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OPTIMIZATION AND QUALITY EVALUATION OF OHMIC-HEATED GLUTEN-FREE BREAD FORMULATED WITH SOYBEAN, SORGHUM, AND RED BEAN FLOURS USING RESPONSE SURFACE METHODOLOGY

[Optimasi dan Evaluasi Mutu Roti Bebas-Gluten dari Tepung Kedelai, Sorgum, dan Kacang Merah dengan Pemanasan Ohmik Menggunakan Response Surface Methodology]

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ABSTRACT

Ohmic heating has emerged as an alternative volumetric baking technology capable of improving the quality of gluten-free bread; however, its effectiveness is strongly influenced by the starch and protein composition of the ingredients used. This study aimed to optimize the formulation of gluten-free bread processed by ohmic heating using soybean flour, sorghum flour, and red kidney bean flour as complementary protein–starch sources. A mixture design Response Surface Methodology (RSM) was applied to evaluate the effects of flour proportions on specific volume and proximate composition. Soybean flour (50-80%), sorghum flour (10-30%), and red kidney bean flour (10-20%) were selected as independent variables. The optimal formulation consisted of 71.39% soybean flour, 10.00% sorghum flour, and 18.61% red kidney bean flour, yielding a specific volume of 2.79 ± 0.07 cm³/g and a protein content of 22.68 ± 0.05%. Model validation confirmed no significant differences between predicted and experimental values ($p>0.05$). The results demonstrate that synergistic interactions between protein-rich and starch-rich flours under ohmic heating significantly enhance bread structure and nutritional quality. This study provides a systematic formulation strategy for developing high-quality gluten-free bread using ohmic heating technology.

Keywords: *Gluten-free bread; legume flour; mixture design; ohmic heating; response surface methodology*

ABSTRAK

Pemanasan ohmik telah berkembang sebagai teknologi pemanggangan volumetrik alternatif yang mampu meningkatkan mutu roti bebas gluten; namun efektivitasnya sangat dipengaruhi oleh pati dan protein dalam komposisi bahan yang digunakan. Penelitian ini bertujuan untuk mengoptimalkan formulasi roti bebas gluten yang diproses menggunakan pemanasan ohmik dengan memanfaatkan tepung kedelai, tepung sorgum, dan tepung kacang merah sebagai sumber protein dan pati yang saling melengkapi. Metode Response Surface Methodology (RSM) dengan rancangan campuran digunakan untuk mengevaluasi pengaruh proporsi tepung terhadap volume spesifik dan komposisi proksimat roti. Tepung kedelai (50-80%), tepung sorgum (10-30%), dan tepung kacang merah (10-20%) ditetapkan sebagai variabel bebas. Formulasi optimum diperoleh pada komposisi 71,39% tepung kedelai, 10,00% tepung sorgum, dan 18,61% tepung kacang merah, yang menghasilkan volume spesifik sebesar 2,79 ± 0,07 cm³/g dan kadar protein sebesar 22,68 ± 0,05%. Validasi model menunjukkan tidak terdapat perbedaan yang signifikan antara nilai prediksi dan nilai eksperimen ($p>0,05$). Hasil penelitian ini menunjukkan bahwa interaksi sinergis antara tepung kaya protein dan tepung kaya pati di bawah pemanasan ohmik secara signifikan meningkatkan struktur dan kualitas gizi roti. Penelitian ini menyediakan strategi formulasi yang sistematis untuk pengembangan roti bebas gluten berkualitas tinggi menggunakan teknologi pemanasan ohmik.

Kata Kunci: Mixture design; pemanasan ohmik; response surface methodology; roti bebas gluten; tepung legum

INTRODUCTION

The application of ohmic heating in bread production has increased substantially in recent years due to its potential to overcome limitations associated with conventional baking methods. Conventional baking often results in gluten-free bread with undesirable characteristics, including low volume expansion, dense crumb structure, surface crust formation, prolonged baking time, and reduced nutritional quality (Bender et al., 2019; Bender & Schönlechner, 2020; Waziiroh et al., 2021). Ohmic heating is a volumetric heating technique in which electrical current passes through electrically conductive food materials, generating internal heat due to electrical resistance.

Baking is a critical stage in bread production because it induces complex physical, chemical, and biochemical transformations, including moisture evaporation, structure formation, volume expansion, protein denaturation, and starch gelatinization. Ohmic heating offers advantages over conventional thermal processing by enabling rapid and uniform heat generation throughout the product matrix, which can enhance baking efficiency and product quality.

Previous studies by Hutasoit et al., (2024) applied ohmic heating to gluten-free bread formulated from corn starch and soybean flour. Their findings demonstrated that among seven voltage treatments (70-120 V), the application of 120 V produced bread with superior quality characteristics compared with conventional baking. These included higher specific volume (3.17 ± 0.17 cm^3/g), lower baking loss (14.58 ± 1.50%), improved textural attributes, and higher crumb porosity. Increasing voltage enhanced electrical conductivity, accelerated temperature rise, shortened baking time, and promoted greater loaf expansion and pore development.

Gluten-free bread dough is typically prepared from non-gluten ingredients such as rice flour, cassava, corn, and tuber-based flours. The absence of gluten generally results in poor gas retention, limited dough expansion, inferior nutritional value, and unsatisfactory appearance. This is primarily due to the lack of a viscoelastic protein network capable of mimicking gluten functionality. In gluten-free systems, structural reinforcement relies largely on starch gelatinization, which is often insufficient to stabilize the dough structure and retain gas bubbles during proofing and baking. Consequently, alternative strategies involving ingredient modification and processing innovations are required.

Further research by Hutasoit et al., (2021) evaluated the addition of transglutaminase (TGase), an enzyme that catalyzes protein cross-linking and promotes protein network formation. The incorporation of TGase significantly improved loaf volume, crumb texture, and pore structure, with the optimal concentration identified at 1.5%, yielding the highest specific volume (3.36 ± 0.17 cm^3/g). However, the effectiveness of TGase depends on protein availability and concentration within the dough matrix (Giosafatto et al., 2018; Miwa, 2020). Excessive protein cross-linking may compromise dough stability when exposed to high baking temperatures due to protein denaturation, indicating the need for complementary structural components such as starch. Several studies by An & King, (2007); Waziiroh et al., (2021) have also reported that the starch content present in flour-based formulations plays a crucial role in the development of gluten-free bread structure. During thermal processing, the swelling and gelatinization of starch granules contribute significantly to the formation of the dough matrix, thereby influencing gas retention capacity and ultimately determining the expansion and final volume of the bread.

Sorghum is a promising cereal crop with favorable nutritional composition, containing approximately 73 g carbohydrates, 3.3 g fat, and 11 g protein per 100 g (Mustika et al., 2019). Several studies have reported that sorghum incorporation improves the technological and sensory properties of gluten-free bread (de Oliveira et al., 2022; Dogruer et al., 2023; Olojede et al., 2020). Soybean (Glycine max) is particularly rich in protein (43.75 ± 0.55%) and fat (21.15 ± 0.04%), making it a valuable ingredient for enhancing dough viscoelasticity and nutritional quality (Filipini et al., 2021; Taghdir et al., 2017). Red kidney beans are also a significant source of protein, carbohydrates, dietary fiber, and lipids (Ramzy & Putra, 2019). Their incorporation into bread formulations has been shown to improve both nutritional composition and physical characteristics of gluten-free bakery products (Ramzy & Putra, 2019).

Based on these considerations, an optimized formulation combining soybean flour, sorghum flour, and red bean flour is required to maximize the structural integrity, expansion, and nutritional quality of gluten-free bread produced using ohmic heating. This study aimed to evaluate and optimize the formulation of gluten-free bread made from soybean flour, sorghum flour, and red bean flour to achieve superior loaf expansion and nutritional quality using Response Surface Methodology

Despite increasing studies on ohmic heating for gluten-free bread production, most previous research has focused on processing parameters such as voltage levels or enzyme modification, while systematic optimization of flour composition remains limited. In particular, the combined use of protein-rich legume flours and starch-based cereals under ohmic heating conditions has not been comprehensively investigated using mixture design approaches. Therefore, this study addresses this gap by optimizing the formulation of soybean flour, sorghum flour, and red kidney bean flour to improve both structural and nutritional properties of gluten-free bread processed by ohmic heating.

MATERIALS AND METHODS

Materials and Equipment

The equipment used in this study included an ohmic heating bread baking system, a mixer equipped with a whisk hook (KLAZ, 220-240 V), a fermentation chamber (Getra Gas Manual Proofer), digital balance, measuring cylinders, stopwatch, mixing bowls, knives, plastic wrap, and a digital laser tachometer. Raw materials consisted of soybeans, sorghum grains, and red kidney beans, which were processed into flours using a dry milling method. The grains were sorted, then milled using a hammer mill, and sieved through an 80-mesh screen. Proximate analyses of the flours were conducted to determine moisture content (gravimetric method), protein content (Kjeldahl method), fat content (Soxhlet extraction), and carbohydrate content (by difference). Additional ingredients, including instant dry yeast, sugar, salt, margarine, liquid milk, eggs, emulsifier, rice flour, and transglutaminase enzyme powder, were obtained from the Sumbawa Central Market.

Experimental Design and Dough Preparation

The primary objective of this study was to optimize the proportions of soybean flour, sorghum flour, and red bean flour in gluten-free bread formulation using Response Surface Methodology with a mixture design. The objective of this optimization was to produce bread with improved expansion characteristics and enhanced nutritional quality. The independent variables were soybean flour (X_1: 50-80%), sorghum flour (X_2: 10-30%), and red bean flour (X_3: 10-20%). The response variables included specific volume, moisture content, fat content, protein content, carbohydrate content, and ash content. Design-Expert® software version 11 was used to generate optimal formulation solutions with varying desirability values. Solutions with desirability values approaching 1 were considered optimal. All experiments were conducted in triplicate, and the order of experimental runs was randomized to minimize systematic bias.

Bread dough preparation followed the method of Sutrisno et al., (2025) with minor modifications. All dry ingredients, except yeast and TGase, were mixed in a mixer bowl. The dough formulation included flour blend, salt (2%), milk (25%), emulsifier (1.5%), and margarine (5%). Yeast was activated by mixing with sugar and 100 mL of water at 25°C and incubated in a proofer for 5 minutes. Tgase 1.5% was dissolved in 80 mL of water prior to incorporation. A TGase concentration of 1.5% was fixed in this study because it was previously shown to produce the highest loaf volume and the most desirable bread texture (Hutasoit et al., 2024). Mixing was performed at 84 rpm for 3 minutes, followed by the addition of yeast suspension and TGase solution, and further mixing until homogeneous. The dough was placed into molds and proofed at 30-35 °C for 40 minutes. Baking was conducted using ohmic heating at a voltage gradient of 120 V for 30 minutes.

Evaluation of Bread Characteristics

Specific Volume Analysis

The specific volume of bread was determined using the seed displacement method according to AACC Approved Method 10-05.01 (AACC, 2000). Briefly, a container of known volume was filled with basil seeds as a substitute for rapeseeds. The bread loaf was placed in the container, and the volume of displaced seeds was recorded as the loaf volume (cm^3). The specific volume (cm^3/g) was then calculated by dividing the loaf volume by its weight (g). All measurements were performed in duplicate, resulting in four observations for each treatment condition.

Proximate Analysis of Raw Materials and Bread Samples

Moisture Content

Moisture content was determined according to AACC methods (AACC, 2001). Empty moisture dishes were first dried in an oven at 105 °C for 30 min and weighed (W_0). Approximately 2 g of sample was placed in the dish and weighed (W_1), then dried at 105 °C for 6 h. After drying, samples were cooled in a desiccator for 15 min and reweighed (W_2). Drying and weighing were repeated until a constant weight was achieved, defined as a mass difference ≤ 0.002 g between successive measurements. Moisture content (%) was calculated as:

$$\text{Moisture (\%)} = \frac{(W1-W2)}{(W1-W0)} x100\% \qquad (1)$$

Fat Content

Fat content was analyzed using the Soxhlet extraction method following AOAC procedures (AOAC, 2005). The extraction flask was sterilized and weighed (W_2). Approximately 5 g of homogenized sample was weighed (W_1), wrapped in filter paper, and placed in the Soxhlet apparatus. Hexane was used as the extraction solvent, and the extraction process was carried out for approximately 6 h until the solvent in the siphon tube became clear.

The extracted fat was recovered using a rotary evaporator set at 50 rpm and 69 °C, followed by oven drying at 105 °C for 1 h. The flask containing extracted fat was weighed (W_3). If the mass difference between successive weighings exceeded 0.0002 g, additional drying was performed until a constant weight was obtained. Fat content (%) was calculated as:

$$\text{Fat (\%)} = \frac{(W3-W2)}{(W1)} x100\% \qquad (2)$$

Protein Content

Protein content was determined using the Kjeldahl method according to AOAC guidelines (AOAC, 2005). Approximately 2 g of sample was weighed and transferred into a 500 mL Kjeldahl flask. A catalyst mixture (K_2SO_4, $CuSO_4$, and SeO_2 in a 5:3:1 ratio) and 20 mL of concentrated H_2SO_4 were added. The mixture was digested at 200-300 °C for 1 h until a clear green solution was obtained and then cooled for approximately 1 h.

The digest was diluted to 100 mL with distilled water. A 10 mL aliquot was distilled after adding 50 mL of 40% NaOH and 50 mL of distilled water. The released ammonia was trapped in 30 mL of 4% boric acid solution. Distillation was conducted at 200°C for 1 h until a blue coloration appeared. The distillate was titrated with 0.1 N HCl using methyl red as an indicator. Nitrogen content (%) was calculated as Equation (3) and crude protein (%) was calculated as Equation 4.

$$\text{N (\%)} = \frac{(V_1 \times N_1 \times F_1 \times MW_N)}{W_S \times 10} \qquad (3)$$

$$\text{Protein (\%)} = \text{N (\%)} \times \text{conversion factor} \times F_2 \qquad (4)$$

Note:
Ws = weight of the sample (g)
V1 = volume of 0.1 N HCl used in the titration, calculated as the difference between the final and initial burette readings (mL)
N1 = normality of HCl solution (0.1 N)
F1 = correction factor (set to 1)
Conversion factor = nitrogen-to-protein conversion factor, 5.70 for soybean protein
MWN = atomic weight of nitrogen (14.007)
F2 = dilution factor

Carbohydrate Content

Carbohydrate content was calculated by difference according to AACC methods (AACC, 2001):

$$\text{Carbohydrates (\%)} = 100 - (\%\ \text{protein} + \%\ \text{fat} + \%\ \text{moisture} + \%\ \text{ash}) \qquad (5)$$

RESULTS AND DISCUSSION

Proximate analysis was conducted to determine the moisture, protein, fat, carbohydrate, and ash contents of the raw materials, namely soybean flour, sorghum flour, and red bean flour. The results are presented in Table 1.

Table 1. Proximate analysis results of raw materials

Raw Material	Moisture Content (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Ash Content (%)
Soybean	3.20	49.90	16.80	19.10	11.00
Sorghum	5.22	23.20	4.60	61.98	5.00
Red kidney bean	4.04	35.46	2.60	49.90	8.00

Soybean flour exhibited the highest protein and fat contents, reaching 49.90% and 16.80%, respectively. High protein and lipid contents play a crucial role in enhancing dough viscoelasticity, which directly influences dough structure and gas retention capacity during proofing and baking. Proteins contribute to the formation of a cohesive network, while lipids improve dough softness and crumb texture (Bender et al., 2019; Pereira et al., 2021). However, excessive lipid content may weaken dough structure by reducing viscoelastic stability.

In contrast, sorghum flour and red bean flour showed higher carbohydrate contents, indicating their potential contribution as starch sources. Carbohydrates, particularly starch, are essential for bread expansion through granule swelling and gelatinization during heating (Waziiroh et al., 2021; Wong et al., 2011). Ohmic heating accelerates starch gelatinization, promoting structural development and volume expansion in gluten-free bread systems. These findings confirm that combining protein-rich and starch-rich flours is a rational strategy to improve gluten-free bread quality.

Mixture Design Optimization of Gluten-Free Bread Formulation

Mixture design was employed to determine the optimal combination of soybean flour, sorghum flour, and red bean flour for gluten-free bread production. The experimental design evaluated soybean flour (50-80%), sorghum flour (10-30%), and red bean flour (10-20%) as independent variables, with specific volume, moisture content, fat content, protein content, carbohydrate content, and ash content as response variables.

As shown in Table 2, the specific volume ranged from 2.17 to 2.78 cm³/g, while moisture content varied between 51.43% and 54.73%. Fat, protein, carbohydrate, and ash contents also showed substantial variation across formulations, indicating strong interactions among the mixture components. Compared with the proximate composition of individual raw materials, the formulated doughs demonstrated synergistic effects, particularly in enhancing specific volume and nutritional attributes.

Table 2. Experimental Design and Response Variables of the Mixture Design

Run	**Soybean Flour (%)**	**Sorghum Flour (%)**	**Red Bean Flour (%)**	**Specific Volume (cm^3/g)**	**Moisture Content (%)**	**Fat Content (%)**	**Protein Content (%)**	**Carbohydrate Content (%)**	**Ash Content (%)**
1	55.60	25.24	19.15	2.17	51.43	6.00	14.07	24.03	4.47
2	80.00	10.00	10.00	2.51	54.70	12.00	24.35	4.43	4.52
3	65.54	18.82	15.64	2.37	52.34	8.00	20.51	14.58	4.37
4	50.00	30.00	20.00	2.22	51.49	4.80	13.32	26.23	4.16
5	75.21	14.79	10.00	2.65	53.73	11.60	22.92	7.16	4.59
6	56.37	30.00	13.63	2.24	51.46	6.20	18.70	19.46	4.18
7	50.00	30.00	20.00	2.28	51.94	4.70	13.39	25.64	4.33
8	70.37	14.34	15.29	2.78	51.97	11.60	23.97	7.79	4.67
9	70.00	10.00	20.00	2.67	52.40	11.60	21.58	9.70	4.72
10	70.31	19.69	10.00	2.56	51.72	11.60	21.07	10.95	4.66
11	70.00	10.00	20.00	2.75	52.50	11.20	21.76	10.12	4.42
12	60.26	19.74	20.00	2.24	51.50	6.60	17.64	19.81	4.45
13	75.14	10.00	14.86	2.78	53.24	12.00	23.09	6.93	4.74
14	65.25	24.75	10.00	2.35	51.50	9.00	17.70	19.17	4.20
15	56.37	30.00	13.63	2.27	51.76	7.00	17.27	20.52	4.05
16	80.00	10.00	10.00	2.75	54.73	12.00	23.09	5.31	4.87

Modeling and Analysis of Bread Specific Volume Response

The results of the response surface analysis indicated that the special cubic model was the most appropriate to describe the relationship between the formulation variables and the specific volume of gluten-free bread. The analysis of variance (ANOVA) for the specific volume response is presented in Table 3.

As shown in Table 3, the selected model was statistically significant ($p < 0.05$), with an F-value of 14.52, indicating that the model adequately explained the variability in specific volume. The coefficient of determination (R^2) was 0.9064, demonstrating a strong correlation between the experimental data and the values predicted by the model. The adjusted R^2 value of 0.8439 further confirmed the robustness of the model after accounting for the number of terms included. Although the predicted R^2 value (0.7152) was slightly lower, it remained within an acceptable range, indicating reasonable predictive capability.

Table 3. ANOVA of the Special Cubic Model for Bread Specific Volume

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	0.7157	6	0.1193	14.52	0.0004	significant
(1)Linear Mixture	0.6061	2	0.3031	36.89	< 0.0001	
AB	0.0116	1	0.0116	1.42	0.2645	
AC	0.0527	1	0.0527	6.41	0.0321	
BC	0.0386	1	0.0386	4.70	0.0584	
ABC	0.0811	1	0.0811	9.88	0.0119	
Residual	0.0739	9	0.0082			
Lack of Fit	0.0397	5	0.0079	0.9270	0.5445	not significant
Pure Error	0.0343	4	0.0086			
Cor Total	0.7896	15				
R^2	0.9064					
Adjusted R^2	0.8439					
Predicted R^2	0.7152					

Model adequacy was further supported by the lack-of-fit test, which was not statistically significant ($p = 0.5445$). This result indicates that the deviation between the experimental data and the model predictions was not significant, confirming that the special cubic model was suitable for describing the effects of the mixture components on bread specific volume. Significant interaction effects were observed among the formulation components. The interaction between soybean flour and red bean flour (AC) as well as the three-component interaction (ABC) significantly influenced specific volume ($p < 0.05$). These interactions suggest a synergistic effect between protein-rich and starch-rich ingredients in enhancing gas retention and structural development during proofing and ohmic baking.

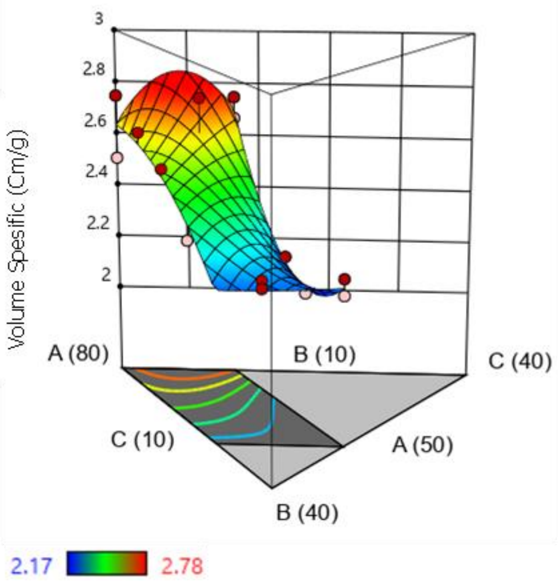

Figure 1. Three-dimensional response surface plot illustrating the effects of soybean flour (A), sorghum flour (B), and red bean flour (C) on the specific volume of gluten-free bread.

The final regression equation for predicting the specific volume of gluten-free bread formulated with soybean flour (A), sorghum flour (B), and red bean flour (C) is expressed as Equation (6), where A, B, and C represent soybean flour, sorghum flour, and red bean flour, respectively.

$$\text{Yvolume spesifik} = 2.64*A + 1.76*B - 1.27*C + 0.8118*A*B + 6.16*A*C + 6.74*B*C - 9.50*A*B*C \qquad (6)$$

Positive coefficients in the model indicate synergistic effects that enhance specific volume, whereas negative coefficients suggest antagonistic interactions that reduce loaf expansion. The surface response plot (Figure 1) illustrates that the specific volume increased with higher proportions of soybean flour, while an excessive increase in sorghum flour tended to reduce loaf volume. This behavior highlights the critical balance between protein-rich and starch-rich components in maintaining gas retention and structural stability during proofing and baking.

In the present study, the optimum specific volume was 2.79 cm^3/g, which was lower than the values reported by Hutasoit et al., (2024), namely 3.17 cm^3/g and 3.36 cm^3/g following TGase optimization. This difference may be attributed to the higher moisture content of the bread produced in the current study. The high starch content enhanced water absorption and retention during the gelatinization process, thereby increasing the final weight of the bread. Since specific volume is calculated as the ratio of loaf volume to loaf weight, increased water retention may result in a lower specific volume despite the occurrence of loaf expansion. Furthermore, excessive moisture content may lead to a denser crumb structure, thereby limiting volume expansion during baking.

Specific volume is a key quality parameter in bread and bakery products, as it reflects the dough's ability to retain gas during fermentation and baking. In gluten-free systems, the absence of gluten necessitates alternative structural networks, primarily provided by protein-starch interactions. Proteins from legume flours are known to contribute to gel formation and gas cell stabilization, thereby improving loaf expansion. Similar findings have been reported in previous studies Waziiroh et al., (2021), where protein enrichment significantly enhanced the volume of gluten-free bread products.

Modeling and Analysis of Moisture Content Response

The response surface analysis for moisture content indicated that a quadratic model best described the relationship between flour composition and bread moisture content. The ANOVA results for moisture content are summarized in Table 4.

Table 4. ANOVA of the Quadratic Model for Moisture Bread

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	17.35	5	3.47	24.91	< 0.0001	significant
(1)Linear Mixture	12.14	2	6.07	43.57	< 0.0001	
AB	1.56	1	1.56	11.19	0.0074	
AC	0.0040	1	0.0040	0.0289	0.8684	
BC	0.0273	1	0.0273	0.1957	0.6676	
Residual	1.39	10	0.1394			
Lack of Fit	1.24	6	0.2070	5.46	0.0612	not significant
Pure Error	0.1517	4	0.0379			
Cor Total	18.75	15				
R^2	0.9257					
Adjusted R^2	0.8885					
Predicted R^2	0.8270					

The quadratic model was highly significant ($p<0.0001$), with an R^2 value of 0.9257, demonstrating a strong correlation between predicted and experimental values. The adjusted R^2 (0.8885) and predicted R^2 (0.8270) values further confirmed the robustness and predictive capability of the model. The lack-of-fit test was not significant ($p = 0.0612$), indicating that the model adequately represented the experimental data. Significant interaction effects were observed between soybean flour and sorghum flour (AB) ($p < 0.05$), whereas interactions involving red bean flour were not statistically significant. The final regression equation for moisture content is expressed as:

$$\text{Moisture content} = 54.63*A + 51.91*B + 48.83C - 6.78*A*B - 1.36*A*C + 4.28*B*C$$

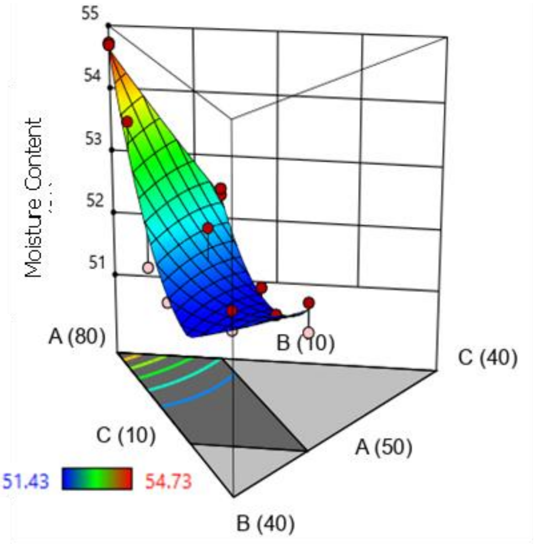

Figure 2. Three-dimensional response surface plot showing the combined effects of soybean flour (A), sorghum flour (B), and red bean flour (C) on the moisture content of gluten-free bread.

The response surface plot (Figure 2) shows that moisture content increased with increasing proportions of soybean flour, while higher levels of sorghum and red bean flour resulted in a decrease in moisture content. This trend can be attributed to differences in water-binding capacity among the flours. Soybean flour, which is rich in protein, exhibits a higher water-holding capacity, contributing to increased moisture retention in the bread matrix (Sutrisno et al., 2025). Conversely, sorghum flour has been reported to reduce moisture content due to its lower protein content and limited ability to bind water effectively. Previous studies (Filipini et al., 2021; Lazo-Vélez et al., 2015; Ribotta et al., 2004) have also demonstrated that gluten-free breads formulated with soybean flour tend to exhibit lower moisture loss during baking compared to those based on starch-dominant flours.

Modeling and Response Analysis of Fat Content

The fat content of gluten-free bread was best described by the special cubic model, as indicated by the ANOVA results presented in Table 5. The model demonstrated excellent predictive performance with an R² value of 0.9711, indicating that more than 97% of the variability in fat content could be explained by the mixture components. The adjusted R² (0.9518) and predicted R² (0.9160) values further support the suitability of the model. The lack-of-fit test was not significant ($p = 0.0509$), confirming model adequacy.

Table 5. ANOVA of the Quadratic Model for Fat Bread

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	118.85	6	19.81	50.40	< 0.0001	significant
(1)Linear Mixture	112.48	2	56.24	143.09	< 0.0001	
AB	0.9010	1	0.9010	2.29	0.1643	
AC	0.8408	1	0.8408	2.14	0.1776	
BC	0.3644	1	0.3644	0.9272	0.3607	
ABC	3.33	1	3.33	8.46	0.0173	
Residual	3.54	9	0.3930			
Lack of Fit	3.13	5	0.6265	6.19	0.0509	not significant
Pure Error	0.4050	4	0.1012			
Cor Total	122.38	15				
R²	0.9711					
Adjusted R²	0.9518					
Predicted R²	0.9160					

A significant three-component interaction (ABC) was observed ($p < 0.05$), highlighting the combined influence of soybean flour, sorghum flour, and red bean flour on fat content. The final regression equation for fat content is expressed as:

$$\mathrm{Yfat} = 12.04{*}A + 3.57{*}B - 6.44C + 7.14{*}A{*}B + 24.62{*}A{*}C + 20.72{*}B{*}C - 60.81{*}A{*}B{*}C$$

The response surface plot (Figure 3) indicates that fat content increased substantially with increasing soybean flour proportion, while higher proportions of sorghum and red bean flour reduced fat content. This trend is consistent with the inherent lipid composition of soybean flour, which contains a relatively high fat content compared to sorghum and red bean flours. Previous studies have reported similar findings, showing that soybean flour incorporation significantly enhances lipid content and improves the fatty acid profile of gluten-free bread (Filipini et al., 2021; Giaretta et al., 2018; Taghdir et al., 2017). Therefore, the high fat content observed in this study is primarily attributed to the contribution of soybean flour within the mixture.

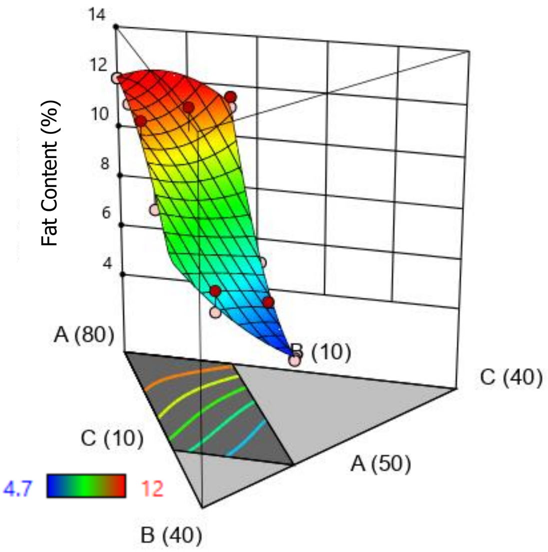

Figure 3. Three-dimensional response surface plot showing the combined effects of soybean flour (A), sorghum flour (B), and red bean flour (C) on the fat content of gluten-free bread

Protein Content Response Modeling and Analysis

The results of the statistical analysis indicated that the quadratic model was the most appropriate model to describe the effect of formulation on the protein content of gluten-free bread. The ANOVA results for protein content are presented in Table 6.

Table 6. ANOVA of the Quadratic Model for Protein Bread

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	198.81	6	33.13	24.77	< 0.0001	significant
(1)Linear Mixture	185.10	2	92.55	69.20	< 0.0001	
AB	0.0669	1	0.0669	0.0501	0.8280	
AC	5.77	1	5.77	4.31	0.0676	
BC	2.37	1	2.37	1.78	0.2155	
ABC	0.0008	1	0.0008	0.0006	0.9809	
Residual	12.04	9	1.34			
Lack of Fit	10.22	5	2.04	4.50	0.0850	not significant
Pure Error	1.82	4	0.4540			
Cor Total	210.84	15				
R^2	0.9429					
Adjusted R^2	0.9049					
Predicted R^2	0.8281					

The quadratic model demonstrated a high coefficient of determination ($R^2 = 0.9429$), indicating a strong agreement between experimental values and model predictions. Model selection based on the sequential sum of squares confirmed that the quadratic model was statistically significant ($p < 0.05$). In addition, the lack-of-fit test was not significant ($p = 0.0850$), suggesting that the model adequately described the experimental data.

The interaction terms between soybean flour and sorghum flour (AB), soybean flour and red bean flour (AC), as well as sorghum flour and red bean flour (BC), showed varying levels of influence on protein content. The final regression equation describing protein content was expressed as:

$$\text{Yprotein} = 23.82*A + 15.03*B - 26.08C - 1.95*A*B + 64.48*A*C + 52.88*B*C - 0.9488*A*B*C$$

The positive coefficients indicate a synergistic effect among formulation components on protein content, whereas negative coefficients suggest antagonistic interactions. These results demonstrate that soybean flour played a dominant role in increasing the protein content of gluten-free bread.

Protein content ranged from 13.32% to 24.34%, depending on the formulation. As shown in the response surface plot (Figure 4), protein content increased with higher proportions of soybean flour, while increasing levels of sorghum and red bean flour tended to reduce protein levels. This trend can be attributed to the high protein concentration of soybean flour compared to sorghum and red bean flours. Although red kidney bean flour contained a relatively high protein content (35.46%), the negative coefficient observed in the mixture model should not be interpreted as indicating that red bean flour reduces bread protein content. In mixture designs, the coefficients represent substitution effects among components under the constraint that the total formulation remains constant (A + B + C = 100%). Therefore, the negative coefficient reflects the replacement of soybean flour, which had the highest protein content, with red kidney bean flour, resulting in a lower overall protein contribution to the bread formulation.

These findings are consistent with previous studies reporting that the incorporation of soybean flour significantly enhances the protein content of gluten-free bakery products (Filipini et al., 2021; Taghdir et al., 2017). The high protein contribution of soybean flour improves both the nutritional value and the structural properties of gluten-free bread.

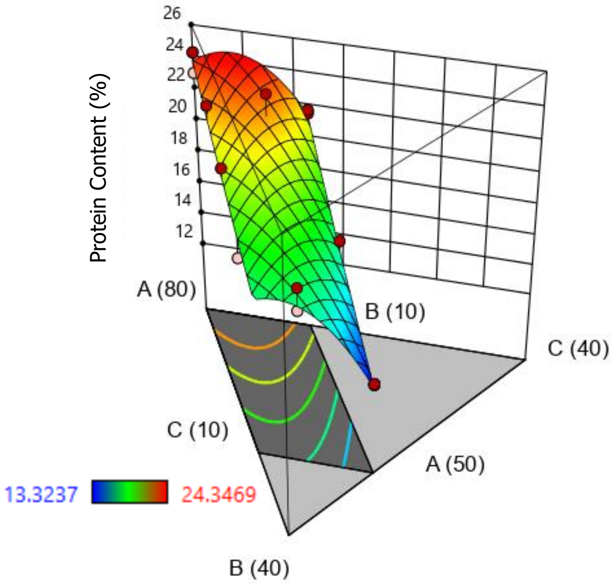

Figure 4. Three-dimensional response surface plot showing the combined effects of soybean flour (A), sorghum flour (B), and red bean flour (C) on the protein content of gluten-free bread

Carbohydrate Content Response Modeling and Analysis

Statistical analysis of carbohydrate content revealed that a cubic model provided the best fit for the experimental data. The ANOVA results for carbohydrate content are summarized in Table 7. The cubic model exhibited an exceptionally high coefficient of determination ($R^2 = 0.9940$), indicating excellent predictive accuracy. The model was statistically significant ($p < 0.05$), and the lack-of-fit test was not significant ($p = 0.0533$), confirming the suitability of the model. The final regression equation describing carbohydrate content was as follows:

$$Y\ \text{carbohydrate} = 4.95*A + 4.95*B - 26.08*C - 260.49*C + 41.64*A*B + 508.43*A*C + 586.90*B*C - 679.03*A*B*C - 57.75*A*B*(A-B) - 264.07*A*C*(A-C) - 350.03*B*C*(B-C)$$

Table 7. ANOVA of the Cubic Model for Carbohydrate Bread

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	855.12	9	95.01	110.69	< 0.0001	significant
(1)Linear Mixture	818.13	2	409.06	476.55	< 0.0001	
AB	0.2620	1	0.2620	0.3052	0.6006	
AC	0.0935	1	0.0935	0.1089	0.7526	
BC	0.1357	1	0.1357	0.1581	0.7047	
ABC	0.1828	1	0.1828	0.2130	0.6607	
AB(A-B)	1.36	1	1.36	1.58	0.2554	
AC(A-C)	0.1000	1	0.1000	0.1165	0.7445	
BC(B-C)	0.1317	1	0.1317	0.1534	0.7088	
Residual	5.15	6	0.8584			
Lack of Fit	3.96	2	1.98	6.66	0.0533	not significant
Pure Error	1.19	4	0.2973			
Cor Total	860.27	15				
R^2	0.9940					
Adjusted R^2	0.9850					
Predicted R^2	-1.5325					

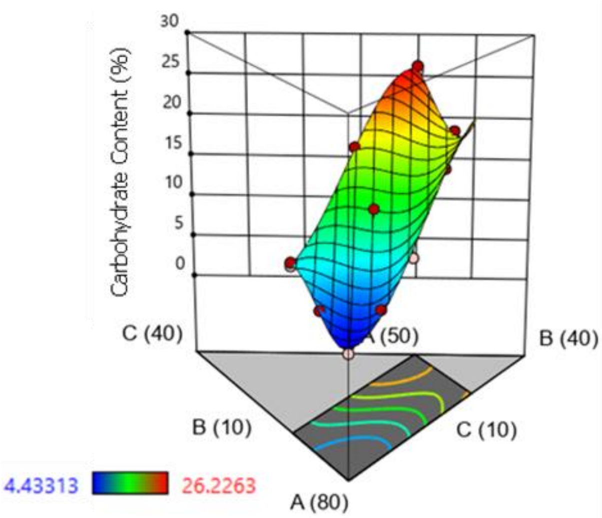

Figure 5. Three-dimensional response surface plot showing the combined effects of soybean flour (A), sorghum flour (B), and red bean flour (C) on the content carbohydrate of gluten-free bread

Carbohydrate content ranged from 4.43% to 26.22%, depending on the formulation. Response surface analysis (Figure 5) showed that carbohydrate content increased with higher proportions of sorghum and red bean flours, while increasing soybean flour resulted in a reduction in carbohydrate content. This behavior is closely related to the intrinsic composition of the raw materials, as soybean flour contains substantially lower carbohydrate levels compared to sorghum and red bean flours. Consequently, substituting soybean flour in higher proportions led to a decrease in total carbohydrate content of the bread. Similar trends have been reported by Filipini et al., (2021), who observed a significant reduction in carbohydrate content when rice flour was partially replaced with soybean flour in gluten-free bread formulations. Although the cubic model provided a high R^2 value, the negative predicted R^2 indicates limited predictive reliability. Therefore, interpretation of carbohydrate response was focused on observed experimental trends rather than model extrapolation.

Ash Content Response Modeling and Analysis

Analysis of ash content indicated that a linear model was sufficient to describe the effect of formulation on ash content. The linear model exhibited an R^2 value of 0.6715, indicating a moderate correlation between experimental and predicted values. Model significance was confirmed by the sequential sum of squares analysis ($p < 0.05$), while the lack-of-fit test was not significant ($p = 0.8118$), demonstrating adequate model performance.

Table 8. ANOVA of the Linear Model for Ash Bread

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	0.5704	2	0.2852	13.29	0.0007	significant
(1)Linear Mixture	0.5704	2	0.2852	13.29	0.0007	
Residual	0.2791	13	0.0215			
Lack of Fit	0.1499	9	0.0167	0.5159	0.8118	not significant
Pure Error	0.1292	4	0.0323			
Cor Total	0.8495	15				
R^2	0.6715					
Adjusted R^2	0.6209					
Predicted R^2	0.4723					

The final regression equation for ash content was expressed as:

$$YAsh = 4.69*A + 3.98*B - 4.63*C$$

The results indicated that ash content was not significantly influenced by the formulation ratios of soybean flour, sorghum flour, and red bean flour. Ash content values ranged from 4.05% to 4.87%, with only minor variations among formulations. Ash content represents the inorganic residue remaining after combustion and is commonly associated with mineral content. The relatively stable ash values observed in this study suggest that variations in flour composition did not substantially alter the mineral profile of the final product. Comparable results were reported by (Taghdir et al., 2017), who observed lower ash content in gluten-free bread enriched with alternative protein sources. The slightly higher ash content observed in the present study may be attributed to the mineral-rich nature of legume-based flours.

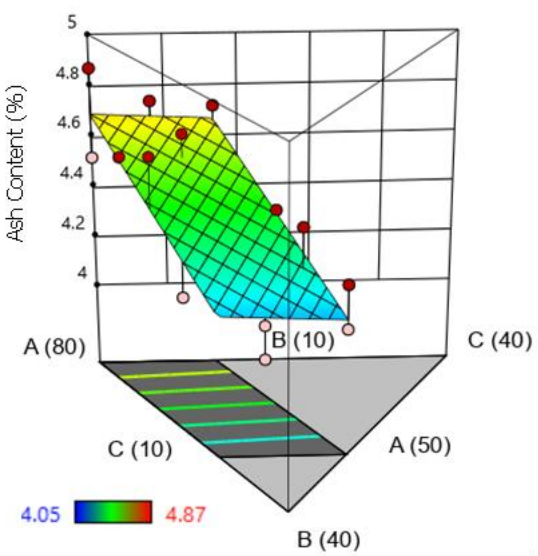

Figure 6. Three-dimensional response surface plot showing the combined effects of soybean flour (A), sorghum flour (B), and red bean flour (C) on the content Ash of gluten-free bread

Optimization of Gluten-Free Bread Formulation Based on Soybean, Sorghum, and Red Bean Flours

The optimization process was conducted to determine the most suitable combination of soybean flour, sorghum flour, and red bean flour that would yield optimal physicochemical and nutritional characteristics of gluten-free bread produced using ohmic heating. The optimization criteria and target responses are presented in Table 9. Soybean flour, sorghum flour, and red bean flour were defined as independent variables within predetermined ranges, while specific volume, moisture content, fat content, protein content, carbohydrate content, and ash content were selected as response variables. The optimization objective for all response variables was set to maximize their values within the acceptable experimental limits.

Statistical analysis using Design-Expert software generated several possible formulation solutions with varying desirability values. The optimal formulation was selected based on the highest overall desirability value, indicating the closest approximation to the ideal response conditions

The predicted optimal formulation consisted of 71.39% soybean flour, 10.00% sorghum flour, and 18.61% red bean flour. To validate the reliability of the optimization model, experimental verification was performed under the predicted optimal conditions. The comparison between predicted and experimental values is summarized in Table 10.

Table 9. Optimization Criteria and Constraint Limits

Criteria	**Target**	**Lower Limit**	**Upper Limit**
Soybean flour	In range	50	80
Sorghum flour	In range	10	30
Red bean flour	In range	10	20
Specific volume	Maximize	2.17	2.78
Moisture content	Minimize	51.43	54.73
Fat content	Maximize	4.70	12.00
Protein content	Maximize	13.32	24.35
Carbohydrate content	Maximize	4.43	26.23
Ash content	Maximize	4.16	4.87

Table 10. Optimal Solution and Verification Result

	Variabels			**Respon**					
	Soybean flour	**Sorghum flour**	**Red bean flour**	**Volume spesific**	**Moisture content**	**Fat content**	**Protein content**	**Carbohydrate content**	**Ash content**
Optimal Solution	71.39	10.00	18.61	2.78	52.68	11.77	22.69	9.78	4.67
Verification				2.79 ±0.07	52.66 ±0.06	11.75 ±0.06	22.68 ±0.05	9.79 ±0.09	4.66 ±0.08
p-value				0.286	0.288	0.308	0.298	0.278	0.282

Note: Values are expressed as mean ± standard deviation (n = 3). No significant differences were observed between predicted and experimental values (p >0.05).

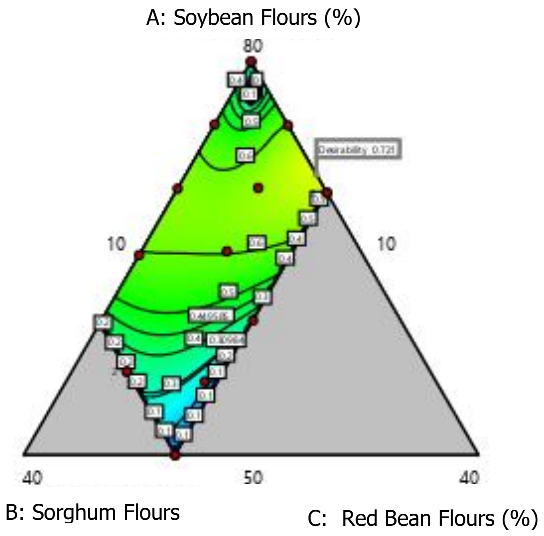

Figure 7. Contour plot of desirability for the optimized gluten-free bread formulation

The experimental verification results showed a specific volume of 2.79 ± 0.07 cm^3/g, moisture content of 52.66 ± 0.06%, fat content of 11.75 ± 0.06%, protein content of 22.68 ± 0.05%, carbohydrate content of 9.79 ± 0.09%, and ash content of 4.66 ± 0.08%. Statistical validation using a T-test (Minitab 17) revealed no significant differences ($p > 0.05$) between the predicted and experimental values for all response variables. These results confirm the adequacy and reliability of the response surface models developed in this study and demonstrate that the optimized mixture design is suitable for producing gluten-free bread with improved structural and nutritional properties. The high desirability value obtained indicates that the optimization objectives were successfully achieved and that the selected formulation provides a balanced contribution of protein and starch sources essential for gluten-free bread quality.

CONCLUSION

An optimal formulation of gluten-free bread was successfully obtained using a mixture design approach, consisting of 71.39% soybean flour, 10.00% sorghum flour, and 18.61% red bean flour. The optimized formulation produced bread with a specific volume of 2.79 ± 0.07 cm^3/g, moisture content of 52.66 ± 0.06%, fat content of 11.75 ± 0.06%, protein content of 22.68 ± 0.05%, carbohydrate content of 9.79 ± 0.09%, and ash content of 4.66 ± 0.08%. The desirability value of 0.721 indicates that the optimization process performed effectively in achieving the targeted physicochemical and nutritional responses. Verification analysis confirmed that there were no significant differences between predicted and experimental values ($p>0.05$), demonstrating the adequacy and reliability of the developed response surface models. Overall, the results confirm that the optimized combination of soybean flour, sorghum flour, and red bean flour is suitable for the production of gluten-free bread using ohmic heating technology, providing improved specific volume and enhanced nutritional quality.

DECLARATION

Authors Contributions

All authors contributed equally as primary authors to this manuscript. All authors have read and approved the final version of the article.

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Conflict of Interest

The authors declare that they have no financial interests or competing personal relationships that could influence the content or results of this research.

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