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FOOD TECHNOLOGY

Physical-chemical characterization, acceptance test, and free-choice profiling of gluten-free bread developed with Brazilian buckwheat starch and flour

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ABSTRACT: Buckwheat is a pseudocereal with a high starch content and compounds of nutritional interest, making it suitable for use in gluten-free baking. A simplex-centroid design was employed to investigate the effects of the mixture components of buckwheat flour, buckwheat starch, and rice flour in nine different proportions. The minimum and maximum limits for the components were 10% and 30% for buckwheat flour, 20% and 40% for buckwheat starch, and 50% and 70% for rice flour, aiming to create diverse gluten-free breads for evaluation. The utilized flours underwent characterization, and their micro- and macronutrient amounts, along with their amino acid compositions, were determined. Specific volume analysis, water loss determination, texture assessment, and sensory evaluations were conducted on the breads. It was observed that the proportion of starch and buckwheat flour directly influenced the specific volume and water loss during product storage. Through sensory analysis, the formulation with the highest acceptance and purchase intention among tasters was identified. Free-choice profiling analysis established the sensory profiles of the developed gluten-free bread formulations. According to tasters, the formulations exhibited characteristics of whole meal bread, noticeable softness, and a homogeneous alveolar distribution. The gluten-free bread was well-received by 124 tasters, with a purchase intention rate of 93.55%.
Key words: buckwheat flour, buckwheat starch, gluten-free bread, free-choice profiling, sensory analysis.

Caracterização físico-química, teste de aceitação e análise descritiva de perfil livre de pão sem glúten desenvolvido com amido e farinha de trigo sarraceno brasileiro

RESUMO: O trigo sarraceno é um pseudocereal com altas quantidades de amido e compostos nutricionais interessantes, tornando-o adequado para aplicação em panificação sem glúten. Um delineamento simplex-centróide foi usado para estudar os efeitos dos componentes da mistura de farinha de trigo sarraceno, amido de trigo sarraceno e farinha de arroz em nove proporções diferentes. Os limites mínimos e máximos dos componentes foram de 10% e 30% para a farinha de trigo sarraceno, 20% e 40% para o amido de trigo sarraceno e 50% e 70% para a farinha de arroz, a fim de criar diferentes pães sem glúten e avaliá-los. As farinhas utilizadas foram caracterizadas, e tiveram as quantidades dos seus micro e macro nutrientes determinadas, além da composição de aminoácidos. Nos pães realizaram-se análise de volume específico, determinação da perda de água, textura e avaliação sensorial. Observou-se que a proporção de amido e farinha de trigo sarraceno, influencia diretamente no volume específico e na perda de água durante a estocagem do produto. Por meio da análise sensorial, foi possível determinar a formulação com maior aceitação e intenção de compra entre os provadores. A análise descritiva de perfil livre estabeleceu os perfis sensoriais das formulações de pão sem glúten desenvolvidas. Segundo os provadores, as formulações apresentaram aspectos de pão integral, maciez aparente e distribuição alveolar homogênea. O pão sem glúten foi bem aceito por 124 provadores, com intenção de compra de 93,55%.
Palavras-chave: farinha de trigo sarraceno, amido de trigo sarraceno, pão sem glúten, análise descritiva de perfil de livre, análise sensorial.

INTRODUCTION

For the food industry, creating gluten-free bread to meet high nutritional and technological standards poses a challenge. Generally, gluten-free bread suffers from a nutritional deficit due to the absence of gluten protein and other micro and macro elements found in cereals like wheat, rye, barley, and triticale, which cannot be used in this product. To compensate for the technological drawbacks caused by gluten-free flour, breads are enhanced with carbohydrates such as the hydrocolloids HPMC and xanthan gum, as well as starch. Consequently, such breads are rich in carbohydrates but deficient in other essential nutrients (ELGETI et al., 2015; MYKHONIK et al., 2022; MUGGAH et al., 2016; NAQASH et al., 2017, ROMAN et al., 2019).

Pseudocereals, such as amaranth (Amaranthus spp.), quinoa (Chenopodium quinoa), and buckwheat (Fagopyrum esculentum), are plants that contain grains with a high starch content, high nutritional value, and no gluten. Their flour has been utilized in gluten-free foods, expanding the product options for those with dietary restrictions (GRAZIANO et al., 2022; SCHOENLECHNER et al., 2008).

Buckwheat (*Fagopyrum esculentum*) is a pseudocereal primarily cultivated in Asia and Europe; Brazil is the world's seventh-largest producer. This grain is among the most valuable pseudocereals in

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terms of nutritional value, containing substantial amounts of phenolic compounds, flavonoids, proteins, and essential amino acids (ALTINDAĞ et al., 2015; GAO et al., 2016; FAOSTAT, 2016; ZHU, 2016).

Buckwheat flour (BF) has been considered interesting for gluten-free bread formulations due to its composition of 59–70% starch (MALIK & SAXENA, 2016; ZHU, 2016). Its incorporation has a positive impact on the technological quality of gluten-free bread (GIMÉNEZ-BASTIDA et al., 2015) and has also been used in various bakery products due to its nutritional quality (BRITES et al., 2022; SCHOENLECHNER et al., 2008).

Bread crafted from BF holds considerable nutritional significance. Buckwheat is a gluten-free whole grain rich in essential nutrients, such as fiber, proteins, and various vitamins and minerals. This grain boasts a notable concentration of antioxidants, including rutin, which contributes to cardiovascular health. Additionally, the high-quality proteins reported in buckwheat are complete, containing all essential amino acids necessary for the body's optimal functioning. Moreover, the inclusion of buckwheat in bread formulations provides a unique earthy flavor profile, making it not only a nutritious but also a flavorful option for those seeking diverse and wholesome dietary choices (KOWALSKI et al., 2022).

Rice flour (RF) is widely utilized in gluten-free bread due to its neutral color and taste, which do not alter the original product characteristics. It has been previously confirmed that bread made with RF (as compared to those made with maize and cassava flour) exhibits superior parameters, resulting in bread with a proper consistency and evenly distributed alveoli (WU et al., 2019).

Therefore, this study developed gluten-free bread using BF, BS, and RF. The evaluation involved physicochemical analysis and the determination of their sensory profiles through free-choice profiling, as well as acceptance through sensory evaluation.

MATERIALS AND METHODS

Starch and flour production

Buckwheat (variety IPR-91 BAILI), provided by IAPAR, Agronomic Institute of Paraná (Brazil), and rice grains (Type 1) were processed into flour using an Ika Werke grinding mill M20 (USA) and sifted through a 42-mesh sieve.

Flours obtained by non-heat treatment are characterized by their preservation of vitamins, macro and micronutrients, enzyme complexes, and antioxidant properties (such as flavonoids like orientin, quercetin, vitexin, and rutin) according to MYKHONIK et al. (2022).

Buckwheat starch (BS) was obtained via the green method according to the method of BET et al. (2016). Green methods do not use acids, bases, or any other solvent to extract starch; they are more environmentally friendly; and they do not cause prior modification of the starch granules. BS was obtained from BF, which was suspended in water at a ratio of 1:3 (flour:water) for 10 minutes using an IKA® RW 20 suspended digital stirrer (Germany). The suspension was sieved through a 170-mesh sieve (A Bronzinox) with an aperture of 90 µm. The upper layer was discarded after 60 minutes, and the decanted starch was centrifuged in a Rotina 420R centrifuge (Germany) at 9,000 rpm for 5 minutes. The recovered starch was dried for 24 hours in an air circulation oven at 40 °C.

Centesimal composition of flours

Centesimal composition determination was carried out on BF, BS, and RF. Moisture, protein, lipids, ashes, and dietary fiber were assessed using protocols 925.10, 960.52, 920.39C, 923.03, 962.0E, respectively, from AOAC (2011). Carbohydrates were calculated by difference (BEMILLER, 2017).

Total amino acid profiles of flours

Total amino acids were determined using the methodology described by VAN KEMPEN & BODIN (1998). For the NIRS assessment, approximately 200 g of flours underwent sieving through a 1-mm mesh. Subsequently, the samples were subjected to 32 scans as they passed through the scanning window, securely positioned in a natural product cell. The NIRS instrument was calibrated and validated using a quartz container. Scans were conducted over a wavelength range from 400 ± 2500 nm with intervals set at 2 nm. Calibrations were established by correlating the true ileal digestible essential amino acid content with spectral data through NIRS II v. 3.00 (Infrasoft International, Port Matilda, PA, USA). Employing the Unscrambler software, a specific calibration was created for the target feedstuff, utilizing exclusively samples from that feedstuff.

Experimental design, formulation, and production of gluten-free breads

To establish the standard formula for gluten-free bread, preliminary tests were conducted, considering previous studies reported in the literature (ALENCAR et al., 2015; APLEVICZ & MOREIRA, 2015; ŚWIECA et al., 2015). It was determined that one of these preliminary tests yielded favorable growth, a suitable taste, and a well-structured gluten-free bread with properties comparable to those of conventional wheat bread.

Following the tests, the minimum and maximum concentrations were defined as follows: 50% to 70% for RF, 10% to 30% for BF, and 20% to 40% for BS.

Breads developed solely with either RF or starch would be nutritionally poor, containing low amounts of vitamins, minerals, proteins, and dietary fiber. Additionally, the technological quality of these breads would be compromised. Therefore, this study proposed to examine flour mixtures. To determine the proportion of the flour mixtures used, a factorial design simplex-centroid was employed with three repetitions at the central point (formulations 7, 8, and 9) using Statistica 10 software (StatSoft Inc., Tulsa, USA). The central point corresponds to the mixture in which the component proportions are the averages of the corresponding vertex proportions.

Every formulation varied only in the composition of the mixed flour used. The ingredients were measured at a 100% mass/mass flour ratio, as shown in table 1.

The bread starter was prepared with yeast, water (27 – 35 °C), and sucrose for the yeast's development (for 15 minutes). Dry ingredients, excluding the salt, were homogenized beforehand and then combined with the others in a planetary mixer (Arno), concluding with the addition of salt. Immediately after mixing, the bread doughs were placed in bread forms (with dimensions of 4.5 × 21.0 × 10.2 cm) and left to ferment for 1 hour at 30 °C in an air-circulating oven MA035 (Marconi).

The bread doughs were baked in an electric oven (Perfecta) for 20 minutes at 180°C. After baking, the gluten-free breads were cooled to room temperature (25 °C) and subsequently stored in low-density polyethylene packaging. They were kept in the dark for further analyses, which were conducted within 72 hours to prevent the effects of starch retrogradation and other changes that can occur in the bread.

To increase the reliability of the analysis results, the formulations were randomly selected for further analysis with the aid of Microsoft Excel software, and each was assigned a 3-digit identification code.

The bread doughs were baked in an electric oven (Perfecta) for 20 minutes at 180 °C. After baking, the gluten-free breads were cooled to room temperature (25 °C) and then stored in low-density polyethylene packaging and kept in the dark for further analyses (which were performed quickly to avoid the effects of starch retrogradation and other changes that occur in the bread).

Specific volume analysis

The specific volumes of gluten-free breads were calculated using the AACC 10-05 method (1999), a methodology that relates the apparent volume of the baked product to its weight.

Gluten-free bread weights were measured on a semi-analytical balance (Mettler Toledo and model PB 8001-S), and the apparent volume was obtained using the millet seed displacement method (Method 10-05, AACC, 1999).

Table 1 - Formulations for gluten-free bread production.

Ingredients	---Formulation (%)[1]---								
	1	2	3	4	5	6	7	8	9
Rice flour	70	50	50	60	60	50	56.66	56.66	56.66
Buckwheat flour	10	30	10	20	10	20	16.67	16.67	16.67
Buckwheat starch	20	20	40	20	30	30	26.67	26.67	26.67
Egg	43.75	43.75	43.75	43.75	43.75	43.75	43.75	43.75	43.75
Sucrose	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9
Vegetable oil	8.75	8.75	8.75	8.75	8.75	8.75	8.75	8.75	8.75
Yeast	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55
Sodium chloride (salt)	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Apple cider Vinegar	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70
Psyllium fiber	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40
Water	91.00	91.00	91.00	91.00	91.00	91.00	91.00	91.00	91.00

[1]The ingredients were measured in relation to 100% of mixed flour.

Determination of weight loss in gluten-free bread

The masses of gluten-free breads were measured before and immediately after baking (while the bread was still hot) and then again after 3, 24, and 72 hours of storage to observe weight loss throughout the shelf life (PURLIS & SALVATORI, 2009).

Water activity

The water activity of gluten-free breads was measured using the direct method at 25 ºC with the Aqualab 3T water activity meter (Decagon Devices), following AOAC-approved methodology 978.18, titled "Water Activity of Canned Vegetables" (2019). To assess changes during storage, measurements were taken in the bread crumbs within 24 and 72 hours after baking to observe any alterations (ABEDFAR et al., 2019).

Texture profiling

Texture profiling was performed on a CT3 texturometer (Brookfield, USA). Through this analysis, the first- and second-cycle hardness (N), elasticity (dimensionless), and chewability (J) were determined (AL-SALEH & BRENNAN, 2012; STURZA et al., 2020).

Gluten-free bread samples were cut into 2.5-cm cubes. They underwent a double compression test at a height of 1.25 cm (50% compression) using a cylindrical acrylic probe with a diameter of 50.8 mm, a velocity of 1 mm/s, and a common interval of 5 seconds between two cycles.

Sensory analysis

Gluten-free breads underwent sensory evaluation (following the approval of the Research Ethics Commission of the Health Sector/ Federal University of Paraná (UFPR), CAAE nº 68934917.2.0000.0102, advice document nº 2.294.264) through application of the following tests: free-choice profiling, acceptance test, and purchase intention.

Free-choice profiling

The methodology used for free-choice profiling was based on that applied by BERNARDI & DAMÁSIO (2004) and developed by WILLIAMS & STEVENS (1984).

SINKINSON (2017) explains that the primary objective of the triangular or triangle test is to discern whether there is a noticeable sensory difference between two products. This test was chosen as the format for selecting tasters for sensory analysis. Then, a descriptor survey was conducted with the 16 selected tasters using the net method proposed by KELLY (1955) and cited by MOSKOWITZ (1983).

After sessions of the descriptor survey, an individual discussion was held to elaborate on the sample evaluation form and provide specific descriptor definitions (a glossary) for each taster.

To analyze the nine gluten-free bread formulations, a sensory analysis was conducted with three repetitions across three sessions for each formulation. During these sessions, the tasters evaluated the block with three samples in a randomized manner.

Acceptance test and purchase intention test

Twenty-four hours after gluten-free bread preparation, 124 untrained tasters participated in both the acceptance test and the purchase intention test. The samples were served monadically (MEILGAARD et al., 2006) using a balanced block design to eliminate first-order and carry-over effects (sensory fatigue). Each sample was identified with a random 3-digit code (MACFIE et al., 1989).

Acceptance was evaluated for color, taste, texture, and overall impression. In each session, every taster received nine formulations. After the fourth sample, a "Consumer survey" was conducted to rest the tasters' palates (MEILGAARD et al., 2006) and gather information about the product's audience.

Following the acceptance test, the purchase intention test was administered. It is worth noting that all the tasters participating in these analyses were selected because they were potential buyers of gluten-free bread and had an interest in this type of product.

Statistical analysis

All physicochemical analyses were performed in triplicate. The results obtained in the physical analysis were submitted to analysis of variance (ANOVA), followed by multiple linear regression using response surface methodology (RSM) to propose mathematical equations that could explain the proportional effect of RF, BF, and BS on the analysis results. The quantity of each flour was expressed as pseudo-components. Equation (1) presents the generalized model applied to shape the experimental data:

$$Y= \beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{123}X_1X_2X_3 \quad (1)$$

where Y is the studied answer; β_1, β_2, β_3, β_{12}, β_{13}, β_{23}, and β_{123} are the regression coefficients; and X_1, X_2 and X_3 are the independent variables (flour types).

The quality adjustments of the model were evaluated by the regression coefficient (R^2) and the adjusted regression coefficient (R^2_{adj}). The obtained responses were visualized in response surface graphics for each dependent variable, based on adjusted models, using Statistica 10 (StatSoft, Inc., Tulsa, OK, USA) software.

For free-choice profiling, a Generalized Procrustes Analysis (GPA) was performed using GENSTAT software (England). The data obtained from free-choice profiling were submitted to analysis of variance (ANOVA) and Principal Component Analysis (PCA). When the samples showed a statistically significant difference, they were compared using Tukey's range test ($P \leq 0.05$) (MEILGAARD et al., 2006).

RESULTS AND DISCUSSION

Centesimal composition of flours

The centesimal composition of the flours and starch that were used in the mixtures for formulating gluten-free breads is presented in table 2.

Table 2 shows that the BS extraction process using the aqueous method was effective because the other micronutrients and macronutrients present in the flour were practically discarded (approximately 3.59 g 100 g^{-1} of them in the sample), which was desired for pure starch. When converting the values in table 2 to a dry basis, it was observed that the degree of purity of the extracted BS was 96%. This value is considered high, especially considering that the starch was extracted using a green method without the use of chemical reagents that could have enhanced extraction efficiency but might have caused modifications in the starch granules.

Buckwheat flour had a higher protein content (14.16 g 100 g^{-1}) than RF (5.07 g 100 g^{-1}), as expected because one of the main attractions of this grain is its high protein content. The literature reports the following values for the protein content of BF: 11.7 g 100 g^{-1} (SYTAR et al., 2016); 13.1 g 100 g^{-1} (MOTA et al., 2016); 13.30 to 15.55 g 100 g^{-1} (WEI et al., 2003); 11.91 to 12.65 g 100 g^{-1} (STEMPIŃSKA & SORAL-ŚMIETANA, 2006). Thus, the obtained results fall within the expected range.

Higher lipid values (3.65 g 100 g^{-1}) were reported for BF than for RF (0.47 g 100 g^{-1}). Other studies on buckwheat have reported lipid contents ranging between 2.29 (KATAR et al., 2016) and 3.94 g 100 g^{-1} (PANDEY et al., 2015), consistent with the values found in this study.

Regarding dietary fiber, a value of 2.36 ± 0.20 g 100 g^{-1} was obtained for BF, while for RF, the most used flour in gluten-free products, it was 0.28 ± 0.01 g 100 g^{-1}. Since fiber fortification of bakery products is of great interest to consumers, the addition of BF or replacement of RF could be ways to add value by meeting the needs of increased daily fiber intake (DEVRIES et al., 1999).

Total amino acid profiles of flours

The total amino acid profiles of RF and BF are shown in table 3.

The quantities of each amino acid can vary significantly based on variety, cultivar, species, and/or subspecies; however, the relative proportion of amino acids is typically maintained. Another factor that can influence the results, significantly impacting the measurement of amino acids, is the use of different extraction and dilution methods (BAI et al., 2015).

All values obtained for BF and RF exceeded the values established by FAO/WHO (2007) as a daily consumption requirement in human food.

The most prominent amino acids in BF (mg AA 100 g^{-1} protein) were three non-essential amino acids: Glutamate (2333 mg 100 g^{-1} protein), Arginine (1362 mg 100 g^{-1} protein, a value very close to that obtained by SYTAR et al., 2018), and Aspartate (1272 mg 100 g^{-1} of protein). When compared with the findings of other researchers, the same three amino acids (Glu, Asp, and Arg) were present in greater quantities. In the case of glutamate, KRUMINA-ZEMTURE et al. (2016) obtained values ranging from 2020 to 2430 mg 100 g^{-1} in their study, while MOTA et al. (2016) reported 2535.3 mg 100 g^{-1}. Regarding aspartate, KRUMINA-ZEMTURE et al. (2016) obtained values ranging from 1240 to 1310

Table 2 - Centesimal composition of flours (g 100 g-1).

Sample	Moisture	Proteins	Lipids	Ash	Dietary fiber	Carbohydrates
Buckwheat starch	8,42 ± 0,28a	2,85 ± 0,04c	0,31 ± 0,08b	0,13 ± 0,002c	0,30 ± 0,02b	87,99± 0,33a
Buckwheat flour	8,18 ± 0,33a	14,16 ± 0,21a	3,65 ± 0,27a	2,21 ± 0,005a	2,36 ± 0,20a	69,44 ± 0,49c
Rice flour	8,33 ± 0,03a	5,07 ± 0,05b	0,47 ± 0,03b	0,30 ± 0,018b	0,28 ± 0,01b	85,55 ± 0,06b
LSD*	0,7929	0,382	0,5196	0,0281	0,3555	1,1115

*LSD: Least Significant Difference.

Table 3 - Total amino acid profile of rice flour and buckwheat flour and FAO/WHO standard for adults.

Amino acids	Rice flour (mg AA 100 g^{-1} protein)	Buckwheat flour (mg AA 100 g^{-1} protein)	FAO/WHO (2007) (mg 100 g^{-1} of sample)
Crude protein	7050	13580	-
Phenylalanine + tyrosine	368	549	-
Histidine	165	333	15
Isoleucine	276	487	30
Lysine	259	765	45
Leucine	562	862	59
Methionine	199	222	16
Threonine	245	502	23
Tryptophan	97	-	6
Valine	399	652	39
Σ Essential amino acids	2570	4372	277
Alanine	390	570	-
Arginine	573	1362	-
Aspartic Acid	640	1272	-
Cysteine	168	317	6
Glycine	313	791	-
Glutamic acid	1205	2333	-
Proline	310	499	-
Serine	354	675	-
Σ Non-essential amino acids	3953	7819	-

mg 100 g^{-1}, and MOTA et al. (2016) reported 1309 mg 100g^{-1}, which are very close to those obtained in the present study.

Buckwheat flour can be classified as a flour of exceptional nutritional quality, particularly in terms of amino acids, especially essential ones. In table 3, a notable abundance of essential amino acids is evident in RF, but particularly in buckwheat, the values significantly exceed those recommended by FAO/WHO (2007) for consumption. The essential amino acids found in the highest quantities in BF are lysine, leucine, and valine. These amino acids play crucial roles in combating infections, forming antibodies, promoting tissue regeneration, and performing various other functions. Substituting RF in various proportions in gluten-free products has significant potential to enhance bakery goods and offer nutritional benefits.

Developed gluten-free bread

The gluten-free breads exhibited heights ranging from 10.30 to 11.10 cm, with formulations 3 and 1 showing higher values and formulations 2 and 5 displaying lower ones. Good alveoli formation, an appealing crumb appearance, and an attractive crust color were observed, all of which are characteristics desired by consumers of this product (Figure 1).

Specific volume

The nine developed formulas for gluten-free bread presented specific volumes ranging from 1.82 ± 0.02 to 1.94 ± 0.05 (cm^3/g).

ALENCAR et al. (2015) developed gluten-free bread with the addition of quinoa and amaranth, achieving a specific volume of 2.30–2.88 cm^3/g. SMITH et al. (2012) created gluten-free bread with 7% carob flour, starch, and HPMC (hydroxymethylpropylcellulose), resulting in bread with a high specific volume (3.5 cm^3/g). The increased specific volume, when compared to the gluten-free buckwheat bread developed, can be attributed to the incorporation of hydrocolloids in the bread composition. ALENCAR et al. (2015) employed xanthan gum, and SMITH et al. (2012) utilized HPMC, both contributing to breads with higher specific volumes. In this article, only psyllium was employed to naturally emulsify the dough.

STORCK et al. (2013) reported in their studies that the addition of transglutaminase enzyme and casein protein significantly increased the specific volume of bread, providing alternative ingredients for developing this specific product.

According to HAN et al. (2019), egg white protein (albumin) exhibited cohesive behavior with aeration retention capacity, enhancing the stability of

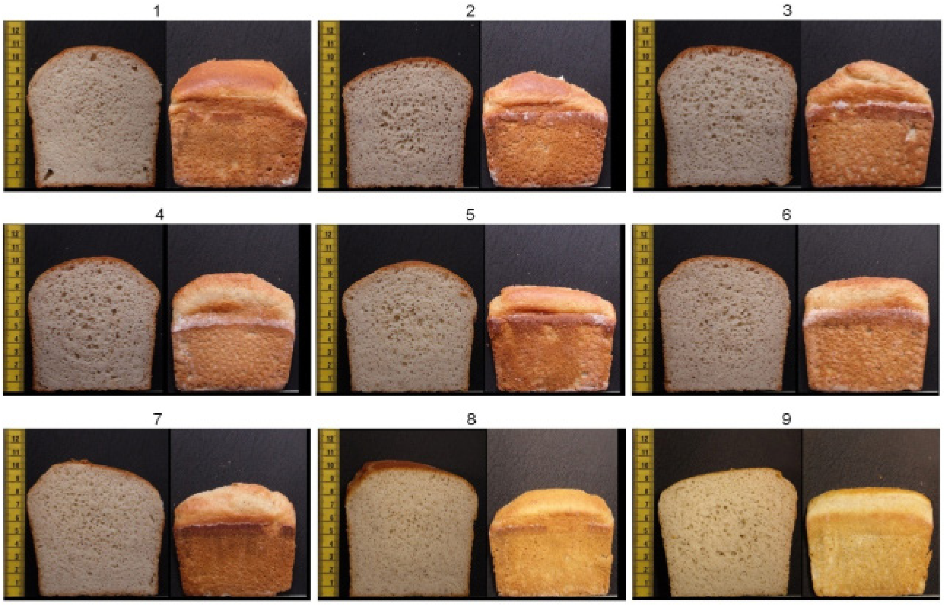

Figure 1 - Images of crumbs and crusts of the nine gluten-free bread formulations.

gluten-free bread and its specific volume to 1.22 cm³/g compared to the known sample (without albumin). Additionally, the texture of gluten-free bread with albumin was more homogeneous, demonstrating that the inclusion of eggs in gluten-free bread dough is crucial for the cohesion of the dough. Therefore, eggs were added to the formulation of the developed gluten-free buckwheat bread.

The authors MYKHONIK et al. (2022) developed various gluten-free breads with a mixture of BF, RF, and corn starch, along with HPMC and xanthan gum as structure-forming additives. The specific volume they obtained ranged from 2.48 to 2.62 cm³/g, higher than the values obtained in this study, possibly due to the presence of gums.

With the absence of gluten, some technological alternatives must be employed to enhance the dough of gluten-free bread. As mentioned earlier, some of these alternatives include the use of hydrocolloids such as HPMC and xanthan gum (ALENCAR et al., 2015; MYKHONIK et al., 2022; SMITH et al., 2012), the addition of albumin (HAN et al., 2019), and the inclusion of transglutaminase enzyme and casein protein (STORCK et al., 2013). Another viable option is the incorporation of psyllium fiber.

As psyllium fiber exhibits a high water absorption capacity, it proves to be an interesting substitute for gluten in baking. It improves dough machinability, stability, and the volume and appearance of the bread. Additionally, it enhances the nutritional quality of gluten-free products (CAPPA et al., 2013; FRANCO et al., 2020; FRATELLI et al., 2018; MARIOTTI et al., 2009); therefore, it was the alternative used in this study.

Determination of weight loss in gluten-free bread

Bread baking is a fundamental process that transforms a dough, primarily composed of flour, water, and yeast, into a high-quality product with unique sensory characteristics. This transformation occurs through the gelatinization of starch and water evaporation induced by heating, resulting in a series of internal reactions (PURLIS & SALVATORI, 2009).

Mass loss in gluten-free bread was measured both before and after baking, with additional assessments conducted at 3, 24, and 72 hours of storage, as detailed in Table 4.

The mass loss during baking varied from 3.66 ± 0.29% (formulation 6, proportion: 0.50 of RF, 0.20 of RF, and 0.30 of BS) to 5.15 ± 0.29% (formulation 2, proportion: 0.50 of RF, 0.30 of RF, and 0.20 of BS). Their values were lower than those reported by PAPASIDERO et al. (2015), who measured water loss due to evaporation as 8.9%.

The post-baking (storage) loss ranged from 7.58 ± 0.73% (formulation 6, proportion: 0.50 of RF, 0.20 of RF, and 0.30 of BS) to 9.72 ± 0.19% (formulation 2, proportion: 0.50 of RF, 0.30 of RF, and 0.20 of BS). Formulation 6 exhibited the least water loss during and after baking, while

Table 4 - Gluten-free bread dough over the production and storage process.

Formulation	Raw Dough (g)	Right after baking (g)	After 3 h (g)	After 24 h (g)	After 72 h (g)
1	420.00 ± 0.00^{a}	400.27 ± 1.33^{bc}	386.30 ± 1.35^{cd}	385.70 ± 1.35^{bc}	380.63 ± 2.83^{d}
2	420.00 ± 0.00^{a}	398.37 ± 1.21^{c}	384.80 ± 0.70^{d}	384.37 ± 0.75^{c}	380.33 ± 0.76^{d}
3	420.00 ± 0.00^{a}	400.07 ± 2.97^{bc}	386.90 ± 3.47^{bcd}	385.93 ± 3.75^{bc}	381.17 ± 3.28^{d}
4	42090 ± 0.10^{a}	400.63 ± 1.42^{bc}	387.57 ± 2.10^{bcd}	387.03 ± 2.15^{bc}	382.60 ± 2.40^{cd}
5	420.33 ± 0.06^{a}	401.07 ± 0.90^{bc}	390.23 ± 0.67^{abcd}	389.30 ± 0.72^{abc}	384.67 ± 0.81^{abcd}
6	420.90 ± 0.72^{a}	405.50 ± 1.92^{a}	395.07 ± 3.92^{a}	394.67 ± 3.86^{a}	389.77 ± 3.59^{a}
7	420.43 ± 0.38^{a}	399.53 ± 1.69^{bc}	387.87 ± 2.73^{bcd}	386.80 ± 2.44^{bc}	383.23 ± 2.40^{bcd}
8	421.00 ± 0.87^{a}	402.30 ± 0.96^{ab}	392.63 ± 2.56^{abc}	391.72 ± 2.79^{ab}	388.47 ± 2.56^{abc}
9	420.50 ± 0.61^{a}	401.67 ± 0.78^{abc}	393.13 ± 1.82^{ab}	392.27 ± 1.80^{ab}	389.03 ± 1.85^{ab}
LSD*	12.499	38.793	64.641	65.898	64.210

Averages followed by the same letter in the column do not statistically differ among each other by Tukey's range test (P < 0.05).
*LSD: Least Significant Difference.
**RF: Rice Flour; BF: Buckwheat Flour, and BS: Buckwheat starch; the proportions of the formulations were: Formulation 1: 0.70 of RF, 0.10 of RF, and 0.20 of BS; Formulation 2: 0.50 of RF, 0.30 of RF, and 0.20 of BS; Formulation 3: 0.50 of RF, 0.10 of RF, and 0.40 of BS; Formulation 4: 0.60 of RF, 0.20 of RF, and 0.20 of BS; Formulation 5: 0.60 of RF, 0.10 of RF, and 0.30 of BS; Formulation 6: 0.50 of RF, 0.20 of RF, and 0.30 of BS; Formulation 7, 8 and 9: 0.56 of RF, 0.17 of RF, and 0.27 of BS.

formulation 2 exhibited the greatest. Considering that both formulations contain the same amount of RF but differ in BS and BF amounts, it can be inferred that higher starch and lower flour quantities result in decreased water loss (as seen in formulation 6).

This is because the starch granules, when heated in the presence of water (present in the bread dough), undergo gelatinization. With gelatinization, a large amount of water is trapped inside the starchy granules, and some studies state that the granules absorb approximately 30% of their weight in water (RATNAYAKE & JACKSON, 2008). In this way, the bread with more starch in the formulation becomes softer and moister, losing this water through retrogradation over a longer period, depending on the conditioning conditions (cooling and packaging), causing the bread to harden. Therefore, the greater the amount of starch, the greater the water retention.

RINALDI et al. (2017) reported very similar values for post-baking mass loss (8.6–12 g/100 g) when assessing the shelf life of bread produced from sourdough fermentation and chestnut flour, where the breads were cooled at room temperature, packaged in alcohol-sprayed sealed air-tight plastic bags, and stored in a temperature-controlled environment at 25 ºC.

The first three storage hours, among the 72 hours analyzed, exhibited the highest mass loss, ranging from 2.12 ± 0.26% for formulation 9 (proportion: 0.56 of RF, 0.17 of RF, and 0.27 of BS) to 3.49 ± 0.32% for formulation 1 (proportion: 0.70 of RF, 0.10 of RF, and 0.20 of BS).

Throughout a product's lifespan, a decrease in moisture content occurs due to various factors, including water migration from the crumb to the crust, water loss to the atmosphere caused by packing permeability, and starch retrogradation (ISHIDA & STEEL, 2014). During storage, the loss of mass caused by evaporation results in both a reduction in weight available for sale (especially significant for products sold by weight, such as bread) and a decline in quality, often leading to hardening. Therefore, it is essential to minimize this effect (PHIMOLSIRIPOL et al., 2011).

To mitigate potential interferences in the analysis results, the bread doughs were assessed at 3, 24, and 72 hours post-baking. To prevent water loss, the breads were carefully packaged in suitable containers. Additionally, the analysis was conducted in a randomized manner, with samples labeled using three-digit codes and organized into three groups, each representing a distinct set of three breads. During the sensory analysis session, random distribution of sample pieces, facilitated by Microsoft Excel software, ensured an unbiased evaluation without any sample identification.

Water activity

The water activity (Aw) of the nine gluten-free breads was measured after a 24-hour storage period, ranging from 0.971 ± 0.003 to 0.980 ± 0.003. The measured Aw values after 72 hours of storage ranged between 0.968 ± 0.003 and 0.978 ± 0.003.

Despite a slight loss of moisture during storage, no significant difference was observed according to Tukey's range test (with a significance level of 5%). Therefore, the product maintained a virtually stable Aw within the 72-hour shelf life.

Texture

An average profile of Texture Profile Analysis (TPA) for the nine gluten-free bread formulations was obtained. The data presented in table 5 were modeled using Response Surface Methodology (RSM). Primary properties (first- and second-cycle hardness, elasticity) and secondary properties (chewability) were evaluated after 1 and 3 storage days.

According to CIVILLE & SZCZESNIAK (1973), texture is "the sensory manifestation of the structure or food's internal composition".

As seen in table 5, the first- and second-cycle hardness increased for all samples after the storage days. Bread is a product that undergoes quality deterioration over time, leading to increasing perceived dryness and crumb rigidity while also losing taste, flavor, and crunchiness (WANG et al., 2018).

The generated model for hardness ($P < 0.05$) was significant, and surface response graphics are displayed in figure 2. The interaction between RF and BS was significant in the first- and second-cycle hardness models. A similar trend between the first and second cycles of hardness was observed, where the interaction influence of RF with BS was more significant when there were lower quantities of BS in the formulation.

A significant interaction (with one day of storage) was found between RF and BF. All binary interactions were significant for chewability (with one day of storage), with less importance when rice and BF were in more isolated concentrations (Figure 2).

The modeling conducted using RSM with the obtained data is presented in table 6.

Sensory evaluation

Free-choice profiling

Free-choice profiling analysis was employed to derive sensory profiles for the nine gluten-free bread formulations based on attributes selected by the tasters (Figure 3).

Five of the nine developed gluten-free bread formulations were categorized by tasters in the quadrant related to integral, integral aspect, and

Table 5 - The nine gluten-free bread formulations' average TPA profile (hardness first- and second-cycle, elasticity, and chewability).

Formulation	---------------1st Cycle hardness (N) ------------		---------------2nd Cycle hardness (N) ------------	
	1st day	3rd day	1st day	3rd day
1	23.90 ± 5.76	29.82 ± 7.77	21.62 ± 5.35	24.20 ± 6.77
2	23.93 ± 5.77	29.46 ± 6.48	20.55 ± 4.37	25.44 ± 4.25
3	27.36 ± 6.38	30.87 ± 4.72	25.11 ± 4.40	27.12 ± 3.16
4	23.28 ± 3.80	26.42± 4.51	19.94 ± 3.88	21.94± 4.22
5	30.49 ± 5.11	33.03± 7.17	26.79 ± 3.07	27.58± 6.69
6	28.71 ± 6.47	30.66± 7.03	20.73 ± 4.35	27.02± 5.79
7	26.49 ± 4.58	29.99 ± 4.15	21.62 ± 4.12	27.70 ± 4.66
8	27.22 ± 4.63	28.17 ± 2.31	22.25 ± 2.41	27.03 ± 4.38
9	27.01 ± 3.32	28.30 ± 1.81	23.22 ± 2.79	26.62 ± 2.27
Formulation	-------------------Elasticity (m)-------------------		------------------Chewability (J) ------------------	
	1st day	3rd day	1st day	3rd day
1	9.16 ± 1.10	9.22 ± 1.46	0.11 ± 0.05	0.11 ± 0.038
2	9.10 ± 0.87	9.10 ± 0.99	0.12 ± 0.03	0.12 ± 0.028
3	9.50 ± 1.23	10.33±1.41	0.17 ± 0.02	0.14 ± 0.032
4	10.37±1.19	8.89 ± 0.68	0.16 ± 0.04	0.11 ± 0.029
5	9.93 ± 1.21	9.23 ± 0.77	0.19 ± 0.03	0.14 ± 0.036
6	9.02 ± 0.77	9.50 ± 1.58	0.12 ± 0.02	0.15 ± 0.049
7	9.74 ± 0.99	9.36 ± 1.19	0.17 ± 0.05	0.16 ± 0.022
8	9.56 ± 1.03	9.41 ± 1.11	0.17 ± 0.05	0.15 ± 0.033
9	9.42 ± 0.60	9.38 ± 0.79	0.16 ± 0.04	0.16 ± 0.024

*RF: Rice Flour; BF: Buckwheat Flour, and BS: Buckwheat starch; the proportions of the formulations were: Formulation 1: 0.70 of RF, 0.10 of RF, and 0.20 of BS; Formulation 2: 0.50 of RF, 0.30 of RF, and 0.20 of BS; Formulation 3: 0.50 of RF, 0.10 of RF, and 0.40 of BS; Formulation 4: 0.60 of RF, 0.20 of RF, and 0.20 of BS; Formulation 5: 0.60 of RF, 0.10 of RF, and 0.30 of BS; Formulation 6: 0.50 of RF, 0.20 of RF, and 0.30 of BS; Formulation 7, 8 and 9: 0.56 of RF, 0.17 of RF, and 0.27 of BS.

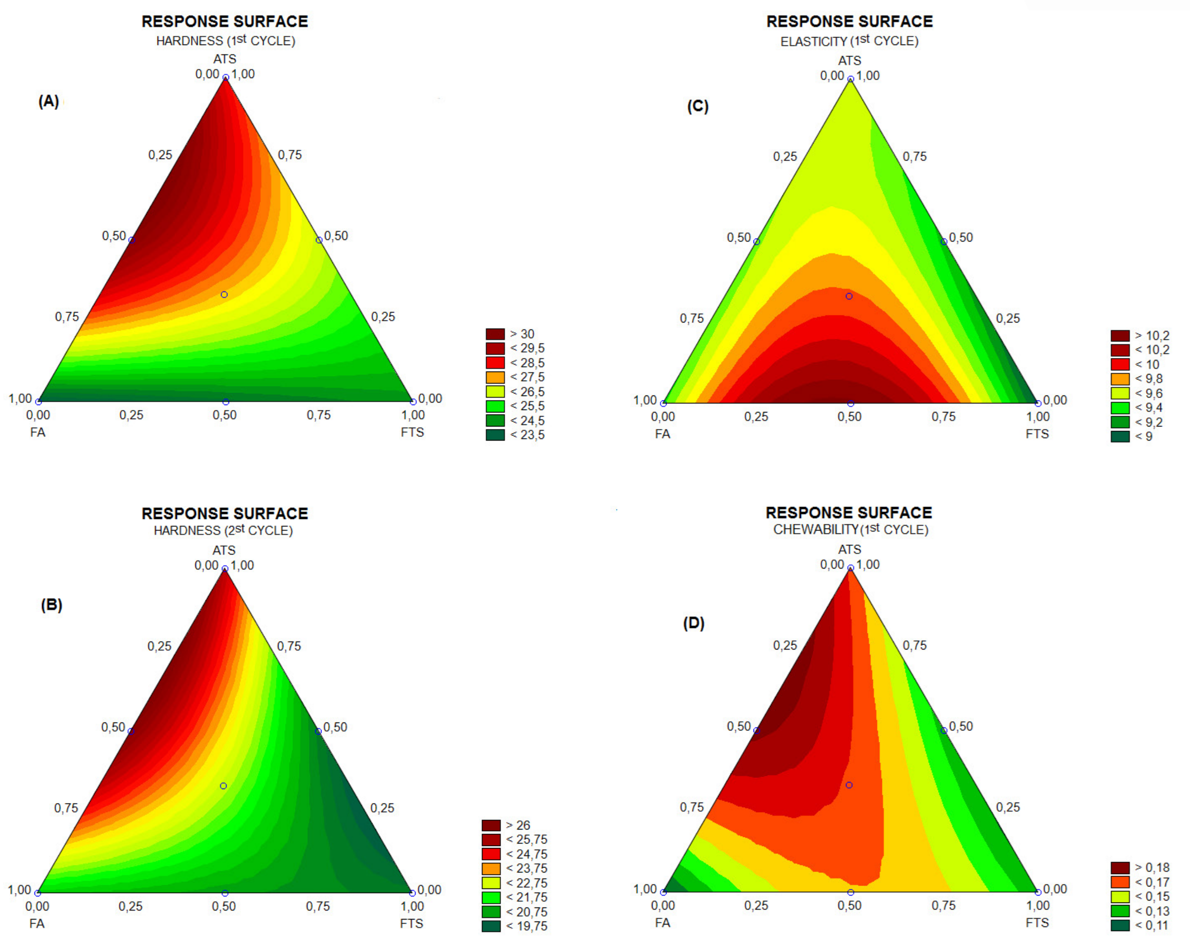

Figure 2 - Response surfaces for first- and second-cycle, elasticity and chewability (after 1 storage day).

bread characteristic. Some aspects, including those mentioned by the research tasters, such as “wet”, “dry”, and “herbaceous”, did not exhibit vector proximity with any of the formulations; therefore, these aspects were not considered characteristics of the studied products. The values obtained from the free-choice profiling of the nine gluten-free breads were organized into a radial graph (Figure 4) for better visualization and understanding.

The parameters with higher values in figure 4 define the characteristics of the developed bread, as they are most prominently perceived by the research tasters. Therefore, the gluten-free bread formulations were classified as soft, exhibiting bread-like characteristics, and featuring a homogeneous distribution of crumb alveoli. Conversely, the less pronounced (and undesirable) characteristics included dryness, a dense texture, a herbal scent, and a fermented taste.

Acceptance test and purchase intention

Using the 9-point Hedonic Scale (ranging from 1 to 9, where 1 is “Dislike extremely” and 9 “Like extremely”), the 124 tasters evaluated the parameters: color, taste, texture, and overall acceptance (Table 7).

It can be observed that all formulations, in every parameter analyzed, received ratings from 6.86 to 7.39, indicating preferences ranging from “like slightly” to “like moderately”.

Among the total of 124 tasters, it was found that 116 (93.55%) expressed an intention to purchase the buckwheat gluten-free bread produced in this study, while 8 (6.45%) would not. The tasters were based on their personal experiences and expectations regarding a tasty, soft gluten-free bread, distinct from what is commonly found on the market, as they were already consumers of this type of product. In the additional comments on the Sensory Assessment form, they expressed that the bread

Table 6 - Regression coefficients obtained by RSM according to the effects of RF, BF and BS in texture parameters.

Properties	Regression coefficient	Standard error	P-value
---------------*Hardness 1st cycle – Day 1*---------------			
(A) RF	23.45207	1.163579	20.15511
(B) BF	24.38777	1.030358	23.66923
(C) BS	28.39885	1.164457	24.38805
AC	16.21254	5.535702	2.92872
R^2	0.8209		
Adjusted R^2	0.7134		
P-value (model)	0.032681	P value (lack of fit)	0.050835
---------------*Hardness 2nd cycle – Day 1*---------------			
(A) RF	21.14055	0.688618	30.69998
(B) BF	20.07737	0.688903	29.14397
(C) BS	25.24681	0.751634	33.58924
AC	12.13291	3.280071	3.69898
BC	-9.91478	3.299679	-3.00477
R^2	0.9427		
Adjusted R^2	0.8853		
P-value (model)	0.024755	P value (lack of fit)	0.103060
---------------*Elasticity – Day 1*---------------			
(A) RF	9.372498	0.264781	35.39719
(B) BF	8.962373	0.264981	33.82275
(C) BS	9.570031	0.234465	40.81641
AB	4.630823	1.259689	3.67616
R^2	0.7477		
Adjusted R^2	0.5964		
P-value (model)	0.014351	P value (lack of fit)	0.276375
---------------*Chewability - Day 1*---------------			
(A) RF	0.107196	0.003735	28.69987
(B) BF	0.122258	0.003734	32.74528
(C) BS	0.168225	0.003734	45.05689
AB	0.177766	0.016296	10.90831
AC	0.221233	0.016296	13.57557
BC	-0.076099	0.016388	-4.64361
R^2	0.9934		
Adjusted R^2	0.9824		
P-value (model)	0.001426	P value (lack of fit)	0.405950

Note: RF: Rice Flour; BF: Buckwheat Flour, and BS: Buckwheat starch.
R^2 = coefficient of determination.
R^2 adjusted = adjusted coefficient of determination.
P value = significance probability.

had surpassed their expectations and was genuinely excellent, in addition to being nutritious.

Internal reference mapping

To obtain the Internal Preference Mapping (IPM) or Multidimensional Preference Analysis (MDPREF), the acceptance data were organized in a matrix composed of samples (rows) and consumers (columns), which was then subjected to Principal Component Analysis (PCA).

The results were expressed in a dispersion graph related to the first two principal components (Figure 5). These factors together explained 40.37% of the total variability among the nine gluten-free bread formulations.

Overall, formulation 3 (proportion: 0.50 of RF, 0.10 of RF, and 0.40 of BS) and formulation 6 (proportion: 0.50 of RF, 0.20 of RF, and 0.30 of BS) were the most preferred, considering the “global acceptance” of the research tasters.

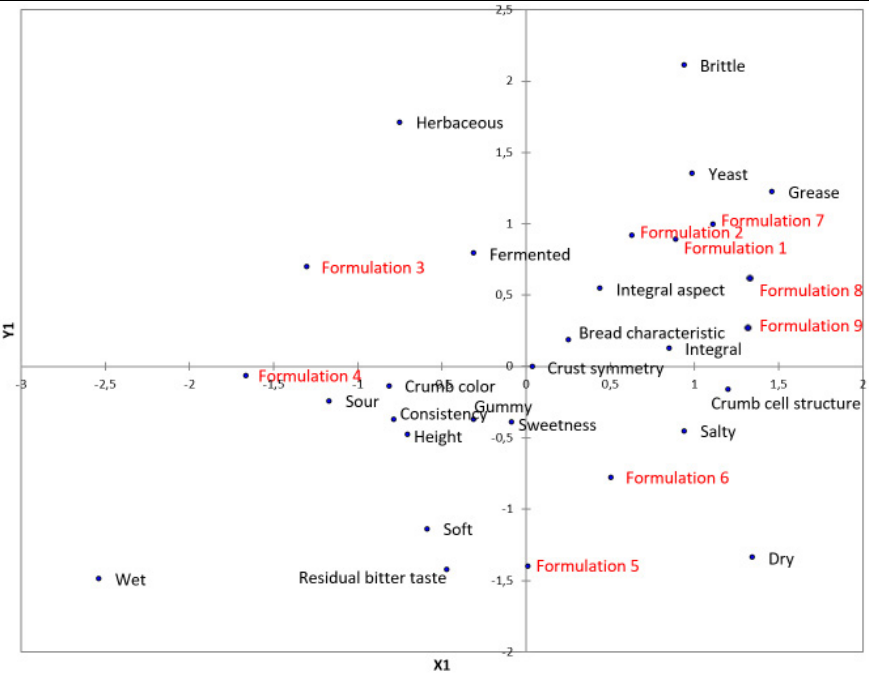

Figure 3 - Developed profiles of the nine gluten-free bread formulations.

As observed in the results, formulations with higher proportions of starch are notably better received, highlighting the significant benefits of this key ingredient in gluten-free products. Starch plays a crucial role in enhancing the softness, palatability, and moisture retention of gluten-free bread. Its positive impact contributes to an overall improvement in the quality of gluten-free bread, demonstrating the pivotal role of starch in formulating products with superior attributes and consumer acceptance.

Customer profile

Some factors impact product consumption, especially the consumer's choice at the time of

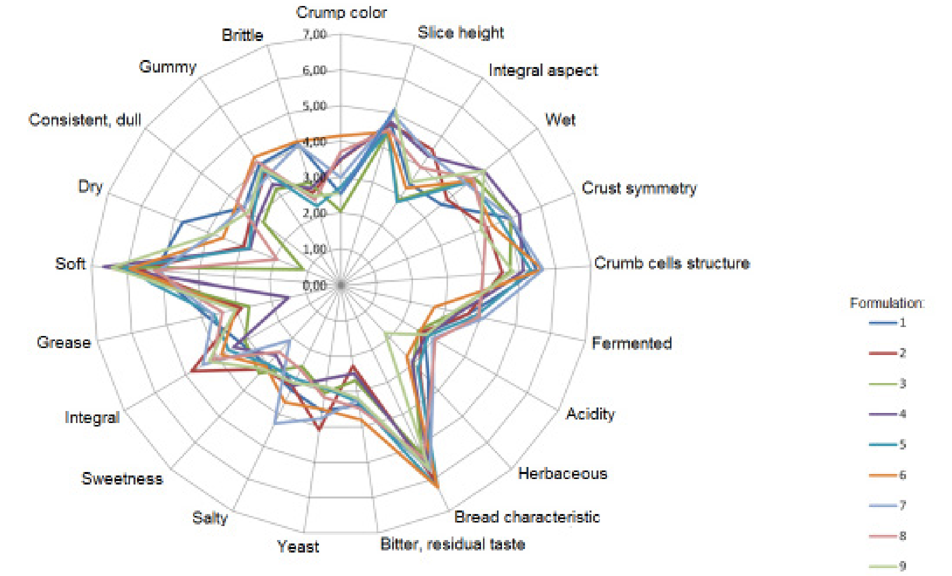

Figure 4 - Sensory profiling radial graph of nine gluten-free bread formulas obtained by free-choice profiling.

Table 7 - Average of the obtained grades of nine gluten-free bread formulations for the parameters: color, taste, texture, and global acceptance.

Formulation	-----------Color----------	----------Taste-----------	---------Texture---------	---Global acceptance---
1	7.23 ± 1.38^{a}	6.99 ± 1.59^{a}	6.90 ± 1.66^{a}	7.12 ± 1.50^{a}
2	7.27 ± 1.40^{a}	6.97 ± 1.61^{a}	7.12 ± 1.61^{a}	7.10 ± 1.46^{a}
3	7.39 ± 1.45^{a}	7.06 ± 1.54^{a}	7.26 ± 1.46^{a}	7.24 ± 1.32^{a}
4	7.33 ± 1.46^{a}	7.05 ± 1.56^{a}	7.42 ± 1.43^{a}	7.31 ± 1.32^{a}
5	7.35 ± 1.43^{a}	7.23 ± 1.43^{a}	7.37 ± 1.37^{a}	7.41 ± 1.29^{a}
6	7.32 ± 1.49^{a}	7.15 ± 1.41^{a}	7.32 ± 1.43^{a}	7.31 ± 1.25^{a}
7	7.35 ± 1.39^{a}	7.10 ± 1.54^{a}	7.27 ± 1.56^{a}	7.35 ± 1.28^{a}
8	7.20 ± 1.56^{a}	6.86 ± 1.70^{a}	7.01 ± 1.75^{a}	7.02 ± 1.61^{a}
9	7.38 ± 1.38^{a}	7.06 ± 1.66^{a}	7.20 ± 1.60^{a}	7.15 ± 1.47^{a}
LSD	0.350012	0.49167	0.51337	0.42671

[*]LSD: Least significant difference.
[**]RF: Rice Flour; BF: Buckwheat Flour, and BS: Buckwheat starch; the proportions of the formulations were: Formulation 1: 0.70 of RF, 0.10 of RF, and 0.20 of BS; Formulation 2: 0.50 of RF, 0.30 of RF, and 0.20 of BS; Formulation 3: 0.50 of RF, 0.10 of RF, and 0.40 of BS; Formulation 4: 0.60 of RF, 0.20 of RF, and 0.20 of BS; Formulation 5: 0.60 of RF, 0.10 of RF, and 0.30 of BS; Formulation 6: 0.50 of RF, 0.20 of RF, and 0.30 of BS; Formulation 7, 8 and 9: 0.56 of RF, 0.17 of RF, and 0.27 of BS.

purchase. The buyer's decision-making process can be influenced by cultural, social, personal, and psychological aspects (SAFRAID et al., 2022).

Cultural aspects influencing consumption are related to the accumulation of values, beliefs, and customs. In other words, if a person already has the habit of eating gluten-free or whole-grain products and is health-conscious, they are likely to appreciate the developed gluten-free buckwheat bread. Another influencing factor is social class or education level; individuals with higher purchasing power or higher levels of education tend to consume more whole and healthy products, aligning more with the consumer profile of gluten-free buckwheat bread.

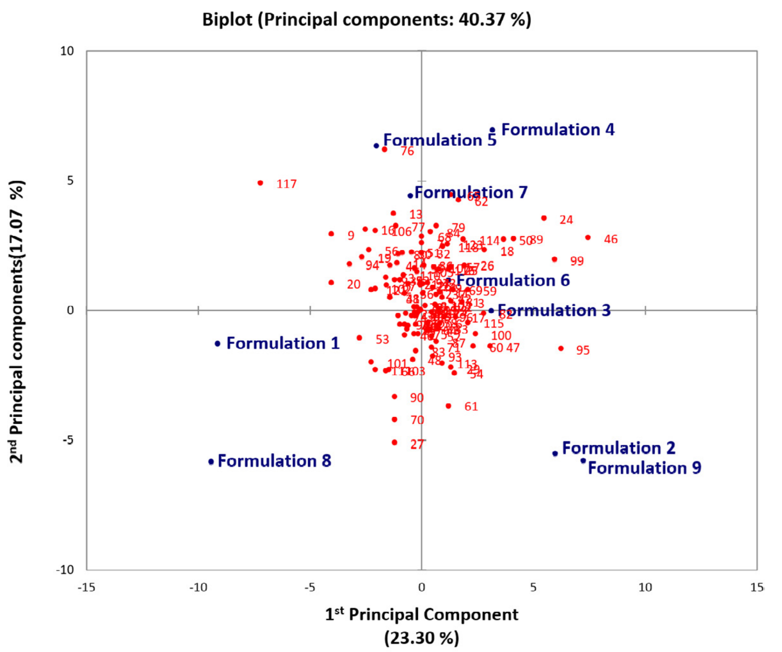

Figure 5 - Intern preference mapping shows the acceptance tendency concerning the global acceptance of gluten-free bread.

That is why it is interesting to outline the consumer profile of the product being developed. The initial questions applied in this study aimed to establish consumers' profiles regarding gender, age, income, level of education, familiarity with the term "gluten", whether they had any food intolerance or allergy, if they were consumers of this type of product, and if they would purchase the product presented during the sensory analysis.

There was a total of 959 responses to the questionnaire, with participants from 22 different Brazilian states and five countries. Among the survey respondents, 78.7% were women, and 21.3% were men, 75.8% self-identified as white, 17.9% as brown, and 6.3% as others.

For a significant and well-represented consumption study, the study population should encompass a diverse audience with different levels of education. In the current study, 8.2% had completed high school, 31.0% had graduated, 24.4% had a specialization, 14.7% had a master's degree, and 20.0% had a doctoral degree (1.7% others).

Out of a total of 959 participants, 94.4% were aware of the meaning of gluten. This demonstrates that the concept of "gluten" is widely acknowledged by the general populace. Additionally, 65.5% declared themselves to be celiac, and 27.8% affirmed feeling any kind of physical discomfort when consuming gluten.

A total of 65.6% of the participants expressed interest in consuming or purchasing gluten-free products, with bread being the most popular choice product (78.6% of the participants), followed by cake, snacks, and flour, among other items. This information is valuable since the developed bread was specifically designed for this audience, either with dietary restrictions, due to illness/discomfort when ingesting gluten, or for personal health care reasons.

Research into the development of nutritious and tasty gluten-free breads, soft and with different ingredients, becomes of great relevance because, as observed, there is great interest in products like this among the interviewed population.

CONCLUSION

Nine mixed flours were developed using the Simplex-Centroid design, with BS, BF, and RF as variables. Upon application of these mixed flours to gluten-free baking, various product parameters were evaluated. The resulting nine gluten-free bread formulations exhibited similar characteristics in terms of weight loss, specific volume, water activity, and texture.

In the sensory analysis, free-choice profiling characterized gluten-free bread as soft, with an integral aspect, featuring a homogeneous distribution of alveoli, a description carried out within the attributes listed by the selected tasters themselves when they created their glossaries. These tasters were selected through the triangular test, demonstrating their ability to perceive differences between highly similar samples.

In the acceptance test and purchase intention tests, the gluten-free bread demonstrated a high level of purchase intention (93.5% of the total participants) and was evaluated between the parameters 6 (like slightly) and over 7 (like moderately).

In conclusion, it can be inferred that incorporating BS, BF, and RF in gluten-free baking products can yield a quality product with widespread acceptance and purchase intention among consumers.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The funding sources had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The study was carried out with the approval of the Research Ethics Committee of the Health Sector/Universidade Federal do Paraná (UFPR), under the consubstantiated opinion nº 2.294.264.

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AUTHORS' CONTRIBUTION

All authors contributed equally to the conception and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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