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# EFFECT OF ENZYMATICALLY MODIFIED PUMPKIN FLOUR ON THE PHYSICAL, CHEMICAL, AND SENSORY PROPERTIES OF WHITE BREAD

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## ABSTRACT

Pumpkin (*Cucurbita moschata*) flour contains valuable nutrients and bioactive compounds but has limited application in bread-making due to its interference with gluten structure and water absorption. To address this, enzymatic modification using α-amylase was applied to improve its functional properties. This study aimed to evaluate the effects of substituting wheat flour with native and enzymatically modified pumpkin flour on the physical, chemical, and sensory characteristics of white bread. Five bread formulations were prepared: control (100% wheat flour), 5% and 10% substitution with native pumpkin flour, and 5% and 10% with enzymatically modified pumpkin flour. Results showed that the bread with 5% enzymatically modified pumpkin flour (A2B1) exhibited optimal bread volume, moisture retention, crumb structure, and sensory acceptance. Increased substitution improved ash and fat content but reduced brightness and volume. The study concludes that enzymatically modified pumpkin flour is a promising functional ingredient for enhancing bread quality while utilizing local agricultural resources. These findings have practical implications for improving bread quality through the utilization of locally available pumpkin flour and support further innovation in composite flour technology.

**Keywords:** pumpkin flour, enzymatic modification, bread quality, sensory evaluation, composite flour

## INTRODUCTION

Indonesia is a country rich in local food resources such as tubers, legumes, and fruits that hold great potential for food diversification. One such local commodity is pumpkin (*Cucurbita moschata*), which is abundant, underutilized, and economically affordable. Pumpkins are often only consumed as vegetables or dessert ingredients, despite being rich in nutrients such as carbohydrates (approximately 6.6 g/100 g), dietary fiber (around 3.07 g/100 g in flour form), and β-carotene (up to 4857.6 µg/100 g), which contribute to their functional food potential. The high nutritional value and availability of pumpkin flour make it a potential alternative to wheat flour in bakery products like white bread. This is especially relevant given the rising consumption of bread among urban populations, driven by its practicality and increasing incorporation into daily meals **(Bayramov *et al.*, 2022; Galenko *et al.*, 2024; Litvynchuk *et al.*, 2022)**.
Indonesia's dependency on imported wheat flour continues to rise, with projections reaching over 18 million tons by 2025. The incorporation of local flour sources, such as pumpkin flour, may reduce reliance on imports and support food security initiatives. However, native pumpkin flour exhibits functional limitations in baking applications, particularly regarding texture and structure **(F. Ge *et al.*, 2021)**. Hence, research into flour modification especially enzymatic modification is essential to enhance the functional performance of pumpkin flour in baked goods like white bread **(Bayramov *et al.*, 2022; Li *et al.*, 2023; Polakova *et al.*, 2025)**.
The main problem addressed in this study is the limited application of pumpkin flour in bread production due to its suboptimal functional properties, which hinder dough structure and bread quality. A general solution to overcome these limitations is the enzymatic modification of pumpkin flour using alpha-amylase to improve its physicochemical behavior and baking performance, defined as its ability to form stable dough, retain gas during fermentation, and produce bread with desirable volume, texture, and crumb structure (Askari *et al.*, 2025).
Enzymatic modification using α-amylase is a well-established method for improving the functionality of starch-based ingredients. Alpha-amylase acts by hydrolyzing α-1,4 glycosidic bonds in starch molecules, producing dextrins and simple sugars that enhance solubility, reduce viscosity, and improve dough handling and crumb texture in bread-making **(Aydin *et al.*, 2023; Karaś *et al.*, 2024; Mendelova *et al.*, 2025)**. The use of enzymes from microbial sources such as *Aspergillus oryzae* or *Bacillus subtilis* under controlled temperature and pH conditions has been shown to be effective for this purpose **(Abdul-Hadi *et al.*, 2025; Ayo *et al.*, 2024; Chikpah *et al.*, 2025; Pandey *et al.*, 2024)**. Moreover, studies have shown that enzymatically modified flours can improve volume expansion, moisture retention, and overall sensory characteristics in bread products. **Hensel, (2025)** demonstrated the potential of α-amylase treatment to enhance starch functionality in various tuber-based flours. Applying this technique to pumpkin flour is a logical extension and holds promise for developing bread with improved technological performance.
Several studies have investigated the partial substitution of wheat flour with pumpkin flour in bakery products. **Alija *et al.*, (2025)** observed increased beta-carotene content and acceptable sensory scores in white bread fortified with up to 7.5% pumpkin flour. **Ozgoren & Yapar, (2022)** also reported favorable results for dough development and organoleptic properties in donuts containing up to 30% pumpkin flour. However, these studies primarily used non-modified pumpkin flour and did not address the structural or textural limitations encountered during baking. To date, limited research has applied enzymatic modification, particularly using α-amylase, to pumpkin flour for bread applications. Studies using acid-modified or lactic-acid-treated flours have shown potential but resulted in undesirable taste attributes **(Asghari *et al.*, 2025; Lakhmili *et al.*, 2025; Tomić *et al.*, 2022)**. Therefore, there remains a significant gap in exploring the functional, sensory, and nutritional impacts of enzymatically modified pumpkin flour in white bread formulations, a gap that this study aims to address. This study aims to evaluate the effect of enzymatically modified pumpkin flour on the physical, chemical, and sensory characteristics of white bread. The novelty of this research lies in the application of α-amylase modification to enhance the functionality of pumpkin flour, an approach that has not been extensively applied in bread production. The scope of the study includes varying substitution levels (5% and 10%) of both non-modified and α-amylase-modified pumpkin flour and analyzing parameters such as slice appearance, color, texture, moisture content, ash, fat content, and consumer acceptability.

## MATERIAL AND METHODS

### Research Location and Equipment

This research was conducted at the Food Processing Engineering Laboratory and the Food Chemistry and Biochemistry Laboratory of Universitas PGRI Semarang, with additional chemical analyses performed at the Integrated Laboratory of

Diponegoro University. Equipment used included a laboratory oven (Memmert, Germany; 60–180°C), dough mixer (Miyako), proofing cabinet, baking oven, analytical balance (Ohaus, USA; ±0.01 g), muffle furnace (Nabertherm), Soxhlet extractor for lipid analysis, and a digital pH meter (Hanna Instruments). The enzymatic reaction was maintained at 55–60°C using a thermostatic water bath.

**Materials**

Pumpkin (*Cucurbita moschata*) was sourced fresh from a traditional market in Semarang. The fruit was selected based on ripeness, firmness, and the absence of spoilage. Additional bread-making ingredients included wheat flour (Segitiga Biru), sugar (Gulaku), margarine (Blue Band), instant yeast (Fermipan), salt (Refina), and mineral water. The enzyme α-amylase, derived from *Aspergillus oryzae*, was used for enzymatic modification of the flour. All chemicals used for physicochemical analysis, such as HCl, NaOH, ether, and aquadest, were analytical grade and obtained from laboratory stocks.

**Preparation of Pumpkin Flour**

Pumpkins were washed, peeled, deseeded, and sliced into thin pieces (3–5 mm thick). These slices were steamed at 100°C for 15 minutes to soften the tissue and inactivate endogenous enzymes, then dried at 60°C for 8–10 hours until completely dehydrated. The dried pumpkin slices were ground using a dry grinder and sieved through an 80-mesh screen to produce pumpkin flour following the method of **Hensel, (2025)** with slight modifications. The flour was stored in airtight containers at room temperature prior to use.

**Enzymatic Modification of Pumpkin Flour**

A suspension was prepared by mixing 100 g of pumpkin flour with 200 mL of distilled water (1:2 w/v). The pH was adjusted to 5.5 using 0.1 M sodium acetate buffer. α-Amylase was added at a concentration of 0.02% (w/w), and the mixture was incubated in a thermostatic water bath at 55°C for 60 minutes. The α-amylase used in this study was a commercial enzyme preparation, and its application followed the manufacturer's recommended specifications and activity range. The enzyme activity was applied according to the manufacturer's specification, as the exact activity (U/g) was not specified on the product label. After incubation, the suspension was dried in an oven at 60°C until a constant weight was reached, then ground and sieved through an 80-mesh sieve to obtain enzymatically modified pumpkin flour, following the method of **F. Ge *et al.*, (2021)** with slight modifications.

**Bread Formulation and Baking Process**

Five bread formulations were developed in this study, including one control formula using 100% wheat flour and four test formulations incorporating 5% and 10% substitution of either native or enzymatically modified pumpkin flour. Each dough was prepared using 250 g of total flour, 50 g of sugar, 35 g of margarine, 11 g of yeast, 5 g of salt, and 170 mL of water. All ingredients were mixed and kneaded for approximately 20 minutes until a smooth, elastic dough was formed. The dough then underwent a first fermentation (proofing) at 30°C for 45 minutes, followed by shaping and a second proofing period of 30 minutes. The final baking process was carried out at 180°C for 25 minutes. The procedure followed the method of **Yang *et al.*, (2024)** with slight modifications. The complete sequence of steps, from raw pumpkin preparation through enzymatic treatment to bread baking, is illustrated in Figure 1, which presents a comprehensive flowchart of white bread production using enzymatically modified pumpkin flour.

**Physicochemical Analysis**

The physical and chemical characteristics of the bread were analyzed after baking. Moisture content was determined using the oven drying method at 105°C, ash content was analyzed by incinerating samples at 550°C in a muffle furnace, and fat content was measured using Soxhlet extraction with petroleum ether as solvent, following previously reported methods **(Yang *et al.*, 2024)**. Bread volume was measured using the seed displacement method, and crumb characteristics were assessed visually. Color was measured using a Chromameter (Konica Minolta, Japan) to determine L* (lightness), a* (red–green), and b* (yellow–blue) values under standard conditions. Texture profile analysis (TPA) was performed using a Texture Analyzer (Stable Micro Systems, UK) to determine hardness and springiness of the bread samples (Hensel, 2025).

**Sensory Evaluation**

Sensory testing was conducted by 25 semi-trained panelists aged 18–22 years. Each bread sample was coded and presented in uniform slices. Panelists evaluated five attributes-color, aroma, texture, taste, and overall acceptability using a 5-point hedonic scale where 1 = "dislike very much" and 5 = "like very much" following previously reported methods **(Wieser *et al.*, 2023)**. The evaluation was conducted under consistent environmental conditions to reduce bias and variability. However, the panel was limited to a specific age group, and future studies are recommended to include a broader demographic to improve representativeness.

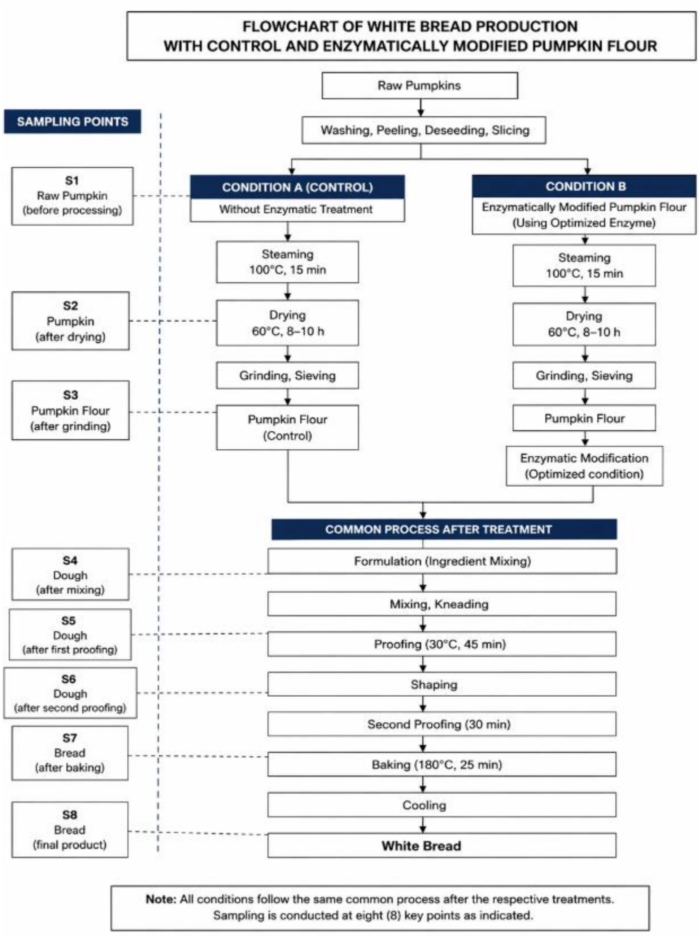


**Figure 1** Flowchart of white bread production using control and enzymatically modified pumpkin flour, including preprocessing, enzymatic treatment, and baking processes.

**Statistical Analysis**

All experimental data were collected in triplicate and reported as means ± standard deviation. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) to determine significant differences between formulations. When significant, post hoc comparisons were conducted using the Least Significant Difference (LSD) method at a 95% confidence level ($\alpha = 0.05$). Data analysis was carried out using Microsoft Excel 2019 and IBM SPSS Statistics version 25. Differences among treatments are indicated by different superscript letters (a, b, ab) in the figures.

**RESULTS AND DISCUSSION**

**Physical Characteristics of Bread**

In this study, each bread formulation was labeled as follows: K refers to the control formulation using 100% wheat flour without pumpkin flour substitution. A1B1 represents bread with 5% native pumpkin flour, A1B2 with 10% native pumpkin flour, A2B1 with 5% enzymatically modified pumpkin flour, and A2B2 with 10% enzymatically modified pumpkin flour. This coding system allows for easy comparison of the effects of both substitution level and enzymatic treatment on bread quality.
The physical characteristics analyzed in this study included bread volume, slice appearance, and texture profile (hardness and springiness). Bread volume, shown in Figure 2, indicates that the bread with 10% enzymatically modified pumpkin flour (A2B2) exhibited a significantly higher value compared to other treatments. This suggests that enzymatic modification improved gluten interaction, enabling better gas retention during fermentation **(Yang *et al.*, 2024; M. Zhang *et al.*, 2023; Y. Zhang *et al.*, 2024)**. Higher bread volume generally results in improved loaf structure and consumer perception of bread quality.

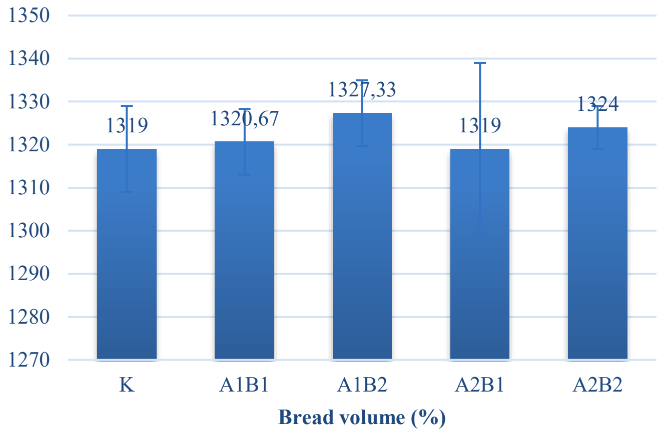


**Figure 2** Bread volume of bread samples K (100% wheat flour), A1B1 (5% native pumpkin flour), A1B2 (10% native pumpkin flour), A2B1 (5% enzymatically modified pumpkin flour), and A2B2 (10% enzymatically modified pumpkin flour)

As shown in Figure 2, bread volume in the control (K) was not significantly different from A1B1, A1B2, and A2B1, but differed significantly from A2B2. Although Figure 3 visually shows that the control bread had a more open crumb structure, slice appearance should not be interpreted as a direct quantitative measure of bread volume. Figure 3 is intended only to illustrate crumb pore structure and compactness. The differences in bread volume are influenced by the variation in the addition of native and enzymatically modified pumpkin flour in wheat formulations. Higher wheat content contributes to increased gluten formation, which enhances gas retention during fermentation **(Wieser *et al.*, 2023)**. In addition, the use of α-amylase can improve dough structure by modifying starch components, thereby supporting gas stability and contributing to improved bread volume. Gluten exhibits elastic and extensible properties that allow the dough to retain $CO_2$ and expand during fermentation, resulting in a more uniform crumb structure **(Gasparre *et al.*, 2023; Madadi *et al.*, 2024a)**.

Bread slice appearance, as shown in Figure 3, demonstrates finer and more compact pores in breads with higher substitution levels of pumpkin flour due to its hygroscopic nature, which competes with gluten for water. The presence of pumpkin fiber also interferes with gluten network development, resulting in denser but moister crumbs **(Alija, Olędzki, Nedelkoska, *et al.*, 2025b; Madadi *et al.*, 2024b)**.

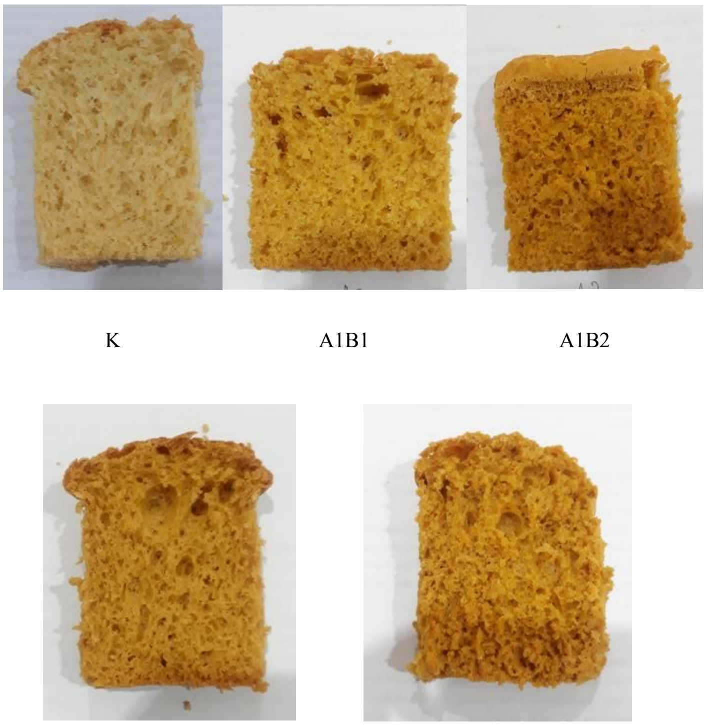


**Figure 3** Visual slice appearance of bread samples showing crumb pore structure and compactness, not quantitative bread volume

The texture profile analysis focused on hardness and springiness as objective parameters of bread structure. Hardness increased with higher substitution levels due to reduced gluten content and higher fiber presence, which limited dough expansion and gas retention. This indicates that pumpkin flour interferes with the gluten network, resulting in a denser crumb structure. However, this effect remained within acceptable limits. Springiness results indicated that enzymatic treatment maintained elasticity, particularly in the A2B2 formulation, which demonstrated favorable recovery properties upon compression. This suggests that α-amylase modification improved the structural flexibility of the dough matrix.

**Color and Chemical Characteristics**

Color analysis using a Chromameter showed that the L* value decreased with increasing pumpkin flour substitution, indicating darker bread crumb, especially in the 10% treatments. This was attributed to natural pigment and Maillard reactions intensified by the presence of reducing sugars released during enzymatic hydrolysis. The b* value, representing yellowness, increased with pumpkin addition, confirming the contribution of carotenoids such as beta-carotene **(H. Ge *et al.*, 2025)**. Although β-carotene and dietary fiber were not directly measured in this study, their presence in pumpkin flour has been widely reported in previous studies. These changes were visually perceptible and contributed to the visual characteristics of the bread.
Chemical characteristics measured included moisture, ash, and fat content. Fat content of the bread samples ranged from 10.67% to 11.85%, with the highest value observed in the A2B2 formulation. Moisture content was higher in breads with enzymatically modified flour due to the higher water-binding capacity of enzymatically modified pumpkin flour **(Gungor & Torun, 2022)**. Ash content increased proportionally with substitution level, suggesting an increase in mineral contribution from pumpkin flour **(Alija, Olędzki, Nikolovska Nedelkoska, *et al.*, 2025)**. These results affirm the role of modified pumpkin flour in improving the functional quality of bread.

**Sensory Evaluation**

Hedonic tests revealed that the most preferred formulation was A2B1, with 5% enzymatically modified pumpkin flour. This treatment offered a favorable balance of softness, taste, and aroma. Although the control bread was still favored in color, A2B1 showed a better overall sensory profile, particularly in texture and aroma. Panelists reported that the pumpkin aroma was subtle and pleasant, while the crumb was soft and moist. These findings confirm that moderate substitution levels are suitable for enhancing bread characteristics without compromising acceptability. The sensory preference is further illustrated in Figure 4, which presents the hedonic scores for each formulation across different attributes. Although texture profile analysis indicated that A2B2 exhibited better structural properties, sensory evaluation showed that A2B1 was the most preferred formulation. This difference may be attributed to the balance of multiple sensory attributes, where moderate substitution levels provide more acceptable overall characteristics for consumers.

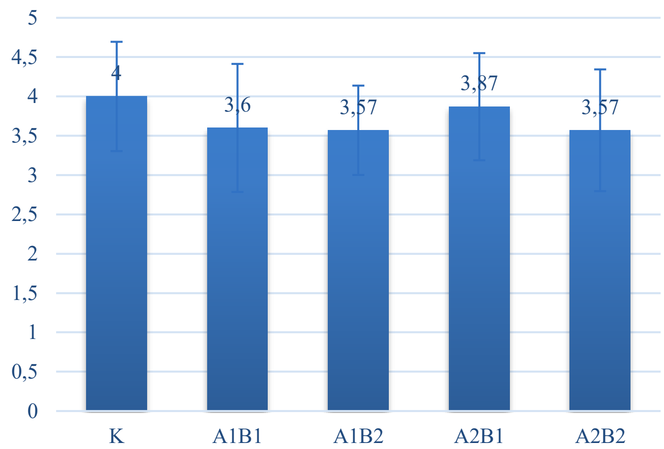


**Figure 4** Organoleptic Aroma Assessment Results of Non-Modified and Modified Pumpkin Flour Substituted White Bread

Based on the hedonic aroma scores of white bread, the addition of native and enzymatically modified pumpkin flour showed a significant effect among treatments, while no significant difference was observed between the control and some formulations. The highest hedonic aroma score was observed in the control bread (4.00), indicating that it was generally liked by the panelists. The average aroma score ranged from 3.57 to 4.00, corresponding to “slightly dislike” to “like.”

Increasing the substitution level of pumpkin flour tended to decrease panelists' preference for aroma.
Aroma in white bread is mainly developed during fermentation as a result of the metabolic activity of Saccharomyces cerevisiae, producing volatile compounds such as organic acids, aldehydes, and esters. Aroma formation is influenced by fermentation conditions such as time and temperature, which affect yeast metabolism and the development of flavor compounds. Additionally, starch hydrolysis during processing can contribute to aroma development through the formation of dextrins and related compounds **(Atencio *et al.*, 2022)**.

**Comparison with Previous Studies**

These findings align with those of **Aljobair, (2024)**, who reported improved nutritional and sensory attributes upon incorporating pumpkin flour in bread. Improvements in texture and cohesiveness in this study are also consistent with research by **Olamiti & Ramashia, (2024)**, who observed similar benefits from enzymatically treated composite flours. Other studies, such as those by **Sheikholeslami *et al.*, (2021)**, have also highlighted the role of enzymatic modification in improving dough structure and softness. These findings are also consistent with previous studies on pumpkin flour in bread products, which reported that moderate substitution levels can maintain acceptable sensory properties while influencing bread quality

**Limitations**

Future studies should explore different enzymatic concentrations and combinations, as well as the use of other hydrocolloids or improvers to offset the reduced gluten strength in pumpkin flour-enriched bread. Investigations on nutrient retention post-baking, antioxidant capacity, and consumer perception in larger populations would strengthen claims for nutritional benefits. Shelf-life testing, packaging optimization, and scaling to pilot production would also be valuable for evaluating feasibility in commercial bakery settings.

**Future Work**

Future research should investigate different enzymatic concentrations and enzyme combinations to further optimize the functional properties of pumpkin flour in bread production. Further studies should also evaluate nutrient retention after baking, antioxidant activity, and consumer acceptance using broader demographic groups. In addition, shelf-life evaluation, packaging optimization, and pilot-scale production should be conducted to assess the feasibility of enzymatically modified pumpkin flour for commercial bakery applications.

## CONCLUSION

This study demonstrated that partial substitution of wheat flour with enzymatically modified pumpkin flour significantly influenced the physical, chemical, and sensory characteristics of white bread. Among the five formulations tested, the bread sample containing 5% enzymatically modified pumpkin flour (A2B1) exhibited the most desirable balance between bread volume, crumb texture, moisture content, and overall sensory acceptance, particularly aroma. These findings suggest that enzymatically modified pumpkin flour can be a valuable functional ingredient in bread making, contributing to improved moisture retention, enhanced color, and acceptable texture while utilizing local agricultural resources. The enzymatic treatment offers a promising approach for improving the functionality of non-wheat flours in bakery applications. Future studies are recommended to evaluate shelf-life, antioxidant activity, consumer preferences across broader demographics, and scalability to commercial production. These findings demonstrate the potential of enzymatically modified pumpkin flour as a local composite flour ingredient for bread production

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