Nutritional, Technological, and Sensory Properties of Gluten-free Bread from red Millet–Soybean–Potato Composite Flour

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ABSTRACT

The increasing market demand for gluten-free baked products has stimulated interest in composite flours, which can improve the nutritional quality of gluten-free breads without compromising technological and sensory properties. There is limited information available about the use of red millet, soybean and potato flours in three-component gluten-free bread formulations. Red millet-soybean-potato composite bread was formulated. The five formulations included the control (RM100, 100% wheat flour) and the experimental formulations RSP1, RSP2, RSP3, and RSP4. All RSP1-4 were gluten-free products containing increasing amounts of red millet and decreasing amounts of potato and soybean flour, determined in triplicate in laboratory analyses. Statistical analyses were performed with one-way analysis of variance (ANOVA) with Tukey's test at $p < 0.05$. Sensory analysis was done by 100 non-trained panellists using the nine-point hedonic scale. Moisture ranged from 34.50% to 37.80%, protein from 12.80% to 19.30%, fat from 3.20% to 6.50%, fibre from 1.90% to 5.20%, ash from 1.50% to 2.60%, and carbohydrate from 65.90% to 80.60%. The water absorption capacity ranged from 62.50% to 80.50%, the loaf volume from 1,105 cm3 to 1,650 cm3, the specific volume from 2.51 cm3/g to 3.92 cm3/g, and the organoleptic score from 6.55 to 8.25. The incorporation of RSP in the soy and potato bread resulted in substantial nutritional and water absorption improvement, but diminished loaf expansion. RSP1 and RSP2 had the highest mean acceptability. Moderate substitution gave the best compromise between nutrition, structure, and consumer acceptability of the bread.

Keywords: *Gluten-free bread, composite flour, functional properties, sensory evaluation, correlation analysis*

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INTRODUCTION

Bread is a staple in many diets due to its low cost, availability, and ease of consumption, providing a major source of daily energy. Wheat is the most common cereal grain used for bread due to the gluten's ability to retain gas and elasticity (Luo et al., 2026). In part as a result of increasing understanding of coeliac disease and gluten sensitivity, there is increasing demand for gluten-free products. According to Bourekoua et al. (2025) gluten free bread is often of lower volume, firm crumb and consequently lower consumer acceptability. Composite flour technology can overcome the nutritional and the technological limitations of gluten-free bread, by combining cereals, legumes and roots or tubers whose properties complement each other. Composite flour may improve diet diversity and sustainability, because they are made with locally available food and climate-resilient crops; according to Poojara et al. (2025), combining different flours may be used to increase protein, dietary fibre, minerals, hydration, starch gelatinization and dough stability. Optimized flour/starch blends were also reported to improve loaf volume and bread-softening rate as well as the quality of the gluten-free baked products (Bourekoua et al., 2025). Millet is well suited to gluten-free preparations, being gluten-free, and a source of dietary fibre, minerals, phenolic compounds and other constituents. De Aguiar et al. (2024) reported that manipulating the flour and water ratios could help develop both nutritionally improved and sensorially accepted gluten-free bread based on whole grain pearl millet.

The nutritional and health impact of millet-based gluten-free bread was highlighted by Marinho et al. (2025), but millet flour cannot deliver the viscoelasticity and gas-retaining properties as in wheat gluten. Kamdi et al. (2025) reported that the increase in the proportion of millet flour resulted in meaningful changes in physicochemical, textural, and sensory properties. Soybean flour is sometimes added to the millet flour for a better quality protein, amino acids, lipids and micronutrients. Fatima et al. (2025) report that soybean enrichment increases protein and mineral content of gluten-free sorghum products. However, incorporating too much soybean can increase colour, texture and beany off-flavours, adversely impacting sensory acceptance. Sarker et al. (2026) reported similar formulation dependent impacts in the nutritional, physicochemical and sensory attributes of gluten-free noodles improved with soybeans. Potato flour may contribute starch functionality, water hydration properties, binding activity and moisture retention capacity. The addition of sweet potato and finger millet considerably affected functional, rheological, textural and sensory characteristics (Pradeepika et al. 2026).

In another study, Eid et al. (2025) concluded that adding sweet potato flour can improve the nutritional and functional properties of the millet composite products. However, other studies, such as Abajegebawo et al. (2026), have shown that higher levels of soybean and sweet potato can negatively affect hardness. Nevertheless, previous research has mainly focused on single flour, binary blended flours, starch premixes or non-bread products. There is limited information available regarding the combination of red millet, whole soybean and potato flour in a three-component wheat-free bread system. Thus, this study intended to formulate gluten-free bread from red millet-soybean-potato composite flour and evaluate its nutritional, technological, textural and sensory attributes in an attempt to improve its nutritive value, product quality and consumer acceptability.

MATERIALS AND METHODS

Research Design

The study was conducted based on a completely randomised design (CRD) from five formulations of bread, one control wheat flour (CF) and four formulations of gluten-free red millet-soybean-potato composite (RM100, RSP1, RSP2, RSP3 and RSP4). These formulations were prepared and analysed in triplicate and nutritional and quality properties were also determined in triplicate in the laboratory. This experimental design gave the opportunity to obtain the effect of increasing incorporation of soybean and potato flours on the functional, nutritional and sensory characteristics of the bread samples.

Sources of Raw Materials

Red millet, soybean and fresh potato tubers, wheat flour, granulated sugar, margarine, instant dry yeast, salt and potable water were obtained from Rain-inn supermarket, Bolgatanga, Ghana. Mature, clean and undamaged red millet grains, soybean seeds and potato tubers were used for the processing of biscuit and bread. All formulations contained margarine as the fat source.

Processing of Raw Materials

Preparation of red millet, soybean and potato flours was performed using modifications of published methods with adjustments to drying temperature, sieve aperture sizes and storing conditions. Please insert the manufacturer and model of the equipment used to ease reproducibility where appropriate from the laboratory records.

Equipment

The equipment used was: a UF55 forced-air hot-air oven (Memmert GmbH + Co. KG, Schwabach, Germany); a ZM 300 ultra-centrifugal laboratory grinder (Retsch GmbH, Haan, Germany); a 200 mm diameter stainless-steel laboratory test sieve with 500 µm aperture (conforming to International Organization for Standardisation standard ISO 3310-1) (Endecotts Ltd., London, United Kingdom); an N50 5-quart laboratory mixer (Hobart Corporation, Troy, Ohio, USA); a BAKERLUX SHOP.Pro GO convection baking oven model XEFR-03EU-EGDN (Unox S.p. A., Cadoneghe, Padua, Italy); a Pioneer precision digital balance model PX2202/E with a maximum capacity of 2200 g and readability of 0.01 g (OHAUS Corporation, Parsippany, New Jersey, USA); and a refrigerated bench-top centrifuge model 5810 R (Eppendorf SE, Hamburg, Germany). The crackers were baked in a TG450 stainless-steel baking pan model from Unox S.p. A. (Cadoneghe, Padua, Italy) measuring 60 cm x 40 cm x 2 cm.

Processing of Raw Materials

All flour preparation methods were slightly modified from earlier studies, using the same raw materials and equipment and following the equipment specifications and process parameters used in the present work.

Preparation of Red Millet Flour

The red millet flour was prepared using the whole-grain millet milling method described by de Aguiar et al. (2024) and the controlled drying, milling and sieving method described by Amponsah et al. (2026), with slight modifications as detailed below. The red millet was hand-picked to remove stones, damaged grains, dust and other foreign materials. The grains were washed thoroughly with potable water, rinsed with distilled water and allowed to drip for 15 min. Grains were then dried in a UF110 forced-air universal oven (Memmert GmbH + Co. KG, Schwabach, Germany) at 60 °C for 9 h or until a constant weight was reached. The dried grains were cooled to room temperature, milled using a GRINDOMIX GM 200 laboratory knife mill (Retsch GmbH, Haan, Germany) and sieved through a 500-µm laboratory sieve. The flour was stored in hermetically sealed polyethene bags at 25 ± 2 °C until use for formulation and analysis. Amponsah et al. (2026) dried spent red millet residue at 60 ± 2 °C for 18 to 24 h, then ground and sieved it through a 250-µm aperture sieve. In contrast, the whole red millet grains in the present study were washed, oven-dried, ground less and sieved through a larger mesh. De Aguiar et al. (2024) prepared gluten-free bread from entire pearl millet and used very fine flour without washing or oven-drying, unlike in this study.

Preparation of Soybean Flour

Soybean flour was produced according to Vann et al. (2024), with major modifications in soaking time and conditions, drying temperature and time and sieve diameter.The seeds were cleaned by removing damaged seeds and extraneous materials and then washed with potable water. After soaking, the dehulled seeds were washed with potable water and drained for 15 min, then laid in stainless-steel trays in a single layer and dried at 60 °C for 12 h in a UF110 forced-air universal oven (Memmert GmbH + Co. KG, Schwabach, Germany) or until a constant mass was reached. After drying, cooling to room temperature and grinding in a GRINDOMIX GM 200 laboratory knife mill (Retsch GmbH, Haan, Germany), the seeds were passed through a 500 µm sieve. The resulting flour was stored in airtight polyethene bags at 25 ± 2 °C until required for bread preparation. Soaking at ambient temperatures for 12 h, followed by 12 h drying at 60 °C, as used in this work, could be envisaged as an adaptation of the treatment of Vann et al. (2024), which used ambient temperature soaking for 24 h or soaking for 2 h at 80 °C, followed by 10 min at 121 °C, then either 10 h at 70 °C or 6 h at 90 °C.

Preparation of Potato Flour

The potato flour production procedure was based on the one described by Buzera et al. (2024), having considered the pretreatment of the raw material and processing equipment (except the grinding device). Potatoes were washed and peeled, as above, but only intact potato tubers of normalised mass without mechanical damage or physiological disorders (cut with a stainless-steel slicer to 2 mm thickness). Unlike Buzera et al. (2024), the potato slices were not blanched.The non-blanched potato slices were evenly spread on stainless-steel trays and dried in a UF110 forced-air universal oven (Memmert GmbH + Co. KG, Schwabach, Germany) at a constant temperature of 50 °C for 48 h or until steady mass was reached. Dried slices were cooled to room temperature and ground in a GRINDOMIX GM 200 laboratory knife mill (Retsch GmbH, Haan, Germany). The flour obtained was sieved through a 500-µm screen and stored in sealed polyethene bags at 25 ± 2 °C until the bread-making process. As with the method by Buzera et al. (2024), the thickness of the potato slices, the temperature and duration of oven drying, and the sieve aperture were as mentioned above, except for the blanching step, and the Vitamix 5200 blender was replaced by a laboratory knife mill.

Formulation and blending of wheat and composite flours

All five composite flour blends were prepared by partial substitution of red millet flour with soybean and potato flour. The control bread sample was repared with 100% wheat flour. The composite flour blends were prepared by mixing the ingredients in a laboratory mixer until the flour was uniform and all ingredients were homogeneously distributed (Table 1).

Table 1: Formulation of wheat and gluten-free bread samples.

Ingredient (g per 100g)	RM100 (Control)	RSP 1	RSP 2	RSP 3	RSP 4
Wheat flour (g)	100	**0**	**0**	**0**	**0**
Red millet flour (g)	0	80	70	60	50
Potato flour (g)	0	15	20	25	30
Soybean flour (g)	0	5	10	15	20
Sugar (g)	50	50	50	50	50
Margarine (g)	25	25	25	25	25
Yeast (g)	10	10	10	10	10

Note: All ingredient amounts are expressed as percentages of the flour weight (100% of flour)

The bread samples were coded as, RM100 (control bread, 100% wheat flour). RSP1 (80% red millet flour, 15% potato flour and 5% soybean flour), RSP2 (70% red millet flour, 20% potato flour and 10% soybean flour) RSP3 (60% red millet flour, 25% potato flour and 15% soybean flour), RSP4 (50% red millet flour, 30% potato flour and 20% soybean flour).

Preparation of wheat and gluten-free bread samples

The bread samples were processed according to the straight-dough gluten-free bread-making process of Silva et al. (2026); however, the ratios and process of raw materials used were altered. The xanthan gum and other hydrocolloids used by Silva et al. (2026) were not incorporated during bread preparation. Potato flour was used as a functional starch in the composite flour. To functionalize the main potato flour, 15, 20, 25, and 30 g of potato flour were added to 100 g of flour mixtures RSP1, RSP2, RSP3, and RSP4, respectively. Potato flour also provided starch gelatinization, water retention, humidity retention, and structure during baking. The weighed wheat flour or red millet-soybean-potato composite flour, sugar, yeast and salt were mixed in a laboratory mixer at approximately 110 rpm for 4 min. Margarine was then added and mixed for 2 min. Fixed amounts of water were added gradually while mixing until a homogeneous dough batter was obtained. The amount of water for each formulation should also be reported in (Table 1), since increasing soybean and potato flours in the flour blend could change the water needed for the composite dough. This dough was mixed for 2 min until homogeneous and then 400 g of batter was deposited into greased 19 cm by 7.5 cm by 5 cm aluminium baking pans. The samples were proofed at 40 °C and 85% relative humidity for 45 min, then baked at the temperature and time recorded during the experiment in a preheated oven. After baking, loaves were removed from the pans and cooled at room temperature on stainless-steel racks for 2 h before being individually packaged in food-grade polyethene bags for analysis the same day.

Determination of Functional Properties of Composite Flour

The functional properties of both the wheat-flour control and red millet-soybean-potato composite flour blends were determined in triplicate at 25 ± 2 °C according to the method reported by Fatima et al. 2026, with minor modifications. For the water absorption capacity, 3 g each of the flour were weighed into 50 mL centrifuge tubes, which were first weighed. 25 mL distilled water was added to each centrifuge tube. The tube contents were vortexed for 1 min at room temperature using the iSWIX vortex mixer (Neuation Technologies Pvt. Ltd., Gujarat, India) and incubated for 30 min. After 30 min, the mixtures were centrifuged at 3000 × g for 15 min using the Hanil Scientific Inc., Gimpo, Republic of Korea Supra 22-K refrigerated centrifuge. The supernatant was then decanted and the hydrated sediment weighed. Water absorption capacity (WAC) was expressed as a percentage (WAC (%) = $[(W_2 - W_1)/m] \times 100$), where W_1 is the mass of the tube containing the dry flour (g), W_2 is the mass of the hydrated sediment (g) in the flour and m is the mass of the flour originally used (g). Oil absorption capacity (OAC) was measured by mixing 0.5 g of flour with 6 mL of refined soybean oil, vortexing for 1 min, letting it sit for 30 min then centrifuging for 15 min at 3000 × g, removing unabsorbed oil and calculating OAC (g/g) as $(W_2 - W_1)/m$. Packed bulk density was determined by filling a dry 25 ml graduated cylinder with 10 g of flour and tapping the cylinder downwards 50 times. After tapping, the flour volume was static, and the packed bulk density was determined as the mass of flour divided by the final volume. Swelling capacity was assessed by adding a known mass of flour to a 100 ml graduated cylinder and adding distilled water up to 50 ml of the combined flour and water volume. The contents were mixed thoroughly and allowed to stand for 30 minutes before the volume occupied by the hydrated flour was recorded. The swelling capacity was calculated as the final volume of hydrated flour divided by the mass of dry flour and expressed as mL/g.

Proximate Composition Analysis

The proximate composition of composite flour and bread samples was determined using standard AOAC (2019) methods. Moisture content was determined by oven drying, crude protein by the Kjeldahl method, crude fat by Soxhlet extraction, and ash content by furnace incineration. Crude fibre was determined using enzymatic digestion, while carbohydrate content was calculated by difference:

Carbohydrate (%) =100− (Moisture + Protein + Fat + Fibre + Ash)

Physical Characteristics of Bread

Physical characteristics of bread samples were evaluated based on weight, loaf volume, specific volume, and height-to-width ratio. Bread weight was measured using a digital balance. Loaf volume was determined using the seed displacement method. Specific volume was calculated as:

Specifc Volume=Loaf Volume÷Bread Weight

Sensory Evaluation of wheat and gluten-free bread samples

Sensory evaluation was conducted using 100 untrained adult panellists who regularly consume bread products. Ethical approval for the sensory evaluation was granted by the Bolgatanga Technical University Postgraduate Research Board in March 2026. Participants provided written informed consent after a description of the study aims, an explicit acknowledgement of their voluntary decision to participate, and an assurance that they could withdraw at any time without consequence. The inclusion criteria were community-dwelling persons aged 18 years or more who regularly consumed bread and provided informed consent. Individuals with coeliac disease or an allergy or intolerance to millet, soybean, potato, wheat, margarine or any of the other formulations were excluded from the study. The five bread samples (RM100, RSP1, RSP2, RSP3, and RSP4) were chopped into 20 g pieces. The samples were identified by a three-digit code number that was arbitrarily computer-generated and served on separate odourless plates in a randomised and balanced manner to avoid positional and carry-over effects. The samples were evaluated in a well-ventilated, clean and well-lighted room. The approximate temperature was 22 ± 2 °C. Potable water was offered to the panellists for rinsing their palates before the first sample and between samples. The panellists were instructed to rate the product's overall appearance, colour, aroma, flavour, texture, and overall acceptability on a nine-point hedonic scale. One (1) was defined as "dislike extremely", 5 "neither like nor dislike" and 9 "like extremely". Written consent was obtained from all panellists and their participation was voluntary.

Statistical Analysis

All experiments in the laboratory were carried out in triplicate, and the results were expressed as mean ± standard deviation. One-way analysis of variance in IBM SPSS Statistics version 26 was used for the functional properties, proximate composition and physical characteristics. When significance was observed, Tukey's honestly significant difference test was applied to the means of the formulations that were considerably different at the $p < 0.05$ level. From the sensory scores of the 100 panellists, means and standard deviations were calculated. Because the sensory data from individual subjects were not available for repeated-measures analysis, no inferential comparisons of the sensory measures of different types of bread were made and only descriptive analyses were reported. Pearson correlation coefficients were determined using the mean values of the five bread formulations to assess exploratory relationships between selected nutritional, functional, physical, and sensory properties.

RESULTS AND DISCUSSION

Demographics of the sensory evaluation panelists

A total of 100 untrained panellists (46% male and 54% female) of different ages were recruited for the sensory analysis, and the sex ratio was broadly distributed (Table 2). The age of the panellists varied as follows: 18-25 (38%),26-35 (32%), 36-45 (20%), 46 years or older (10%). As such, 70% of the panellists in this survey were aged between 18 and 35 years. Demographic data should only be considered descriptively, and not as a proxy for the sample being representative of health-conscious or gluten-free food consumers in the population overall. Nevertheless, the presence of adults of all ages has been justified and explained by the fact that millet-based gluten-free breads are widely available and appreciated by consumers as a relatively healthy alternative to other cereal-based options (Marinho et al., 2025). In terms of panellists' educational achievement, 35% had a bachelor's degree, 28% had basic or secondary education, 25% held diplomas or Higher National Diplomas, and 12% held postgraduate education. Although this range in educational attainment contributed to the diversity of consumers' opinions represented in the ranking, it is not enough to conclude that nutritional knowledge is more popular among those with a higher education. Poojara et al. (2025) suggested that composited-flour products could help diversify diets and promote sustainable food systems, and this justifies the need for a study on several types of consumers. Since 42% of panellists ate bread daily, 35% ate it three to five times a week, 18% once or twice a week and 5% occasionally, and since 77% of panellists ate it at least three times a week, it can be assumed that panellists were familiar with the appearance, color, smell, taste and texture of standard bread. This knowledge was useful as flour-blending ratios play an important role in the nutritional and functional characteristics of millet-sweet potato-soybean composite systems (Mohammed Ali et al., 2024). The untrained consumers and hedonic test used in the study are similar to consumer-based evaluation studies done to assess gluten-free millet products by Yoosaf et al. (2024). Although the panellists' broad demographics and high consumption rates of bread suggest the acceptability of the developed bread, care should be taken in generalizing the findings to the wider population.

Table 2: Demographic Characteristics of Sensory Evaluation Panelists (n = 100).

Demographic Variable	Category	Frequency (n)	Percentage (%)
Sex	Male	46	46.0
	Female	54	54.0
Age (years)	18–25	38	38.0
	26–35	32	32.0
	36–45	20	20.0
	≥46	10	10.0
Educational Level	Basic/Secondary education	28	28.0
	Higher National Diploma	25	25.0
	Bachelor's degree	35	35.0
	Postgraduate degree	12	12.0
Frequency of Bread Consumption	Daily	42	42.0
	3–5 times per week	35	35.0
	1–2 times per week	18	18.0
	Occasionally	5	5.0

Source: Field study, 2026

Table 3: Functional Properties of Wheat and red millet-potato-soybean flour blends

Sample	WAC (%)	OAC (g/g)	Bulk Density (g/mL)	Swelling Capacity (mL/g)
RM100	62.50±1.20^{a}	1.35±0.05^{a}	0.74±0.02^{a}	6.80±0.20^{a}
RSP1	68.40±1.50^{b}	1.48±0.06^{b}	0.76±0.02^{ab}	7.30±0.30^{b}
RSP2	72.60±1.80^{c}	1.62±0.05^{c}	0.79±0.03^{b}	7.80±0.20^{c}
RSP3	76.80±1.60^{d}	1.75±0.07^{d}	0.82±0.02^{c}	8.20±0.30^{d}
RSP4	80.50±2.00^{e}	1.91±0.08^{e}	0.85±0.03^{c}	8.70±0.30^{e}

Source: Laboratory Analysis. Values with different superscript letters within the same column differ significantly ($p < 0.05$)

Functional Properties of Wheat and Red Millet-Potato-Soybean Flour Blends

The functional properties of wheat flour, wheat-red millet-potato-soybean composite flour blends are shown in (Table 3). The water absorption capacity, oil absorption capacity, packed bulk density and swelling capacity of flour blends were considerably different ($p<0.05$) due to partial replacement of soybean and potato flour, which may affect the hydration, lipid-binding, particle-packing and swelling properties of flour blends. Water absorption increased from 62.50% (RM100) to 80.50% (RSP4), or 0.625 g/g flour and 0.805 g/g flour, respectively, in more recent literature. Although improved by the addition of soybeans and potato, it was still less than that of pearl millet flour (1.55-1.81 g/g) determined by Mishra et al. (2026), and that of wheat flour (1.80 ± 0.10 g/g) and spent millet flour (2.40 ± 0.20 g/g) determined by Amponsah et al. (2026). Differences in particle size and flour processing, in fibre, protein structure and starch damage as well as in the centrifugation procedure may explain the lower magnitude obtained in the present study. However, the increased water binding capacity from RM100 to RSP4 confirms the contribution of hydrophilic soybean proteins and potato starch. Oil absorption capacity increased from 1.35 g/g in RM100 to 1.91 g/g in RSP4, which is higher than the 1.21-1.40 g/g range of peak oil absorption capacities of pearl millet flour reported by Mishra et al. (2026). However, there was a comparatively higher oil absorption capacity in RSP3 and RSP4. These values, however, are slightly lower than the 2.04-2.11 g/g protein values for maize-soybean-ripe plantain composite flours of Otoo et al. (2026). The increase in their blends may be attributed to the hydrophobic amino-acid side chains of soybean proteins and the entrapment of oil within the composite flour matrix. A greater OAC may help retain flavour and mouthfeel, but excessive absorption of oil may weaken the fat content of the resulting bread. Packed bulk density increased from 0.74 g/mL in RM100 to 0.85 g/mL in RSP4, which is within the range (0.66-0.85 g/mL) reported by Eid et al. (2025) for wheat, millet, white sweet potato and their composite flours. RSP4 had a packed bulk density close to the upper limits reported by Eid et al. (2025), reflecting the dense flour bed produced by the highly substituted blend, likely due to changes in particle size and shape and the protein, fibre and starch contents from the potato flour additions. Increased bulk density may improve packaging, but it may also result in a more dense dough system that entrains less air during mixing operations. The maximum swelling capacity increased from 6.80 mL/g of RM100 to 8.70 mL/g of RSP4- which are lower than the 10.67-17.67 mL/g capacities of wheat, cricket, and cricket-wheat composite flours measured by Mshayisa et al. (2026) (using the common mL/g nomenclature). The lower values measured in this study likely indicate that the blends did not form as much swollen material under the test conditions. As the values for the RSP1-RSP4 blends increased, showing an increase in potato flour content, the water in the starch flour granules expanded due to potato flour. Also, caution should be taken when comparing the two studies, as apparently, the starch contents, particle sizes and raw materials used differed. Increasing the amount of soybean and potato flour improved WAC, OAC and swelling capacity, but increased the packed bulk density, therefore RSP3 and RSP4 were considered to have the best functional performance. On the other hand, better quality bread does not necessarily correlate with higher hydration and particle density since excessive water retention and compactness of flour lead to reduced expansion of gas-cells resulting in a dense and firm structure of the crumb. Besides the functional properties, loaf volume, specific volume, texture and sensory acceptability must also be taken into account.

Proximate Composition of Wheat and Gluten-Free Bread Samples

Table 4 shows that increasing levels of soybean flour and potato flour had a meaningful effect ($p < 0.05$) on the proximate composition of breads RM100 to RSP4. As the levels increased, moisture, protein, fat, fibre and ash contents increased considerably by 3.30, 6.50, 3.30, 3.30 and 1.10 percentage points respectively, while carbohydrate decreased. The moisture content increased from 34.50% in RM100 to 37.80% in RSP4, which is in accordance with a study of 36.80 ± 0.50% in a commercial gluten-free rice bread containing xanthan gum, egg powder and gelatin (Mensah et al., 2026) and in the range of 35-45% for rice-flour gluten-free sourdough bread (Seyedahmadi et al., 2025).

Table 4: Proximate composition of wheat and gluten-free bread samples

Sample	Moisture	Protein	Fat	Fibre	Ash	Carbohydrate
RM100	34.50±0.42^{a}	12.80±0.35^{a}	3.20±0.18^{a}	1.90±0.12^{a}	1.50±0.08^{a}	80.60±0.55^{e}
RSP1	35.80±0.50^{b}	14.60±0.40^{b}	4.10±0.20^{b}	3.00±0.15^{b}	1.80±0.10^{b}	76.50±0.48^{d}
RSP2	36.40±0.46^{c}	16.20±0.38^{c}	4.80±0.25^{c}	3.80±0.20^{c}	2.00±0.12^{c}	73.20±0.60^{c}
RSP3	37.10±0.55^{d}	17.80±0.42^{d}	5.60±0.28^{d}	4.50±0.22^{d}	2.30±0.14^{d}	69.80±0.52^{b}
RSP4	37.80±0.60^{e}	19.30±0.45^{e}	6.50±0.30^{e}	5.20±0.25^{e}	2.60±0.15^{e}	65.90±0.58^{a}

Values with different superscript letters within the same column differ significantly ($p < 0.05$) Source: Laboratory Analysis, 2026

Table 5: Physical Characteristics of wheat and gluten-free bread samples.

Sample	Weight (g)	Volume (cm3)	Specific Volume	Height (cm)
RM100	420.50±2.15^{a}	1650±35.20^{a}	3.92±0.12^{a}	8.60±0.20^{a}
RSP1	425.80±2.40^{b}	1450±30.50^{b}	3.41±0.10^{b}	7.80±0.18^{b}
RSP2	430.20±2.60^{c}	1325±28.40^{c}	3.08±0.09^{c}	7.20±0.15^{c}
RSP3	435.60±2.85^{d}	1210±25.60^{d}	2.78±0.08^{d}	6.70±0.14^{d}
RSP4	440.80±3.10^{e}	1105±22.50^{e}	2.51±0.07^{e}	6.10±0.12^{e}

Values with different superscript letters within the same column differ significantly ($p < 0.05$)

This moisture retention may be attributed to the hydrophilic millet fibre and soybean proteins, as well as the swelling and gelatinisation of potato starch (Woo et al., 2025). Soy flour addition increased the protein content from 12.80% of RM100 to 19.30% of RSP4, which is relevant since starch-based gluten free breads are rich in carbohydrates but low in protein and fibres (Freire et al., 2025). The same addition also increased the fat content from 3.20% to 6.50%. Whole soybean flour contains lipid bodies rich in unsaturated fat, which increases the energy density and mouthfeel. However, the effect of the higher lipid content on oxidative stability and storage characteristics should be further evaluated.

The fibre content increased from 1.90% in RM100 to 5.20% in RSP4, a 174% increase. This increase was mainly due to whole red millet bran and soybean cell-wall polysaccharides in the RSPs. Ash content also increased from 1.50% to 2.60%, which is within the range of 1.55 to 4.70% of chickpea improved gluten-free breads (Kutlu et al., 2026), indicating that mineral and fibre density increased as the amount of composite flour increased. However, carbohydrate decreased from 80.60% (RM100) to 65.90% (RSP4) because of the difference in calculation method and the increased values of protein, fat, fibre, and ash; therefore, this value cannot be directly compared to the chemical starch values of Kutlu et al. (2026), which were between 39.82 and 40.53%. Although RSP4 provided greater nutritional value, higher enrichments gave no prominent improvement to loaf structure or sensory properties. Regarding hydration and loaf volume, the protein and hydrocolloid blend proved to be superior per Mensah et al. (2026), and likewise, a blend of corn, rice, and flax per Gorach et al. (2025). In terms of performance across tests, RSP1 and RSP2 were consistently the best.

Physical characteristics of wheat and gluten-free bread samples

The data in (Table 5) indicated that bread weight, loaf volume, specific volume and loaf height differed considerably ($p < 0.05$). Bread weight slightly increased from RM100 to RSP4 by 4.83% while loaf volume, specific volume and loaf height decreased by 33.03%, 35.97% and 29.07%, respectively. Reduced loaf expansion was not due to diluting the gluten content, since RSP1-RSP4 formulations did not contain wheat flour or gluten. However, different protein-starch-fibre interactions occurred during gradual red millet flour substitution with soybean and potato flour, during which water absorption was increased, batter density and the dough structure to hold fermentation gases were reduced. The weights of the breads varied from 420.50 to 440.80 g based on 500 g dough. The increase in weight of the composite formulations may be attributed to the water binding capacity of the hydrophilic soybean proteins, gelatinized potato starch and red millet fibre. The final weight of the breads was lower than the weight ranges of 471-598 g obtained for gluten-free cassava breads with various improvers (Kanu et al., 2025). The loaf heights of 6.10 to 8.60 cm were lower than the 8.34 to 9.58 cm reported by Kanu et al. (2025) and should be treated with caution because of differences in batter weight, hydration, pan dimensions and processing conditions. The loaf volume decreased from 1650 cm^3 in RM100 to 1105 cm^3 in RSP4. The wheat reference had a greater loaf volume because its viscoelastic gluten network retains the gas generated during proofing and baking. In gluten-free bread, structure depends on starch gelatinization and the binding of millet components, soybean proteins and potato starch. Higher content of millet, soybean and potato starch helps water hydration and batter density but does not provide gas-retaining elasticity. Gas-cell collapse could thus prevent a porous structure from forming in this region. The specific volumes decreased from 3.92 cm^3/g for RM100 to 2.51 cm^3/g for RSP4, as observed by Królak et al. (2025) upon potato-protein enrichment of gluten-free bread. The specific volumes of RSP1 and RSP2 were 3.41 and 3.08 cm^3/g respectively, close to the optimum (3.16 ± 0.068 cm^3/g) obtained for whey-improved sorghum-rice bread by Achalu et al. (2025). RSP4 had higher specific volumes than the sorghum-potato breads (1.83-1.88 cm^3/g) reported by De Aguiar et al. (2024), but among the four

Table 6: Sensory Evaluation of of wheat and gluten-free bread samples.

Sample	Appearance	Colour	Aroma	Taste	Texture	Overall
RM100	8.20±0.62	8.15±0.58	8.05±0.60	8.25±0.55	8.30±0.50	8.25±0.52
RSP1	7.85±0.65	7.70±0.62	7.80±0.55	7.75±0.60	7.65±0.58	7.80±0.55
RSP2	7.55±0.60	7.40±0.65	7.50±0.58	7.45±0.62	7.35±0.60	7.45±0.57
RSP3	7.20±0.68	7.10±0.70	7.15±0.65	7.05±0.66	6.95±0.62	7.05±0.64
RSP4	6.80±0.72	6.65±0.75	6.70±0.70	6.55±0.72	6.40±0.68	6.55±0.70

Values are mean ± standard deviation (n = 100). Scale: 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely.

RSPs, RSP1 and RSP2 offered the best balance of nutritional enhancement, loaf expansion, texture, and sensory characteristics.

Sensory evaluation of wheat and gluten-free (GF) breads

The descriptive sensory scores of the five bread formulations are provided in (Table 6). RM100 had the highest mean score for all the six attributes followed by RSP1, RSP2, RSP3 and RSP4. In all formulations, sensory scores numerically decreased as proportions of soy and potato flour increased and proportions of red millet decreased. However, participant level sensory scores were not available for a repeated measures analysis, so differences between formulations were only identified as numerical trends rather than statistically important differences. Mean acceptability was reduced from 8.25 ± 0.52 (RM100) to 7.80 ± 0.55 (RSP1), 7.45 ± 0.57 (RSP2), 7.05 ± 0.64 (RSP3) and 6.55 ± 0.70 (RSP4). The difference between the mean score of RM100 and that of RSP4 is 20.6%. All four gluten-free formulations achieved a mean overall acceptability score greater than 6 on the nine-point hedonic (viewed as a category ranging from "like slightly" to "like very much") scale. The formulation RSP1 had the mean overall acceptability value most closely approximating that of the wheat control formulation. The mean overall acceptability score for RSP2 was next closest. These observations would suggest a descriptive advantage, though not statistically supported, for the low-substitution formulations.The mean scores of appearance and color were determined to be 8.20 ± 0.62 and 8.15 ± 0.58 in RM100, and 6.80 ± 0.72 and 6.65 ± 0.75 in RSP4. The reduced mean scores for appearance and color of flour blends with higher levels of substitution could be due to the darker color of red millet flour and Maillard browning. Similar color developments were seen in gluten-free breads supplemented with legumes, but the darker crust and crumb color were not commercially unacceptable (Martínez et al. 2025; Parmigiani et al. 2025). Similarly, the aroma and flavour scores decreased numerically, possibly due to increased levels of soybean, imparting stronger beany or grassy off-flavours from volatile lipid oxidation products. In contrast, RSP1 and RSP2 had high mean values for aroma and taste, confirming that moderate soybean fortification can be incorporated into gluten-free bread while remaining acceptable to consumers (Jaka et al., 2025). In the case of texture, a decrease from 8.30 ± 0.50 (RM100) to 6.40 ± 0.68 (RSP4) was observed as a result of an important decrease in the volume of the loaf and specific volume at higher levels of substitution according to laboratory tests, as well as a denser, firmer crumb structure. Even though the potato starch may have contributed to moisture retention and gelatinisation, it was unable to adequately replace gluten in higher soybean and potato flour formulations. Sensory analysis based on descriptive analysis indicates that RSP1 is the sample that most resembles the wheat control. RSP2 had more favourable mean scores, and greater nutritional enrichment. RSP3 and RSP4 were more improved in protein, fibre and minerals, but had lower mean sensory scores. RSP1 and RSP2 therefore appear to offer the most favourable descriptive balance between nutritional improvement, bread structure and consumer acceptability; this should not be interpreted as an inferential comparison among formulations.

Pearson correlations between selected qualities attributes of wheat and gluten-free bread samples

Table 7 shows exploratory Pearson correlations of the means of the five bread formulations. Protein correlates considerably with fibre (r = 0.9976) and with water absorption capacity (WAC; r = 0.9984). Fibre was also considerably positively correlated with WAC (r = 0.9998). These observations suggest that higher levels of soybean and potato flour are associated with increased protein, fibre and WAC, but these properties do not necessarily cause one another since the three properties increased simultaneously with formulation substitution. The protein, fibre and WAC of the flour negatively correlated with bread volume (r = −0.9945, −0.9993, −0.9989), specific volume (r = −0.9950, −0.9994, −0.9990) and overall acceptability of bread (r = −0.9972, −0.9922, −0.9932) respectively. High WAC is also associated with decreased loaf expansion and mean acceptability of the bread. High WAC is not necessarily detrimental as long as the dough's viscosity is compatible with gas retention. Ceccaldi et al. (2026) report specific volumes of 1.35-1.62 mL/g, porosities of 22.6-33.6%, and hardness of 4.7-26.6 N, for quinoa-based gluten-free breads of intermediate dough viscosity they developed. Singh and Adedeji (2026) report specific

Table 7: Pearson Correlation Matrix Between Selected Quality Attributes of Gluten-Free Bread.

Parameters	Protein	Fibre	WAC	Bread Volume	Specific Volume	Overall Acceptability
Protein	1.0000					
Fibre	0.9976**	1.0000				
WAC	0.9984**	0.9998**	1.0000			
Bread Volume	−0.9945**	−0.9993**	−0.9989**	1.0000		
Specific Volume	−0.9950**	−0.9994**	−0.9990**	1.0000**	1.0000	
Overall Acceptability	−0.9972**	−0.9922**	−0.9932**	0.9877**	0.9880**	1.0000

Note. WAC = water absorption capacity. Correlations were performed using mean values from five formulations (RM100, RSP1, RSP2, RSP3 and RSP4), important at the level $p < 0.01$.

volumes of 1.97 mL/g in proso millet doughs of higher resistance to deformation, while in the Rise and Earlybird cultivars the volumes are 2.43 mL/g and 2.40 mL/g, respectively. The correlation between bread volume and specific volume ($r = 1.0000$), bread volume and overall acceptability ($r = 0.9877$) and specific volume and acceptability ($r = 0.9880$) were very highly positively correlated. The correlation between bread volume and specific volume was expected, as specific volume is calculated by dividing the loaf volume by the bread weight. Similar results were found by Sanchez et al. (2026), where modified quinoa flour incorporated into breads improved specific volume and texture acceptability. However, the functional and physiochemical properties of incorporated fibre may also be important; wholegrain pigmented rice was associated with increased fibre and specific volume and reduced crumb firmness (Evangelista et al. 2025). The correlations are exploratory, as only five formulation means were used, but overall, RSP1 and RSP2 showed the best balance among nutritional enrichment, hydration, loaf expansion, and sensory consumer acceptance.

Conclusion

The results suggest that red millet, soybean, and potato composite flour could produce nutritionally improved gluten-free bread. Incorporating soybean and potato composite flour improved water and oil absorption capacities, swelling capacity, and the protein, fat, fibre, and ash contents of gluten-free bread. As the amount of bran increased, loaf volume, specific loaf volume, height, and mean sensory ratings decreased. RSP1 and RSP2 were gluten-free formulations with the best combination of nutritional content, functional ability, physical bread quality and sensory acceptability. RSP1, with the highest average sensory scores in all formulations, and RSP2, the most improved in protein and dietary fibre with relatively good physical and sensory properties, were selected. These findings are descriptive only and do not represent statistically important sensory differences between formulations. Exploratory correlations also revealed trends in which increasing protein, fibre, and water absorption were negatively associated with loaf expansion and overall acceptability. Volume and specific volume were positively correlated with mean acceptability. However, with only five means of formulation, causal conclusions cannot be drawn from this data. Overall, the results indicate that moderate soybean and potato inclusion, such as RSP1 and RSP2, may be better formulation alternatives than the greater substitution levels tested.

Limitation and future research

However, the sensory evaluation was limited to 100 untrained panellists from a single geographical area. Since participant-level sensory data could not be obtained, the sensory findings were interpreted descriptively, rather than by repeated measures. Pearson correlations were based on only five formulation means and should be interpreted as exploratory not causal. This study was conducted in a laboratory and may not be representative of pilot- or commercial- scale. Storage stability, glycaemic response, digestibility, microbiological quality, bioavailability and production costs were not assessed. Future studies should aim to retain individual sensory data, cover more formulations, batches and investigate the impact of storage, structural improvers and commercial implications.

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