET. Amaranth produces a nutty flavor at high temperatures, which might be unpleasant in some baked products (Ayo *et al*., 2001). Similarly, GAB decreases with an increase in the SS of the extruder. Dalbhagat *et al*. (2019) reviewed the effect of the extrusion process on rice-based products. They mentioned that at any temperature and with a long residence time (i.e., low screw speed) inside the extruder, starch damage increases, which in turn increases the water absorption index of the food product. Therefore, it can be stated that extruded amaranth flour at low SS could negatively affect the

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volume and sensory attributes of the bread due to its high-water absorption capacity. It can also be observed that at intermediate extrusion temperatures, GAB decreases with the EAF content, starting from a 15 % EAF content (**Figure 1O**). This could be attributed to the fact that at intermediate ET, the addition of high EAF contents (greater than 15 %) increases the hardness of the crust and the number of cracks. It is important to note that such characteristics were classified as undesirable by the judges. The analysis of the GAB graphs [**Figure 1 (M-O)**] indicates that using EAF as an ingredient for GFB production favors the overall sensory acceptance of GFB at ET below 120 °C and low SS.

Table 1. Experimental results of the response variables evaluated for gluten-free bread produced with different inclusion levels of extruded amaranth flour under variable extrusion conditions**.**

	Factors			Response variables								
Treatment[1]	**ET[2]**	**SS[3]**	**EAF[4]**	**PC[5]**	**SV[6]**	**CmC[7]**	**CsC[8]**	**CmT[9]**	**CsT[10]**	**BS[11]**	**BF[12]**	**GAB[13]**
1	90	88	5.5	7.88	**2.42**	72.32	71.25	65.95	67.05	66.91	63.85	67.12
2	150	88	5.5	7.97	2.34	75.32	69.65	**71.15**	68.98	69.48	61.55	70.40
3	90	201	5.5	7.61	1.84	73.80	66.22	68.97	67.34	72.03	69.05	67.12
4	150	201	5.5	8.65	2.00	69.34	65.98	67.67	63.86	70.38	66.33	66.19
5	90	88	20	9.04	2.05	71.52	67.50	69.92	66.55	70.98	**72.22**	71.25
6	150	88	20	8.54	1.86	62.81	65.92	**57.41**	60.04	60.78	60.69	58.13
7	90	201	20	8.45	1.75	68.53	65.47	64.20	68.60	67.38	67.32	66.23
8	150	201	20	9.73	1.92	59.35	**63.22**	57.85	58.20	**58.75**	**57.98**	**55.28**
9	**70**	145	12.75	**6.66**	2.41	73.45	**73.38**	70.43	**70.25**	71.50	68.41	69.21
10	**170**	145	12.75	8.24	2.13	**57.93**	70.37	60.85	**56.67**	64.22	59.42	58.38
11	120	**50**	12.75	8.54	2.13	73.94	67.75	68.33	69.96	70.08	69.29	69.33
12	120	**240**	12.75	9.31	1.41	74.86	64.32	70.97	69.07	68.91	66.95	66.13
13	120	145	**0.5**	8.13	1.90	72.88	64.23	68.60	67.52	68.27	66.33	66.75
14	120	145	**25**	**10.74**	**1.66**	64.45	63.32	59.43	62.98	65.22	65.12	63.05
15	120	145	12.75	8.35	1.94	75.03	69.73	68.09	66.28	72.91	65.83	66.60
16	120	145	12.75	9.23	1.81	**76.60**	71.16	70.11	70.20	**75.17**	69.76	**73.12**
17	120	145	12.75	8.23	1.76	72.67	69.73	68.55	69.52	72.31	70.93	71.40
18	120	145	12.75	8.58	1.90	75.60	70.12	68.66	68.68	73.32	67.13	68.67
19	120	145	12.75	8.75	1.96	74.98	70.28	69.91	66.09	71.55	70.11	69.88
20	120	145	12.75	8.85	1.99	72.73	68.62	66.18	67.62	70.72	67.62	67.82

[1]Rotatable composite central experimental design with three factors and five coded levels of variation; 20 experiments. [2]ET = extrusion temperature (ºC), [3]SS = screw speed (rpm) and [4]EAF = extruded amaranth flour (%), [5]PC = protein content (%, db), [6]SV = specific volume of bread (mL/g), [7]CmC = crumb color, [8]CsC = crust color, [9]CmT = crumb texture, [10]CsT = crust texture, [11]BS = bread softness, [12]BF = bread flavor, [13]GAB = global acceptance of bread.

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Table 2. Coded regression coefficients and analysis of variance of experimental prediction models showing the relationship between process variables (ET, SS and EAF) and response variables (PC, SV, CmC, CsC, CmT, CsT, BS, BF and GAB).

Coefficients	PC[1]	SV[2]	CmC[3]	CsC[4]	CmT[5]	CsT[6]	BS[7]	BF[8]	GAB[9]
Intercept									
β_0	9.72	1.85	74.52	69.85	68.76	68.36	72.68	68.31	69.04
Linear									
β_1	0.3340**	-0.0310^{NS}	-3.33**	-0.7860*	-2.27**	-3.02**	-2.21**	-3.00**	-2.92**
β_2	0.1675^{NS}	-0.1737**	NS	-1.41**	NS	-0.4486^{NS}	-0.1151^{NS}	-0.1142^{NS}	-1.28*
β_3	0.5874**	-0.1045**	-3.13**	-0.9188**	-2.91**	-1.57**	-1.91**	-0.3379^{NS}	-1.91**
Quadratic									
β_{11}	-0.4597**	0.1548**	-3.17**	0.6811*	-1.25*	-1.85**	-1.84**	-1.80**	-1.98**
β_{22}	NS	NS	NS	-1.38**	NS	NS	-1.26*	NS	NS
β_{33}	0.2428*	NS	-2.12**	-2.18**	-1.83**	-1.21*	-2.23**	-1.16*	-1.59**
Interaction									
β_{12}	0.3417*	0.0765^{NS}	NS	NS	NS	-1.16^{NS}	NS	NS	NS
β_{23}	NS	0.0844*	NS	NS	NS	NS	-1.46*	-2.20**	NS
β_{13}	NS	NS	-2.05	NS	-2.85**	-1.92**	-2.47**	-1.98**	-3.30**
p model	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
p lack of fit	0.6674	0.3483	0.1996	0.3780	0.2230	0.5646	0.4424	0.8973	0.8338
CV (%)	3.85	5.20	2.98	1.45	2.76	2.48	2.33	2.37	2.96
R^2	0.8929	0.8842	0.8920	0.9221	0.8702	0.8915	0.9140	0.8964	0.8778
R^2 ajust.	0.8435	0.8308	0.8534	0.8862	0.8239	0.8282	0.8515	0.8360	0.8215
R^2 pred.	0.7085	0.6927	0.7044	0.7661	0.7192	0.6597	0.6725	0.7628	0.7376

[1]PC = protein content (% db), [2]SV = specific volume of bread (mL/g), [3]CmC = crumb color, [4]CsC = crust color, [5]CmT = crumb texture, [6]CsT = crust texture, [7]BS = bread softness, [8]BF = bread flavor, [9]GAB = global acceptance of bread. * Significant at $p \leq 0.05$. ** Significant at $p \leq 0.01$. NS Not significant (P > 0.05).

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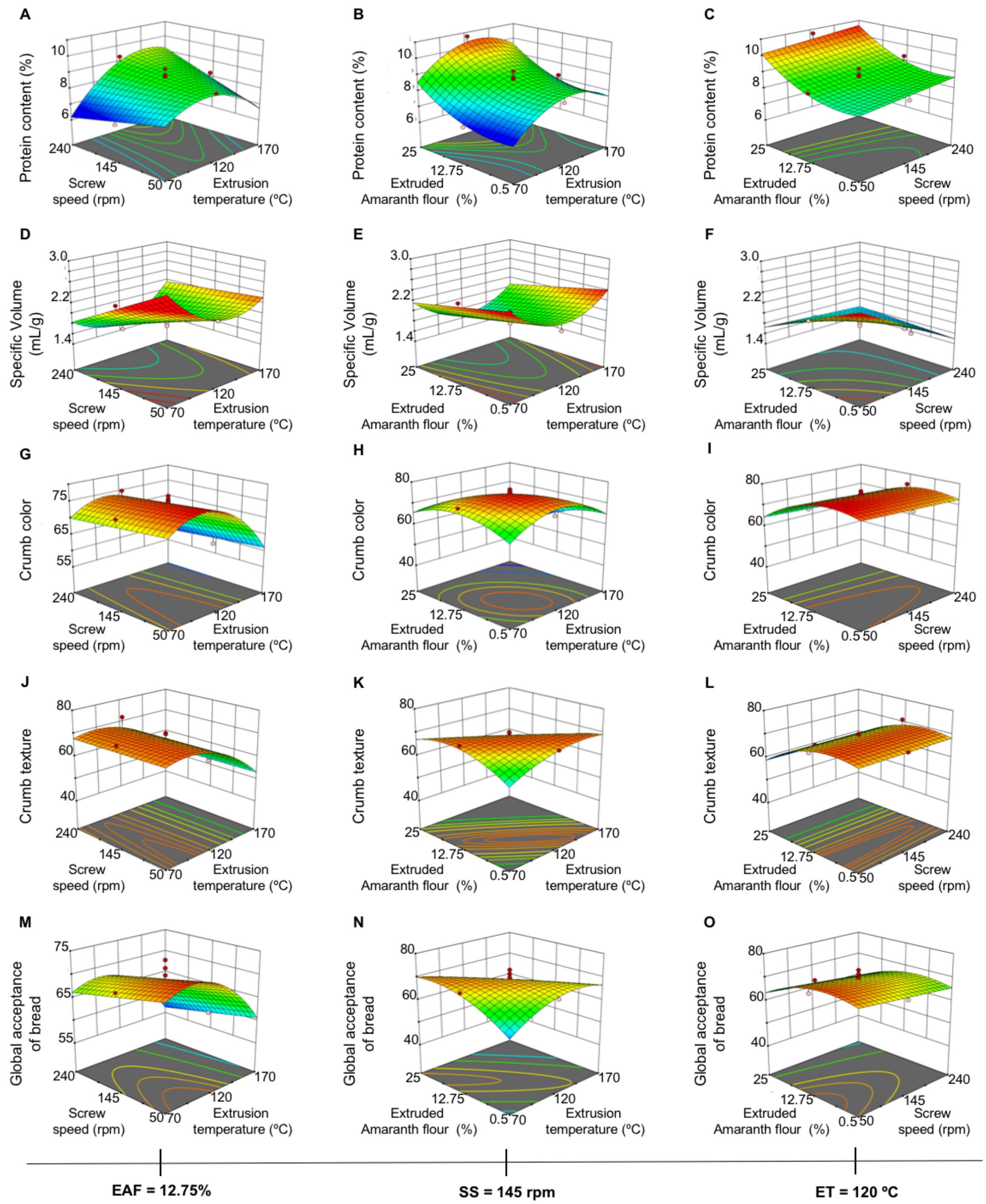

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Figure 1. Response surface and contour graphs showing the effect of the process variables [extrusion temperature (ET), screw speed (SS), extruded amaranth flour (EAF)] on the response variables (A-C) protein content, (D-F) specific volume, (G-I) Crumb color, (J-L) Crumb texture and (M-O) Global acceptance of bread.

3.3. Optimization

The impact of the process variables (ET, SS, and EAF) on the optimization variable Global Desirability (D) can be observed in the contour plots (**Figure 2**). D is a reasonable choice, as if any di(x) = 0, the global desirability would be D = 0, indicating that the bread is not acceptable.

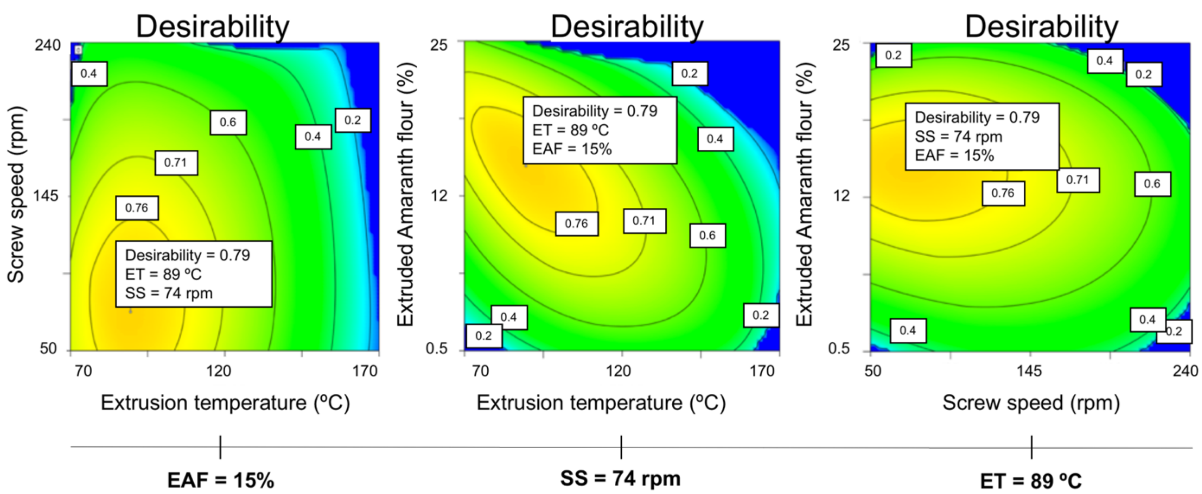

Figure 2. Contour plots showing the effect of process variables (extrusion temperature, ET; screw speed, SS; extruded amaranth flour, EAF) on Global Desirability. The highest Global Desirability (D = 0.79) was selected to obtain the best combination of process variables (ET = 89 ºC/ SS =74 rpm/ EAF = 15 %).

The logical procedure for carrying out the optimization process consisted of estimating response variable values at multiple points within the experimental region by using their respective regression models obtained from the previous regression and variance analysis. The optimal conditions [best values of the process conditions (ET, SS, and EAF)] are obtained using the following criterion: Global desirability (D) values between 0.6 and 0.8 are appropriate for food systems, with the ideal optimal value being D = 1. In this figure, it can be observed that the region with the highest global desirability (D) values is found at extrusion temperatures below 120 °C, extruder screw speeds below 150 rpm, and amaranth flour contents in the formulation, used to make the GFB, between 10 and 20 %. The optimal conditions were obtained by selecting the central point of the mentioned optimization region. The selected optimal Global Desirability value was D = 0.79, which corresponds to the best process conditions. The selected optimal values for

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ET, SS, and EAF to obtain gluten-free bread with the highest possible values of PC, SV, CmC, CsC, CmT, CsT, BS, BF, and GAB were ET = 89 °C, SS = 74 rpm, and EAF = 15 %.

The individual desirability values for each of the response variables associated with the selected optimal Global Desirability were: d^{PC} = 0.41, d^{SV} = 0.88, d^{CmC} = 0.91, d^{CsC} = 0.71, d^{CmT} = 0.89, d^{CsT} = 0.88, d^{BS} = 0.79, d^{BF} = 0.91, d^{GAB} = 0.93, where it can be observed that most of the individual desirabilities were above 0.63, which is considered acceptable and good (López-Ríos & Rudnykh, 2018). The optimal Global Desirability obtained with these individual desirabilities was 0.79. In this case, the global desirability is close to 0.8, indicating that the combination of process variables seems to achieve favorable results for all responses. However, the individual desirability shows that the combination was more effective in maximizing SV, CmC, CsC, CmT, CsT, BS, BF, and GAB than in maximizing PC. Additionally, the optimal values of the process variables were found based on the values predicted by the mathematical models.

The predicted values for protein content (PC) and specific volume (SV) of the GFB (gluten-free bread) with the optimal content (15 %) of extruded amaranth flour (EAF), prepared under the best extrusion conditions (ET and SS), were 9.34 % and 2.30 mL/g, respectively. Meanwhile, the predicted sensory quality attributes for this GFB, prepared with the optimal process conditions, were: crumb color (CmC) = 74.9, crust color (CsC) = 70.4, crumb texture (CmT) = 69.7, crust texture (CsT) = 68.6, bread softness (BS) = 71.7, and bread flavor (BF) = 71.0. The global acceptability of the bread (GAB) was positively correlated with the aforementioned sensory attributes, achieving a value of 71.9 for this sensory attribute, which falls between “like moderately” and “like very much” on a LAM scale.

Validation

Table 3 shows the predicted values from the mathematical models, the 95 % confidence intervals obtained from such models, and the results of the response variables evaluated for the bread obtained from the five experimental replicas using the found optimal condition. When comparing the experimental results with the predicted values, it was observed that the latter fell within the theoretical range, suggesting that the optimal conditions were appropriate and reproducible. This indicates that the model used was able to accurately predict the results observed experimentally, which supports the validity and usefulness of the established conditions.

Díaz-Corona *et al*. (2024) evaluated the effect of optimized hot water treatment on the preservation of fresh eggplants, finding that the optimal condition application was the most suitable for maintaining the eggplant quality. In this article, they performed optimization and validation similar to what we reported in the present manuscript. Similarly, Félix-Medina *et al*. (2020) conducted the optimization and validation of extrusion process variables and the inclusion level of bean flour to develop second-generation snacks (directly expanded); these researchers also validated the optimal process conditions, finding them to be appropriate and reproducible. Menchaca-Armenta *et al*. (2020) used optimization to determine the best extrusion conditions for producing extruded nixtamalized corn flours and to prepare tortillas with good techno-functional and sensory properties. In this study, the optimization results also passed the validation test. The results of these studies demonstrate the robustness of the response surface methodology as an optimization technique for finding the best process conditions in various fields (e.g., hydrothermal treatment, extrusion, etc.), which were appropriate and highly reproducible, as the optimal conditions passed the validation test.

Conclusions

In this study, it was found that both extrusion temperature and screw speed are crucial factors in the extrusion cooking process of amaranth flour. These parameters significantly influence the final characteristics of gluten-free bread (GFB). The optimal extrusion conditions for amaranth flour and its optimal inclusion percentage of extruded amaranth flour (EAF) in the mixture were determined to produce GFB with improved nutritional, techno-functional, and sensory properties. Additionally, this mixture represents an alternative flour not only for celiac patients but also for those with other adverse food reactions, such as non-celiac gluten intolerance and wheat allergy.

Author Contributions

Work conceptualization: López Olivas, Z.V., Gutiérrez Dorado, R.; Methodology development: López Olivas, Z.V., Madrigales Reátiga, L.F., Gutiérrez Dorado, R., Perales Sánchez, J.X.K., Pineda Hidalgo, K.V.; Software supervision: Gutiérrez Dorado, R.; Experimental validation: López Olivas, Z.V.; Result analysis: López Olivas, Z.V., Gutiérrez Dorado, R., Perales Sánchez, J.X.K.; Data management: López Olivas, Z.V., Gutiérrez Dorado, R., Perales Sánchez, J.X.K.; Manuscript writing and preparation: López Olivas, Z.V.; Writing, reviewing, and editing: Gutiérrez Dorado, R., Perales Sánchez, J.X.K., Reyes Moreno, C.

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Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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