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Wheat Germ in Sourdough Fermentation: A Sustainable Approach to Reducing Food Waste

Nguyet Huynh Thai, Phuong Pham Trieu, Nguyet Nguyen Thi Minh*

Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao Street, Ward 4, Go Vap District, Ho Chi Minh City, Vietnam
nguyenthiminhnguyet@iuh.edu.vn

Wheat germ (WG), a nutrient-rich by-product of wheat milling, contains high levels of protein, lipids, fibre, and bioactive compounds but is often underutilized due to its susceptibility to rancidity. This study explored the potential of incorporating WG into sourdough (SD) fermentation to produce nutrient-dense sandwich bread. The research objectives included evaluating the physicochemical properties of WG, assessing its impact on SD fermentation, analyzing bread texture and structure, and conducting sensory and nutritional assessments. At a 25 % substitution level, WG promoted lactic acid bacteria growth, reduced pH, and increased organic acid content during fermentation. SD bread with fermented WG exhibited a lower pH, higher organic acid content, and improved dough rheology. Compared to industrial yeast bread, SD bread exhibited a denser structure, darker crust colour due to increased Maillard reactions, and a more complex flavour profile. Sensory evaluation revealed that while consumers appreciated the nutritional benefits, the characteristic sour taste was less preferred. Nutritional analysis revealed higher protein and fibre content, making the product suitable for health-conscious consumers. The findings highlight the feasibility of valorizing WG in SD bread production, offering a sustainable approach to reducing food waste and supporting the circular economy.

1. Introduction

WG, accounting for approximately 2.5 – 3.8 % of the total wheat kernel weight, is a highly nutritious by-product of the milling industry. It contains 10 – 15 % lipids, 26 – 35 % proteins, 17 % sugars, 10 – 14 % dietary fibre, and 4 % minerals, along with various bioactive compounds such as tocopherols, phytosterols, policosanols, carotenoids, thiamin, riboflavin, and enzymes. These components contribute to numerous health benefits, including cholesterol reduction, enhanced physical endurance, anti-ageing effects, fertility improvement, and cancer prevention (Zhang et al., 2023). Although WG is highly nutritious, approximately 25 × 10^6 t are produced annually (Liu et al., 2024), most are discarded or used as animal feed due to lipid oxidation and enzymatic activity (Skendi et al., 2020). The valorization of WG through fermentation could provide a sustainable solution to reduce food waste and promote circular economy practices in the food system. In light of global efforts to reduce food waste and enhance sustainability, the valorization of such by-products has gained increasing attention. Recent studies have investigated bioconversion, fermentation, and green processing techniques to upcycle agro-industrial residues into high-value functional foods and nutraceuticals (Nguyen et al., 2024).
The incorporation of WG into sourdough (SD) fermentation represents a promising biotechnological strategy that not only enhances the stability of its bioactive compounds but also supports cleaner production, waste minimization, and the development of circular food system models. This approach is aligned with global objectives related to climate action, sustainable food systems, and low-emission food technologies. SD fermentation, an ancient bread-making method, involves the natural fermentation of cereal flour and water by lactic acid bacteria (LAB) and yeast. LAB produce lactic and acetic acids, lowering the pH and creating stable fermentation conditions, while yeast generate carbon dioxide and ethanol, contributing to dough rise and bread structure (De Vuyst et al., 2023). SD fermentation improves the nutritional and sensory properties of bread by enhancing antioxidant capacity, reducing glycemic index, and decreasing antinutritional factors. It also

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represents a sustainable approach to valorising legumes, pseudocereals, and milling by-products, contributing to food security and environmental sustainability (Gobbetti et al., 2020).
Teterycz et al. (2023) demonstrated that incorporating WG into wheat flour (WF) products not only enhances their nutritional value, achieving complete protein quality but also improves sensory properties, particularly by increasing product firmness. Despite the promising benefits, limited studies have explored the utilization of WG by-products from milling plants in combination with traditional SD fermentation to enhance the nutritional quality of sandwich bread, particularly in Vietnam. The application of SD fermentation as an alternative to commercial yeast in Vietnamese bakery products remains under-researched. This study aimed to integrate WG with traditional SD fermentation to develop nutrient-enriched sandwich bread, addressing nutritional enhancement and food waste reduction. The specific research objectives included: 1) Investigating the influence of ingredients on the properties of SD; 2) Assessing the physical properties of sandwich bread formulations; 3) Conducting sensory evaluation of sandwich bread; and 4) Analyzing the nutritional composition of sandwich bread.

2. Materials and methods

2.1 Raw materials

ASW - Australia Standard White WG is supplied by Mekong Flour Company. Its chemical composition includes 10.74 % moisture, 3.42 % ash, 1.446 % fibre, 28.20 % protein, 10.8 % lipids and 45.39 % carbohydrates, providing 391.56 Kcal / 100g of energy. The ingredients included: WF No. 13 (Interflour Vietnam Company), pure palm sugar paste (provided by a traditional palm sugar production facility in Tinh Bien district, An Giang province), salt (produced by Bac Lieu Salt Joint Stock Company), and VIETCOCO Premium Organic pure coconut oil (made by Luong Quoi Coconut Processing Company Limited).

2.2 Prepare SD

The mixed formula of the research SD samples consisted of three variations. Sample S1 was prepared using 100% wheat flour No.13 (WF No.13). Sample S2 contained a blend of 75 % WF No.13 and 25% whole grain flour (WG). Sample S3 included 50% WF No.13 and 50% WG. The mixture was then combined with water at a flour-to-water ratio of 62.5 %: 37.5 % using a Venko dough kneader (VK35E8, Japan) at level 2 for 4 m. Fermentation was carried out at room temperature (32.9 ± 0.5 °C) with a relative humidity (RH) of 50.7 ± 3.8 %. Every 24 h, the starter was "fed" by using 10 % of the previously fermented dough and adding a fresh mixture of flour and water in the same 62.5 %: 37.5 % ratio, followed by thorough mixing. On the sixth day of fermentation, the mixture was used for bread production.
The bread samples were formulated according to the composition designed in Table 1, where all additional ingredients were calculated as a percentage of the WF weight. After thorough mixing, the dough was allowed to rest for 10 m. The dough was then divided into 300 g portions, rounded, sheeted, and rolled into shape. The shaped dough was placed in a non-stick aluminium mould (19 × 9.5 × 8 cm). The samples were proofed at room temperature (32.6 ± 0.9 °C) with a humidity of 56.1 ± 6.9 % for 3 h. Baking was conducted in a Sunhouse Mama oven (SHD4250S, China), which was preheated to 180 °C for 10 min in convection mode with both upper and lower heat settings. The proofed samples were then baked for 20 min. After baking, the bread was cooled for 2 h at 34.7 ± 0.2 °C with a humidity of 50.5 ± 5.9 %, after which structural analysis, colour measurement, and nutritional composition assessment were performed.

Table 1: Ingredients and formula for mixing research cake samples

Ingredients (g)	CS_Y	FM1	FM2	FM3	CS1	CS2	CS3
Flour No.13	100	100	100	100	100	100	100
Salt	1 %	1 %	1 %	1 %	1 %	1 %	1 %
Oil	2 %	2 %	2 %	2 %	2 %	2 %	2 %
Sugar	10 %	10 %	10 %	10 %	10 %	10 %	10 %
Instant dry yeast Mauri	1 %	-	-	-	-	-	-
SD	-	20 % S1	20 % S2	20 % S3	20% S1	20 % S2	20 % S3
WG	20 %	20 %	20 %	20 %	-	-	-
Water	65%	65 %	65 %	65 %	60 %	60 %	60 %

2.3 Determination of SD Properties

Total titratable acidity (TTA) and pH analysis were performed as indicated by Sevgili et al. (2021). The microbial composition of the SD was determined as follows: 0.5 g of each sample was aseptically weighed and mixed with 4.5 mL of sterile distilled water, then homogenized using a Vortex Mixer (MX-S, USA). Serial tenfold dilutions were prepared up to 10^{-5}, and the following microbiological analyses were performed: The Total

Bacterial Count (TBC) was determined according to ISO 4833-1:2013 using Plate Count Agar (PCA), the pour plate technique, incubation at 30 °C for 48 h, and counting the total bacterial cells. The Yeast Count was determined according to ISO 21527-2:2008 by incubating on Dichloran Glycerol 18 % (DG 18) at 30 °C for 48 h, followed by yeast enumeration. The LAB Count was determined according to ISO 15214:1998 using MRS medium, incubating at 30 °C for 48–72 h.

2.4 Methods for Analyzing Bread Properties

We measured loaf volume using the standard AACC 10-05 method, modified by replacing rapeseed with millet for the displacement test (American Association of Cereal Chemists, 2000). Texture Profile Analysis (TPA) was performed following the method of Marti et al. (2014) using a CT3 Brookfield texture analyzer (Brookfield Ametek, USA). The samples were cooled at 35 ± 10 °C and 51 ± 19 % humidity for 2 h before testing. Three central slices, each 1.5 cm thick, were used for analysis. The testing parameters included: a cylindrical probe (TA11/1000), a test speed of 2 mm/s, 40 % strain, two compression cycles, and a compression force of 0.5 N. The TPA analysis was conducted at room temperature (25 °C), and the following parameters were recorded: Hardness, Fracturability, and Resilience. Each analysis was performed in triplicate, and the average values were reported. Colour measurement was conducted at nine different positions on the crumb surface using a Konica Minolta colourimeter (CR - 400, Japan). The CIE Lab* colour space method was applied, where L* represents brightness (white – black), while a* and b* indicate chromatic coordinates (red – green and yellow – blue). The browning index (BI) was used to describe colour changes during the baking process: dL, da, and db represent the differences in L, a, and b values between the sample and the control sample (a white ceramic standard colour plate with L = 61.87, a = 3.24, and b = 26.27) with Eq(1):

$$BI = \frac{100\times(x-0{,}31)}{0{,}17} \quad (1)$$

$where\ x = \frac{a^*+1{,}75L^*}{5{,}645L^*+a^*-3{,}012b^*}$.

The sensory evaluation method was adapted from the study by Mariotti et al. (2014). The sensory quality of the product was assessed by a panel of nine students majoring in Nutrition and Food Science at the Institute of Biotechnology and Food Technology, Ho Chi Minh City University of Industry. Each panellist received prior training to familiarize themselves with sensory analysis methods, sample characteristics, and evaluation techniques. They rated their preference using a 9-point hedonic scale, ranging from 1 (extremely dislike) to 9 (extremely like). The prepared samples included seven types of bread, cooled for 2 h before evaluation. The samples were coded and served in random order. Each bread slice (1.5 cm thick) was cut into two pieces, and each panel received one piece per sample. The nutritional composition of the bread samples rated highly by the sensory panel was determined by inputting the ingredient composition ratios into the Eiyokun software, which calculated the nutritional content and energy values.

3. Results and discussion

3.1 Investigation of the influence of ingredients on the properties of SD

Figure 1 shows that the initial pH value increases gradually in the order S1 < S2 < S3 due to the presence of (WG), which contains the enzyme phytase and minerals that create an alkaline environment.

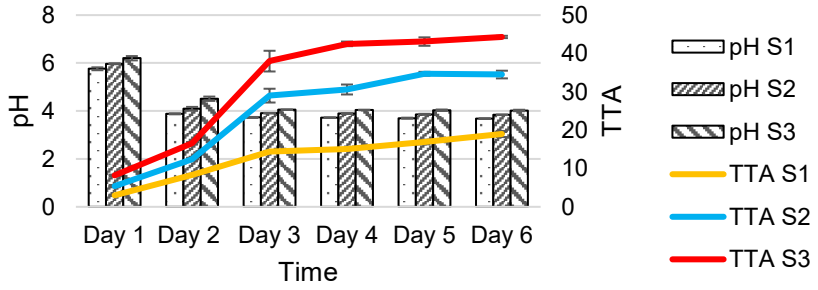

Figure 1: pH and TTA of samples over time (Data expressed in mean ± SD, at significance level p<0.05)

Phytase in WG hydrolyzes phytic acid, an acidic compound, reducing the acidity in the system, and leading to an increase in pH (Wang et al.,2002). At the same time, minerals such as calcium, magnesium, potassium and sodium in WG can neutralize acid, contributing to making the environment more alkaline. Because S3 contains

the most WG, the degree of phytic acid decomposition and alkalizing effect is also the strongest, leading to a higher pH than S2 and S1. During fermentation, pH decreases rapidly in the first 24 h (~2 units), then declines more slowly, stabilizing from days 3 to 6 due to the formation of organic acids. TTA values increase from S1 to S3, reflecting the accumulation of organic acids, and range from 19.0 ± 3.6 to 44.3 ± 1.1, influenced by the raw materials. TTA rises sharply in the first three days, then slightly decreases, correlating with pH changes.
The total aerobic microorganisms (see Table 2) in the initial raw materials vary significantly due to contamination, harvesting, and storage conditions. No yeast or LAB was detected, indicating that the raw materials were "clean." In SD the total aerobic microorganism count varies based on flour type and fermentation conditions, aligning with Adepehin (2020), who reported a range of 7.70 to 10.52 CFU/g. The yeast count also changed significantly, showing a diversity in microbial numbers consistent with Gabriel's (1999) study on traditional Chinese SD bread from 10^5 to 10^7 CFU / g. The total aerobic microorganisms and yeast increased in the order of S3 < S1 < S2, while the highest TTA was observed in S3. After six days, LAB dominated in S2 and S3 due to favourable temperature and WG supplementation providing essential nutrients, creating optimal conditions for this bacterium to grow more strongly than in S1. In particular, in S3 with the dominance of LAB, more acid was produced, which inhibited the growth of yeast. This finding is consistent with Boreczek (2020) study, which reported that the LAB count was three times higher than the yeast count in SD.

Table 2: Results of total microbial count (LAB, Yeast and bacteria) of raw materials and samples S1, S2 and S3

	Total LAB count	Total yeast count	Total Bacterial Count
Flour	0	0	$2.30 \pm 0.12 \times 10^5$
WG	0	0	$2.35 \pm 0.19 \times 10^5$
S1	$4.13 \pm 0.96 \times 10^7$	$3.90 \pm 0.37 \times 10^5$	$1.74 \pm 0.13 \times 10^8$
S2	$8.27 \pm 0.38 \times 10^7$	$3.03 \pm 0.75 \times 10^7$	$2.40 \pm 0.10 \times 10^8$
S3	$15.77 \pm 0.84 \times 10^7$	0	$1.61 \pm 0.24 \times 10^6$

Data are presented as mean ± standard deviation (SD).

3.2 Assessing the physical properties of sandwich bread formulations

An examination of Table 3 reveals that the CSY sample exhibited the highest pH. This phenomenon is explained by the lactic acid fermentation process in SD, which lowers the pH of bread made with SD. Similarly, fermented WG can reduce the pH of bread by producing more organic acids, such as lactic acid, during fermentation due to the activity of LAB. In this survey, the pH of SD bread ranged from 4.27 to 4.96. While the pH of SD bread ranged from 4.9 to 5.6 (Flander et al., 2011), or a very low pH of only about 3.9 (Schober et al., 2003). From the data obtained, it can be concluded that the pH of bread can vary based on the specific recipe and fermentation process.
SD bread has a smaller volume (Figure 2) but a higher weight compared to bread made with industrial yeast, as natural fermentation produces CO_2 more slowly. Increasing the proportion of WG in SD improves bread volume due to phytase enzymes breaking down phytic acid, enhancing fermentation. Among the formulations and control samples, FM3 and CS3 had the largest volumes, while FM1 and CS1 had the smallest. These results indicate that WG affects bread structure, opening up new research directions for SD made from WG.
The difference in bread colour is mainly due to the formation of air holes during baking, which affects the Maillard reaction. The CS_Y sample has larger air holes, which increases the degree of browning, while the FM samples are lighter in colour with higher L* values. The b* index indicates that the samples with WG are yellower due to xanthophyll pigments. The BI is higher as the percentage of WG increases, due to the higher sugar and amylase enzyme content, which promote the Maillard reaction. FM3 has the highest browning degree, confirming the important role of WG in determining bread colour.

Figure 2: Cross-sectional images of sandwich bread samples

Hardness reflects the degree of hardness/softness of the bread, while breakability represents the force required to break the bread. The results of the study showed that SD bread was harder than industrial yeast bread because lactic acid increased gluten strength. The addition of WG made the bread harder because the water-absorbing fibre and glutathione weakened the gluten network. Hardness decreased as the amount of fermented

germ increased, making the bread softer. In terms of breakability, breads with fewer air holes had a stronger structure and required a higher breaking force. Conversely, breads with more air holes became spongy but more brittle. Elasticity was similarly affected: SD bread had a higher elasticity due to lactic acid, while the addition of raw WG reduced elasticity due to the breakdown of the gluten structure. Overall, the hardness, breakability, and elasticity of bread depended on the type of yeast, the amount of WG, and the structure of the air holes.

Table 3: Properties of bread samples in different formulations

FM	pH	Volume (ml)	Weight (g)	L*	a*	b*	BI	Hardness (N)	Fracturability (N)	Resilience
CSY	5.53 ± 0.01^{e}	673 ± 6^{g}	277.3 ± 1.4^{a}	57.2 ± 1.0^{a}	3.2 ± 0.2^{ab}	28.1 ± 0.3^{c}	69.3 ± 2.2^{d}	8.86 ± 0.05^{b}	8.86 ± 0.05^{b}	0.26 ± 0.02^{c}
FM1	4.96 ± 0.1^{d}	453 ± 6^{a}	289.5 ± 1.5^{d}	64.0 ± 0.6^{d}	3.3 ± 0.2^{abc}	29.0 ± 0.5^{d}	62.4 ± 1.5^{b}	25.46 ± 0.73^{f}	25.46 ± 0.73^{f}	0.19 ± 0.04^{ab}
FM2	4.64 ± 0.06^{c}	507 ± 6^{c}	285.4 ± 1.7^{c}	61.9 ± 0.8^{c}	3.4 ± 0.2^{bc}	28.7 ± 0.6^{d}	64.3 ± 1.9^{c}	22.42 ± 0.69^{e}	22.42 ± 0.69^{e}	0.17 ± 0.02^{a}
FM3	4.52 ± 0.06^{b}	527 ± 6^{d}	286.0 ± 0.8^{c}	59.2 ± 0.5^{b}	4.0 ± 0.2^{d}	29.0 ± 0.5^{d}	69.9 ± 1.5^{d}	18.58 ± 0.80^{d}	18.58 ± 0.80^{d}	0.22 ± 0.04^{abc}
CS1	4.58 ± 0.06^{bc}	467 ± 6^{b}	283.8 ± 0.6^{c}	62.6 ± 1.2^{c}	3.4 ± 0.2^{bc}	26.7 ± 0.4^{b}	58.1 ± 2.3^{a}	10.37 ± 0.25^{c}	10.37 ± 0.25^{c}	0.23 ± 0.01^{bc}
CS2	4.35 ± 0.04^{a}	547 ± 6^{e}	280.3 ± 1.6^{b}	62.6 ± 1.1^{c}	3.2 ± 0.1^{a}	26.3 ± 0.5^{a}	56.6 ± 0.9^{a}	6.68 ± 0.80^{a}	6.68 ± 0.80^{a}	0.26 ± 0.02^{c}
CS3	4.27 ± 0.02^{a}	573 ± 6^{f}	281.0 ± 0.9^{b}	62.5 ± 1.2^{c}	3.5 ± 0.3^{c}	26.6 ± 0.5^{ab}	58.0 ± 2.4^{a}	5.76 ± 0.18^{a}	5.76 ± 0.18^{a}	0.31 ± 0.03^{d}

Different letters in the same column indicate significant differences (ANOVA, α = 0.05)

3.3 Sensory evaluation of bread

According to Figure 3a and 3b, the total reliability reached 57.82 %, indicating that the sensory evaluation results were meaningful and usable. The products were divided into three groups: group 1 (CSY) had elasticity and a light germ odour; group 2 (FM1, FM2, FM3) had a sour odour, sour taste and sour aftertaste; group 3 (CS1, CS2, CS3) had a brown colour, sour taste, sour aftertaste and germ odour. Adding WG reduced the sour odour, and increased the germ odour and characteristic brown colour. According to Figure 3 (c), the CSY sample was the most popular (≈ 8 points), followed by FM1, FM2, and CS2 (≈ 6 points), while FM3, CS1, and CS3 had a lower level of popularity (≤ 5 points). Consumers were not familiar with SD bread but tended to accept it more when using S2 SD (75 % WF, 25 % WG), this showed that the bread samples supplemented with S2 had a good balance between sour taste, wheat germ aroma and color, making them more acceptable. Samples FM1 and FM2 will be selected for further nutritional analysis.

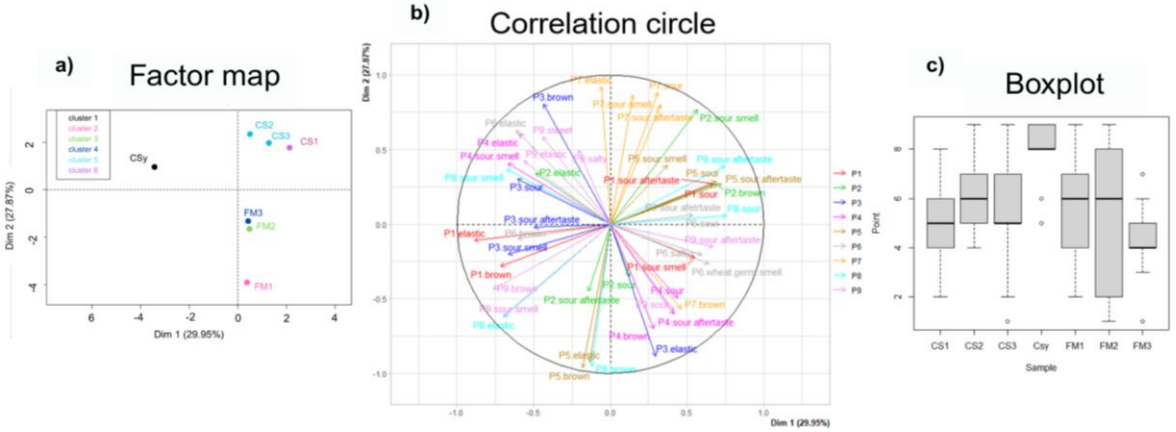

Figure 3: (a) and (b) show the correlation in assessments between samples and properties, while (c) represents the council's preference for the samples

3.4 Nutritional assessment of bread

According to Table 4, Otto bread provides a lot of energy and carbohydrates, suitable for heavy workers or athletes, but has a high lipid content. In contrast, FM1 and FM2 have lower calories and lipids, suitable for dieters. FM2 and FM1 have a higher protein content, which is good for muscle recovery. Overall, FM1 and FM2

are good choices for those who want to control calories, are rich in protein and balance carbohydrates, FM2 is superior thanks to its highest protein content and slightly lower lipids.

Table 4: Nutritional composition of bread samples

	Energy (Kcal)	Protein (g)	Lipid (g)	Carbohydrate (g)
FM1	220.52 ± 0.4^{a}	9.52 ± 0.03^{b}	3.45 ± 0.02^{a}	40.35 ± 0.05^{b}
FM2	221.8 ± 0.44^{b}	9.74 ± 0.02^{c}	3.56 ± 0.01^{b}	40.13 ± 0.06^{a}
Otto bread	290^{c}	9^{a}	4^{c}	54^{c}

Different letters in the same column indicate significant differences (ANOVA, $\alpha = 0.05$)

4. Conclusion

This study introduces a novel approach to valorizing wheat germ (WG)—a nutrient-dense milling by-product—through its incorporation into sourdough (SD) fermentation. The addition of 25% WG enhanced microbial growth, optimized pH and TTA levels, and contributed to a distinct flavour and improved dough rheology. Bread made with fermented WG showed higher volume, softer texture, and deeper crust colour due to intensified Maillard reactions. Importantly, WG enrichment increased protein and fibre content while reducing caloric density, aligning with consumer health trends. However, sensory data indicated limited acceptance of sour taste, highlighting the need for tailored formulation. These findings demonstrate a sustainable, functional application of WG in bakery products, supporting circular economy goals and advancing green food processing strategies.

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