DEVELOPMENT AND OPTIMIZATION OF LOW GLUTEN BREAD USING MULTI GRAIN FLOUR BLEND WITH FLAXSEED POWDER USING RESPONSE SURFACE METHODOLOGY

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Abstract

There is a rising prevalence of gluten-related issues among consumers that drives the need for nutritious altered-gluten products. Often, gluten-free alternatives are made with refined starches that are low in vital nutrients and negatively affect the product's texture. This study aimed to create a reduced-gluten bread from a multi-grain blend of wheat, corn, black rice, and brown rice, as well as flaxseed powder to improve the bread's texture and nutrition. Moreover, the flaxseed fortified the dough with omega-3s, Fiber and protein which made the dough more elastic. Further, xanthan gum helped to improve the dough stability and the overall crumb structure of the enhanced multi-grain bread.The Response Surface Methodology (RSM) conducted optimization on the levels of flaxseed (0-10% of flour weight) and xanthan gum (0-2%) with the parameters of loaf volume, density, moisture loss, and sensory evaluation. A Central Composite Design (CCD) was used to look in depth at the interactions among the variables. It was possible to observe

from the output that flaxseed enhanced the protein, fiber and healthy fats proportion while softening crumb texture. Improvement of the bread's elasticity and crumbliness, as well as the retention of the loaf volume was xanthan gum's contributions.The mouthfeel, aroma, and taste of the bread were maintained and the taste was even reduced by 40%, compared to the standard wheat bread. The sensory tests conducted confirmed the consumers acceptance of the product, thus the bread is viable as a reduced-gluten alternative.

Keywords: Reduced-gluten bread, multi-grain flour blend, Response Surface Methodology (RSM), gluten intolerance, bread optimization.

1. Introduction

Bread is a staple food enjoyed around the world, and it's mostly made from wheat flour. This is because wheat contains proteins like gliadin and glutenin, which give dough its elasticity and structure (Shewry & Hey, 2016). Recently, there's been a noticeable increase in the demand for gluten-free options, largely due to the rise in gluten-related health issues such as celiac disease, non-celiac gluten sensitivity, and wheat allergies. Gluten, a protein found in wheat, barley, and rye, is crucial for the texture and structure of bread, helping it rise and maintain its shape during fermentation. Unfortunately, for those with gluten sensitivities, eating gluten can lead to serious health problems, including digestive issues, nutrient malabsorption, and autoimmune reactions. This has created a growing need for reduced-gluten bread that meets these dietary needs while still tasting good and providing the nutrition of traditional bread (Singh et al., 2022).

Typically, reduced-gluten breads use starches and refined flours, which often lack important nutrients and can result in products that don't taste as good or have a shorter shelf life. To overcome these challenges, researchers are looking into alternative flours and functional ingredients that can boost the nutritional value and sensory qualities of reduced-gluten bread. Some promising options include corn flour, black rice flour, brown rice flour, and flaxseed powder, all of which have unique nutritional benefits (Masure et al., 2019). Corn flour, made from maize, is packed with dietary fiber, vitamins, and antioxidants, making it a great choice for enhancing the nutritional quality of bread (Giuberti et al., 2022).

Black rice flour is notable for its high anthocyanin content, which gives it antioxidant properties and a unique color, while brown rice flour provides extra fiber, vitamins, and minerals compared to more refined options. Flaxseed powder is also a fantastic addition, as it's rich in omega-3 fatty acids, lignans, and dietary fiber, has been shown to improve heart health, reduce inflammation, and enhance the texture of baked goods. By mixing these ingredients with wheat flour, we can whip up a reduced-gluten bread that not only caters to dietary needs but also packs a nutritional punch (Marpalle et al., 2022).

However, creating reduced-gluten bread using a blend of different flours comes with its own set of hurdles. Without enough gluten, the dough can lose its elasticity, affecting the crumb structure and loaf volume, which often results in a denser, crumbly product. Plus, adding alternative flours and other functional ingredients can change the flavor, texture, and overall appeal of the bread. To tackle these issues, optimization techniques like Response Surface Methodology (RSM) have become popular in food product development. RSM is a statistical and mathematical approach that helps researchers explore how various factors interact, allowing them to pinpoint the best ingredient ratios and processing conditions (Manley et al., 2020).

This research aims to develop and fine-tune a reduced-gluten bread recipe using RSM, focusing on the impact of blends of wheat, corn, black rice, and brown rice flours, along with the addition of flaxseed powder. By harnessing the nutritional advantages of these ingredients and utilizing RSM for optimization, the goal is to create a reduced-gluten

bread that satisfies the dietary requirements of gluten-sensitive individuals while offering excellent sensory and nutritional qualities. The results of this study could significantly enhance our understanding of functional food development and provide a practical solution for improving the quality of reduced-gluten bread products.

4. Objective

- To Develop a low-gluten bread formulation using black-brown rice flour, wheat flour, corn starch and flax seed powder.
- To Optimize the effect of flax seed powder and total flour in low gluten bread using Response Surface Methodology (RSM)
- To Analyze the sensory characteristics, nutritional composition and shelf life study of the optimized bread
- To Analyze the cost Economics

2. Materials and methodology

2.1 Materials required

2.1.1 Wheat Flour

Wheat flour, which is the main source of gluten, was used in smaller amounts to decrease the overall gluten content while keeping some structural integrity. Gluten, a protein complex present in wheat, plays a crucial role in giving dough its elasticity and ability to hold gas, which ultimately affects the texture and rise of bread. By cutting back on the amount of wheat flour, this study aimed to develop a bread recipe that would be suitable for those with gluten sensitivity, all while preserving some of gluten's functional benefits.

2.1.2 Black Rice Flour

Black rice flour, a key ingredient in this study, is rich in fiber, antioxidants (especially anthocyanins), and essential micronutrients. Being gluten-free, it is ideal for those with gluten intolerance or celiac disease. Its slightly sweet, nutty flavor and striking dark purple color enhance both taste and visual appeal. Black rice flour offers health benefits, including reduced inflammation, heart health support, and protection against oxidative stress due to its high antioxidant content. Though less common than white or brown rice flour, it is available in specialty stores and should be stored in an airtight container in a cool, dark place to preserve freshness.

2.1.3 Brown Rice Flour

Brown rice has a light to medium brown color due to its bran layer, germ, and endosperm. The grains should appear uniform with minimal damage. It has a firm, chewy texture neither too hard nor too soft when properly cooked. Nutritionally, it provides complex carbohydrates, fibre, B vitamins, magnesium, selenium, and antioxidants like phenolic compounds. These nutrients support various health benefits, including improved digestion and heart health. To maintain freshness, store brown rice in a cool, dry place away from sunlight and moisture to prevent spoilage.

2.1.4 Flax Seed Powder

Incorporating flaxseed powder into gluten-free black-brown rice flour bread enhances its nutritional profile and texture. Flaxseed powder adds a rich source of omega-3 fatty acids, dietary fiber, and protein, increasing the bread's nutritional value. The fiber content improves digestive health, while omega-3 fatty acids support heart health. Flaxseed's gel-like properties help replace gluten, enhancing texture and structure.

2.1.5 Xantham Gum

Xanthan gum replaces gluten in rice bread, improving dough structure, texture, and rise. It traps air bubbles for better leavening, prevents crumbling, and enhances moisture retention keeping bread softer longer. This extends shelf life, crucial for gluten-free bread that stales quickly. It also supports gas retention during fermentation, ensuring an even rise and preventing collapse. In this study, xanthan gum levels ranged from 2% to 5%, as exceeding 15% can be harmful

2.2 methodology

2.2.1 ingredient selection

- Wheat flour, Black-brown rice flour, and Corn flour were selected with a proportion of 30%, 25%,25% and Flax seed powder with 2-10%
- Select active dry yeast or instant yeast, here it used active dry yeast.
- Additional ingredients like salt, warm water, oil/ fat (sunflower), and Powdered sugar as sweeteners were used.

2.2.2 yeast preparation

Here, Active dry yeast was used so it needs to be dissolved in water before use. It serves as a leavening agent and contributes to texture and flavor development. Yeast grows in warm water(20℃-40℃), So 4g gets mixed with warm water and a pinch of icing sugar as feed it becomes activated within 10-15 minutes which produces carbon dioxide gas that seems foamy bubbling form.

2.2.3 preparation flow chart

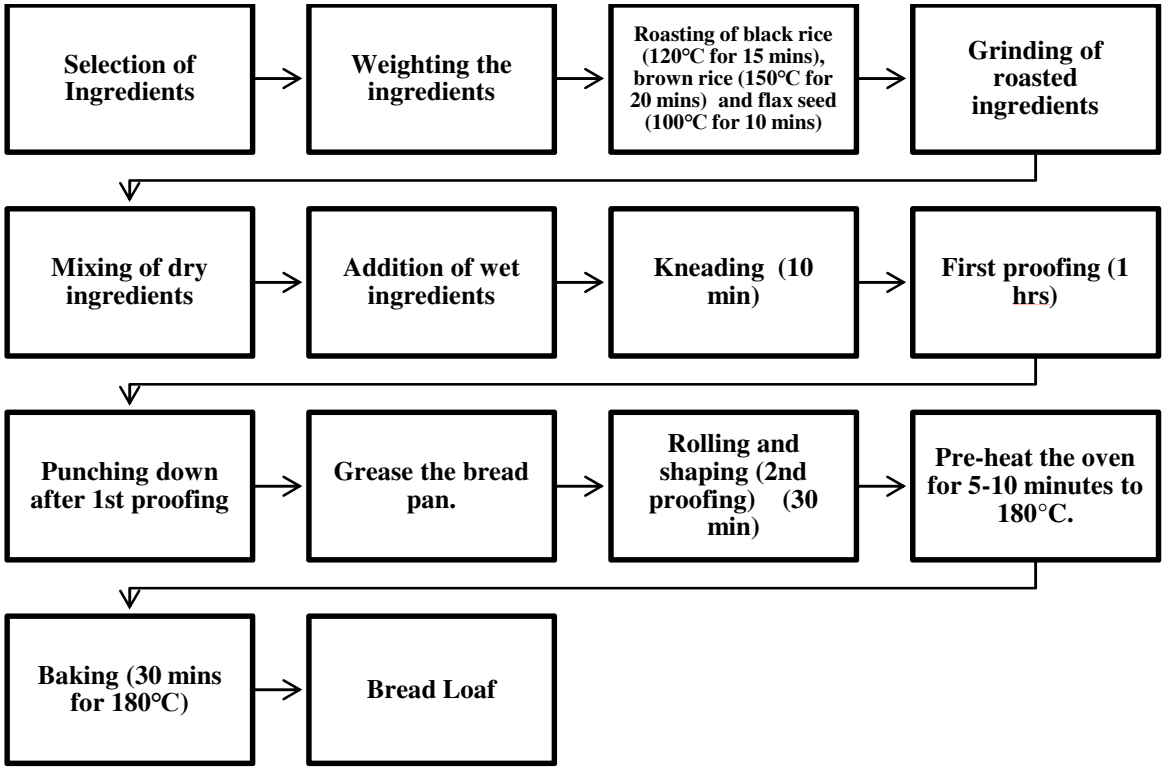


Fig no. 3.7 Flowchart for Low Gluten Bread Making

2.3Analytical methods

2.3.1 Nutritional Analysis.

The chemical characterization of the reduced-gluten bread encompassed comprehensive evaluation of its nutritional profile through standardized analytical procedures. pH measurement was conducted on a 10% (w/v) aqueous suspension using a calibrated pH meter, providing insight into the product's acidity and potential shelf-life stability. Protein content was determined following the SSFTL/CH/SOP/015:2023 protocol, which employs the Kjeldahl nitrogen analysis method with appropriate conversion factors for cereal-based matrices. Total dietary fiber quantification utilized enzymatic digestion (amylase, protease, and amyloglucosidase treatment) followed by gravimetric analysis to differentiate soluble and insoluble fiber fractions. Gluten content

assessment implemented an ELISA-based immunoassay with sensitivity below 5 ppm, verifying compliance with international low-gluten standards (<100 ppm). Mineral analysis (zinc, iron, magnesium, and phosphorus) was performed using inductively coupled plasma optical emission spectrometry (ICP-OES) after microwave-assisted acid digestion, while vitamin E (α-tocopherol) quantification employed reverse-phase high-performance liquid chromatography (HPLC) with fluorescence detection. All analyses were conducted in triplicate with appropriate method blanks and certified reference materials to ensure analytical precision, with results reported on both fresh weight and dry matter basis to account for moisture variability.

2.3.1.1 Moisture loss:

Moisture loss is a key parameter to assess the impact of multi-grain flour blends and flaxseed powder incorporation on the baking performance of low-gluten bread.

$$Moisture\ loss = W_1 - W_2 / W_1$$

Were,

W_1 : Weight of dough before baking

W_2: Weight of product after baking

2.3.2 Physical properties

2.3.2.1 **Loaf volume**:

It is a critical parameter in evaluating the quality and characteristics of baked bread, often used in both commercial and research settings to assess the effectiveness of ingredients, formulations, and baking processes.The volume of a loaf can be calculated using the formula:

$$Loaf\ Volume = Loaf\ Length \times Loaf\ Width \times Loaf\ Height.$$

This method provides a straightforward and reproducible way to quantify the expansion and structural development of the bread during baking. Accurate measurement of these dimensions ensures consistency in quality control and allows for comparative analysis across different batches or experimental conditions.

2.3.2.2 Density:

Determined by the ratio of the weight of the bread loaf to its volume (g/cm^3). In the context of the study density serves as a key indicator of the structural integrity and porosity of the bread. Lower density values typically correlate with a more open crumb structure and softer texture, which are desirable attributes in low-gluten bread formulations.

By optimizing the multi-grain flour blend and flaxseed powder incorporation, the study aims to achieve an ideal balance between nutritional enhancement and sensory properties, ensuring the final product maintains an acceptable density while meeting low-gluten dietary requirements. This parameter is crucial for understanding the impact of ingredient variations and processing conditions on the overall bread quality.

Density = Weight of bread loaf / vol of bread loaf

2.3.3 Sensory evaluation

Sensory analysis of lower gluten bread was conducted using a 9-point hedonic scale to evaluate consumer acceptability and preference for attributes such as appearance, aroma, texture, taste, and overall liking. The sensory evaluation was a critical step in assessing the impact of multi-grain flour blends and flaxseed powder incorporation on the sensory profile of the final product. Panelists rated each attribute on a scale ranging from 1 (dislike extremely) to 9 (like extremely), providing quantitative data to identify the optimal formulation that balances nutritional enhancement with desirable sensory properties. The results of the sensory analysis were used to refine the formulation, ensuring the low-gluten bread met consumer expectations for taste, texture, and overall quality.

2.4 Optimize the effect of flax seed powder and Total flour in lower gluten Bread Using Response Surface Methodology

Optimize the formulation of a bread product to achieve the desired loaf volume, density, and moisture loss. Response Surface Methodology (RSM) was employed to

investigate the effects of three key ingredients: flaxseed powder, total rice flour, and xanthan gum, on these responses. A Central Composite Design (CCD) was utilized to systematically vary the levels of these factors. The CCD was chosen due to its ability to efficiently estimate both linear and quadratic terms in the model, allowing for a comprehensive understanding of the factor interactions and curvature in the response surface. The Design-Expert software was used for experimental design and data analysis. The findings of this study will provide valuable insights into the optimal formulation for achieving the desired bread quality.

2.4.1 Experimental Design

1. Selection of Factors: Two independent variables (factors) were selected for optimization:

- **Flaxseed powder (X1)**: Varied from 0% to 10% of the total flour weight.
- **Xanthan gum (X2)**: Varied from 0% to 2% of the total flour weight.

2. Response variables: The following dependent variables (responses) were measured:

- **Loaf volume (LV)**: Measured using the seed displacement method.
- **Density (D)**: Calculated as the ratio of bread weight to volume.
- **Moisture loss**: Determined by measuring the weight loss before and after baking.

3. **Central composite design (ccd)**: A CCD was used to design the experiments. CCD is an efficient design for studying quadratic responses and interactions between factors.

2.4.2 Data Collection

1. **Bread preparation**: The bread was prepared according to the methodology outlined in Section 3, using the factor levels specified in the CCD.

2. **Measurement of Responses**: For each experimental run, the following responses were measured:
    - **Loaf volume**: Measured using the seed displacement method.
    - **Density**: Calculated as the ratio of bread weight to volume.
    - **Moisture loss**: Determined by measuring the weight loss before and after baking.

3.4.3 statistical analysis

Statistical Analysis of Variance (ANOVA), The lack-of-fit test (LOF), Sequential Mean Sum of Squares (SMSS), and multiple regressions of the RSM were performed using Design-Expert version 13 software.

3.4.4 Analysis of Variance (ANOVA):

1. ANOVA was performed to determine the significance of the factors and their interactions.
2. A p-value < 0.05 was considered statistically significant.
3. The goodness of fit was evaluated using R^2 (coefficient of determination) and Adjusted R^2 values. A value closer to 1 indicates a better fit.

Name	Units	Low	High
Total flour	gm	30	75
Xanthum gum	gm	4	13
Flax seed powder	gm	2	10

Table 3.1 Actual levels of factors

A Central composite design (CCD) with 20 trials was used to develop a quadratic model for each response. The trials are shown in Table 3.2;

RUN	Factor 1 A- Flaxseed Powder	Factor 2 B- Total Rice Flour	Factor 3 C- Xanthan Gum	Response 1 Loaf Volume(LV)	Response 2 Density(D)	Response 3 Moisture Loss(ML)
1	6	52.5	8.5	86.25	1.44	0.074
2	6	52.5	0.931932	72	1.56	0.28
3	10	30	13	74.46	1.26	0.35
4	10	75	13	75	2.42	0.205
5	2	30	4	50	1	0.375
6	6	52.5	8.5	86.25	1.44	0.074
7	6	52.5	8.5	86.25	1.44	0.074
8	2	75	4	94.5	1.67	0.15
9	6	14.6597	8.5	49.59	1.2	0.35
10	5	25	4	86.25	0.18	0.17
11	12.7272	52.5	8.5	88.25	2.44	0.174
12	10	75	4	75	1.42	0.105
13	6	52.5	8.5	86.25	1.44	0.074
14	6	52.5	8.5	86.25	1.44	0.074
15	6	52.5	8.5	86.25	1.44	0.074
16	2	30	13	42	1.8	0.17
17	6	52.5	8.5	86.25	1.44	0.074

18	10	30	4	40	2.5	0.25
19	6	52.5	8.5	86.25	1.44	0.074
20	6	52.5	8.5	86.25	1.44	0.074

Table 3.2 Trials

Pictorial presentation results of bread in different 13 trials are shown as;

Trial – 1

Trial – 2

Trial – 3

Trial – 4

Trial – 5

Trial – 6

Trial – 7

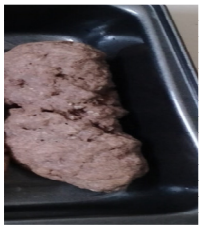
Trial – 8

Trial – 9

Trial – 10

Trial – 11

Trial – 12



Trial – 13

Fig no. 3.8 Trials

4. Result and discussion

4.1 Optimization of Lower gluten bread formulation

The ANOVA tables from the Response Surface Methodology (RSM) analysis provide critical insights into the optimization of a lower-gluten bread formulation, focusing on three key responses: Leaf Volume (LV), Density (D), and Moisture Loss (ML). Each model's significance and the individual contributions of factors such as Flaxseed Powder (A), Total Rice Flour (B), and Xanthan Gum (C) are evaluated.

4.1.1 response 1: loaf volume (lv)

Source	SumofSquares	df	Mean Square	F-value	p-value
Model	4157.68	9	461.96	3.75	0.0255 (significant)
A-Flaxseed Powder	167.16	1	187.16	1.36	0.2709
B-Total Rice Flour	209.53	1	209.53	1.70	0.212
C-Xanthan Gum	241.21	1	241.21	1.96	0.1918
AB	0.6722	1	0.6722	0.0055	0.9425
AC	741.40	1	741.40	6.02	0.0340
BC	175.32	1	175.32	1.42	0.2602
A^2	230.96	1	230.96	1.88	0.2007
B^2	365.83	1	365.83	2.97	0.1154
C^2	547.35	1	547.35	4.45	0.0611
Residual	1230.57	10	1230.56		
Lack of Fit	1230.57	2	615.29		
Pure Error	0.0000	8	0.0000		
Cor Total	5388.25	19			

Table: 4.1

The model shows some interesting results, with an F-value of 3.75 and a p-value of 0.0255. Notably, the interaction between Flaxseed Powder and Xanthan Gum (AC) stands out as significant, with a p-value of 0.0340. This suggests that these two factors together have a real impact on leaf volume. The R^2 value of 0.7716 tells us that the model accounts for about 77.16% of the variability in leaf volume. However, the negative Predicted R^2 of -48.8300 raises a red flag, indicating that the model might struggle to predict new observations accurately. This points to a need for improvement, perhaps by

exploring higher-order terms or incorporating additional factors.

Statistic	Value
Std. Dev.	11.09
R^2	0.7716
Mean	76.17
Adjusted R^2	0.5661
C.V. %	14.56
Predicted R^2	-48.8300
Adequate Precision	6.7065

Table: 4.2 Fit Statistics

4.1.2 response 2: density(d)

When we dive into the analysis of Density (D), we find that the model holds some weight, with an F-value of 4.01 and a p-value of 0.0206. Both Total Rice Flour (B) and Xanthan Gum (C) show significant individual effects, with p-values of 0.0124 and 0.0133, respectively. Plus, their interactions with Flaxseed Powder (AB and AC) are noteworthy too. The R^2 value of 0.7829 indicates a decent fit, but the negative Predicted R^2 of -36.8035 raises some red flags about the model's predictive accuracy.

Source	Sumof Squares	df	Mean Square	F-value	p-value
Model	3.93	9	0.459	4.01	0.0206 (significant)
A-Flaxseed Powder	0.0835	1	0.038	0.7596	0.4039
B-Total Rice Flour	1.01	1	1.01	9.26	0.0124
C-Xanthan	1.01	1	1.01	9.29	0.0133

Source	Sumof Squares	df	Mean Square	F-value	p-value
Gum					
AB	0.8141	1	0.8141	7.47	0.0211
AC	1.08	1	1.08	9.91	0.0104
BC	0.7451	1	0.7451	6.83	0.0259
A^2	1.15	1	1.15	10.54	0.0088
B^2	0.0050	1	0.0050	0.0463	0.8340
C^2	0.2702	1	0.2702	2.48	0.1465

Table:4.3

4.1.3 response 3: moisture loss

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Model	0.1908	9	0.0212	9.59	0.0008	significant
A-Flaxseed Powder	0.0047	1	0.0037	2.12	0.1793	
B-Total Rice Flour	0.0320	1	0.0320	14.48	0.0055	
C-Xanthan Gum	0.0096	1	0.0096	0.3972	0.5427	
AB	0.0007	1	0.0007	0.3211	0.5834	
AC	0.0208	1	0.0208	9.42	0.0118	
BC	0.0011	1	0.0011	0.5070	0.4927	
A^2	0.0037	1	0.0037	1.69	0.2225	
B^2	0.0083	1	0.0083	3.73	0.0822	
C^2	0.0219	1	0.0219	9.88	0.0105	

Table: 4.4

The ANOVA for Moisture Loss (ML) reveals a statistically significant quadratic model (p=0.0008, F=9.59), effectively explaining response variability. Key significant factors include: B-Total Rice Flour (p=0.0055), AC interaction (p=0.0118), and C^2 term

(p=0.0105). In contrast, A-Flaxseed Powder, C-Xanthan Gum, and most interactions (AB, BC) showed no significant effects (p>0.05). The negative Predicted R^2 (-20.7752) suggests potential model overfitting.

Metric	Value
Std. Dev.	0.0470
R^2	0.8961
Mean	0.1622
Adjusted R^2	0.8026
C.V.%	28.99
Predicted R^2	-20.7752
Adequate Precision	8.6534

Table: 4.5 Fit Statistics

4.1.4 3d surface plots

3D surface and contour plots are powerful tools for analysing complex data in food science. These visualizations clearly show how ingredient combinations (like flaxseed powder, rice flour, and xanthan gum) affect bread quality parameters (density, moisture loss) in gluten-reduced formulations. Using Response Surface Methodology (RSM), the plots reveal optimal ingredient ratios by displaying response variables across the experimental design space. For instance, 3D plots demonstrate how flaxseed and rice flour quantities influence bread density (at fixed xanthan gum levels) and moisture retention. The graphical and numerical optimization features help identify ideal formulations that minimize moisture loss while achieving target texture. These visualization techniques are indispensable for developing consistent, high-quality reduced-gluten bread products.

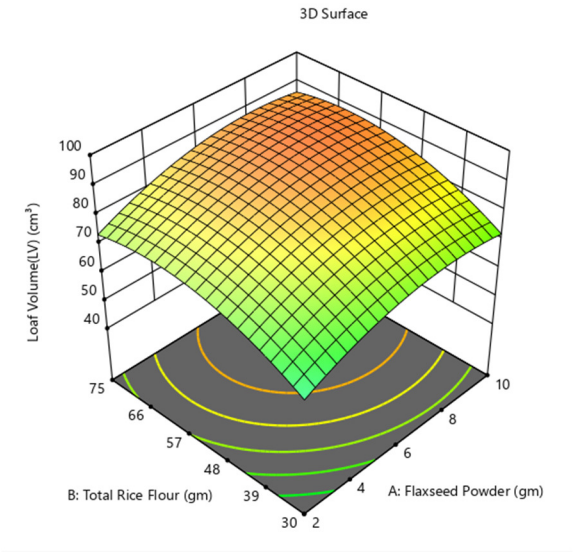
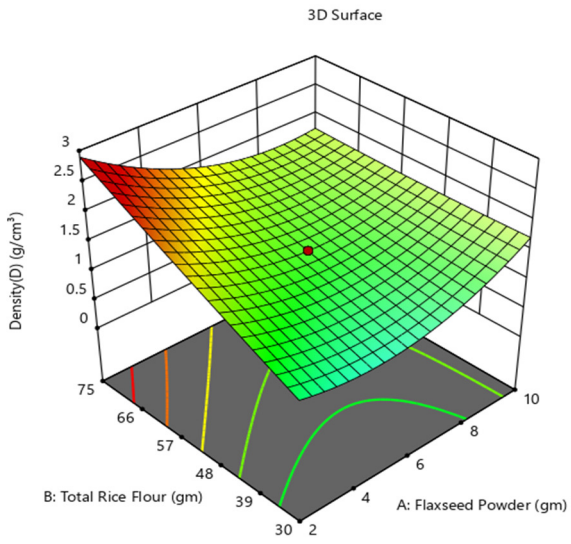


Fig: 4.1 Loaf Volume



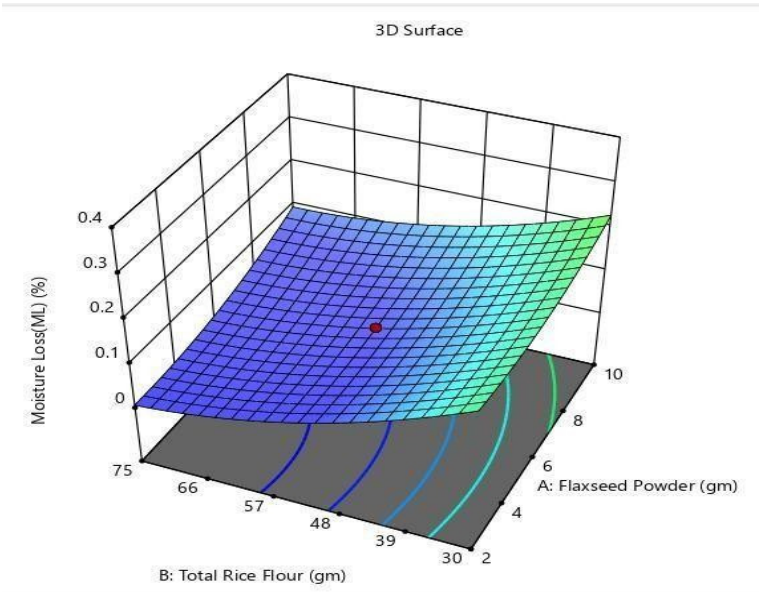


Fig: 4.3 Moisture Loss

4.2 Optimization of Chemical Analysis

Chemical analysis followed standard methods (e.g., SSFTL/CH/SOP/015:2023 for protein). Key results: pH 6.10 (acceptable range), 9.73% protein, <0.5% gluten (low-gluten compliant), and 0.45% dietary fiber. Nutrient levels included Vitamin E (2.60 mg/kg), iron (2.21 mg/100g), zinc (2.06 mg/100g), magnesium (79.7 mg/100g), and phosphorus (152 mg/100g). Fiber content may need enhancement for improved nutrition.

Sl. No.	PARAMETERS	TEST METHOD	UNIT	RESULT
1	pHof10%Solution	Inhouse Method	-	6.10
2	Protein	SSFTL/CH/SOP/015:2023	%	9.73
3	Dietary Fibre	Inhouse Method	%	0.45
4	Gluten	Inhouse Method	%	<0.5
5	Vitamin E	Inhouse Method	mg/kg	2.60
6	Iron	Inhouse Method	mg/100g	2.21
7	Zinc	Inhouse Method	mg/100g	2.06
8	Magnesium	Inhouse Method	mg/100g	79.7
9	Phosphorous	Inhouse Method	mg/100g	152

Table: 4.6

4.3 Optimization of Sensory Analysis

In the sensory evaluation of bread using a 9-point hedonic scale (1 = extremely dislike, 9 = extremely like), results from 13 different trials indicated that the 8th trial was

rated significantly higher compared to the others. Panelists consistently scored the 8th trial with higher acceptability, suggesting superior attributes in taste, texture, aroma, or overall liking.

TrialNo.	Appearance (Avg.)	Taste/Flavor (Avg.)	Mouthfeel (Avg.)	Aroma/Smell (Avg.)	Overall Acceptance (Avg.)
1	5.2	5.0	4.8	5.1	5.0
2	6.0	5.5	5.3	5.4	5.5
3	5.8	5.7	5.5	5.6	5.6
4	6.1	6.0	5.8	5.9	6.0
5	6.3	6.2	6.0	6.1	6.2
6	6.5	6.4	6.2	6.3	6.3
7	6.7	6.5	6.3	6.4	6.5
8	7.8	7.9	7.7	7.8	7.8
9	6.6	6.5	6.4	6.5	6.5
10	6.4	6.3	6.1	6.2	6.2
11	6.2	6.1	5.9	6.0	6.0
12	6.0	5.8	5.7	5.8	5.8

TrialNo.	Appearance (Avg.)	Taste/Flavor (Avg.)	Mouthfeel (Avg.)	Aroma/Smell (Avg.)	Overall Acceptance (Avg.)
13	5.9	5.7	5.6	5.7	5.7

Table: 4.7

4.4 Optimization of Microbial and Shelf-life Analysis

Microbial analysis tested for total plate count, *E. coli*, yeast/mould, *Salmonella*, and *S. aureus* using standard methods (IS 5402:2012, IS 5887:1976, IS 5403:1999, ISO 6579-1:2017). Initial counts (Day 0) were 1.4×10^3 CFU/g (total plate) and 7.0×10^1 CFU/g (yeast/mould), with no pathogens detected. By Day 7, counts rose to 6.2×10^4 CFU/g and 4.0×10^2 CFU/g, respectively, but remained free of harmful microbes, confirming safety despite microbial growth.

SI.NO	TEST PARAMETERS	UNIT	TEST PROTOCOL	RESULT
1.	Total plate count	Cfu/g	IS 5402:2012	1.4×1031.4×103
2.	E.coli	/g	IS 5887(I):1976	Absent
3.	Yeast & Mould	Cfu/g	IS 5403:1999	7.0×1017.0×101
4.	Salmonella	/25g	ISO 6579-1:2017	Absent
5.	Staphylococcus aureus	/25g	IS 5887-II: 1976	Absent

Table: 4.8

Conclusion

The study successfully developed and optimized a multi-grain flour blend with flaxseed powder for low-gluten bread using Response Surface Methodology (RSM). The experimental design enabled the identification of optimal ingredient proportions, balancing nutritional enhancement with acceptable sensory and textural properties. The incorporation of multi-grain flour and flaxseed not only improved the dietary fiber, protein, and omega-3 fatty acid content but also maintained satisfactory loaf characteristics despite reduced gluten content. The model's validation confirmed the reliability of RSM in predicting the best formulation, offering a viable approach for producing healthier, functional bread. This research provides a foundation for further exploration of gluten-reduced bakery products with enhanced nutritional profiles, catering to health-conscious consumers and individuals with gluten sensitivities.

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