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Research article

QUALITY AND FUNCTIONAL EVALUATION OF GLUTEN-FREE BREAD FORMULATED WITH BUCKWHEAT AND RICE FLOURS

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Abstract
This research aimed to develop a gluten-free bread by optimizing a buckwheat flour bread recipe and partially replacing it with rice flour, varying the flour proportions, the amount and type of hydrocolloids, or replacing the hydrocolloids with psyllium husk. Six samples were analyzed, evaluating the sensory properties, specific volume, crumb porosity, moisture, color, textural parameters, as well as the polyphenol content and antioxidant activity. The results revealed that the sample with 50% buckwheat flour, 50% rice flour, and 10% psyllium husk (Sample_5) was the most appreciated from a sensory point of view, also presenting the highest specific volume. The porosity of the crumb was maximum for Sample_2, while Sample_6 presented the weakest values. Differences were observed for lightness, L*, which increased with partial replacement of buckwheat flour with rice flour. Polyphenol content and antioxidant activity were higher in samples with higher proportions of buckwheat flour, decreasing with its substitution with rice flour and with increasing water content. The formulation corresponding to Sample_5 demonstrated an optimal balance between sensory acceptability and technological properties, being selected as the optimal gluten-free bread variant for subsequent fortification with flours obtained from by-products.

1. Introduction

Celiac disease is a chronic immune-mediated disorder triggered by gluten ingestion in genetically susceptible individuals, leading to intestinal inflammation, villous atrophy, and nutrient malabsorption (Monsuur & Wijmenga, 2006). Other wheat-related disorders, including wheat allergy and non-celiac gluten sensitivity, further highlight the importance of developing suitable gluten-free food alternatives (Sapone et al., 2012; Catassi et al., 2015; Elli et al., 2017). However, producing gluten-free bread with desirable technological, nutritional, and sensory characteristics remains challenging because the

absence of gluten compromises dough structure, gas retention, loaf volume, and crumb texture (Capriles et al., 2021; Djeukeu et al., 2024).

The selection of appropriate flour combinations is essential for improving gluten-free bread quality. Buckwheat (*Fagopyrum esculentum* Moench), an underutilized pseudocereal, represents a promising ingredient due to its nutritional composition and potential contribution to more diversified and sustainable food systems (Adelabu & Franke, 2023; Li et al., 2020). From a broader sustainability perspective, the integration of alternative agricultural resources into food production aligns with circular economy principles, which emphasize resource efficiency and sustainable production practices (Bătușaru & Sbârcea, 2023). Moreover, incorporating sustainability into organizational strategies can contribute to long-term value creation by integrating economic performance with social and environmental considerations (Rădoiu & Bătușaru, 2022). Buckwheat grains contain approximately 65–75% carbohydrates, 10–12.5% proteins, and 2–2.5% lipids, as well as polyphenols, vitamins, and other bioactive compounds (Zhu, 2020). Nevertheless, its incorporation into bread formulations may influence water absorption, dough rheology, and product texture (Biduski et al., 2024). Rice flour is widely used in gluten-free products because of its neutral taste, light color, and easily digestible carbohydrates (Fratelli et al., 2021; Pecyna et al., 2023). However, rice-based formulations often require additional structure-forming ingredients to compensate for the absence of gluten (Adewole et al., 2025). Combining buckwheat and rice flours may therefore provide an opportunity to balance nutritional characteristics and technological performance while improving the acceptability of gluten-free bread.

Hydrocolloids and dietary fibers are commonly incorporated into gluten-free formulations to improve water retention, dough viscosity, gas retention, and crumb characteristics (Mir et al., 2016; Djeukeu et al., 2024). Xanthan gum and carboxymethyl cellulose (CMC) are hydrocolloids capable of modifying dough rheology and influencing bread structure and quality (Lazaridou et al., 2007). Their combined incorporation represents a potential strategy for improving the structural properties of gluten-free dough. Burešová et al. (2016) investigated the effects of xanthan gum and CMC on the rheological, textural, and sensory characteristics of rice–buckwheat bread, highlighting the relevance of these hydrocolloids in such formulations. Alternatively, psyllium husk, a dietary fiber with high water-holding capacity, can contribute to dough cohesiveness and structural development, partially compensating for the absence of gluten (Fratelli et al., 2018, 2021; Pecyna et al., 2023). The effectiveness of these ingredients depends on their concentration, flour composition, and hydration conditions, emphasizing the importance of formulation-specific evaluation.

Although previous studies have demonstrated the potential of gluten-free flours, hydrocolloids, and dietary fibers to improve bread quality, formulation development requires consideration of the relationships among flour composition, hydration, and structure-forming ingredients. Further investigation of buckwheat–rice bread formulations incorporating either a xanthan gum–CMC blend or psyllium husk under different hydration conditions may provide useful information on their technological, sensory, and antioxidant characteristics. Evaluating these attributes together is particularly relevant for selecting a suitable base formulation for subsequent enrichment with ingredients derived from food-processing by-products.

Therefore, this study aimed to develop and evaluate six gluten-free bread formulations based on different buckwheat-to-rice flour ratios, water contents, and the incorporation of either a xanthan gum–CMC blend or psyllium husk. The formulations were characterized in terms of specific volume, crumb porosity, color, sensory acceptability, total polyphenol content, and antioxidant activity. The objective was to identify a formulation providing a

favorable balance between technological quality, sensory characteristics, and bioactive compound content, suitable for subsequent fortification with flours obtained from food-processing by-products.

2. Materials and methods

2.1. Materials

2.1.1. Plant material and sample preparation

Buckwheat flour, rice flour, and psyllium husk were obtained from a commercial online retailer. Fresh yeast, sugar, and salt were purchased from a local retail supplier. Hydrocolloids were provided by an authorized distributor.

Preliminary trials were conducted to establish suitable ingredient proportions for the development of gluten-free bread based on buckwheat and rice flours. Different hydrocolloid combinations, hydration levels, and amounts of yeast and sugar were initially evaluated. The incorporation of xanthan gum and hydroxypropyl methylcellulose (HPMC) at concentrations of 1% and 2% (based on flour weight) did not provide the desired bread characteristics. Consequently, a combination of 1% xanthan gum and 1% carboxymethyl cellulose (CMC) was selected for further formulation development. A sugar concentration of 3% was established during preliminary trials to support dough fermentation.Six bread formulations were subsequently developed, as presented in Table 1. The first three formulations (Sample_1–Sample_3) contained buckwheat-to-rice flour ratios of 100:0, 75:25, and 50:50, respectively, with a constant hydration level of 90 g water/100 g flour and the addition of 1% xanthan gum and 1% CMC. Sample_6 contained 50% buckwheat flour and 50% rice flour, with the same hydrocolloid concentrations but a reduced water content of 80 g/100 g flour.

Table 1. Description of gluten-free bread samples

Raw materials	Sample_1	Sample_2	Sample_3	Sample_4	Sample_5	Sample_6
Buckwheat flour	100	75	50	100	50	50
Rice flour	0	25	50	0	50	50
Sugar	3	3	3	3	3	3
Yeast	2	2	2	2	2	2
Salt	2	2	2	2	2	2
Cellulose gum (CMC)	1	1	1	0	0	1
Xanthan gum	1	1	1	0	0	1
Water	90	90	90	110	110	80
Psyllium husk	0	0	0	10	10	0

Note: All ingredient quantities are expressed as g per 100 g of total flour. Sample_6 has the same composition as Sample_3, except for the reduced water content (80 instead of 90 g/100 g flour).

Two additional formulations (Sample_4 and Sample_5) were prepared using psyllium husk at 10% of the total flour weight instead of the xanthan gum–CMC blend. Sample_4 contained 100% buckwheat flour, whereas Sample_5 contained 50% buckwheat flour and 50% rice flour. Due to the high water-holding capacity of psyllium husk, the hydration level was increased to 110 g water/100 g flour in both formulations.

The amounts of yeast, sugar, and salt were maintained constant at 2%, 3%, and 2% of the total flour weight, respectively, across all formulations. The complete ingredient compositions are provided in Table 1.

The samples were prepared using a professional planetary mixer model MZPL007 equipped with a 7 L bowl. Mixing was performed at speed level 3 (337 rpm) for 5 minutes. All dry ingredients, except for yeast, were added to the mixing bowl and blended for

1 minute. The yeast was dissolved in part of the formulation water and then added to the bowl together with the remaining water, after which kneading was initiated. The temperatures of the water, flour, and dough were monitored throughout the mixing process. The added water had a temperature of 43 °C, while the flour temperature was 21 °C.

After kneading, the dough was allowed to rest for 10 minutes, then divided into 450 g portions, rounded manually, and placed into baking molds. The dough pieces were proofed at 30 °C and 70% relative humidity for 1 hour, followed by baking at 240 °C for 40 minutes.

2.2. Methods

2.2.1. Dough Firmness Determination

Dough Firmness Determination was determined using a TA-XT plus texture analyzer (Stable Micro Systems, Surrey, UK). A CTX texture analyzer with a 5 kg load cell and a 6 mm cylindrical probe (TA-41) was used for dough penetration. The prepared dough was placed in a Berzelius beaker and flattened to even out the dough, and air bubbles were removed. The compression test parameters were as follows: pre-test speed: 1.0 mm/s, test speed: 1.0 mm/s, post-test speed: 1.0 mm/s. Target type: deformation, target distance: 15 mm, trigger force: 5 g. Six tests were performed for each dough sample, rotating the bowl to a new position for each determination. After the determination, the dough was flattened, and air bubbles were expelled, and the sample was kept covered at room temperature for a new determination after one hour (Ametek, 2024).

2.2.2. Dough Stickiness Determination

Dough stickiness was determined using a TA-XT Plus texture analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with a 25 mm cylindrical Plexiglas probe (SMS P/25). The probe was brought into contact with the dough surface and then withdrawn at a defined speed to measure the detachment force. The maximum force (stickiness/adhesiveness), the positive area (work of adhesion), and the separation distance were recorded as characteristic parameters. Before testing, the dough was placed in the dedicated sample cell and allowed to rest for 30 s to prevent moisture loss. All measurements were performed in triplicate (Smewing, 2015).

2.2.3. Sensory analysis of gluten-free bread samples

Sensory evaluation was performed by 10 trained assessors using coded bread samples and the Romanian 20-point bread evaluation scheme (Bordei, 2007). Five attributes were assessed: external appearance (3 points), including loaf shape and volume; crust appearance (3 points), considering color, thickness, and surface defects; crumb appearance (7 points), evaluating porosity, uniformity, elasticity, and texture; odor (3 points), assessing characteristic aroma and the absence of undesirable odors; and taste (4 points), evaluating characteristic flavor and the absence of off-flavors. Points were deducted for identified defects according to their severity, with a maximum total score of 20 points. Higher scores indicated better sensory quality. Individual scores were recorded and subsequently processed for each formulation.

2.2.4. Specific Volume of gluten-free bread samples

The determination consisted of measuring the volume of bread displaced in a medium composed of rapeseeds. The specific volume was calculated using the following formula:

$$\text{Specific volume}\left(\frac{\text{cm}^3}{100\text{g}}\right) = \frac{\text{V}}{\text{m}} * 100 \quad (1)$$

Where, V= difference between the volume read after the bread was introduced and the bread was turned over, cm3; m= mass of the product, g (SR 91:2007, 2007) (AACC International, 2000)

2.2.5. Determination of the crumb porosity of gluten-free bread

The method is based on determining the volume of the compact bread crumb after the pores have been eliminated by pressing. A certain volume of bread is pressed by hand until the air gaps disappear, forming spherical lumps about the size of a hazelnut. Approximately 150 cm³ of oil is poured into a graduated cylinder, and the volume is measured

precisely. The dough lumps are then introduced into the cylinder, and the volume is read again (Bordei, 2007).

$$Crumb\ porosity\ (\%) = \frac{V-(V_2-V_1)}{V} * 100 \quad (2)$$

Where, V= core volume, cm^3; V1= oil volume before sample introduction, cm^3; V2= oil volume after sample introduction, cm^3

2.2.6. Determining the moisture content in gluten-free bread samples

Approximately 5 g of crumbled bread was placed in a clean, dry crucible (dried for 30 min at 130 °C in an oven), cooled (20 min in a desiccator), and pre-weighed. The samples, along with the crucible, were weighed and then placed in an oven at 130 °C for 50 minutes for dehydration, with the vessel left open. After the time elapsed, the samples were removed from the oven and placed in a desiccator for cooling. After cooling, the crucible with the dehydrated sample was weighed. The moisture content is calculated using the following formula:

$$Moisture\ (\%) = \frac{m_1-m_2}{m_1-m_0} * 100 \quad (3)$$

Where, m_o= mass of the empty crucible, m_1= mass of the crucible with the sample, m_2= mass of the crucible with the dehydrated sample. (AACC, 1999)

2.2.7. Determination of the color of gluten-free bread samples

The determination of the color of flour samples was carried out using a HunterLab Agera colorimeter. The results were expressed in terms of L^* (lightness), a^*, and b^* values, according to the CIELab system, with reference to the D65 illuminant and a 10° observer angle.

2.2.8. Determination of the texture of gluten-free bread samples

The purpose of this method is to quantitatively determine the force required to compress a bakery product to a predetermined force using a texture analyser (TA-XT plus, Stable Micro Systems, UK). For this purpose, 12.5 mm thick slices of bread were compressed using a 21 mm (3,464 cm^2) aluminium piston and a 5 kg load cell. The sample was compressed approximately 10 mm (40% compression), and the force reading was measured at 25% compression. The curve was plotted from 25% to 40%, speeds being set in mm/min (100 mm/min for compression, 500 mm/min for charting). (AACC, 1999)

2.2.9. Extraction of compounds from bread samples for analysis

0.5 g of the product is subjected to the extraction process using 10 mL of a solvent composed of methanol and distilled water in a 70:30 (v/v) ratio. The extraction is carried out in a stoppered Erlenmeyer flask by maintaining it in an ultrasonic water bath at 40 °C for 30 minutes. After cooling, the solution is filtered into a 10 mL volumetric flask, and the volume is adjusted to the mark with the same solvent.

2.2.10. Determination of the polyphenol content in the obtained extracts

A 0.4 mL aliquot of the hydroalcoholic extract was transferred into a test tube. To this, 1 mL Folin–Ciocalteu reagent, 15 mL purified water, and 2 mL sodium carbonate solution (290 g/L) were added. The mixture was shaken for 10 minutes and incubated at 40 °C for 20 minutes. After cooling, the absorbance was measured at 760 nm against a blank prepared under the same conditions using the same reagent volumes (Georgescu, Bratu, & Tamas, 2005)(Frum et al., 2018). A calibration curve was constructed using gallic acid (1–3 µg/mL), yielding the equation: y =0.1328 x − 0.0099 (R^2=0.999) where y is the absorbance at 760 nm and x is the concentration in mg/mL. Results were expressed as mg gallic acid equivalents (GAE) per g of dry weight.

2.2.11. Determination of the antioxidant activity of the obtained extracts

25 µg/mL DPPH stock solution was prepared in methanol and kept in the dark at low temperature for 2 hours. For the assay, 970 µL of DPPH solution was mixed with 30 µL of sample, and the absorbance was measured at 515 nm using a Shimadzu UV-1900 spectrophotometer. The calibration curve was linear from 0.25 to 250 µg DPPH/mL: y = 0.0533x + 0.1293 (R^2 = 0.9688) y=0.0533x+0.1293(R^2=0.9688) where y is the absorbance and x the DPPH concentration

(µg/mL). The radical scavenging activity (RSA) was calculated as:

$$RSA\ (\%) = \frac{C_0 - C_1}{C_0} * 100 \quad (4)$$

with C_0 representing the blank absorbance and C_1 the sample absorbance (Constantinescu & Tit, 2020; Georgescu et al., 2022; Popovici & Saykova, 2017).

2.3. Data analysis

All bread formulations were prepared in three independent batches (n = 3), and the results were expressed as mean ± standard deviation (SD). Differences among formulations were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, with statistical significance set at $p < 0.05$. The assumptions of normality and homogeneity of variances were assessed before interpreting the ANOVA results. Pearson correlation analysis was performed using the mean values of the six bread formulations (n = 6) to investigate relationships among technological, textural, color, and antioxidant parameters. Hierarchical cluster analysis (HCA) was conducted using standardized formulation-level mean values, Euclidean distance, and Ward's linkage method. The results were visualized using a Pearson correlation heatmap and an HCA dendrogram. Statistical analyses were performed using Minitab software.

3. Results and discussions

3.1. Dough firmness determination

Dough firmness was significantly influenced by formulation at both measurement times (Figure 1). One-way ANOVA revealed significant differences among samples immediately after preparation ($F(5,30) = 167.05$, $p < 0.001$) and after one hour ($F(5,30) = 1016.42$, $p < 0.001$). No significant differences were observed between D1 and D2, indicating that replacing 25% of buckwheat flour with rice flour did not substantially affect firmness under the investigated conditions. However, increasing the rice flour proportion to 50% (D3) reduced firmness, possibly due to differences in water absorption and the resulting dough structure. Reducing hydration from 90% in D3 to 80% in D6, while keeping flour composition and hydrocolloid concentrations the same, increased initial firmness from 30.03 to 98.08, highlighting the importance of water availability in determining dough resistance.

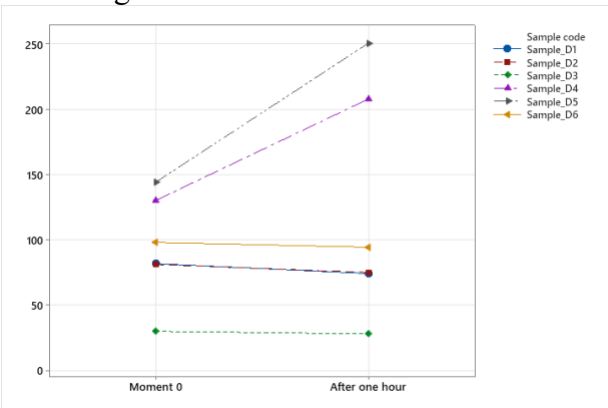

Figure 1. Firmness (compression resistance) of gluten-free dough formulations (D1–D6), measured immediately after preparation and after one hour. Values are expressed as mean ± standard deviation (n = 6).

The psyllium-containing formulations (D4 and D5) exhibited significantly higher firmness than the xanthan gum–CMC formulations, with the highest values recorded for D5. After one hour, mean firmness increased from 130.22 to 208.19 in D4 and from 144.16 to 251.03 in D5, corresponding to increases of approximately 60% and 74%, respectively. This behavior may

be associated with the progressive hydration of psyllium and the formation of a more resistant fiber-based network. In contrast, the xanthan gum–CMC formulations showed slight reductions in mean firmness during resting. Nevertheless, the effects of psyllium cannot be fully separated from those of hydration, as both factors differed between the formulation groups.

3.2. Dough stickiness determination

As shown in Figure 2, dough stickiness, adhesion to a surface, and cohesiveness also differed significantly among formulations ($p < 0.001$ for each parameter, one-way ANOVA). D3 exhibited the highest stickiness (30.71 g), adhesion (4.21 g·s), and cohesiveness (3.21 mm). In the xanthan gum–CMC formulations containing 90% water, increasing the proportion of rice flour was associated with higher stickiness and adhesion. Reducing hydration to 80% in D6 substantially decreased all three parameters compared with D3, suggesting that water availability contributed to the observed differences. The psyllium-containing formulations exhibited comparatively low stickiness despite their higher hydration levels, potentially reflecting the water-binding capacity of psyllium and the reduced availability of mobile water at the dough surface.

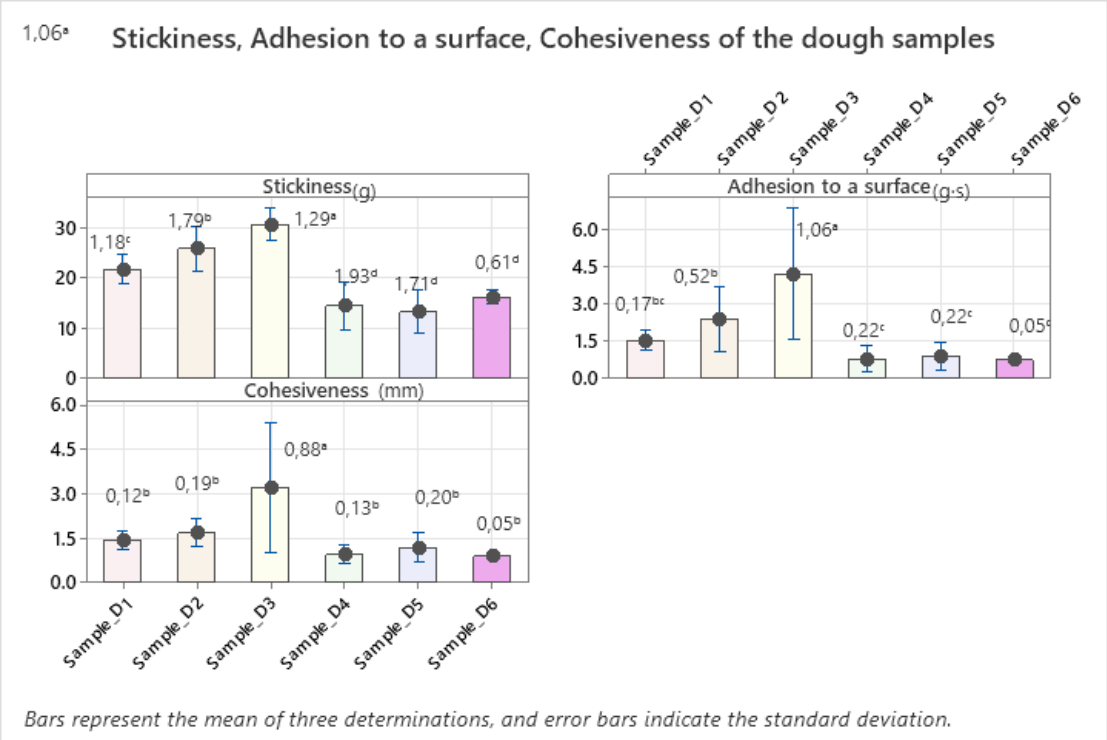

Figure 2. Stickiness, adhesion to a surface, and cohesiveness of gluten-free dough formulations (D1–D6). Values re presented as mean ± standard deviation (n = 3). Different lowercase letters indicate statistically significant differences among formulations ($p < 0.05$).

Water content plays an important role in determining the rheological and textural characteristics of gluten-free dough (Arendt & Dal Bello, 2008). Dough stickiness and adhesion may also be affected by formulation composition and processing conditions, including mixing time and sugar concentration (Tan et al., 2016). The present findings support the importance of hydration and structure-forming ingredients in modifying dough behavior. However, because several formulation variables changed simultaneously, the observed differences should not be attributed exclusively to a single ingredient.

Pearson correlation analysis was performed using the mean values of the six formulations

to evaluate the relationships among stickiness, adhesion to a surface, and cohesiveness (table 2). Strong positive correlations were identified between stickiness and adhesion ($r = 0.946$, $p = 0.004$), stickiness and cohesiveness ($r = 0.888$, $p = 0.018$), and adhesion and cohesiveness ($r = 0.985$, $p < 0.001$). These findings indicate that formulations exhibiting greater surface adhesion generally also presented higher stickiness and cohesiveness. The relationships may reflect common influences of hydration and dough structure on the measured textural properties. Nevertheless, the correlations do not establish causality and should be interpreted cautiously given the limited number of formulations.

Table 2. Pearson correlation coefficients between stickiness, adhesion to a surface, and cohesiveness of gluten-free dough formulations.

Parameters	Pearson r	p-value
Stickiness – Adhesion to a surface	0.946	0.004
Stickiness – Cohesiveness	0.888	0.018
Adhesion to a surface – Cohesiveness	0.985	< 0.001

Note: Correlation coefficients (r) were calculated using the mean values of the six dough formulations (n = 6). The corresponding p-values indicate the statistical significance of each correlation (two-tailed test). Statistical significance was considered at $p < 0.05$. P-values were not adjusted for multiple comparisons.

Overall, the results demonstrate that flour composition, hydration level, and the selected structure-forming system collectively influenced dough textural behavior. The comparison between D3 and D6 provides particularly clear evidence of the influence of hydration within the investigated formulation range, while the higher firmness of the psyllium-containing samples suggests differences in the development of the dough structure during resting.

3.3. Sensory analysis of gluten-free bread samples

The external appearance and crumb structure of the gluten-free bread samples are illustrated in Figure 3, while their sensory profiles are presented in Figure 4. Differences in crumb color and structure were observed among formulations, reflecting variations in flour composition, hydration, and structure-forming ingredients.



Figure 3. External appearance (upper row) and crumb structure (lower row) of gluten-free bread samples (own archive). Numbers 1–6 correspond to Sample_1–Sample_6, respectively, as described in Table 1.

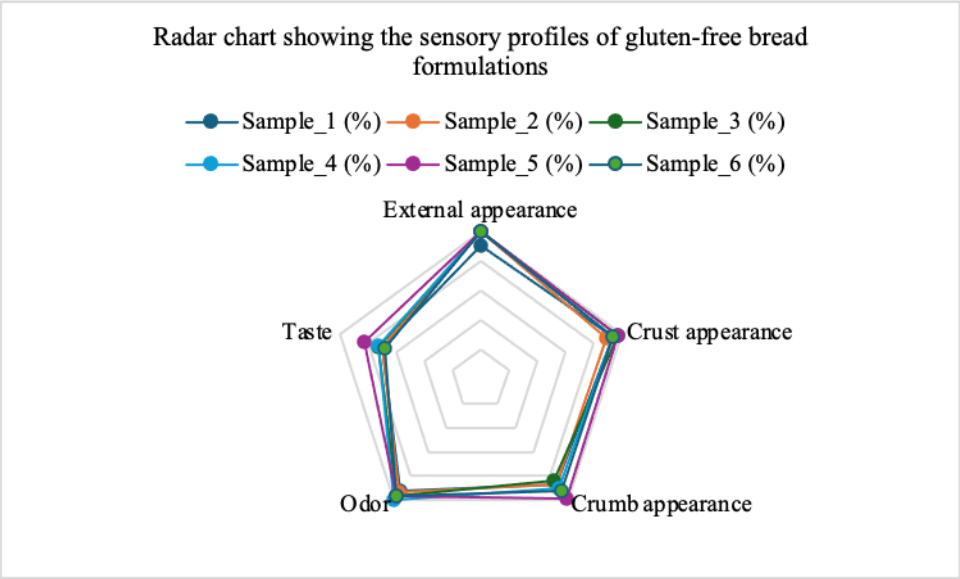

Figure 4. Radar chart showing the sensory profiles of gluten-free bread formulations (Sample_1–Sample_)6). The mean scores for external appearance, crust appearance, crumb appearance, odor, and taste were normalized to the maximum score assigned to each attribute and expressed as percentages.

Sensory evaluation revealed generally high scores for external appearance (2.71–3.00/3 points) and crust appearance (2.69–2.91/3 points). Crumb appearance showed greater variation, with Sample_5 receiving the highest score (6.89/7 points), followed by Sample_6 (6.49/7 points), whereas Sample_3 obtained the lowest score (5.84/7 points). The favorable crumb characteristics of Sample_5 may be related to the water-binding and gel-forming properties of psyllium husk.

Odor scores were relatively similar among samples (2.78–2.98/3 points), with Sample_4 achieving the highest value. Taste scores ranged from 2.71/4 points in Sample_6 to 3.29/4 points in Sample_5, indicating more noticeable differences in flavor perception. These variations may reflect the combined effects of flour composition, hydration, and structure-forming ingredients.

Overall, Sample_5 (50% buckwheat flour, 50% rice flour, and 10% psyllium husk) achieved the highest total sensory score (18.99/20 points), mainly due to its favorable crumb appearance and taste. This formulation therefore exhibited the most favorable sensory profile among the investigated samples, although the differences should be interpreted descriptively because statistical significance has not been established.

3.4. Physicochemical, Textural, and Antioxidant Characterization of Gluten-Free Bread

The physicochemical, textural, and antioxidant properties of the six gluten-free bread formulations are presented in Table 3. One-way analysis of variance (ANOVA) revealed a significant effect of formulation on all investigated parameters ($p < 0.001$), including specific volume, crumb porosity, moisture content, color coordinates (L*, a*, and b*), crumb firmness, stickiness, total phenolic content, and DPPH radical scavenging activity. Tukey's post hoc test identified significant differences between individual formulations, as indicated by the superscript letters in Table 3. These findings demonstrate that changes in flour composition, hydration level, and structure-forming ingredients were associated with differences in bread quality. The individual parameters are discussed in the following subsections.

Table 3. Physicochemical, textural, and antioxidant properties of gluten-free bread formulations

Parameter	Sample_1	Sample_2	Sample_3	Sample_4	Sample_5	Sample_6
Specific volume (mL/g)	1.617 ± 0.000^{b}	1.629 ± 0.017^{b}	1.633 ± 0.021^{b}	1.665 ± 0.001^{b}	1.805 ± 0.019^{a}	1.624 ± 0.037^{b}
Crumb porosity (%)	44.44 ± 0.00^{b}	53.70 ± 0.00^{a}	44.44 ± 0.00^{b}	33.95 ± 1.07^{c}	43.70 ± 0.64^{b}	25.93 ± 0.01^{d}
Crumb moisture (%)	51.14 ± 0.35^{c}	50.91 ± 0.08^{c}	51.04 ± 0.04^{c}	53.35 ± 0.10^{b}	54.40 ± 0.01^{a}	48.56 ± 0.39^{d}
L*	50.51 ± 0.09^{cd}	51.65 ± 0.17^{bc}	56.56 ± 0.35^{a}	49.74 ± 0.64^{d}	52.82 ± 0.75^{b}	56.83 ± 0.11^{a}
a*	4.39 ± 0.04^{c}	4.41 ± 0.07^{c}	4.18 ± 0.03^{d}	5.36 ± 0.05^{b}	5.63 ± 0.08^{a}	4.21 ± 0.04^{d}
b*	13.94 ± 0.23^{c}	14.26 ± 0.17^{bc}	14.69 ± 0.18^{ab}	14.21 ± 0.16^{bc}	14.16 ± 0.11^{bc}	15.06 ± 0.30^{a}
Crumb firmness (g)	2316.53 ± 42.30^{a}	1630.90 ± 37.44^{b}	1621.27 ± 29.64^{b}	1475.82 ± 12.21^{c}	1098.52 ± 2.62^{e}	1216.05 ± 37.61^{d}
Stickiness (g·s)	3900.64 ± 7.79^{a}	2874.31 ± 72.21^{c}	2749.58 ± 90.51^{c}	3121.99 ± 18.67^{b}	2368.61 ± 41.30^{d}	2837.90 ± 78.67^{c}
TPC (mg GAE/g)	1.555 ± 0.029^{a}	0.955 ± 0.035^{c}	0.859 ± 0.014^{d}	1.061 ± 0.035^{b}	0.809 ± 0.004^{d}	1.081 ± 0.025^{b}
DPPH (%)	28.43 ± 0.47^{a}	22.03 ± 0.39^{c}	18.60 ± 0.26^{d}	25.10 ± 0.57^{b}	18.09 ± 0.51^{d}	20.91 ± 0.19^{c}

Note: Values are expressed as mean ± standard deviation (n = 3 independent batches). Different superscript letters within the same row indicate statistically significant differences among formulations according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

3.4.1. Specific Volume

The specific volume of gluten-free bread varied among formulations (table 3), with the highest value recorded for Sample_5 (1.805 mL/g), containing equal proportions of buckwheat and rice flour and 10% psyllium husk. Sample_4 also exhibited a relatively high specific volume (1.665 mL/g), whereas the xanthan gum–CMC formulations showed lower values (1.617–1.633 mL/g). These results suggest that psyllium-containing formulations may favor loaf expansion under the investigated conditions, although the simultaneous differences in hydration must also be considered. Similar improvements in gluten-free bread volume with psyllium addition have been reported by Fratelli et al. (2018, 2021). Conversely, Mancebo et al. (2015) observed that increasing psyllium concentration could reduce specific volume, highlighting the importance of balancing fiber content and hydration.

3.4.2.Crumb porosity

Crumb porosity ranged from 25.93% in Sample_6 to 53.70% in Sample_2. The highest porosity was obtained in Sample_2, containing 75% buckwheat flour and 25% rice flour with xanthan gum and CMC. In contrast, Sample_6 exhibited the lowest porosity, suggesting that reducing hydration from 90% to 80%, while maintaining the same flour and hydrocolloid composition as Sample_3, adversely affected crumb structure. These findings are consistent with previous reports describing the contribution of hydrocolloids, including xanthan gum and CMC, to the development of gluten-free bread crumb structure (López et al., 2004; Lazaridou et al., 2007).

3.4.3. Crumb moiture content

Crumb moisture content differed significantly among formulations ($p < 0.05$), ranging from 48.56% in Sample_6 to 54.40% in Sample_5. The psyllium-containing formulations (Sample_4 and Sample_5), prepared with 110% hydration, exhibited higher moisture contents than the formulations containing xanthan gum and CMC. Sample_5 showed the highest moisture content, followed by Sample_4 (53.35%). Conversely, reducing hydration from 90% in Sample_3 to 80% in Sample_6 decreased crumb moisture from 51.04% to 48.56%. These findings indicate that hydration level, together with the water-binding properties of the structure-forming ingredients, influenced moisture retention. Similar effects of hydrocolloids and psyllium on moisture retention in gluten-free bread have been discussed by Mohammadi et al. (2014) and Filipčev et al. (2021).

3.4.5. Crumb color

Crumb color was significantly influenced by formulation ($p < 0.05$). The highest lightness (L*) values were recorded for Sample_6 (56.83) and Sample_3 (56.56), both containing equal proportions of buckwheat and rice flour, whereas Sample_4 exhibited the lowest value (49.74). This suggests that incorporating rice flour contributed to a lighter crumb, while the combination of buckwheat flour and psyllium husk in Sample_4 resulted in a darker appearance. All formulations exhibited positive a* and b* values, indicating reddish and yellow color components. Sample_5 showed the highest a* value (5.63), while Sample_6 exhibited the highest b* value (15.06). These differences may be associated with flour composition and the presence of dietary fibers, which have previously been reported to influence bread color (Cappa et al., 2013).

3.4.6. Crumb texture

Crumb firmness and stickiness differed significantly among formulations ($p < 0.05$). Sample_1 exhibited the highest firmness (2316.53 g), whereas Sample_5 showed the lowest value (1098.52 g), indicating a considerably softer crumb. Partial replacement of buckwheat flour with rice flour reduced firmness, while the psyllium-containing Sample_5 combined low firmness with the highest specific volume and moisture content. This behavior may be associated with the water-binding and structure-forming properties of psyllium, although differences in hydration and flour composition must also be considered. Previous studies have reported that the influence of psyllium on crumb texture depends strongly on its concentration and the

available water (Mancebo et al., 2015; Fratelli et al., 2021).

Crumb stickiness followed a broadly similar pattern, with the highest value in Sample_1 (3900.64 g·s) and the lowest in Sample_5 (2368.61 g·s). Sample_4 exhibited intermediate stickiness (3121.99 g·s), while Sample_2, Sample_3, and Sample_6 did not differ significantly from one another. Overall, Sample_5 displayed the most favorable instrumental texture profile, combining the lowest firmness and stickiness values among the investigated formulations..

3.4.7. Total phenolic contents and antioxidant activity

Total phenolic content (TPC) and radical scavenging activity differed significantly among formulations ($p < 0.05$). sample 1, containing 100% buckwheat flour, exhibited the highest TPC (1.555 mg gae/g) and dpph activity (28.43%). The lowest values were observed in sample 5 (0.809 mg gae/g and 18.09%, respectively), although its TPC and PPH activity did not differ significantly from those of sample 3. The generally lower antioxidant values in formulations containing rice flour may reflect the partial replacement of buckwheat flour, which contributes phenolic compounds to the product. compared with sample 3, sample 6 exhibited higher TPC (1.081 versus 0.859 mg gae/g) and DPPH activity (20.91% versus 18.60%), despite having the same flour and hydrocolloid composition. This difference coincided with reduced hydration and may also reflect differences in the concentration of compounds in the finished bread. overall, the antioxidant results indicate a trade-off between the technological advantages observed for sample_5 and the higher phenolic content and radical scavenging activity of sample 1.3.5 Pearson correlation analysis

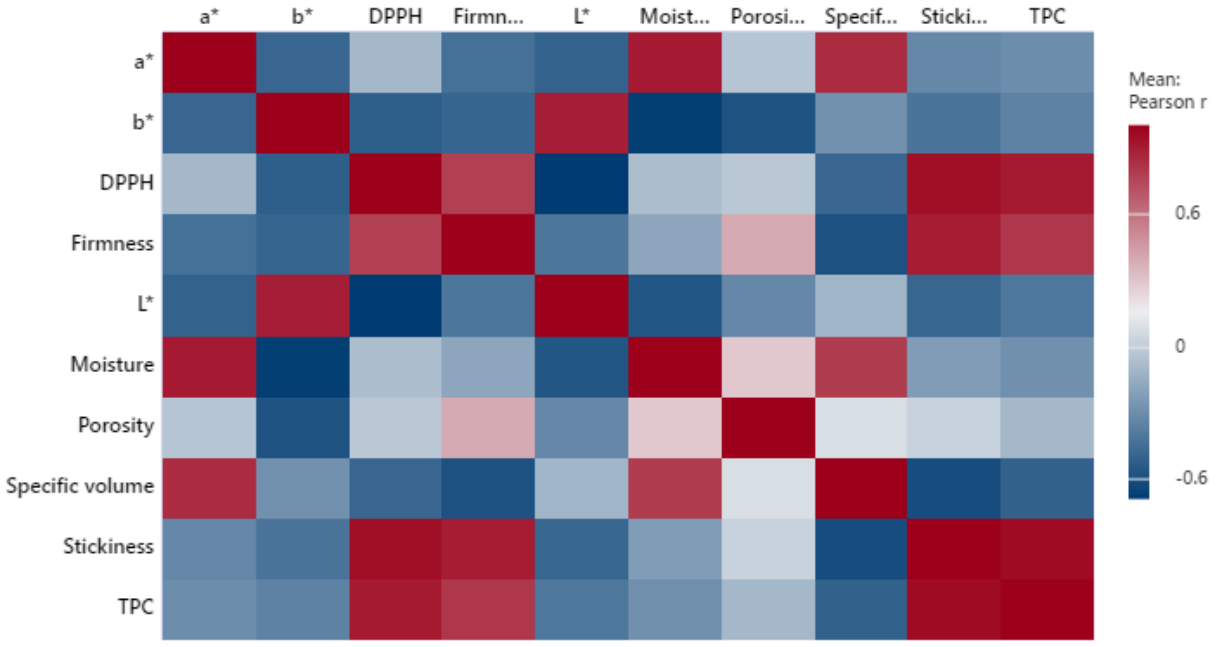

Figure 5. Pearson correlation heatmap illustrating the relationships among specific volume, crumb porosity, moisture, color parameters (L*, a*, b*), crumb firmness, stickiness, total phenolic content (TPC), and DPPH radical scavenging activity of gluten-free bread formulations. Correlation coefficients were calculated using formulation-level mean values (n = 6). Red and blue indicate positive and negative correlations, respectively.

Table 4. Pearson correlation coefficients among technological, textural, color, and antioxidant properties of gluten-free bread formulations.

	Specific volume	**Porosity**	**Moisture**	**L***	**a***	**b***	**Firmness**	**Stickiness**	**TPC**
Porosity	0.076								
Moisture	0.786	0.289							
L*	-0.117	-0.331	-0.570						
a*	0.847	-0.042	0.907	-0.510					
b*	-0.291	-0.584	-0.679	0.892	-0.494				
Firmness	-0.592	0.397	-0.187	-0.418	-0.446	-0.500			
Stickiness	-0.610	0.021	-0.237	-0.495	-0.333	-0.425	0.900		
TPC	-0.515	-0.102	-0.293	-0.414	-0.309	-0.359	0.806	0.961	
DPPH	-0.491	-0.027	-0.080	-0.693	-0.103	-0.528	0.773	0.948	0.908

Note: Pearson correlation coefficients were calculated using the mean values of the six bread formulations (n = 6).

Pearson correlation analysis was performed using the mean values of the six gluten-free bread formulations **(Figure 5**). Table 4 indicates the correlation parameters. Strong positive correlations were observed between total phenolic content and DPPH radical scavenging activity (r = 0.908), suggesting an association between phenolic compounds and antioxidant capacity. Crumb firmness was positively correlated with stickiness (r = 0.900), while stickiness was strongly associated with total phenolic content (r = 0.961). In contrast, specific volume showed a moderate negative correlation with crumb firmness (r = −0.592). These relationships indicate that the technological, textural, and antioxidant characteristics of the bread samples were interrelated. However, the correlations should be interpreted as exploratory findings due to the limited number of formulations (n = 6) and do not establish causal relationships.

3.6. Hierarchical Cluster Analysis

Hierarchical cluster analysis (HCA) was performed using standardized mean values of ten bread quality parameters, applying Ward's linkage method and Euclidean distance (Figure 6). At the selected three-cluster partition, Sample_1 formed a separate cluster, indicating a distinct overall quality profile. Sample_2, Sample_3, and Sample_6 were grouped together, suggesting similarities in their combined technological, textural, color, and antioxidant characteristics. Sample_4 and Sample_5 formed another cluster, corresponding to the formulations containing psyllium husk.

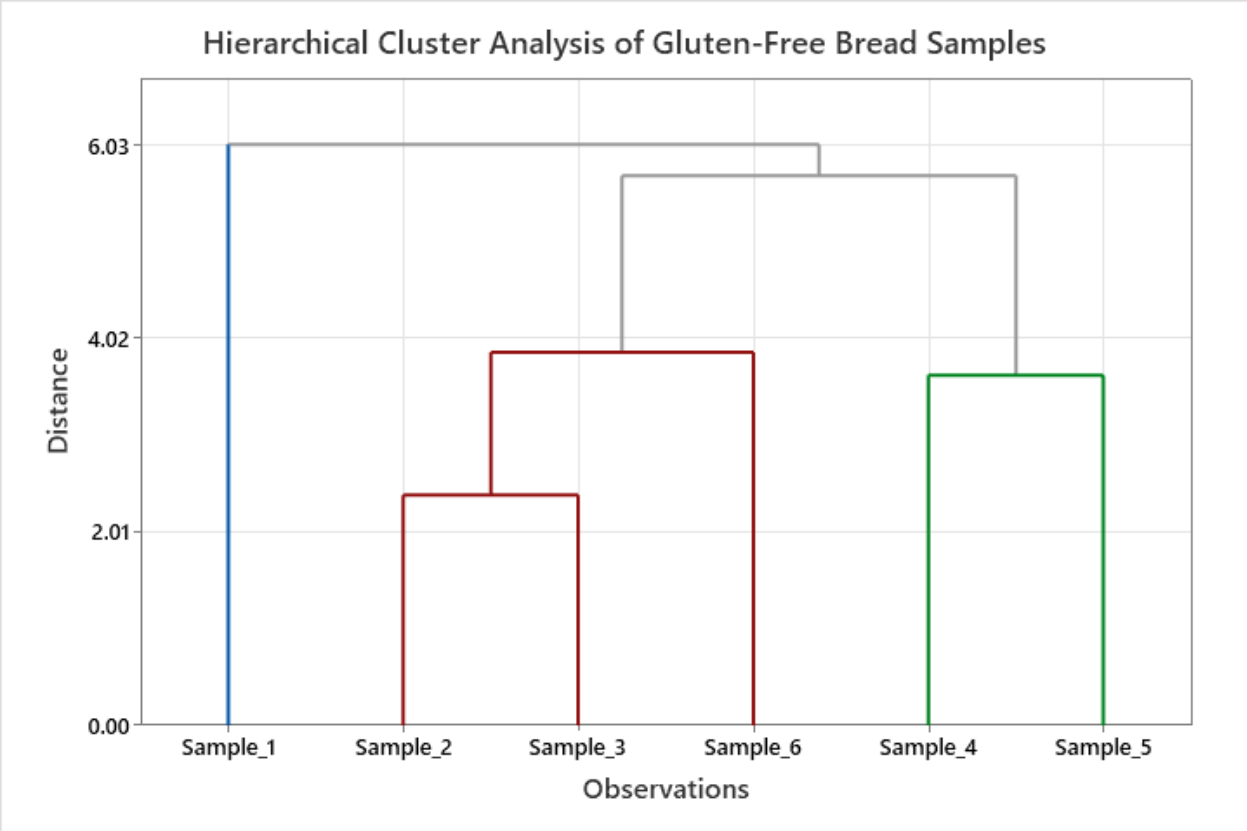

Figure 6. Hierarchical cluster analysis (HCA) dendrogram of gluten-free bread formulations (Sample_1–Sample_6) based on ten standardized technological, textural, color, and antioxidant parameters. Clustering was performed using Euclidean distance and Ward's linkage method. Colors indicate the three clusters selected for exploratory interpretation.

The separation of Sample_1 may be associated with its comparatively high crumb firmness, stickiness, total phenolic content, and DPPH radical scavenging activity. The grouping of Sample_4 and Sample_5 suggests similarities between the psyllium-containing formulations despite differences in flour composition. Overall, the HCA results indicate that the investigated formulations exhibited distinct multivariate quality profiles. However, the clustering should be interpreted as exploratory, given the limited number of formulations and the simultaneous variation of several formulation factors.

4. Conclusions

The present study demonstrated significant differences ($p < 0.05$) among the six gluten-free bread formulations in terms of physicochemical, textural, and antioxidant properties. Partial replacement of buckwheat flour with rice flour was associated with increased crumb lightness and reduced total phenolic content and antioxidant activity. Differences in hydration level and structure-forming ingredients were also associated with changes in specific volume, crumb moisture, porosity, and texture.

Among the investigated formulations, Sample_5, containing 50% buckwheat flour, 50% rice flour, and 10% psyllium husk, exhibited the highest specific volume and the lowest crumb firmness and stickiness. This formulation also received the highest overall sensory score, although sensory differences were evaluated descriptively. In contrast, Sample_1, containing 100% buckwheat flour, showed the highest total phenolic content and DPPH radical scavenging activity. These findings highlight the need to balance technological and sensory properties with

antioxidant characteristics when developing gluten-free bread.

Based on its overall technological and sensory profile, Sample_5 was selected as a promising base formulation for further investigation. Future research will focus on incorporating flours derived from agro-food by-products to improve the nutritional and sensory characteristics of gluten-free bread, particularly its dietary fiber and protein content, taste, and texture. Further studies should evaluate the effects of these ingredients on bread quality, nutritional composition, and consumer acceptance.

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