



Galactomannan-Rich Ingredients from *Caesalpinia Pulcherrima* Seeds Reorganize Wheat Bread Microstructure and Enhance Technological and Nutritional Quality

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Abstract

Galactomannan-rich ingredients from tropical legumes are promising biomaterials to design bakery products with improved structure and nutritional quality. This study evaluated the structure–function relationships of *Caesalpinia pulcherrima* seed flour (FSF) and seed gel (GSF) in wheat bread. A central composite design assessed the effects of FSF (3.96–11.04%) and GSF (0.17–5.82%) on technological properties, proximate composition and microstructure. FSF and GSF increased bread specific volume (from 2.16 to 3.87 mL g⁻¹), alveoli number and circularity, while maintaining expansion index values comparable to the control, indicating improved gas retention without impairing dough expansion. FSF-enriched breads showed higher protein (up to 17.28%), lipid (up to 10.11%) and ash contents than the control, while crude fiber increased more modestly, indicating that FSF was the main contributor to nutritional enrichment, whereas GSF mainly affected hydration and crumb structure. pH, moisture and water activity remained within typical ranges for bread and compatible with standard yeast fermentation. SEM and CLSM images suggested a more open and organized crumb structure in enriched breads, with thinner cell walls and more uniform gas cells, consistent with the improved technological performance. Overall, FSF and GSF act as functional biomaterials that valorize an underutilized tropical legume and support the development of nutritionally enhanced, structurally tailored wheat breads.

Keywords Bakery reformulation · Crumb porosity · Bread volume · Seed flour · Seed gel · Tropical legume

Introduction

Wheat bread is widely consumed and represents an important vehicle for nutritional improvement through formulation strategies. However, conventional bread made with refined wheat flour is typically limited in fiber, minerals, vitamins and some bioactive components. For this reason, the partial replacement of wheat flour with plant-derived ingredients has been increasingly explored to improve the

nutritional profile and technological functionality of bakery products [1, 2].

A common strategy to improve nutritional quality is the partial replacement of wheat flour with plant-based ingredients such as whole grains, legume flours, by-products or natural hydrocolloids. Yet, due to their gluten-free and fiber-rich nature, these ingredients often impair dough rheology, gas retention and bread volume when used at high levels [3]. Legume-derived materials are particularly attractive because they combine proteins, lipids, minerals and dietary fiber with polysaccharides capable of modifying the dough matrix.

Among tropical legumes, *Caesalpinia pulcherrima*, known as flamboyant-mirim, dwarf poinciana or Barbados pride, is widely distributed in tropical regions such as Brazil, India, Malaysia and Sri Lanka and is well adapted to the Brazilian Cerrado. Various plant parts exhibit biological activities [4].

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Its seeds are rich in galactomannans, soluble dietary fiber with thickening, stabilizing and film-forming properties [5, 6]. These polysaccharides are applied in dairy desserts, edible films and fiber-enriched foods and strongly influence water binding, viscosity and phase behavior. In bread systems, galactomannans may contribute to water binding, gas-cell stability and crumb organization, thereby affecting bread quality and staling behavior [7, 8]. Additionally, *C. pulcherrima* seed flour has been explored for biofuel and animal feed due to its protein- and lipid-rich matrix, suggesting nutritional density coupled with functional macromolecules.

Despite these attributes, no studies have evaluated *C. pulcherrima* seed derivatives in breadmaking. Thus, the relevance of the present work lies not simply in adding a legume-derived ingredient to bread, but in comparing two structurally distinct fractions obtained from the same underutilized tropical seed: a flour fraction (FSF), which retains proteins, lipids, minerals and fiber-rich particles, and a gel fraction (GSF), which behaves predominantly as a hydrated galactomannan-rich hydrocolloid.

We hypothesized that these fractions would play different roles in the wheat bread matrix. Specifically, FSF was expected to mainly promote compositional enrichment and modify crumb structure through partial flour replacement and particle–matrix interactions, whereas GSF was expected to primarily affect water distribution, dough viscosity and gas-cell stability because of its hydrocolloid nature. Their combined use was therefore expected to modulate the gluten–starch matrix differently from studies evaluating only legume flours or only hydrocolloid addition.

In this context, the present study investigated the structure–function relationships of *C. pulcherrima* seed flour (FSF) and seed gel (GSF) in wheat bread using response surface methodology. The objective was to determine how these two seed-derived ingredients influence technological quality, proximate composition and crumb microstructure, while discussing their effects considering flour replacement, hydration and gas-cell stabilization mechanisms.

Materials and Methods

Raw Material Collection

Pods of *Caesalpinia pulcherrima* containing mature seeds were collected at the Federal University of Ceará, Campus Pici, Fortaleza, Brazil (3°44'38.6"S; 38°34'47.3"W), under SisGen authorization ABF331B. Pods were selected based on dry texture, natural dehiscence, and brown coloration. A voucher specimen was deposited in the Prisco Bezerra Herbarium (EAC 67246).

Preparation of *C. Pulcherrima* Seed Flour (FSF)

Pods were manually opened, and seeds were selected for integrity and absence of defects. Seeds were ground in an industrial blender (Li1.5, Skymesen, Brazil) for 5 min in 1-min intervals and then milled in a knife mill (Luca-226/5, Lucadema, Brazil) for 5 min under the same conditions. The powder was sieved through a 0.250 mm mesh using an electromagnetic shaker (Rotachoc Chopin®, France) for 10 min. The resulting seed flour (FSF; 0.250 mm) was vacuum-packed, stored at room temperature.

Preparation of *C. Pulcherrima* Seed Gel (GSF)

For the preparation of GSF, 20 g of seeds were mixed with 200 mL of distilled water and heated on a magnetic hot plate (Q310-22B, Quimis, Brazil) at 70 °C for 5 h. After cooling to room temperature, the cooked mixture was homogenized with a vertical mixer (Black & Decker, Brazil) and filtered through a fine mesh sieve (0.6 mm) to obtain the gel. The GSF was stored in polyethylene containers at 4 °C (Electrolux, Brazil). The gel yield was approximately 69.7%.

Bread Formulation and Experimental Design

The control bread formulation, without FSF or GSF, followed Guimarães et al. [7], and consisted of wheat flour (100%), water (58%), hydrogenated vegetable fat (10%), refined sugar (5%), instant dry yeast (Dr. Oetker, Brazil) (3.3%) and salt (2%). Ingredients were purchased from a local market in Teresina, Piauí, Brazil.

Because GSF showed high moisture content (95.24%), the water contributed by the gel was considered in each formulation. Thus, the amount of added water was reduced according to the GSF level so that total dough hydration remained comparable among formulations on a wet basis. FSF replaced wheat flour proportionally, whereas GSF was incorporated according to the experimental design, with its endogenous water discounted from the added water.

The experimental design consisted of eleven runs combining different concentrations of FSF and GSF according to the Central Composite Rotational Design. Run 1 was formulated with low levels of both factors ($X_1 = -1$; 5.0% FSF and $X_2 = -1$; 1.0% GSF), while Run 2 maintained the same FSF level (5.0%) combined with the high GSF level ($X_2 = +1$; 5.0%). Runs 3 and 4 used the high FSF concentration ($X_1 = +1$; 10.0%), combined with low GSF (1.0%) in Run 3 and high GSF (5.0%) in Run 4. The axial points included Run 5 ($X_1 = -1.41$; 3.96% FSF and $X_2 = 0$; 3.0% GSF) and Run 6 ($X_1 = +1.41$; 11.04% FSF and $X_2 = 0$; 3.0% GSF). Runs 7 and 8 were formulated with the central FSF level ($X_1 = 0$; 7.5%) combined with the lowest axial GSF

level ($X_2 = -1.41$; 0.17%) in Run 7 and the highest axial GSF level ($X_2 = +1.41$; 5.82%) in Run 8. Finally, Runs 9, 10 and 11 corresponded to the central point of the design, containing 7.5% FSF and 3.0% GSF in all replicates ($X_1 = 0$; $X_2 = 0$).

Bread-Making Process

Dough ingredients were mixed in a planetary mixer (Arno, Brazil) for 7 min, divided into 250 g portions and manually shaped. For each experimental run, three breads were prepared under the same processing conditions, and these breads were used as biological replicates for quality analysis. Doughs were placed in single-sheet pans and proofed at 32 °C and 75% RH for 90 min in a fermentation chamber (Frilux, Brazil). Baking was performed in an electric oven (Venâncio, Brazil) at 220 °C for 20 min, followed by cooling at room temperature (25 ± 2 °C). Bread quality analyses were conducted 2 h after baking [9].

Expansion Index (EI)

The EI was determined according to Zambelli et al. [10]. Cylindrical dough portions (~10 g) had their diameter and height measured with a calibrated caliper before fermentation and after baking. EI was calculated using Eq. (1):

$$EI = \frac{(D_P/H_P)}{(D_M/H_M)} \quad (1)$$

where:

D_p — diameter of the bread after baking; H_p – height of the bread after baking; D_m —diameter of the molded dough before fermentation; H_m — height of the molded dough before fermentation.

Specific Volume of Bread

Bread volume was measured by the millet seed displacement method (AACC Method 72–10) [11]. For each experimental run, specific volume was determined for the three breads produced, and each bread was analyzed in triplicate. Specific volume (mL.g^{-1}) was calculated as the ratio between bread volume and weight.

Crumb Structure Image Analysis

The crumb structure was analyzed by digital image processing. Central bread slices were scanned at 550 dpi using an HP ScanJet 2400, and images (900×900 pixels) were processed in ImageJ® 1.47v (National Institutes of Health, USA). Images were converted to 8-bit grayscale, thresholded

using Otsu's method, and analyzed for alveoli number and circularity following [12].

Crumb Color

Crumb color was determined in the CIELAB system and expressed as L (lightness), a (red green coordinate), and b (yellow-blue coordinate). The total color difference relative to the control bread was calculated according to Eq. (2). Color measurements were performed using a colorimeter NR0000 (3NH, China).

$$\Delta E = \left[(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2 \right]^{1/2} \quad (2)$$

where L_0 , a_0 , and b_0 correspond to the color coordinates of the control bread.

Physicochemical and Proximate Analyses

Physicochemical and proximate analyses, including pH, titratable acidity, moisture, protein, ash, crude fiber, lipids and carbohydrates, were determined for FSF, GSF and bread samples according to Adolfo Lutz Institute [13] and AOAC [14] methods. Water activity (a_w) was measured using a LabSwift- a_w analyzer (Novasina, Switzerland), and carbohydrate content was calculated by difference. For each experimental run, physicochemical and proximate analyses were carried out using the three breads produced as biological replicates, and each determination was performed in triplicate for each bread.

Scanning Electron Microscopy (SEM)

The microstructure of FSF, GSF, and bread crumb samples was examined by SEM. Samples were fixed on aluminum stubs with carbon tape and sputter-coated with gold (~20 nm) using a Bal-Tec SCD 050 coater. Micrographs were obtained in an INSPECT S50 (FEI Company, Hillsboro, OR, USA) operated at 15 kV, with magnification $1000 \times$ to visualize surface morphology, starch granules, and the protein–starch network.

Confocal Laser Scanning Microscopy (CLSM)

CLSM was used to visualize crumb microstructure and the distribution of starch and protein. Samples were mounted on glass slides and analyzed using a Zeiss LSM 710 confocal laser scanning microscope (Carl Zeiss AG, Oberkochen, Germany). Protein and starch were double stained using 20 μL of Rhodamine B solution (0.1%, w/v, in ethanol) and 20 μL of FITC solution (1%, w/v, in ethanol), respectively.

Images were acquired and processed using ZEN software (Zeiss, Oberkochen, Germany) to assess the three-dimensional organization of the protein–starch matrix.

Statistical analysis

Statistical differences among treatments, control bread, and raw materials were assessed by ANOVA followed by Tukey's test at 5% significance using Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA). The effects of FSF (x_1) and GSF (x_2) on bread quality variables were modeled using second-order polynomial regression, as shown in Eq. (2).

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 \quad (3)$$

where Y is the dependent variable; β_0 is the intercept; β_1 and β_2 are the linear coefficients; β_{11} and β_{22} are the quadratic coefficients; and β_{12} represents the interaction coefficient between the independent variables.

Model significance was assessed using the F-test, lack-of-fit, and determination coefficients (R^2). Validated models were then used to generate response surface and contour plots, illustrating how FSF and GSF levels influenced the technological and nutritional attributes of the breads.

This article does not contain any studies with human participants or animals performed by any of the authors.

Results and Discussion

The experimental values obtained for alveoli circularity and count, specific volume and expansion index are shown in Table 1, together with the corresponding measurements of the control bread for comparison.

The incorporation of FSF and GSF increased the specific volume of all enriched breads compared with the control, indicating improved gas retention during proofing and baking. The highest specific volume (3.87 mL g^{-1}) was observed in run 6 (11.04% FSF + 3% GSF), whereas the control showed the lowest value (2.16 mL g^{-1}). Crumb image analysis further supported these macroscopic differences: breads containing FSF and GSF had higher alveoli numbers (1034–1383) and circularity (0.832–0.952) than the control (728; 0.639), resulting in a more homogeneous crumb with well-distributed and rounded gas cells [15]. Despite these structural changes, the EI remained statistically unchanged (1.24 – 1.35 cm ; $p > 0.05$; Table 1), suggesting that the partial replacement of wheat flour with FSF and GSF preserved dough expansion capacity while allowing higher bread volume and a finer crumb structure.

The observed increases in bread volume and crumb aeration are consistent with the structuring role of the galactomannans naturally present in the seeds in wheat bread. In the present study, the higher specific volume, the improved alveoli distribution and circularity, and the more continuous crumb organization observed by SEM and CLSM indicate that FSF and GSF positively influenced bread structure. These results show that, even under partial flour replacement, the incorporation of both ingredients promoted better gas retention and crumb organization, leading to breads with superior technological quality compared with the control. In addition, the slightly higher lipid contents measured in FSF- and GSF-enriched breads (8.78–10.11%) compared with the control (8.28%) may also have contributed to this behavior by favoring gas-cell stability during proofing and baking. Taken together, these results support a positive structuring effect of FSF and GSF on wheat bread, as evidenced by technological parameters and qualitative microstructural observations.

Comparable improvements in bread volume and crumb uniformity have been reported in breads enriched with plant-based flours or hydrocolloids, which contribute to gas retention and crumb organization in bakery systems [8, 16–18]. Consistent with these findings, the present results show that galactomannan-rich legume ingredients can act as structuring components in wheat bread. In this sense, FSF and GSF contributed to a more aerated and organized crumb structure, which agrees with the improved bread performance observed in the enriched formulations.

Table 1 Mean values and Tukey's test results for technological properties of breads supplemented with FSF and GSF

Runs	Alveoli circularity	Alveoli number (cells)	EI	Specific Volume (mL.g^{-1})
1	$0.928 \pm 0.02^{\text{ab}}$	$1034 \pm 58.00^{\text{c}}$	$1.26 \pm 0.01^{\text{a}}$	$3.27 \pm 0.13^{\text{ab}}$
2	$0.900 \pm 0.04^{\text{b}}$	$1120 \pm 45.00^{\text{b}}$	$1.32 \pm 0.11^{\text{a}}$	$3.51 \pm 0.23^{\text{ab}}$
3	$0.903 \pm 0.03^{\text{b}}$	$1199 \pm 38.00^{\text{b}}$	$1.32 \pm 0.05^{\text{a}}$	$3.55 \pm 0.12^{\text{ab}}$
4	$0.939 \pm 0.02^{\text{ab}}$	$1244 \pm 29.00^{\text{ab}}$	$1.35 \pm 0.05^{\text{a}}$	$3.59 \pm 0.25^{\text{ab}}$
5	$0.832 \pm 0.04^{\text{d}}$	$912 \pm 31.00^{\text{c}}$	$1.28 \pm 0.01^{\text{a}}$	$3.00 \pm 0.13^{\text{b}}$
6	$0.952 \pm 0.01^{\text{a}}$	$1383 \pm 19.00^{\text{a}}$	$1.30 \pm 0.04^{\text{a}}$	$3.87 \pm 0.55^{\text{a}}$
7	$0.908 \pm 0.03^{\text{b}}$	$1093 \pm 2.00^{\text{b}}$	$1.24 \pm 0.04^{\text{a}}$	$3.44 \pm 0.15^{\text{ab}}$
8	$0.898 \pm 0.03^{\text{c}}$	$1012 \pm 32.00^{\text{bc}}$	$1.25 \pm 0.05^{\text{a}}$	$3.37 \pm 0.35^{\text{ab}}$
9	$0.910 \pm 0.02^{\text{b}}$	$1183 \pm 14.00^{\text{b}}$	$1.31 \pm 0.02^{\text{a}}$	$3.60 \pm 0.24^{\text{ab}}$
10	$0.905 \pm 0.03^{\text{b}}$	$1179 \pm 18.00^{\text{b}}$	$1.32 \pm 0.03^{\text{a}}$	$3.16 \pm 0.08^{\text{ab}}$
11	$0.908 \pm 0.03^{\text{b}}$	$1190 \pm 21.00^{\text{b}}$	$1.29 \pm 0.03^{\text{a}}$	$3.53 \pm 0.01^{\text{ab}}$
Control	$0.639 \pm 0.03^{\text{e}}$	$728 \pm 30.00^{\text{d}}$	$1.31 \pm 0.04^{\text{a}}$	$2.16 \pm 0.05^{\text{c}}$

Different lowercase letters in the same column indicate significant differences according to Tukey's test ($p < 0.05$)

The experimental results for pH, titratable acidity, moisture and water activity are presented in Table 2 for the control bread, FSF, GSF and enriched breads. FSF and GSF showed slightly acidic pH values (6.29 and 6.25, respectively), which are typical of bakery ingredients and within the range reported for *C. pulcherrima* seed mucilage (pH 5.7) [19]. Although near-neutral pH foods may favor spoilage microorganism growth [20], the pH values observed for FSF and GSF remain compatible with their incorporation into bread formulations.

The pH of breads containing FSF and GSF ranged from 5.40 to 5.55, while the control presented a slightly lower value (5.38). Formulations 1–3 (5.40–5.43) were similar to the control, whereas runs 4–11 exhibited higher pH values. This range is technologically adequate, since yeast fermentation is optimal between pH 4.5 and 6.5 and conventional breads typically show pH 4.7–5.4 due to organic acid formation during fermentation [1]. Previous studies have also reported only minor pH changes when plant-based ingredients are incorporated into wheat doughs [3, 21], indicating that FSF and GSF slightly increased pH without impairing fermentation.

Titratable acidity, which is related to freshness and shelf stability [15], was lowest in FSF and GSF (0.12%) and ranged from 0.16% to 0.30% in breads. Among the breads, formulation 2 exhibited the lowest acidity, whereas the control showed the highest value, in agreement with reports of reduced acidity when plant ingredients partially replace wheat flour [22]. The lower acidity of enriched breads, combined with essentially unchanged water activity values (Table 2), suggests that the observed differences were driven by the intrinsic composition of FSF and GSF rather

than by deviations in fermentation conditions, supporting process reproducibility and compatibility with standard breadmaking.

Moisture values reflected the contrasting nature of the raw materials. FSF showed low moisture (5.92%), whereas GSF presented very high moisture (95.24%), in agreement with previous reports for *C. pulcherrima* seeds and mucilage [19, 23, 24]. FSF complied with Brazilian standards for flours (maximum 15% moisture; RDC 263/2005), confirming its suitability as a dry ingredient. Bread moisture ranged from 32.33% to 37.09%, with the control displaying the highest value and formulation 4 (10% FSF and 5% GSF) the lowest. Since the water contributed by GSF was considered during formulation, the moisture differences observed among breads are more likely related to differences in water-binding behavior and baking losses than to uncontrolled dilution effects. All breads met regulatory limits for bread moisture ($\leq 38\%$; RDC 90/2000), indicating that FSF and GSF were compatible with the compositional and regulatory requirements of this product category. Although several studies have reported increased moisture when plant-based ingredients are incorporated into breads [3, 16, 18, 25], the present results show that, under the conditions tested, FSF and GSF levels were compatible with moisture contents typical of conventional breads.

Water activity followed a complementary pattern. FSF exhibited low a_w (0.496), whereas GSF showed a very high value (0.986), which explains the need for refrigeration of the gel. Bread a_w ranged from 0.943 to 0.956, without significant differences ($p > 0.05$) relative to the control. These values confirm that all breads are high- a_w products, which accounts for their naturally short shelf life and the need for appropriate packaging and storage conditions.

The lack of significant changes in a_w after FSF and GSF incorporation indicates that the microenvironment of available water in the crumb was not markedly altered, despite differences in formulation and moisture. Similar trends of essentially unchanged a_w in enriched breads have been reported when plant-based ingredients are added to wheat-based formulations [22, 26, 27].

Although some of these variables differed statistically among formulations, the magnitude of change was limited, and all breads remained within ranges typically reported for bread or within applicable regulatory limits. Therefore, these results should be interpreted primarily as evidence of formulation compatibility with breadmaking rather than as indicators of superior technological quality.

The proximate composition results (Table 3) highlighted marked differences in lipid content between the seed-derived ingredients. GSF had a very low lipid content (0.08%), whereas FSF exhibited a substantially higher value (9.90%), consistent with previous reports for *C. pulcherrima* seeds

Table 2 pH, titratable acidity, moisture, and water activity of breads with FSF and GSF, control, and raw materials

Runs	pH	Titratable acidity (%)	Moisture (%)	Water Activity
1	5.40 ± 0.01 ^{de}	0.21 ± 0.00 ^b	37.00 ± 0.03 ^b	0.952 ± 0.00 ^b
2	5.41 ± 0.01 ^{cde}	0.16 ± 0.00 ^g	35.03 ± 0.27 ^c	0.955 ± 0.00 ^b
3	5.43 ± 0.01 ^{cde}	0.20 ± 0.01 ^{bc}	32.78 ± 0.25 ^{gh}	0.955 ± 0.00 ^b
4	5.51 ± 0.01 ^b	0.20 ± 0.01 ^{bcd}	32.33 ± 0.04 ^h	0.956 ± 0.00 ^b
5	5.48 ± 0.01 ^{bcd}	0.17 ± 0.00 ^{fg}	32.88 ± 0.14 ^g	0.952 ± 0.00 ^b
6	5.52 ± 0.04 ^b	0.18 ± 0.00 ^{cde}	34.11 ± 0.01 ^{ef}	0.954 ± 0.00 ^b
7	5.55 ± 0.02 ^b	0.19 ± 0.00 ^{cde}	32.52 ± 0.11 ^{gh}	0.943 ± 0.00 ^c
8	5.48 ± 0.02 ^{bc}	0.19 ± 0.02 ^{cde}	33.79 ± 0.09 ^f	0.951 ± 0.00 ^{bc}
9	5.55 ± 0.02 ^b	0.17 ± 0.00 ^{efg}	34.82 ± 0.08 ^{cd}	0.950 ± 0.00 ^{bc}
10	5.55 ± 0.03 ^b	0.18 ± 0.00 ^{cdef}	34.54 ± 0.07 ^{de}	0.950 ± 0.00 ^{bc}
11	5.54 ± 0.01 ^b	0.18 ± 0.00 ^{def}	34.86 ± 0.38 ^{cd}	0.948 ± 0.00 ^{bc}
Control	5.38 ± 0.03 ^c	0.30 ± 0.00 ^a	37.09 ± 0.10 ^b	0.951 ± 0.00 ^{bc}
FSF	6.29 ± 0.07 ^a	0.12 ± 0.00 ^h	5.92 ± 0.12 ⁱ	0.496 ± 0.00 ^d
GSF	6.25 ± 0.02 ^a	0.12 ± 0.00 ^h	95.24 ± 0.06 ^a	0.986 ± 0.00 ^a

Different lowercase letters in the same column indicate significant differences according to Tukey's test ($p < 0.05$)

Table 3 Proximate composition and Tukey's test for breads with FSF and GSF, control, and raw materials

Runs	Lipids (%)	Proteins (%)	Crude Fiber (%)	Ash (%)	Carbohydrate (%)
1	8.78±0.03 ^d	14.59±0.23 ^{gh}	0.24±0.02 ^{de}	1.62±0.02 ⁱ	37.75±0.45 ^{def}
2	9.44±0.31 ^{bc}	14.81±0.08 ^{fgh}	0.25±0.04 ^{de}	2.03±0.04 ^g	38.43±0.52 ^{cde}
3	9.70±0.06 ^{abc}	16.51±0.24 ^{bc}	0.33±0.01 ^{de}	2.19±0.03 ^f	38.47±0.45 ^{cde}
4	9.91±0.02 ^{ab}	16.41±0.22 ^{bcd}	0.35±0.02 ^{de}	2.33±0.02 ^e	38.65±0.20 ^{cd}
5	8.86±0.23 ^d	14.90±0.08 ^{fgh}	0.23±0.03 ^e	1.77±0.02 ^h	41.34±0.30 ^b
6	10.11±0.07 ^a	17.28±0.29 ^b	0.53±0.04 ^c	2.97±0.04 ^b	35.00±0.29 ^h
7	9.58±0.09 ^{bc}	15.95±0.13 ^{cde}	0.37±0.02 ^d	2.65±0.02 ^d	38.93±0.37 ^c
8	9.70±0.01 ^{abc}	16.34±0.15 ^{cde}	0.34±0.02 ^{de}	2.79±0.05 ^c	37.04±0.16 ^{fg}
9	9.76±0.10 ^{abc}	16.17±0.26 ^{cde}	0.36±0.02 ^{de}	2.76±0.02 ^c	36.12±0.21 ^{gh}
10	9.64±0.11 ^{abc}	15.47±0.42 ^{efg}	0.36±0.03 ^{de}	2.72±0.01 ^{cd}	37.28±0.36 ^{defg}
11	9.37±0.06 ^c	15.57±0.19 ^{def}	0.37±0.01 ^d	2.74±0.02 ^{cd}	37.19±0.21 ^{efg}
Control	8.28±0.12 ^e	14.20±0.24 ^h	0.25±0.00 ^{de}	1.57±0.05 ⁱ	38.59±0.29 ^{cd}
FSF	9.90±0.40 ^{ab}	27.34±0.78 ^a	1.32±0.11 ^a	3.85±0.00 ^a	51.66±1.27 ^a
GSF	0.08±0.01 ^f	2.11±0.04 ⁱ	0.67±0.05 ^b	0.15±0.01 ^j	3.18±0.06 ⁱ

Different lowercase letters in the same column indicate significant differences according to Tukey's test ($p < 0.05$)

[22]. This lipid fraction is intrinsic to the seed and, when transferred to the flour, represents a natural source of fats for bakery formulations, offering the possibility of improving fatty acid profiles compared with conventional breads.

In the breads, lipid contents ranged from 8.78% to 10.11%, while the control presented the lowest value (8.28%). The highest lipid level was observed in run 6 (11.04% FSF+3% GSF), mirroring the higher FSF inclusion. Similar increases in lipid content have been reported in breads enriched with plant flours [17, 27]. From a technological perspective, these lipids interact with gluten and starch, improving gas retention, bread volume and crumb elasticity, which reinforces the dual role of FSF as both a nutritional and structuring ingredient.

Protein content also differed markedly between the seed ingredients. GSF showed low protein content (2.11%), whereas FSF exhibited a much higher value (27.34%), like that reported by Omode et al. [23], but lower than values described by Oderinde et al. [24], and Yusuf et al. [22]. In breads, protein contents ranged from 14.59% to 17.28%, all above the control (14.20%), with run 6 again presenting the highest level. This pattern agrees with studies demonstrating that protein-rich plant flours increase the protein content of breads [24]. Taken together, these results indicate that FSF functions as a protein-dense biomaterial capable of enriching breads without compromising gluten functionality, since dough expansion and bread volume were maintained or improved.

Crude fiber contents were low in GSF (0.67%) and higher in FSF (1.32%), although still slightly below values reported for *C. pulcherrima* seeds in some studies [22, 24]. In the breads, crude fiber ranged from 0.23% to 0.53%, with run 6 presenting the highest value, whereas most formulations did not differ significantly from the control ($p > 0.05$). Therefore, within the formulation range evaluated, fiber enrichment was limited and should be regarded as a

secondary nutritional effect when compared with the more pronounced increases observed for protein, lipids and ash. Similar outcomes have been reported in breads formulated with moderate levels of plant-based ingredients, where fiber enrichment is constrained by both the intrinsic composition of the ingredient and the technological limits of incorporation [18, 25, 27].

FSF had a high ash content (3.85%), whereas GSF showed a much lower value (0.15%). Reported ash levels for *C. pulcherrima* seeds vary in the literature [22–24], but the present values confirm that the flour fraction concentrates most of the minerals. In breads, ash contents ranged from 1.62% to 2.97%, all above the control (1.57%), with run 6 presenting the highest value. These findings are in line with the Brazilian Food Composition Table and with studies showing that plant flours increase the mineral content of breads [18, 28], reinforcing FSF as a nutritionally advantageous additive.

Carbohydrate contents also reflected the distribution of macronutrients between the seed fractions. FSF exhibited a high carbohydrate content (51.66%), whereas GSF showed a much lower value (3.18%) (Table 3), confirming the predominance of carbohydrates in the flour fraction [22, 24, 25]. Bread carbohydrates ranged from 35.00% to 41.34%. Formulations 1, 2, 3, 4 and 7 were statistically similar to the control ($p > 0.05$), while the remaining formulations differed significantly, with run 5 presenting the highest value (41.34%) and run 6 the lowest (35.00%). These variations result from simultaneous changes in moisture, ash, protein, lipid and fiber contents, since carbohydrates were calculated by difference. Similar patterns have been reported in breads containing plant flours [8, 18, 25], indicating that FSF and GSF modify macronutrient distribution without deviating from typical ranges for bread.

Table 4 shows that crumb color was significantly influenced by the incorporation of seed flour and seed gel.

Table 4 Crumb color parameters (L^* , a^* , b^*) of breads containing seed flour and seed gel from *Caesalpinia pulcherrima*

Runs	L	a	b	ΔE
1	55.64±2.65 ^{abc}	2.60±0.17 ^d	15.80±1.22 ^{cd}	12.43
2	57.06±1.10 ^{abc}	2.31±0.06 ^d	14.78±0.57 ^d	11.50
3	48.22±10.11 ^c	3.81±0.24 ^{abc}	20.00±0.73 ^{ab}	19.25
4	52.13±3.20 ^c	4.65±0.10 ^a	19.84±0.26 ^{ab}	15.62
5	57.50±3.53 ^{abc}	3.01±0.31 ^{cd}	15.43±0.67 ^{cd}	10.96
6	59.02±1.14 ^{abc}	4.67±0.19 ^a	21.03±0.15 ^a	9.23
7	64.72±1.49 ^{ab}	4.21±0.53 ^{ab}	19.44±1.42 ^{ab}	4.47
8	56.14±4.80 ^{abc}	4.33±0.67 ^{ab}	18.30±1.25 ^{abc}	11.80
9	59.15±3.71 ^{abc}	3.65±0.37 ^{bc}	19.51±1.44 ^{ab}	8.64
10	54.13±4.94 ^{bc}	2.65±0.43 ^d	17.31±1.76 ^{bcd}	13.49
11	58.17±0.57 ^{abc}	3.64±0.02 ^{bc}	19.46±0.63 ^{ab}	9.55
Control	67.19±0.57 ^a	0.52±0.03 ^c	19.91±0.54 ^{ab}	-

Means followed by the same lowercase letters in the same column do not differ significantly by Tukey's test ($p < 0.05$)

Crumb color was significantly influenced by the incorporation of seed flour and seed gel (Table 4). Compared with the control bread, all enriched formulations tended to show lower lightness values, indicating a darker crumb. This effect was particularly evident in formulations containing higher levels of seed flour, such as runs 3 and 4, which presented the lowest L^* values.

In parallel, the increase in a^* values observed in the enriched breads indicates a shift toward redder tones, while variations in b^* suggest that the intensity and saturation of yellow hues were also modified depending on the formulation. These results show that the incorporation of the seed-derived ingredients not only altered bread structure and composition, but also visibly affected crumb appearance. Such changes are likely related to the intrinsic pigmentation of the flour and gel fractions, as well as compositional

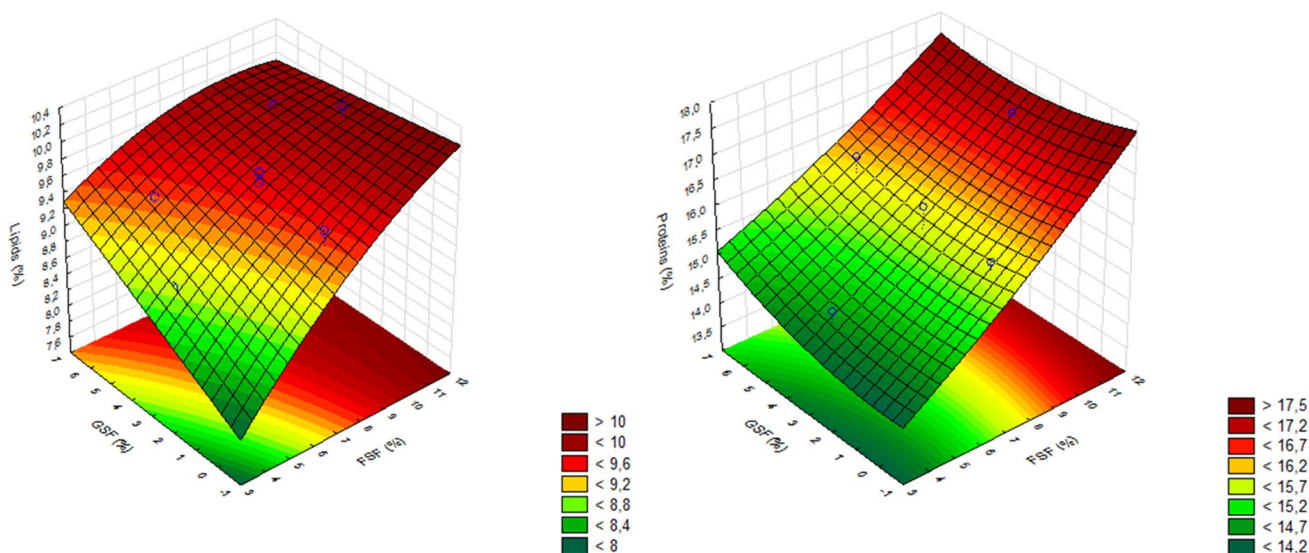
modifications in the bread matrix during baking. The visual differences observed among formulations were consistent with the instrumental color measurements and reinforced the impact of these ingredients on the overall quality attributes of the breads. The total color difference (ΔE) confirmed an overall visual change in crumb color relative to the control bread, with the highest values observed for runs 3 and 4, indicating the greatest deviation from the control crumb color.

The response surface plots in Fig. 1 illustrate how FSF and GSF concentrations affect bread lipid and protein contents. Increasing FSF levels consistently raised both lipid and protein values, confirming FSF as the main contributor to the nutritional enrichment of the breads, whereas GSF had little effect within the evaluated range. Based on the significant regression coefficients ($p < 0.05$), the lipid content (YLIPIDS) was described by the following second-order model:

$$Y_{(LIPIDS)} = 6.99 + 0.41x_1 - 0.01x_1^2 + 0.23x_2 - 0.0003x_2^2 - 0.02x_1x_2 \quad (4)$$

The positive linear term for x_1 and the negative quadratic coefficient indicates that lipid content increases with FSF concentration up to the upper region of the experimental domain (10–11.04% FSF; $x_1 = +1$ to $+1.41$). This trend is consistent with runs 3 (10% FSF, 1% GSF), 4 (10% FSF, 5% GSF) and 6 (11.04% FSF, 3% GSF), which presented the highest lipid contents (9.70–10.11%). In contrast, the non-significant or small coefficients for x_2 confirm that GSF did not markedly influence lipid content within the studied range (up to 5.83%).

Protein content followed a similar pattern and was modelled as:

**Fig. 1** Response surface plots showing the effects of FSF and GSF levels on (a) lipid and (b) protein contents of breads

$$Y_{(\text{PROTEINS})} = 13.20 + 0.29x_1 + 0.006x_1^2 + 0.05x_2 + 0.01x_2^2 - 0.01x_1x_2 \quad (5)$$

where: $\times 1 = \text{FSF} (\%)$, $\times 2 = \text{GSF} (\%)$.

As shown in Fig. 1, increasing FSF concentration resulted in a proportional rise in protein content. Formulations containing 10–11.04% FSF ($x_1 = +1$ to $+1.41$) yielded the highest protein values, with runs 3, 4 and 6 reaching 16.41–17.28%. GSF concentration did not significantly affect protein content within the evaluated range. Together, these models highlight FSF as the key driver of nutritional enhancement, while GSF plays a minor role in macronutrient enrichment but contributes structurally via its hydrated galactomannan matrix.

Figure 2 shows the SEM micrographs of FSF, GSF, control bread crumb and enriched bread crumb (Run 4: 10% FSF and 5% GSF). The micrographs revealed visible

morphological differences among the samples. FSF showed irregular particles with rough surfaces and compact aggregates, whereas GSF exhibited a more compact and continuous gel-like structure. In the bread crumbs, the control sample displayed a denser matrix with lower porosity, while the enriched bread showed a more open structure with thinner cell walls and larger void spaces [27, 29]. These observations are consistent with the higher specific volume and improved alveoli characteristics of the enriched breads [8]. Therefore, the SEM images should be interpreted as qualitative morphological evidence of altered crumb organization rather than as direct proof of specific molecular interactions within the dough matrix.

As shown in Fig. 3, the CLSM micrographs revealed qualitative differences in the microstructural organization of FSF, GSF, control bread crumb and enriched bread crumb.

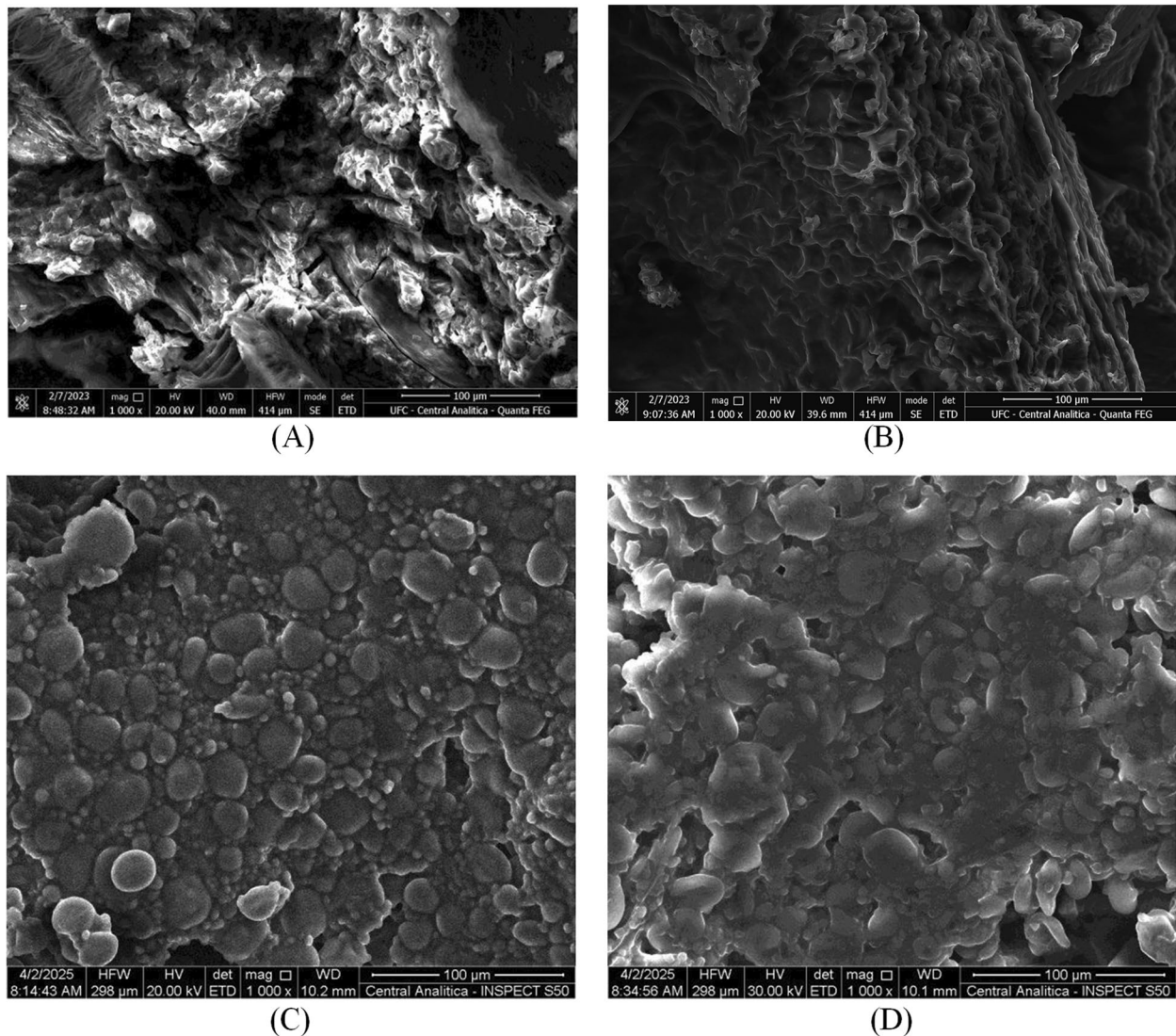


Fig. 2 Scanning electron micrographs of FSF, GSF, and the bread crumb samples enriched with these ingredients. (A)—FSF; (B)—GSF; (C)—Control bread; (D)—Bread enriched with 10% FSF and 5% GSF (Run 4)

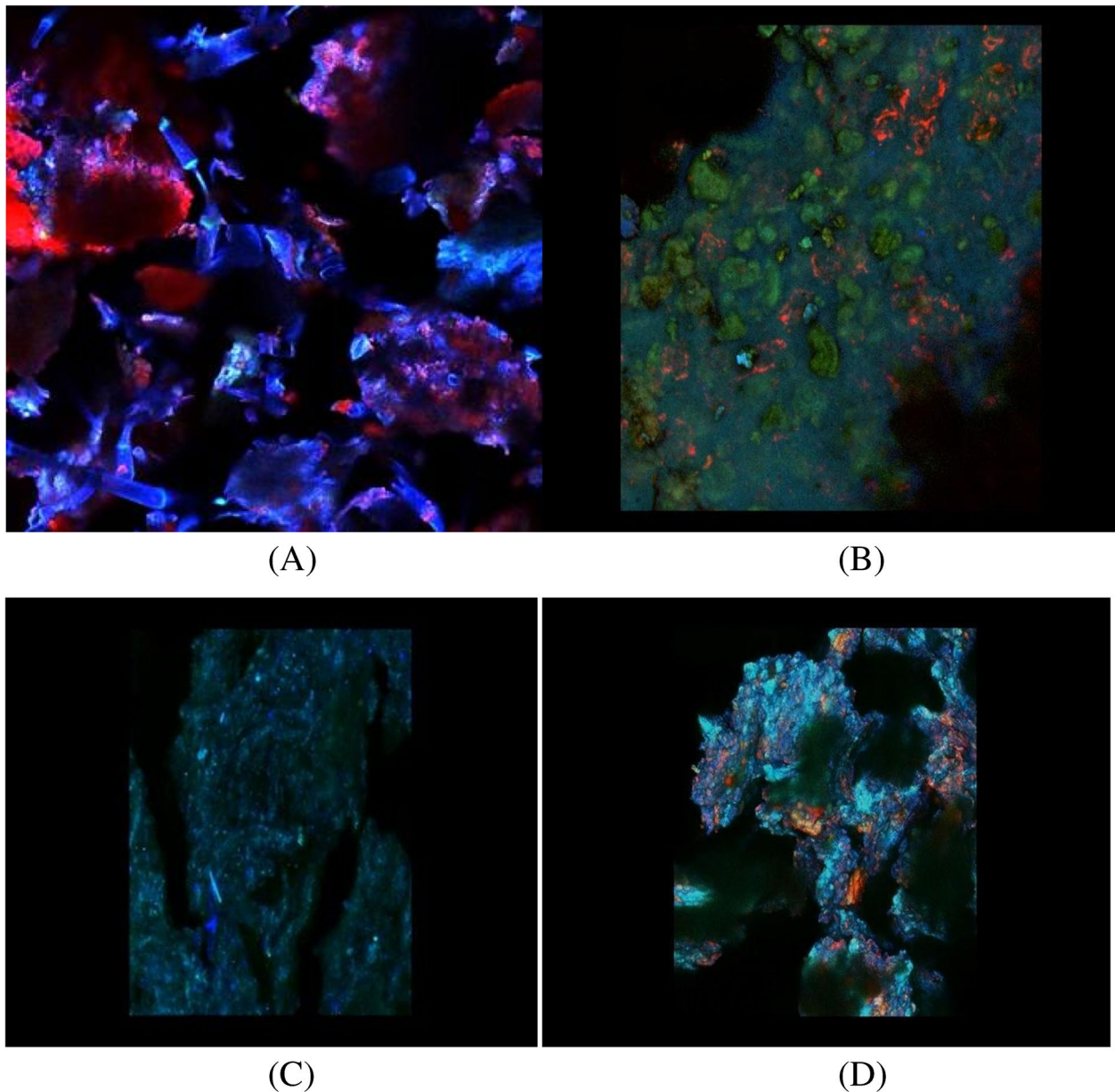


Fig. 3 Confocal laser scanning micrographs of *Caesalpinia pulcherrima* seed flour (FSF), seed gel (GSF), and the crumb of breads enriched with these ingredients. (A) – FSF; (B) – GSF; (C) – Control

bread; (D) – Run 4 (10% FSF, 5% GSF). Images were acquired using a Plan-Apochromat 20×/0.8 M27 objective (zoom 1.0) on a Zeiss LMS 710 confocal microscope

FSF (A) showed a heterogeneous pattern with distinct domains, whereas GSF (B) exhibited a more continuous and cohesive matrix-like organization. The control crumb (C) displayed a denser and less differentiated organization, with weaker contrast and a more compact appearance, consistent with its lower specific volume and reduced aeration. In contrast, the enriched bread (D) showed a more open and continuous structure, with more evenly distributed domains, in agreement with the improved macroscopic crumb characteristics observed [30, 31]. Taken together, the CLSM observations support the complementary roles of FSF and

GSF in bread structuring: FSF contributed to matrix modification through its particulate composition, whereas GSF contributed to hydration and structural continuity. Their combined incorporation was therefore associated with a more organized and aerated crumb, consistent with the improved physicochemical and technological performance of the enriched breads.

The SEM and CLSM analyses revealed visible differences between the control and enriched breads, with the enriched formulations showing a more open and continuous crumb organization, in agreement with the higher specific

volume and improved alveoli parameters. These observations provide qualitative support for the structural role of the seed-derived ingredients in bread organization. Although galactomannan content was not directly quantified in the final bread samples, the role attributed to this fraction is supported by the known composition of the seed-derived gel together with the technological and microstructural responses observed in the breads [32].

Conclusion

Ingredients rich in galactomannans obtained from *Caesalpinia pulcherrima* seeds were successfully incorporated into wheat bread as seed flour and seed gel. Within the evaluated formulation range, these ingredients mainly affected bread specific volume and crumb structure, increasing porosity and circularity while maintaining expansion values comparable to the control. The enriched breads also showed higher protein, lipid and ash contents, whereas crude fiber increased only modestly. Moisture, water activity, pH and titratable acidity remained within typical or regulatory ranges for bread, indicating compatibility with standard breadmaking conditions. Microstructural observations supported the interpretation of a more open and organized crumb in the enriched breads. Overall, *Caesalpinia pulcherrima* seeds represent a promising underutilized tropical resource for the development of wheat breads with improved structural and nutritional characteristics. Future studies should further explore sensory acceptance, shelf-life behavior and broader bakery applications of these seed-derived ingredients.

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Data Availability The datasets generated and/or analyzed during the current study are available from the corresponding author on

reasonable request.

Declarations

Ethics and Consent to Participate The collection of plant material used in this study complied with applicable institutional, local, and national guidelines and legislation. Pods/seeds of *Caesalpinia pulcherrima* (L.) Sw. (Fabaceae) were collected from cultivated ornamental trees located at different sites on the campus of the Federal University of Ceará (UFC), Fortaleza, Ceará, Brazil (Latitude: 3°44'38.6"S; Longitude: 38°34'47.3"W). The plant material was taxonomically identified by Prof. Dr. Maria Iracema Bezerra Loiola (Curator, Herbarium Prisco Bezerra – EAC). Voucher specimen(s) were deposited in the Herbarium Prisco Bezerra (EAC), Federal University of Ceará, Fortaleza, Ceará, Brazil, under accession number EAC 67246. This study did not involve human participants or animals; therefore, ethics approval and consent to participate are not applicable.

Consent to Publish Not applicable.

Competing Interests The authors declare no competing interests.

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