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Functional tortillas made from blue corn, mesquite and hibiscus calyces

Tortillas funcionales elaboradas a base de maíz azul, mezquite y cálices de jamaica

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ABSTRACT

In Mexico, cardiovascular diseases, diabetes mellitus, and cancerous tumors are the leading causes of death. A viable option to minimize the harmful effect of these diseases is the consumption of whole grains and edible plant parts, alone or in combination, for example, the mixture of blue corn, mesquite, and jamaica calyces, in the preparation of functional foods; this, due to its high content of bioactive compounds. An optimized composite mix of extruded blue corn flour (EBCF), fermented-extruded mesquite pods (FEMPF), and hibiscus calyces (HCF) was developed to produce functional tortillas. Using an L-Optimal experimental design, 16 mixtures, with different inclusion levels of the three flours were tested. The mixture was optimized to obtain tortillas with the best nutraceutical characteristics (high content of total phenolic compounds, anthocyanins, flavonoids, and antioxidant activity) and adequate sensory acceptability. The optimized mixture comprised 82.8 % EBCF, 10 % FEMPF, and 7.2 % HCF. The tortillas produced with this mixture showed high values of total phenolic compounds (356 mg eq. of gallic acid/100g), anthocyanins (11.7 mg eq. of cyanidin 3-glucoside/100g), flavonoids (37.7 mg eq. of catechin/100g), and antioxidant activity (5,912 μmol eq. of Trolox/100g), along with a sensory acceptability falling between liking slightly and liking moderately on an eleven-point hedonic scale. These functional tortillas represent a novel strategy to reduce chronic degenerative diseases in Mexico.

KEY WORDS: *Zea mays* L, *Prosopis laevigata, Hibiscus sabdariffa*, extrusion, optimization, phytochemicals, functional foods.

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RESUMEN

En México, las enfermedades cardiovasculares, la diabetes mellitus y los tumores cancerosos son las principales causas de muerte. Una opción viable para minimizar el efecto nocivo de estas enfermedades es el consumo de granos integrales y partes comestibles de plantas, solas o en combinación, por ejemplo, la mezcla de maíz azul, mezquite y cálices de jamaica, en la elaboración de alimentos funcionales; esto, debido a sus altos contenidos de compuestos bioactivos. En esta investigación se desarrolló una mezcla compuesta optimizada de harinas de maíz azul extrudido (EBCF), vainas de mezquite fermentado-extrudido (FEMPF) y cálices de jamaica (HCF) para elaborar tortillas funcionales. Se usó un diseño experimental L-Optimal, se probaron 16 mezclas con diferentes niveles de inclusión de las tres harinas. Se optimizó la mezcla para obtener tortillas con las mejores características nutracéuticas (contenidos altos de compuestos fenólicos, antocianinas, flavonoides, actividad antioxidante) y aceptabilidad sensorial adecuada. La mezcla optimizada consistió en 82.8 % EBCF, 10 % FEMPF y 7.2 % HCF. Las tortillas producidas con esta mezcla mostraron altos valores de compuestos fenólicos totales (356 mg eq. de ácido gálico/100g), antocianinas (11.7 mg eq. de cianidina 3-glucósido/100g), flavonoides (37.7 mg eq. de catequina/100g) y actividad antioxidante (5,912 µmol eq. de Trolox/100g) y una aceptabilidad sensorial ubicada entre me gusta ligeramente y me gusta moderadamente en una escala hedónica de once puntos. Estas tortillas funcionales representan una estrategia novedosa para reducir las enfermedades crónicas degenerativas en México.

PALABRAS CLAVE: *Zea mays* L, *Prosopis laevigata, Hibiscus sabdariffa*, extrusión, optimización, fitoquímicos, alimentos funcionales.

Introduction

Nutrition is a fundamental process for the organism. It involves the assimilation of nutrients to carry out basic life functions, including growth, reproduction, and the preservation of health (Serón-Arbeloa *et al*., 2022).

In this regard, “malnutrition” involves imbalances (deficits or excesses) in human caloric and/or nutritional intake. This condition is one of the major global health issues and can result from poverty, poor food choices, or illness (Serón-Arbeloa *et al*., 2022; WHO, 2024).

Malnourished individuals and those overweight, obese, and elderly are populations with compromised immune systems and highly vulnerable to infectious and chronic degenerative

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diseases. For this reason, malnutrition, overweight, obesity, and chronic degenerative diseases (hypertension, heart diseases, diabetes mellitus, and malignant tumors) are the primary health conditions and leading causes of death in Mexico (Thun *et al*., 2017).

The tortilla is the main maize-based food product in our country, with an average daily consumption of 1.4 billion tortillas. Corn kernels provide macronutrients (proteins, starch, dietary fiber, and lipids) and micronutrients (vitamins and minerals). White and yellow corn are the most commonly used varieties for making tortillas, but in some regions, blue corn is also used. In recent decades, blue corn-derived products have gained attention for their potential health benefits, including antioxidant, antimutagenic, anti-inflammatory, hypoglycemic, hypocholesterolemic, anti-atherosclerotic, anti-obesogenic, anti-aging, and anticancer properties due to the presence of compounds such as phenolic acids and anthocyanins (Serna-Saldívar *et al*., 2013).

It has been reported that the pods of the Prosopis species are a source of bioactive compounds with antioxidant, anti-inflammatory, and antihypertensive activities (Díaz-Batalla *et al*., 2018). Additionally, *Prosopis laevigata* pod flour is a good source of lysine, sulfur-containing amino acids, and total phenolic compounds, with greater free radical scavenging capacity than soy and common beans (Díaz-Batalla *et al*., 2018). However, mesquite pods contain a high quantity of simple sugars, making it necessary to eliminate or significantly reduce them to avoid a high-calorie product with elevated hyperglycemic potential. For this reason, Diaz-Batalla (2019) conducted a study in which whole flour from mesquite pods (*Prosopis laevigata*) was fermented, obtaining a low-simple sugar product. The fermentation was carried out with *Saccharomyces cerevisiae* yeast at 27 °C for 5 days until the sugars were depleted. The fermented mesquite pod flour showed improved nutritional characteristics compared to the non-fermented one.

Mexico is one of the leading producers of hibiscus (*Hibiscus sabdariffa*) in the Americas, and the dried calyx of the fruit is particularly notable in the production of concentrates and decoctions (SIAP, 2023). Hibiscus calyx contains around 40 % dietary fiber, offering significant potential for food fortification to increase its nutraceutical value (Ariza & Flores, 2014). Various studies have reported that hibiscus calyces are a source of phenolic compounds, primarily flavonoids, and anthocyanins, which provide antioxidant and pharmacological potential related to the prevention of chronic-degenerative diseases (Agüero *et al*., 2014; Ariza & Flores, 2014).

The combination and incorporation of cereals, legumes, and plant anatomical parts in food production, such as blue corn, mesquite, and hibiscus calyces, can be considered a viable option to minimize the impact of chronic-degenerative diseases due to their high bioactive compound levels.

Extrusion is an alternative to nixtamalization that does not generate polluting effluents, consumes less water, time, and energy, and results in higher retention of nutrients and phytochemicals. Alkaline extrusion (an eco-friendly process) produces flour for tortillas with better nutraceutical and nutritional characteristics compared to the traditional alkaline cooking process (Milán-Carrillo *et al*., 2006; Gutiérrez-Dorado *et al*., 2008; Mora-Rochín *et al*., 2010; Reyes-Moreno *et al*., 2018). Tortillas prepared through the extrusion process may improve the nutritional status of

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Mexicans and reduce the incidence of chronic degenerative diseases. Fortifying tortillas made from native blue corn with legume and fruit flours, such as mesquite and hibiscus, is a feasible option to enhance their nutraceutical properties (Gámez-Valdez *et al*., 2021; León-Murillo *et al*., 2021; Bon-Padilla *et al*., 2022). However, ingredients other than corn for tortilla fortification compromise its sensory acceptability, which should be minimized as much as possible. In this regard, it is necessary to estimate the inclusion levels of ingredients (flours) in the mixture to produce tortillas with acceptable sensory properties. Statistical designs for mixtures and optimization techniques like response surface methodology (Montgomery, 2009) are useful for this task. It is important to note that during the optimization of food processes, it is often ideal to optimize using more than one response variable, which allows the description of the quality and performance characteristics of the food systems under study. Some variables are meant to be maximized, while others are minimized. In many cases, these responses are competitive, meaning that improving one response may have an opposite effect on another (Milán-Carrillo *et al*., 2012). In this research, an optimized mixture suitable for producing functional tortillas was developed. The mixture was based on extruded blue corn flour (EBCF), fermented-extruded mesquite pod flour (FEMPF), and hibiscus calyces flour (HCF).

Materials and Methods

Materials

To obtain the tortillas, batches of 500 g of blue corn flour (*Zea mays* L.), mesquite pods (*Prosopis laevigata*), and hibiscus calyces (*Hibiscus sabdariffa*) were used. The mentioned materials were obtained from Puebla, Mezquital Valley in Hidalgo, and Guerrero, respectively.

Methods

Flour obtention

The extruded blue corn flour (EBCF) was obtained with the optimized processing conditions from our laboratory (Milán-Carrillo *et al*., 2006). Batches of 500 g of blue corn grains were placed in a household blender to obtain semolina and then passed through a US40 screening mesh (0.425 mm). Before extrusion, the semolina was tempered (25 °C) and mixed with lime (0.21 g per 100 g of semolina) and water to achieve a water content of 28 g per 100 g of wet semolina. All batches were packaged and stored at 4-8 °C for 12 hours. The fermented-extruded mesquite pod flour (FEMPF) was obtained by fermenting the mesquite pods and then extruding them. For this, a batch of one kilogram of whole mesquite pod flour was subjected to a fermentation process, in which the flour was dispersed in 2 liters of water, pasteurized, and inoculated with *Saccharomyces cerevisiae* at a constant temperature of 27 °C for 5 days or until the sugars were depleted (Díaz-Batalla, 2019). Once fermentation was completed, the fermented mixture was dehydrated in a convection oven (Thermo Scientific, MA, USA) at 65 °C for 4 hours. The dehydrated fermented mesquite pods were extruded following the methodology described by Díaz-Batalla *et al*. (2018) with some modifications. Batches of 500 g of dehydrated fermented mesquite pods were placed

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in a household blender to obtain semolina, which was then passed through a US40 screening mesh (0.425 mm). The semolina was mixed with water to achieve a water content of 16 g per 100 g of wet semolina. All batches were packaged and stored at 4-8 °C for 12 hours. Before extrusion, the semolina was tempered (25 °C). The extrusion processes for blue corn and fermented mesquite pods were carried out in a laboratory single-screw extruder Model 20 DN (CW Brabender Instruments, Inc, NJ, USA). For blue corn, the optimal operating conditions for the extruder (extrusion temperature, ET, and screw speed, SS) were ET = 85 °C and SS = 240 rpm (Milán-Carrillo *et al*., 2006). Meanwhile, for the fermented mesquite pods, the optimal operating conditions were ET = 150 °C and SS = 170 rpm (Díaz-Batalla *et al*., 2018). During the extrusion of the fermented mesquite, the barrel temperature was set at 110 °C, 130 °C, and 150 °C for the heating zones 1, 2, and 3, respectively, while for blue corn, the barrel temperature for all three heating zones remained the same (85 °C). The extruded material was allowed to cool and equilibrate under ambient conditions; it was then milled, passed through a US80 screening mesh (0.180 mm), and packaged. The extruded flour from blue corn and fermented mesquite pods were stored at 4-8 °C. For hibiscus calyx flour (HCF) obtention, the calyces were dehydrated (65 °C, 4 hours). Batches of 500 g of dehydrated hibiscus calyces were placed in a household blender to obtain semolina, which was then passed through a US80 screening mesh (0.180 mm). All batches were packaged and stored at 4-8 °C until use.

Tortilla obtention

To produce the tortillas, 500 g batches of each mixture based on EBCF (extruded blue corn flour), FEMPF (fermented-extruded mesquite pod flour), and HCF (hibiscus calyx flour) were used in the proportions indicated by the experimental design. The dough for each mixture was prepared by combining each mixture with water at 60 °C until a suitable consistency was achieved. The dough was then flattened using a manual press. Then, smaller and uniform discs were cut (15 cm in diameter, 28-30 g). The tortillas were cooked on heated griddles at temperatures between 400 and 450 °C for 40 to 60 seconds. The tortillas obtained from each treatment (different mixtures) were evaluated for the following response variables: overall tortilla acceptance (sensory test), antioxidant activity, total phenolic compounds, total flavonoids, and total anthocyanins (nutraceutical tests).

Evaluation of response variables

Sensory evaluation: Tortilla overall acceptability (OA)

For the sensory evaluation, a panel of 30 untrained judges of both sexes (18-35 years old) who were regular tortilla consumers was selected. The judges recorded their results on an evaluation sheet for each tortilla sample, where the attributes to be assessed were flavor, aroma, color, texture, and overall acceptability. A labeled affective magnitude (LAM) scale was used, which is a bidirectional 100 mm scale with verbal descriptors ranging from -100 (maximum imaginable disgust) to +100 (maximum imaginable liking), with zero as the neutral point (neither like nor dislike). These values were then transformed into a scale from 0 to 100 [0 = maximum imaginable disgust, 100 = maximum imaginable liking, and 50 = neutral (neither like nor dislike)] (Cardello & Schutz, 2004). This transformation facilitated the regression analysis of each acceptability response for the

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tortilla samples. Finally, the remaining tortilla samples were dried, ground, passed through a US80 screening mesh (0.180 mm), and stored in plastic bags at 4-8 °C for later determination of the nutraceutical response variables (antioxidant activity, total phenolic compounds, total flavonoids, and total anthocyanins).

Evaluation of nutraceutical variables

Extraction of free and bound phenolic compounds

Free phenolic compounds extraction was performed by suspending 0.5 g of the sample in 10 mL of an ethanol-water mixture (80:20, v/v) (Dewanto *et al*., 2002). The suspended sample was agitated in a circular tube rotator (50 rpm, 10 min), then centrifuged (8000 rpm, 10 °C, 10 min). The residual pellet was subjected to a second extraction with 80 % ethanol. The supernatants from both extractions were concentrated at 45 °C using low-pressure equipment (Apud Vac Concentrator, Thermo Electron Corporation), and the recovered extracts were evaporated to dryness at 45 °C (1-2 nights) and stored at -20 °C until use. The sediments obtained after the extraction of free phenolic compounds were defatted with hexane, then hydrolyzed with 10 mL of NaOH (2 M) at 95 °C and 25 °C for 30 and 60 minutes, respectively, and neutralized with HCl. The bound phenolic extracts were obtained by performing four extractions with 10 mL of ethyl acetate (Adom & Liu, 2002; Adom *et al*., 2003). The extracts were then evaporated and stored until use. Phenolic compound extractions were performed quadruplicate.

Total phenolic content

The total phenolic content (TPC) was determined by preparing a standard gallic acid solution. Then, 20 µL of the standard solution (25–600 ppm) and the free and bound extracts were injected into a 96-well microplate. Each point of the standard curve and extract reacted with the Folin-Ciocalteu reagent and $NaCO_3$ (7 %, v/v), adding 180 µL and 50 µL, respectively, followed by incubation at 25 °C for 90 minutes. The samples were then placed in a microplate reader (Multiskan Skyhigh model, Thermo Scientific, Singapore) to measure absorbance at 750 nm using ethanol as a blank. TPC results were calculated by summing the phenolic compound values obtained for both the free and bound extracts and expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of dry sample (mg GAE/100 g, dw) (Singleton *et al*., 1999). The determinations were performed in triplicate.

Total flavonoid content

The measurement of total flavonoids (TF) was performed using the colorimetric assay described by Xu & Chang (2007). For this, 20 µL of extract was combined with 80 µL of distilled water, followed by the addition of 6 µL of $NaNO_2$ and then 12 µL of $AlCl_3$, allowing the mixture to rest for 5 minutes after adding each reagent. Finally, 40 µL of 1M NaOH and 20 µL of distilled water were added. The extracts were incubated for 30 minutes, and then absorbance was measured at 415 nm using a Multiskan Skyhigh microplate reader (Thermo Scientific, Singapore). Catechin was used as the standard for the calibration curve. The measurements were performed in quadruplicate, and the results were calculated by summing the free and bound flavonoids and expressed as milligrams of catechin equivalents per 100 grams of dry sample (mg CAE /100 g, dw).

Total anthocyanin content

The total anthocyanin content (TA) was evaluated according to the method proposed by Abdel-Aal & Huel (1999). For this measurement, 0.5 g of the sample was homogenized with 10 mL of an acidified methanol solution (95 % methanol and 1 N HCl, 85:15, v/v) in a 10 mL centrifuge tube. Nitrogen gas was injected into the tube to remove oxygen, and the mixture was shaken for 30 minutes. Subsequently, it was centrifuged (3,000g / 4°C / 10 min) using a Sorvall RCSC centrifuge (Sorvall Instruments, Dupont, Wilmington, DE, USA) and the supernatant was collected. The absorbance of the extracts was measured at 535 nm and 700 nm using a UV-vis Genesys 10 UV spectrophotometer (Thermo Electron Corporation, Madison, WI, USA). The anthocyanin content was calculated using the following formula:

$$TA = [(A_{535nm} - A_{700nm})/ (£)] \times [(\text{Total extract volume, L}) \times (MW)] \times [1/(\text{Sample weight, g})] \times [1/(\text{path length, cm})]$$

Where: TA = Total anthocyanin content (mg of Cyanidin 3-Glucoside equivalents (C3GE) / 100 g of dry sample).

A = Absorbance.

£ = Molar absorption coefficient of Cyanidin 3-Glucoside (25,965/cm/M).

MW = Molecular weight of Cyanidin 3-Glucoside (449.2 g/mol).

Antioxidant Activity

The antioxidant activity (AoxA) was evaluated using the decolorization assay described by Re *et al.* (1999). An ABTS solution was prepared by mixing 0.0192 g of the radical with 5 mL of $K_2S_2O_8$ at 2.45 mM. The mixture was incubated in the dark for 16 hours. Then, 500 μL of the ABTS solution was diluted in 45 mL of a phosphate-buffered saline (PBS) solution (pH = 7.4) to adjust its absorbance (0.7 - 1.0 at 734 nm). Once the absorbance of the radical was adjusted, 1980 μL of the radical solution was added to 20 μL of extract, and its absorbance was measured 6 minutes after at 734 nm using a cuvette spectrophotometer, model Multiskan Skyhigh (Thermo Scientific, Singapore). Measurements were performed in quadruplicate, and the results were expressed as micromoles of Trolox equivalents per 100 g of sample on a dry weight basis (μmol TE / 100 g, dw).

Experimental design, statistical analysis, optimization, and validation

Experimental Design: For mixtures with three components, the experimental space is represented by a triangle where the vertices correspond to pure component formulations (i.e., 100 % of a single ingredient). However, in some research cases, restrictions must be applied

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to the mixture components. In our study, the following restrictions were applied to the three components: 0.550 ≤ X_1 (EBCF) ≤ 0.850; 0.100 ≤ X_2 (FEMPF) ≤ 0.300; 0.050 ≤ X_3 (HCJ) ≤ 0.150; where, at any point in the experimental area, $X_1 + X_2 + X_3 = 1.0$ (100 %). It is important to note that each point on the graph corresponds to a specific combination of component proportions. In mixture designs with constraints, the situation is simplified by introducing pseudocomponents, which are related to the original components of the mixture. In such cases, the experimental region does not have a standard shape but forms an irregular polytope (a polytope is a geometric figure in a multidimensional Euclidean space; an irregular polytope, unlike regular polytopes, has faces, vertices, or angles that are not all equal). In these cases, optimal designs or "Optimal" type designs stand out, which are better than classical designs using restriction criteria. In this type of mixture design, the original levels X_i of the components are transformed into a convenient scale X'_i for statistical analysis. That is, the original scale used [proportions of the components (EBCF, FEMPF, and HCJ) expressed as fractions of the original mixture (X_1, X_2, and X_3)] is converted to a "Pseudo" scale, where each component represents a fraction of the "active" part of the mixture, such that the total sum of these "Pseudo" fractions adjusts to 1.0. L-Pseudo is the default scale in response surface methodology (RSM), and designs that use this scale are called lower-bounded Pseudo-value designs, L-Optimal or Optimal L. This design was used for this research. In these designs, the minimum value of each component in the active mixture is converted to zero, and the maximum value approaches one. How close it is to one depends on the imposed restrictions. The equation used to calculate the lower-bounded Pseudo-values is $X`_i = (X_i – L_i) / (1 - \sum L_i)$, where L_i is the minimum level of component "i" in the original base mixture. In this research, the mix of the three components [X_1 (EBCF), X_2 (FEMPF), and X_3 (HCJ)] will always contain at least 55 % of EBCF (X_1 = 0.55), at least 10 % of FEMPF (X_2 = 0.10), and at least 5 % of HCJ (X_3 = 0.05). Therefore, 70 % of the flour mixture ($X_1 + X_2 + X_3 = 0.7$) is predetermined and is known as the original base mixture. Thus, the experiment was conducted to determine the remaining 30 % (the "active" part of the mixture). The original values of the mixture, which are within the ranges defined by the restrictions mentioned earlier for each component, were converted into L-Pseudo values as follows: L-pseudo component of EBCF ($X`_1$) = [fraction of EBCF in the original mixture ($X_1$) – 0.55] / 0.30; L-pseudo component of FEMPF ($X`_2$) = [fraction of FEMPF in the original mixture (X_2) – 0.10] / 0.30; and L-pseudo component of HCJ ($X`_3$) = [fraction of HCJ in the original mixture ($X_3$) – 0.05] / 0.30. Additionally, the L-Pseudo scale must also meet the condition: $X`_1 + X`_2 + X`_3 = 1.0$. Statistical analysis, including regression, variance, and optimization, is carried out by the transformed L-Pseudo values. Later, the specified formulations (e.g., the optimal mixture or any other specified mixture in the experimental design) in the L-Pseudo scale for the Pseudo-components of the mixture are transformed into formulations for the original components as follows: $X_i = L_i + X`_i (1 - \sum L_i)$.

In summary, to obtain the proportion of the three flours (EBCF, FEMPF, and HCF) in the optimized composite mixture, an L-Optimal experimental design (design for mixtures with restrictions for the 3 components mentioned earlier) from the RSM was used. Based on the restrictions imposed on the three components of the mixture, the inclusion levels of the three flours in the composite mixtures were: $X1$ (EBCF) = 0.5650, 0.6123, 0.6460, 0.6501, 0.6698, 0.6982, 0.7227, 0.7442, 0.7580, 0.7803, 0.8294; $X2$ (FEMPF) = 0.1000, 0.1206, 0.1273, 0.1537, 0.1802, 0.2058, 0.2112, 0.2377, 0.2573, 0.3000; and $X3$ (HCF) = 0.0500, 0.0883, 0.0906, 0.0967, 0.1196, 0.1350, 0.1500. The experimental design consisted of 16 different mixtures (16 experiments) (Table 1). The response variables were total phenolic compounds (TPC), total anthocyanins (TA), total flavonoids (TF), antioxidant activity (AoxA), and overall acceptability (OA) of the tortillas (sensory acceptance variable).

Statistical regression and variance analysis: Multiple least squares regression of the RSM for mixtures was applied to fit appropriate and reproducible regression models. The multiple least squares regression method for mixture models is a statistical technique used to model and analyze systems where the response or dependent variable (Y_i) is influenced by a combination of independent variables (X_1, X_2, and X_3), which represent the proportions of the components in a mixture. Mixture models differ from the usual polynomials used in response surface designs due to the constraint $\sum X_i = 1.0$. The fitted models for mixtures can be linear ($Y=\beta_1X_1+\beta_2X_2+\beta_3X_3$), quadratic ($Y=\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3$), or higher order (full cubic or special cubic), which are often necessary in mixture models where the phenomena studied are complex or because the experimental region compromised the whole operability region. In linear models, β_1 represents the expected response for the mixture with the maximum inclusion level of component 1 (EBCF) and the minimum levels of the other two components (FEMPF and HCF): X_1 = 0.85, X_2 = 0.10, X_3 = 0.05. β_2 represents the expected response for the mixture with the maximum possible (theoretical) inclusion level of component 2 (FEMPF) and the minimum levels of the other two components (EBCF and HCF): X_2 = 0.45, X_1 = 0.55, X_3 = 0.05. β_3 represents the expected response for the mixture with the maximum possible (theoretical) inclusion level of component 3 (HCF) and the minimum levels of the other two components (EBCF and FEMPF): X_3 = 0.35, X_1 = 0.55, X_2 = 0.10. In quadratic models, β_1, β_2, and β_3 represent the linear portion of the mixture model, while β_{12}, β_{13}, and β_{23} represent a synergistic or antagonistic mixture when there is curvature derived from a nonlinear mixture (Montgomery, 2009). The significance of the model parameters was verified (regression model coefficients, β_i) with an analysis of variance (ANOVA) (α = 0.1). Additionally, the significance of the full regression model was estimated using α = 0.05. To assess the goodness of fit of the regression models, the following statistical parameters were used: coefficient of determination (R^2), adjusted coefficient of determination (adjusted R^2), predicted coefficient of determination (predicted R^2), coefficient of variation (CV), and probability of lack of fit ($p_{\text{lack of fit}}$). The obtained prediction models were used to graphically represent the system, enabling the graphical analysis of the effect of the independent variables (mixture components X_1, X_2, X_3) on the response variables (Y_i).

Optimization: The overall desirability method (D) from the RSM was used as an optimization technique to obtain tortillas with the best nutraceutical characteristics (maximum values for TPC, TA, TF, AoxA) and suitable sensory acceptability (maximum possible value for OA). For this purpose, the five mathematical regression models obtained for the studied response variables were used to estimate theoretical values for the five response variables at different randomly selected points in the experimental region. These predicted values were transformed into individual desirability values for each response variable [$d_i(X)$] using a transformation equation proposed by response surface methodology to maximize each response variable value. This desirability method involves transforming the predicted values from the mathematical models $\hat{Y}_i(X$ into an individual desirability value [$d_i(X)$], which ranges from 0 to 1 and measures the degree of desirability of the response with the optimal value being targeted (maximum). The individual desirabilities were combined using the geometric mean to obtain the overall desirability [$D = (d_1 \times d_2 \times d_3 \times d_4 \times d_5)^{(1/5)}$]. An overall desirability is a logical option because if any $d_i(X)$ = 0, the overall

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desirability would be D = 0, indicating that the tortillas are unacceptable. Values of D between 0.6 and 0.8 are considered suitable for food systems, with the ideal optimal value being D = 1.

Validation: To validate the optimal conditions, three replicates of composite mixtures with the optimal proportion of the three components (X_1 = EBCF, X_2 = FEMPF, and X_3 = HCJ) were prepared, and tortillas were then made from these mixtures. Each of the studied response variables was experimentally evaluated in triplicate. Subsequently, a theoretical confidence interval was constructed for each response variable based on the optimization zone and using a 95 % confidence level. For a model to pass the validation test, the average of the five replicates for each response must fall within the theoretical range previously established.

The experimental design, variance and regression analysis, optimization, and validation were carried out using the Design Expert Software version 11 (Design Expert, 2018; Stat-Ease, Inc., 1300 Godward Street Northeast, Suite 6400, Minneapolis, MN 55413, USA).

Results and Discussion

Obtention of the optimized mixture based on EBCF, FEMPF, and HCF

Mathematical regression models were used to study the behavior of the response variables as a function of the mixture components (proportions of the flours used in the mixture). The regression models were also used to generate random theoretical values for the response variables within the experimental area and convert these values into individual desirability values for each response.

Based on the obtained individual desirability values, the overall desirability was calculated for each randomly selected point within the experimental region. Finally, the overall desirability value with the highest possible value was selected. The mixture corresponding to this maximum desirability was identified as the optimized mixture based on EBCF, FEMPF, and HCF. This optimized composite mixture was suitable for preparing functional tortillas that are both sensorily acceptable and have high levels of antioxidant activity, phenolic compounds, flavonoids, and total anthocyanins.

Prediction models

Table 1 presents the experimental results for each of the studied response variables corresponding to the various combinations of proportions of each type of flour used (EBCF, FEMPF, and HCF) in the development of the mixture (16 treatments indicated in the experimental design). Based on the experimental data, multiple linear least squares regression was performed to obtain prediction models for each of the studied response variables.

Antioxidant Activity (AoxA)

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Table 1 shows that the AoxA values of the tortillas ranged from 3005 to 6076 µmol equivalents of Trolox/100g, dry weight (dw). The regression and variance analysis revealed a significant linear model (p < 0.05) concerning the EBCF, FEMPF, and HCF proportions. The prediction model for AoxA using coded variables was:

AoxA= 5948*EBCF + 2168*FEMPF + 5461*HCF

This prediction model explained 90.09 % of the total variability (p < 0.0001) in the tortillas AoxA values. The adjusted coefficient of determination (adjusted R^2) for the model was 0.8857, and the lack of fit was not significant (probability equal to 0.7570). Additionally, the relative dispersion of the experimental points for the values predicted by the model (CV = 6.88 %) was less than 10 %. These values indicate that the prediction model for the AoxA of the tortillas was adequate and reproducible.

From this prediction model, a three-dimensional graph was constructed (Figure 1A). In a typical mixture design graph, the axes represent the proportions of the mixture components. Depending on how many components are being analyzed, the graph can be two-dimensional (for two components) or three-dimensional (for three components). In this study, it involves three components, resulting in a triangular shape graph. In the case of the L-Optimal design, the graphs consist of triangles bounded by the maximum and minimum levels of the three components of the mixture, as previously mentioned in the regression and variance analysis section (0.550 ≤ X_1 (EBCF) ≤ 0.850; 0.100 ≤ X_2 (FEMPF) ≤ 0.450; 0.050 ≤ X_3 (HCJ) ≤ 0.350). However, it can be observed in Figure 1 that the graphed experimental region is bounded and does not correspond to the entire area of the triangle in the graph. This is caused by the original restrictions mentioned in the experimental design section (0.550 ≤ X_1 (EBCF) ≤ 0.850; 0.100 ≤ X_2 (FEMPF) ≤ 0.300; 0.050 ≤ X_3 (HCJ) ≤ 0.150). As a result, the graphed experimental region (contour plot) does not have a standard shape (e.g., a triangle) but takes the form of an irregular polytope. It should be noted that a polytope is a figure in a multi-dimensional Euclidean space. On the other hand, an irregular polytope unlike regular polytopes, has faces, vertices, or angles that are not all congruent. This contour plot, which represents the different values of the AoxA response, is employed to interpret the AoxA results as affected by the mixture components. In the triangular graph, for the three components in the mixture, the axis of the component i, which represents the increase in the response variable, is the line or ray that extends from the base point X_i = minimum value of the inclusion level of the component i, X_j = (the difference between the maximum and minimum possible theoretical values of the inclusion level of the component j) /(p – 1), for all j ≠ i and p = number of components in the mixture, at the opposite vertex where X_i = maximum possible theoretical value of the inclusion level of component i, X_j = minimum value of the inclusion level of the component j for all j ≠ i. In the triangle, the base point is located at the centroid of the boundary of (p – 2) dimensions of the design that is opposite the corresponding vertex (vertex coordinates: X_i = maximum possible theoretical value of the inclusion level of the component i, X_j = minimum value of the inclusion level of component j for all j ≠ i). The length of the component axis is equal to the average of the maximum and minimum theoretical values of the inclusion level of component i. However, the contour surface for each response is bounded by the restriction criteria employed in the research study. This is why, in the actual contour surface plotted in **Figure 1**, the components

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X_2 (FEMPF) and X_3 (HCJ) are restricted in their upper inclusion levels, contrasting with the upper levels considered in the triangular area.

Table 1. L-Optimal experimental design[1] of the response surface methodology to obtain different mixtures based on extruded blue corn flour (EBCF), extruded fermented mesquite pod flour (EFMPF), and hibiscus calyx flour (HCF).

Order		Mixture components			Response variables				
Std	Run	X_1: EBCF	X_2: EFMPF	X_3: HCF	AoxA	TPC	TF	TA	OA
1	16	**0.8294**	0.1206	**0.0500**	5860.5	334.9	37.91	9.22	57.5
2	2	**0.8294**	0.1206	**0.0500**	5715.51	347.5	38.75	8.99	56.11
3	3	0.7442	0.2058	**0.0500**	4355.76	334.2	**39.73**	6.60	55.85
4	1	0.6501	**0.2999**	**0.0500**	3595.32	**318.9**	25.62	**4.56**	47.96
5	7	0.7580	0.0154	0.0883	4997.6	365.7	38.25	11.47	51.36
6	10	0.6982	0.2112	0.0906	4214.06	337.1	30.83	9.41	52.06
7	9	0.6982	0.2112	0.0906	4454.57	366.5	25.65	9.91	53.45
8	8	0.6982	0.2112	0.0906	4966.83	358.6	29.04	10.07	47.9
9	12	0.6460	0.2573	0.0967	3720.65	347.5	23.14	9.30	48.33
10	11	0.7803	**0.1000**	0.1196	5548.03	377.9	37.38	14.84	**57.6**
11	14	0.7803	**0.1000**	0.1196	**6076.19**	**389.4**	37.22	15.72	53.98
12	15	0.7803	**0.3000**	0.1350	3696.45	376.6	**17.29**	9.60	41.11
13	5	**0.5650**	**0.3000**	0.1350	**3044.85**	366.2	20.38	8.45	**43.65**
14	6	0.7227	0.1273	**0.1500**	5869.23	384.9	38.76	**15.84**	50.56
15	4	0.6123	0.2377	**0.1500**	4135.18	379.4	23.11	13.81	44.56
16	13	0.6698	0.1802	**0.1500**	4273.87	377.0	22.93	12.09	45.15

[1] L-Optimal experimental design with three mixture components; 16 experiments (mixtures). EBCF = extruded blue corn flour, FEMPF = fermented-extruded mesquite pods flour, HCF = hibiscus calyces' flour, AoxA = antioxidant activity (μmol Trolox eq. /100 g), TPC = total phenolic compounds (mg gallic acid eq. /100 g), TF = total flavonoids (mg catechin eq. /100 g), TA = total anthocyanins (mg cianidin-3-glucoside eq. / 100 g), OA = overall acceptability of tortillas. *The bolded values of the process and response variables correspond to the minimum and maximum values used and obtained, respectively.

In the graphs of Figure 1, component X_1 (EBCF) increases from the centroid of the lower boundary of the triangle with a value of X_1 = 0.55 to the corresponding vertex with a value of X_1 = 0.85, while component X_2 (FEMPF) increases from the centroid of the right boundary of the triangle with a value of X_2= 0.10 to the corresponding vertex with a value of X_2 = 0.45. Component X_3 (HCJ) increases from the centroid of the left boundary of the triangle with a value of X_3 = 0.05 to the corresponding vertex with a value of X_3 = 0.35.

In Figure 1A, it can be observed that the AoxA values in the tortillas are higher (desirable values) when the proportion of component X_1 [content of extruded blue corn flour (EBCF)] is high, and the proportions of component X_3 [content of hibiscus calyx flour (HCF)] range from low to high (from 0.05 to 0.15), while the proportions of component X_2 [content of fermented-extruded mesquite pod flour (FEMPF)] is low. According to the analysis of variance conducted on the terms of the linear regression model fitted to the AoxA data, the components of the mixture that had a significant effect ($p < 0.05$) on this response were X_1 (EBCF) and X_2 (FEMPF), while component X_3 (HCJ) did not have a significant effect ($p > 0.05$) on the AoxA response (effects = 2682.37, -2479.54, and 117.75 for X_1, X_2, and X_3, respectively). It can be observed in Figure 1A that while the increase in the inclusion level of EBCF in the mixture caused higher AoxA, the increase in the inclusion level of FEMPF led to a decrease in AoxA. This may be due to the higher content of phenolic compounds in the EBCF and HCF ingredients compared to FEMPF. Various studies have reported a strong correlation between antioxidant activity and phenolic compound content (Gámez-Valdez *et al*., 2021; Félix-Medina *et al*., 2024). Mora-Rochín *et al*. (2010) concluded that ferulic acid is the most abundant phenolic acid in corn and the main responsible for its antioxidant activity. Similar results have been reported by Aguayo-Rojas *et al*. (2012), who found that the phenolic compounds in extruded tortillas made from pigmented corn are the main contributors to antioxidant capacity. On the other hand, Félix-Medina *et al*. (2021) evaluated extruded snacks made from corn and beans, finding that antioxidant capacity is primarily associated with phenolic compounds. According to Figure 1A, the highest theoretical values (predicted by the prediction model) of AoxA were in the range of 5526 to 5893 µmol equivalents of Trolox/100g, dw.

Total phenolic compounds (TPC)

Table 1 shows that the tortillas TPC values ranged from 318.9 to 389.4 mg gallic acid equivalents/100g, dw. The analysis of variance showed a significant linear model ($p < 0.05$) for the proportion of EBCF, FEMPF, and HCF. The predictive model for TPC using coded variables was:

TPC= 345*EBCF + 314*FEMPF + 494*HCF

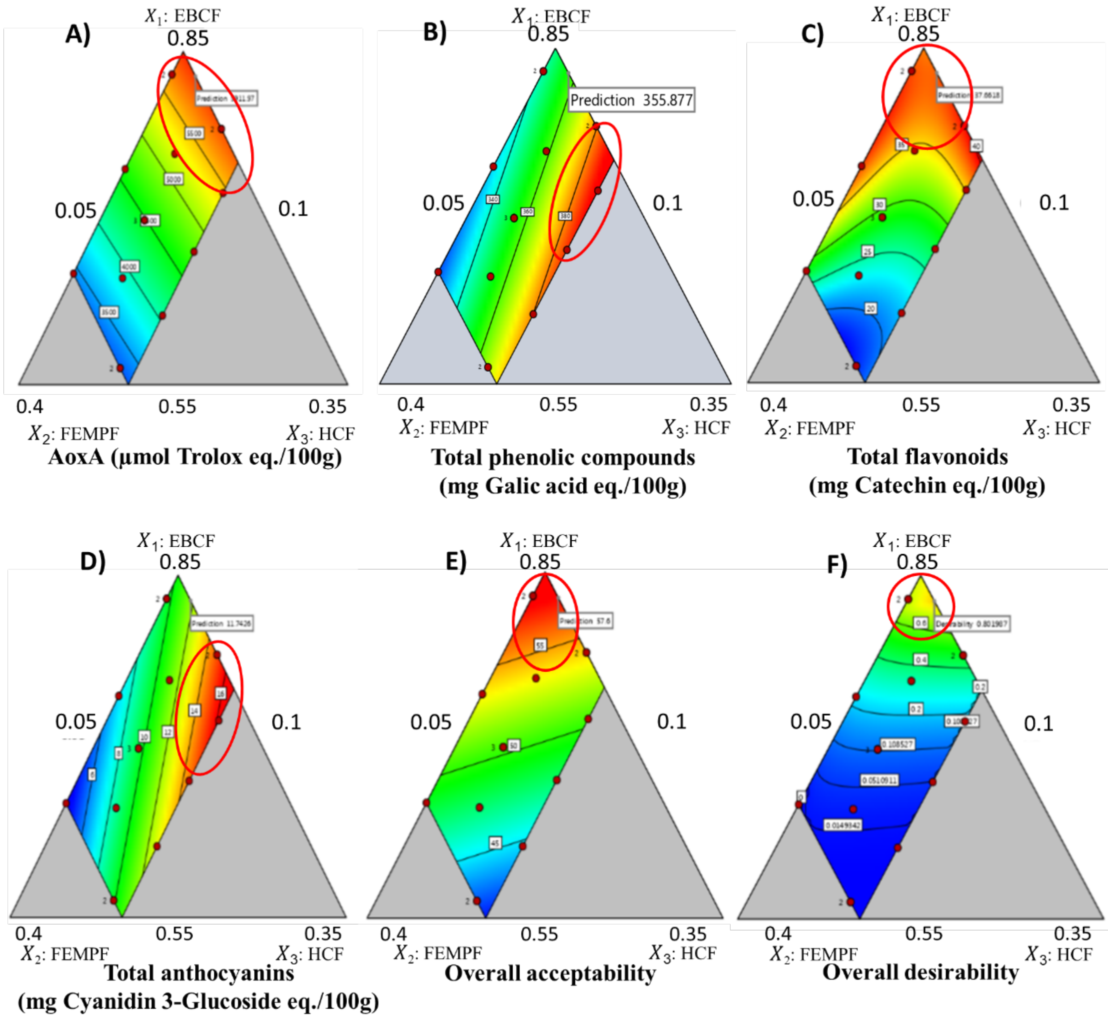

Figure 1. Contour graphs of the prediction of each of the response variables and overall desirability under optimal conditions.

A) Antioxidant activity (AoxA), B) Total phenolic content (TPC), C) Total flavonoids (TF), D) Total anthocyanins (TA), E) Overall acceptability of tortillas (OA), and F) Overall desirability (D).

This predictive model explained 85.28 % of the total variability (p < 0.0001) of the TPC values in the tortillas. The adjusted R^2 coefficient of determination for the model was 0.8301, and the lack of fit was not significant (p = 0.9333). Additionally, the relative dispersion of the experimental points for the values predicted by the model (CV = 2.41 %) was less than 10 %. These values indicate that the predictive model for TPC in the tortillas was adequate and reproducible.

Based on this predictive model, a three-dimensional graph with a triangular shape was constructed (Figure 1B). It is worth mentioning that this graph behaves similarly to the one previously mentioned for AoxA. In the results section corresponding to the AoxA response, it was previously stated how the inclusion levels of the three components of the mixture increase, and this also applies to all the response variables studied in this research.

TPC values in the tortillas are higher (desirable values) in the area with high inclusion levels of components X_1 [extruded corn flour content (EBCF)] and X_3 [hibiscus calyx flour content (HCF)] (Figure 1B). In contrast, the inclusion level of component X_2 [fermented-extruded mesquite pod flour content (FEMPF)] decreased the TPC values. According to the analysis of variance performed on the terms of the linear regression model fitted to the TPC data, the three components of the mixture (X_1, X_2, and X_3) had a significant effect ($p < 0.05$) on this response (effects = -28.79, -33.22, and 51.92 for X_1, X_2, and X_3, respectively). It can be observed in Figure 1B that increasing the inclusion level of EBCF and FEMPF in the mixture caused decreased TPC values. On the other hand, increasing the inclusion level of HCF caused an increase in TPC values. Unlike the AoxA response, the increase in the inclusion level of EBCF had a negative effect on TPC. This may be due to the higher phenolic content in HCF compared to EBCF and FEMPF. It is relevant to note that the HCF component (X_3) had the most significant effect on the TPC response. The highest TPC values are found in the same area where the highest AoxA values are located, confirming a high correlation between TPC and AoxA, as previously reported in the literature (Mora-Rochín *et al*., 2010; Félix-Medina *et al*., 2024). On the other hand, Colín *et al*. (2020) evaluated tortillas made from blue and white corn. Authors noticed that tortillas made from blue corn have higher antioxidant capacity than those produced from white corn. This increment in antioxidant capacity has been associated with blue corn being a potential source of phenolic compounds (polyphenols) (Astorga-Gaxiola *et al*., 2023; Menchaca-Armenta *et al*., 2021). Additionally, Gaxiola *et al*. (2017) observed that the insoluble phenolic compound fraction contributes around 85 % of the total antioxidant capacity in corn. In our research, it was also found that the bound phenolic compound fraction represented the highest TPC content. According to Figure 1B, the highest theoretical TPC values were in the range of 380 to 394 mg gallic acid equivalents/100g, dw.

Total flavonoids (FT)

Table 1 shows that the TF values ranged from 17.29 to 39.73 mg catechin equivalents/100g, dw. The regression and variance analysis showed a significant model ($p < 0.05$) for the proportion of EBCF, FEMPF, and HCF. Additionally, the model significantly depended on the double EBCF*FEMPF and triple interaction EBCF*FEMPF*HCF. The predictive model for TF using coded variables was:

TF= 37*EBCF + 6*FEMPF + 48*HCF + 54*EBCF*FEMPF – 309*EBCF*FEMPF*HCF

This predictive model explained 90.83 % of the total variability ($p < 0.0001$) of the TF values in the tortillas. The adjusted R^2 coefficient of determination for the model was 0.8750, and the lack of fit was not significant ($p = 0.1341$). Additionally, the relative dispersion of the experimental points for the values predicted by the model (CV = 9.12 %) was less than 10 %. These values

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indicate that the predictive model for TF in the tortillas was adequate and reproducible.

Based on this predictive model, a three-dimensional triangular-shaped graph was constructed, containing the irregularly shaped response surface contour (Figure 1C). The increase in the inclusion levels of the components (X_1, X_2, and X_3) of the mixture has already been explained in the AoxA response section.

Figure 1C shows that the TF values in the tortillas are higher (desirable values) when the value of component X_1 [extruded blue corn flour content (EBCF)] in the mixture is high and the values of component X_3 [hibiscus calyx flour content (HCF)] range from low to high (from 0.05 to 0.15). In contrast, the TF values increase when the component X2 [fermented-extruded mesquite pod flour content (FEMPF)] ranges from a low level to an intermediate level. According to the analysis of variance performed on the terms of the cubic regression model fitted to the TF data, the linear part of the regression model referred to as the linear mixture portion, which groups the three components of the mixture (X_1, X_2, and X_3) into a single term, had a significant effect ($p < 0.05$) on this response. The analysis of variance found that the effects of the double interaction X_1*X_2 and the triple interaction X_1*X_2*X_3 of the mixture components were significant ($p < 0.05$). In these cases, the effects of the mixture components analysis should be conducted by the highest-order interaction, in this case, the triple interaction. From the interpretation of the triple interaction, the behavior shown in Figure 1C indicated that the TF content increased significantly in mixtures with a higher inclusion level of component X_1 (EBCF) and a lower inclusion level of component X_2 (FEMPF), while the content of component X_3 (HCF) was maintained in an intermediate to high range (X_3 = 0.10 – 0.15). On the other hand, when the inclusion level of component X_3 in the mixture remained fixed at a low level (X_3 = 0.05), the TF content increased very little in mixtures with a higher inclusion level of component X_1 (EBCF) and a lower inclusion level of component X_2 (FEMPF). This may be because the lower TF values in the second case were much higher than in the first one, while the high TF values in both cases were similar. In general, the interpretation of this triple interaction indicates that high inclusion levels of EBCF and low inclusion levels of FEMPF and HCF in the mixture favor high TF values in the tortillas. This suggests that although all three flours (EBCF, FEMPF, and HCF) are an important source of flavonoids, the results indicate that EBCF flour contains the highest flavonoid content. It is widely known that flavonoid compounds represent a fraction of the total phenolic compounds. That is why higher TF values are found near the area where the highest TPC and AoxA values are located. As included in Figure 1C, the highest theoretical TF values were between 35 to 37 mg catechin equivalents/100g, dry basis.

Total anthocyanins (TA)

Table 1 shows that the tortillas' TA values ranged from 4.56 to 15.84 mg cyanidin-3-glucoside equivalents/100g, dw.

The regression and variance analysis showed a significant linear model ($p < 0.05$) for the EBCF, FEMPF, and HCF proportion. The predictive model for TA using coded variables was:

$$TA= 10*EBCF + 1.0*FEMPF + 31*HCF$$

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This predictive model explained 96.34 % of the total variability ($p < 0.0001$) of the TA values in the tortillas. The adjusted R^2 coefficient of determination for the model was 0.9577, and the lack of fit was not significant ($p = 0.2012$). Additionally, the relative dispersion of the experimental points compared to the values predicted by the model (CV = 6.27 %) was less than 10 %. These values indicate that the predictive model for TA in the tortillas was adequate and reproducible.

Based on this predictive model, a three-dimensional triangle graph was constructed, containing the real experimental region. The triangle had an irregular shape (Figure 1D) due to the restrictions imposed on the components' inclusion levels. The increment ray of the inclusion levels for each of the components in the triangular graph was explained in the AoxA response section.

Figure 1D shows that TA values are higher (desirable values) in the area where the inclusion levels of components X_1 [extruded corn flour content (EBCF)] and X_3 [hibiscus calyx flour content (HCF)] in the mixture are high, whereas the inclusion level of component X_2 [fermented-extruded mesquite pod flour content (FEMPF)] is low. According to the analysis of variance performed on the terms of the linear regression model adjusted to the TA data, components of the mixture that had a significant effect ($p < 0.05$) on this response were X_2 (FEMPF) and X_3 (HCF), while component $X1$ (EBCF) did not have a significant effect ($p > 0.05$) on the TA response (effects = -0.6397, -7.83, and 7.46 for X_1, X_2, and X_3, respectively). An increased HCF inclusion level caused an increase in TA; meanwhile, the increased FEMPF inclusion level decreased this parameter. Unlike the responses previously discussed, the increase in the inclusion level of EBCF in the mixture did not affect the TA content, and it did have a significant effect on AoxA, TPC, and TF. This may be due to the higher total anthocyanin content on the HCF ingredient compared to the EBCF and FEMPF ingredients, as the HCF component (X_3) in the mixture had a significant positive effect on the TA response. On the other hand, the EBCF component (X_1) did not have a significant effect, and the FEMPF component (X_2) had a significant negative effect. The low or null anthocyanin content in the FEMPF ingredient may cause the decreased TA content in tortillas made with high inclusion levels of this ingredient. On the other hand, the EBCF ingredient is a good source of TA, which may explain why increasing or decreasing this component in the mixture did not significantly affect the TA content in the tortillas. The highest TA values are in the same area as the highest AoxA, TPC, and TF values, indicating a possible correlation between TPC and TA, as well as TF and TA, which has not been previously reported in the literature. Anthocyanins are part of the generalized class of phenolic compounds known as flavonoids, and they play a protective role against oxidative stress by acting as radical scavengers (Astorga-Gaxiola *et al*., 2023). Aguayo-Rojas *et al*. (2012) evaluated tortillas made from pigmented Mexican corn, finding that the highest antioxidant activities in these tortillas may be related to the concentration of anthocyanins and total phenolics present in the different corn varieties. Furthermore, Mora-Rochín *et al*. (2016) reported that the pigments present in blue corn are mainly derived from anthocyanins and phenolic compounds, which are responsible for providing antioxidant properties. According to Figure 1D, the highest theoretical TA values were between 14 and 17 mg cyanidin-3-glucoside equivalents/100g, dw."

Overall Acceptability (OA) of tortillas

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In Table 1, we observe that the OA (overall acceptability) of the tortillas presented values with a minimum of 43.65 and a maximum of 57.6; these values were found on the LAM scale between 'slightly dislike' (43.65) and 'moderately like' (57.6).

The analysis of variance showed a significant linear model for the proportion of EBCF, FEMPF, and HCF. The predictive model for OA using coded variables was:

OA= 59*EBCF + 44*FEMPF + 37*HCF

This predictive model explained 86.12 % of the total variability ($p < 0.0001$) of the OA values in the tortillas. The adjusted R^2 coefficient of determination for the model was 0.8399, and the lack of fit was not significant ($p = 0.7254$). Additionally, the relative dispersion of the experimental points compared to the values predicted by the model (CV = 4.10 %) was less than 10 %. These values indicate that the predictive model for OA in the tortillas was adequate and reproducible.

Like the other response variables, a three-dimensional triangular-shaped graph (Figure 1E) was constructed from the predictive model for OA. The graph contained the real experimental area in the form of an irregular polytope, delineated by the restrictions imposed on each of the mixture components.

Figure 1E shows that the OA values are higher (desirable values) when the inclusion level of component X_1 [extruded corn flour content (EBCF)] in the mixture is high, while the inclusion levels of components X_2 [fermented-extruded mesquite pod flour content (FEMPF)] and X_3 [hibiscus calyx flour content (HCF)] cause a significant decrease in the overall acceptability (OA) of the tortillas. According to the analysis of variance, performed on the terms of the linear regression model fitted to the OA data, all three components (X_1, X_2, and X_3) had a significant effect ($p < 0.05$) on this response (effects = 17.83, -8.65, and -6.18 for X_1, X_2, and X_3, respectively). The increase in the inclusion level of FEMPF and HCF in the mixture caused a decrease in the OA of the tortillas, and the increase in the inclusion level of EBCF caused an increase in the OA of the tortillas; this is reaffirmed by the negative effects of the FEMPF and HCF components and the positive impact of the EBCF component (-8.65, -6.18, and 17.83 for X_2, X_3, and X_1, respectively) on the OA of the tortillas. The positive effect of the EBCF component was the most influential on the OA response. This may be because the fermented mesquite pods and hibiscus calyces may contain some compounds that impart undesirable sensory acceptance in the functional tortillas. The highest OA values are near the area with the highest AoxA, TPC, TF, and TA values. According to Figure 1E, the highest theoretical OA values were in the range of 54 to 59 on a scale of 0 to 100. These values were found on the LAM scale between 'I slightly like it' (54) and 'I moderately like it' (59)."

Optimization

Once the prediction models for each of the response variables (AoxA, TPC, TF, TA, and OA) were obtained, the numerical desirability method was used to find the optimal inclusion levels for each flour (EBCF, FEMPF, and HCF) to produce functional tortillas with high nutraceutical values and adequately sensory properties.

Figure 1F shows the effect of the inclusion levels (percentages) of EBCF, FEMPF, and HCF flours on the optimal overall desirability (D) variable. The overall desirability is obtained from the geometric mean [$D = (d_1 \times d_2 \times d_3 \times d_4 \times d_5)^{1/5}$] of the individual desirabilities of the five response variables used to carry out the optimization. An overall desirability is a logical option because if any $d_i(X) = 0$, the overall desirability would be D = 0, indicating that the tortilla is unacceptable. The logical procedure for optimization consisted of estimating values of the response variables at multiple points in the experimental region using their respective regression models obtained from the previously performed regression and variance analysis. Subsequently, the estimated values of the response variables were transformed into individual desirability values $d_i(X)$; this variable takes values between 0 and 1 through transformation equations established by the response surface methodology to maximize the value of each response variable. From the equation shown, overall desirability (D) values were calculated at each of the selected experimental points. Once the overall desirability values were calculated, overall desirability graphs were obtained, showing the behavior of this variable (D) as a function of the independent variables (inclusion level of EBCF, FEMPF, and HCF).

The optimal overall desirability value selected was D = 0.8019. The optimal values selected for the inclusion percentages of the flours were X_1 (EBCF) = 0.828 (82.8 %), X_2 (FEMPF) = 0.10 (10 %), and X_3 (HCF) = 0.072 (7.2 %).

Similarly, Figure 2 shows the individual desirability values associated with the selected overall desirability (D). These values were $d_{AoxA} = 0.94$, $d_{TPC} = 0.52$, $d_{TF} = 0.90$, $d_{TA} = 0.63$, and $d_{OA} = 1$. This indicates that most of the desirabilities obtained were above 0.63, which is considered acceptable and good (López-Ríos *et al.*, 2018). The optimal overall desirability value was 0.8. It is important to remember that a favorable overall desirability must have values ≥ 0.8. This indicates that the optimal combination of independent variables (optimal inclusion levels of EBCF, FEMPF, and HCF flours) achieves favorable results for all responses. Although most responses indicated that the combination of individual desirabilities was effective, it could be suggested to find another combination for $d_{TPC} = 0.52$.

Validation of optimal conditions

To validate the optimal conditions three replicates of the mixture composed of EBCF, FEMPF, and HCF flours were produced (extrusion, drying, and milling of blue corn in triplicate, fermentation, extrusion, drying, and milling of mesquite pods in triplicate, and drying and milling of hibiscus calyces in triplicate) with the best inclusion percentages for each one [(EBCF (82.8 %), FEMPF (10 %), and HCF (7.2 %)] to obtain functional tortillas. These tortillas were experimentally evaluated in triplicate for AoxA, TPC, TF, TA, and OA. Table 2 shows the results of the experimentally assessed response variables for the three replicates of tortillas. It is worth mentioning that the averages of the experimental results (Table 2) were contrasted with the mean values provided by the mathematical prediction models for each of the response variables [AoxA = 5912 μmol Trolox equivalents/100g (dw), TPC = 356 mg gallic acid equivalents/100g (dw), TF = 37.7 mg catechin

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equivalents/100g (dw), TA = 11.7 mg cyanidin-3-glucoside equivalents/100g (dw), and OA = 57.6]. The experimental values of the response variables were similar to the values predicted by the mathematical regression models, indicating that the optimal conditions (optimal mixture of EBCF, FEMPF, and HCF flours) were adequate and reproducible. Furthermore, the average experimental values for each analyzed response variable fell within the 95 % theoretical confidence intervals created from the optimal region in Figure 1F.

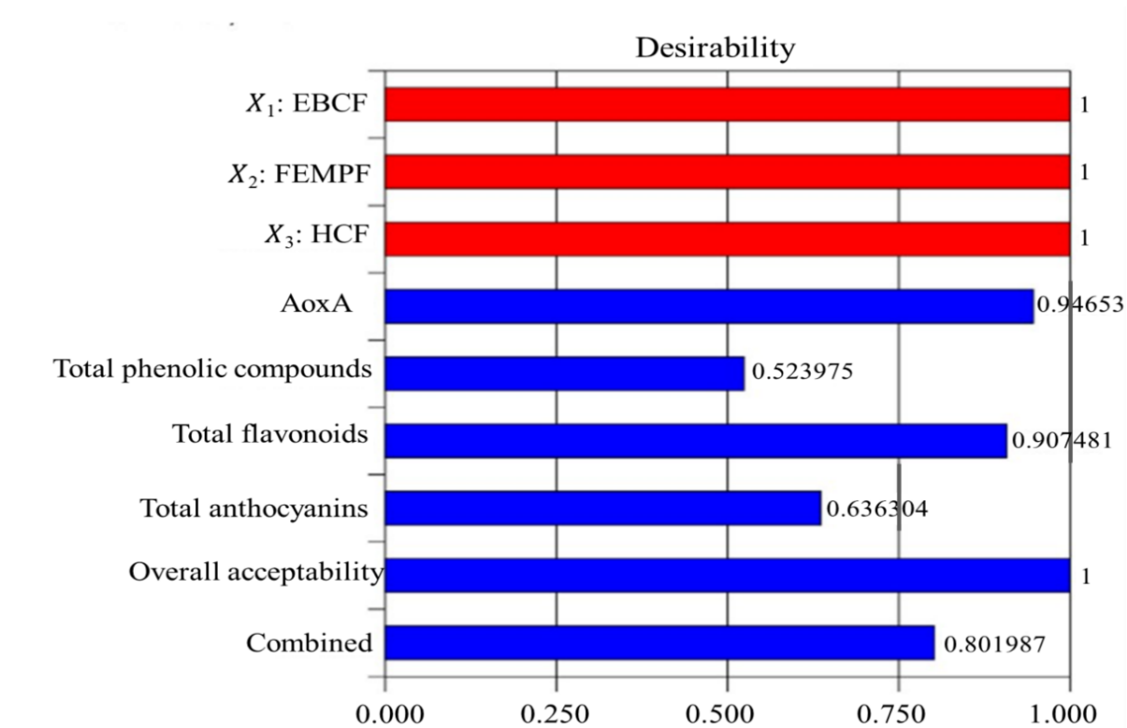

Figure 2. Individual desirabilities of responses and overall desirability of the optimized composite mixture.

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Table 2. Experimental results of the response variables evaluated from three replicates of functional tortillas preparation using optimal inclusion levels of EBCF (82.8 %), FEMPF (10 %), and HCF (7.2 %).

Response variables[1]	Experimental results	Average	Standard deviation	Coefficient of variation (CV, %)
AoxA	5960 5847 5901	5902.7	56.52	0.96
TPC	356.3 355.5 356.0	355.9	0.40	0.11
TF	36.8 37.9 38.1	37.6	0.70	1.86
TA	11.8 11.9 11.6	11.8	0.15	1.30
OA	59.2 57.2 56.3	57.6	1.48	2.58

[1]AoxA = antioxidant activity (μmol Trolox eq. /100 g), TPC = total phenolic compounds (mg gallic acid eq. /100 g), TF = total flavonoids (mg catechin eq. /100 g), TA = total anthocyanins (mg cianidin-3-glucoside eq. /100 g), OA = overall acceptability of tortillas.

Conclusions

The obtention of the optimal inclusion levels of extruded blue corn flour (EBCF), fermented-extruded mesquite pod flour (FEMPF), and hibiscus calyx flour (HCF) in a flour mixture allowed the production of functional tortillas with high values of antioxidant activity, total phenolic compounds, flavonoids, and total anthocyanins, as well as acceptable sensory acceptance. Therefore, due to their sensory, nutritional, phytochemical, and antioxidant characteristics, the functional tortillas developed in this research are a viable alternative to tortillas made solely from corn, which are poor in nutrients, particularly in protein quality. Moreover, these functional tortillas represent a novel strategy to reduce chronic degenerative diseases in Mexico. However, in the future, it is necessary to conduct a deeper characterization of the functional tortillas to validate their potential to impact consumer health positively.

Author contributions

Conceptualization: Marco Cesar Villanazul Verdugo, Roberto Gutiérrez Dorado, Carlos Alberto Gómez Aldapa, Janitzio Xiomara Korina Perales Sánchez, Cuauhtémoc Reyes Moreno; methodology development: Marco Cesar Villanazul Verdugo, Grissel Reyna Fuentes, Ana Luisa Félix Sámano; software management: Roberto Gutiérrez Dorado, Janitzio Xiomara Korina Perales Sánchez; experimental validation: Roberto Gutiérrez Dorado, Carlos Alberto Gómez Aldapa, Janitzio Xiomara Korina Perales Sánchez; results analysis: Marco Cesar Villanazul Verdugo, Roberto Gutiérrez Dorado, Grissel Reyna Fuentes; data management: Roberto Gutiérrez Dorado, Carlos Alberto Gómez Aldapa; writing and manuscript preparation: Marco Cesar Villanazul Verdugo, Roberto Gutiérrez Dorado; drafting, reviewing, and editing: Roberto Gutiérrez Dorado; funding acquisition: Roberto Gutiérrez Dorado, Carlos Alberto Gómez Aldapa, Janitzio Xiomara Korina Perales Sánchez, Cuauhtémoc Reyes Moreno. All authors of this manuscript have read and approved the published version

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Conflict of interest

The authors declare no conflict of interest.

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