Enhancing Antioxidant Properties of Sourdough Bread through the Incorporation of Indonesian Green Leaf Flours: A Multilevel Evaluation

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Abstract

Green leaf flours such as *Moringa oleifera*, *Sauropus androgynus*, and *Centella asiatica* are rich in bioactive compounds and dietary fiber, offering potential as functional ingredients in bakery products. Their effects on sourdough bread quality, however, remain underexplored. In this study, sourdough breads were prepared by incorporating the formulations with 5%, 10%, and 15% of each green leaf flour. Physical properties were assessed through texture analysis and loaf volume. Chemical properties included pH and antioxidant activity (DPPH assay). Sensory evaluation was performed with 50 untrained panelists using a 5-point hedonic scale. Increasing the percentage of green leaf flours generally increased hardness and chewiness while reducing springiness, with cohesiveness remaining stable except at 15% *Centella asiatica* flour. Bread volume decreased in sourdough breads containing *Moringa oleifera* and *Sauropus androgynus*, but *Centella asiatica* showed equal or higher loaf volume than the control, except at 15%. Antioxidant activity was significantly enhanced in all 15% formulations, with the highest value observed in *Sauropus androgynus*. pH increased across all samples, exceeding the recommended range only in high-level *Sauropus androgynus*. Sensory evaluation indicated a preference for the control, yet *Centella asiatica* sourdough breads received more favorable scores than *Moringa oleifera* and *Sauropus androgynus*. Among the tested formulations, *Centella asiatica* at 5–10% incorporation demonstrated the greatest potential for further development, balancing improved nutritional and antioxidant properties with acceptable sourdough bread quality. Future studies should optimize *Centella asiatica* incorporation through processing strategies to enhance sensory attributes and confirm consumer acceptability.

Keywords: Functional, Local food, Sourdough bread, Green leaf flours

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Introduction

The rising trend for sourdough bread has been accompanied by an increasing interest in its potential health benefits, making sourdough a focal point in functional foods. Sourdough bread is fermented using naturally occurring yeast and lactic acid bacteria (LAB), which results in not only distinctive flavors but also numerous health advantages compared to conventionally leavened bread (Lau et al., 2021; Sakandar et al., 2019). The fermentation process enhances the bioavailability of minerals, such as iron, calcium, magnesium, and zinc, which are crucial for human health (Alkay et al., 2024; Ribet et al., 2023). Additionally, reducing antinutritional factors like phytic acid further improves nutrient absorption, promoting overall gastrointestinal health (Fernández-Peláez et al., 2020; Zhang et al., 2016). Furthermore, sourdough bread demonstrates a lower glycemic index, which could potentially aid in managing postprandial glucose levels, thereby benefiting those with diabetes (Alkay et al., 2024; Ribet et al., 2023; Rizzello et al., 2019).

Moringa oleifera has a long history in traditional Indonesian medicine, particularly for enhancing well-being and as a nutritional supplement for postpartum women. It is consumed in various forms, cooked, powdered, or in teas, to boost milk production due to its galactagogue properties (Fungtammasan & Phupong, 2021; Silalahi et al., 2020). The leaves are particularly noted for their high concentrations of antioxidants, vitamins A, C, and E, and essential amino acids (Fungtammasan & Phupong, 2021). Moringa's extracts have demonstrated significant galactagogue properties, enhancing prolactin levels and thus positively contributing to milk production in lactating mothers (Mustofa et al., 2020).

Sauropus androgynus, commonly known as Katuk, is widely consumed in Indonesia, primarily for its reputed ability to enhance breast milk production among lactating mothers. Its use is prevalent in traditional recipes, often prepared as a vegetable dish (Silalahi et al., 2020). Ethnomedicinal practices report that *Sauropus androgynus* serves as a staple food with therapeutic benefits, including serving as an anti-anemia and anti-obesity agent (Anju et al., 2022). The leaf extracts of *Sauropus androgynus* have shown anti-inflammatory, antioxidant, and potential estrogenic effects, which may enhance breast milk production during lactation (Andari et al., 2022; Kurniawan et al., 2024). The variety of secondary metabolites found in the leaves, including tannins, saponins, and steroids, contributes to its array of health benefits (Yunita et al., 2019, 2022).

Centella asiatica is also utilized in various traditional remedies in Indonesia. It is often consumed in salads or as a herbal medicine for improving skin health and cognitive function (Bunawan et al., 2015). *Centella asiatica*, recognized for its medicinal properties, possesses active compounds such as asiaticoside and madecassoside, known for their anti-inflammatory and cognitive enhancement abilities (Fungtammasan & Phupong, 2021). This plant's extracts have traditionally been used to treat various ailments, including skin disorders and for general health improvement (Bunawan et al., 2015).

Incorporating green leaf flours, such as *Moringa oleifera*, *Sauropus androgynus*, and *Centella asiatica*, into sourdough bread formulations presents an innovative opportunity to further enhance the nutritional profile of sourdough bread. *Moringa oleifera*, known for its rich nutrient density, including vitamins, minerals, and antioxidants, has been studied for its role in controlling various health conditions (Ozulku, 2024). Similarly, *Sauropus androgynus*, traditionally used for its high nutrient content and potential health benefits, holds promise for enhancing the functional aspects of food products (Beltrão Martins et al., 2022). *Centella asiatica*, recognized for its medicinal properties and ability to promote cognitive health, can complement the health benefits of sourdough through its bioactive compounds. However, studies on its integration with sourdough are limited (Lau et al., 2021).

Research in this area is vital as it addresses the dual concerns of nutrition and health in modern diets. As consumers become more health-conscious the demand for foods that deliver health benefits through enhanced nutritional profiles is surging. Exploring these unique flour combinations could lead to the formulation of sourdough breads that cater to a broader audience while promoting health outcomes. Therefore, this study aims to investigate the impact of green leaf flours on the physicochemical and sensory characteristics of sourdough bread. This endeavor holds the potential for creating innovative and nutritious food products and addresses public health issues linked to diet and nutrition.

Materials and methods

1. Sourdough bread making

Sourdough bread was prepared using Rahardjo and Sihombing (2023) instructions, with some adjustments. Table 1 shows the sourdough bread formulation used in this investigation. C was the control formulation for sourdough bread, TM was the *Moringa oleifera* flour formulation, TS was the *Sauropus androgynus* flour formulation, and TC was the *Centella asiatica* flour formulation. The treatment variations in this study were at a percentage of 5%w/w (TM1, TS1, TC1), 10%w/w (TM2, TS2, TC2), and 15%w/w (TM3, TS3, TC3). Green leaf flours of (a) *Moringa oleifera*, (b) *Sauropus androgynus*, and (c) *Centella asiatica* (Berbubuk, Blora, Central Java, Indonesia) can be seen in Fig. 1. The products were supplied in sealed packages, and no further processing was applied before use.

(a) (b) (c)

Fig. 1 Green leaf flours. (a) Moringa oleifera flour, (b) Sauropus androgynus flour and (c) Centella asiatica flour

The other ingredients used in this study included commercial wheat flour (Cakra Kembar, PT Bogasari Flour Mills, Indonesia), sugar (Gulaku Hijau Premium, PT Sugar Group Companies, Indonesia), salt (Dolphin, PT Susanti Megah, Indonesia), unsalted butter (Anchor, Fonterra Co-operative Group Ltd., New Zealand), and mineral water (Aqua, PT Tirta Investama, Indonesia). The sourdough starter was obtained from a previous research conducted by Rahardjo and Sihombing (2023), developed through the fermentation of wheat flour and water yeast with daily refreshments for 14 d. The mature starter was maintained at 100% hydration (1:1 flour-to-water ratio, w/w). All ingredients were used as supplied, without further modification. Bread production was carried out using a planetary mixer (SM-101, Sinmag Bakery Machinery Co., Ltd., Taiwan) equipped with a dough hook and with a gas convection oven (SM-705G, Sinmag Bakery Machinery Co., Ltd., Taiwan). Internal bread temperatures were monitored with a central thermometer (AD-5625, A&D Company Ltd., Japan).

Table 1 Sourdough bread formulation

Ingredients	C	TM1	TM2	TM3	TS1	TS2	TS3	TC1	TC2	TC3
Wheat flour (g)	200	190	180	170	190	180	170	190	180	170
Green leaf flour (g)	0	10	20	30	10	20	30	10	20	30
Sugar (g)	30	30	30	30	30	30	30	30	30	30
Salt (g)	4	4	4	4	4	4	4	4	4	4
Butter (g)	30	30	30	30	30	30	30	30	30	30
Sourdough starter (g)	50	50	50	50	50	50	50	50	50	50
Water (g)	80	80	80	80	80	80	80	80	80	80

The dry and wet ingredients, except for salt and butter, were initially mixed using a planetary mixer at 148 rpm for 5 min to ensure homogeneity and sugar dissolution. Salt and butter were then added, and mixing was continued at 307 rpm for 2 min until a cohesive dough was formed. The dough was transferred into a fermentation basin and subjected to bulk fermentation at 30±1°C and 75±2% relative humidity (R.H.) for 180 min. After fermentation, the dough was divided into 250 g portions, manually molded into oval shapes (approximately 12 cm length × 7 cm width × 5 cm height), placed on a silicone baking mat, and proofed for 360 min at 30±1°C and 75±2% R.H. Proofed dough samples, which had approximately doubled in volume, were baked in a gas convection oven at 180°C for 30 min, with an internal temperature target of 90±1°C. The baked sourdough breads were placed on cooling rack and cooled to room temperature (25°C) for approximately 1 h before further analysis.

2. Physical analysis

Physical analysis included texture analysis using a Texture Analyzer (TA Plus, Lloyd Instruments Ltd., United Kingdom) and bread volume. Texture analysis was in accordance with the procedure performed by Li et al. (2013), with the following analysis results: crumb hardness, cohesiveness, springiness, and chewiness. Bread volume was analyzed through the millet seed displacement method, where the volume of the loaf is measured by the volume of seeds displaced by the bread (Różyło et al., 2014). Each sample was analyzed 3 times.

3. Chemical analysis

Chemical analysis included antioxidant activity and pH analysis. Antioxidant activity was measured according to Seo et al. (2021) using DPPH methods, and pH analysis was in accordance with the procedures performed by Soares et al. (2010). Each sample was analyzed 3 times.

4. Sensory analysis

Sensory analysis was carried out using the acceptance test of preference rating (Meilgaard et al., 2016) of 3 parameters, namely appearance, taste, and overall acceptability. The rating used was a rating with 5 scales (scale 1 was the least preferred scale to scale 5, which was the most preferred scale). The participants used for the sensory test were 50 untrained panelists. The sensory evaluation was performed following standard ethical guidelines for research involving human participants. All panelists participated voluntarily after being informed about the nature of the study and subsequently provided their consent. Ethical approval was not required because the study did not involve any invasive procedures or collection of personal, sensitive data.

5. Data analysis

The study was conducted using a Completely Randomized Design (CRD), where all treatments were randomly assigned to experimental units. Data was analyzed using analysis of variance (ANOVA) ($\alpha = 5\%$) on each test parameter. After analyzing ANOVA data, Duncan's Multiple Range Test at $\alpha = 5\%$ was used to identify the treatment level that made a significant impact. All statistical tests were supported by IBM SPSS Statistics 29 software.

Results and discussion

1. Physical characteristics

Texture analysis of bread is critical for determining quality attributes that directly influence consumer acceptability. Bread texture parameters in this research include hardness, cohesiveness, springiness, and chewiness. These textural attributes provide crucial insight into the bread's structural composition and sensory qualities. Table 2 shows the texture analysis results for sourdough bread and bread incorporated with green leaf flours.

Table 2 Texture analysis results

Parameter	Hardness (gf)	Cohesiveness	Springiness (mm)	Chewiness (Nmm)
C	129.19 ± 12.25^{d}	0.05 ± 0.02^{b}	12.29 ± 0.29^{a}	0.83 ± 0.27^{b}
TM1	153.30 ± 48.81^{cd}	0.05 ± 0.03^{ab}	6.35 ± 3.47^{ab}	0.45 ± 0.28^{b}
TM2	213.42 ± 29.13^{bc}	0.06 ± 0.03^{ab}	4.19 ± 2.35^{b}	0.54 ± 0.50^{b}
TM3	252.04 ± 16.40^{bc}	0.06 ± 0.02^{ab}	4.71 ± 1.06^{b}	0.58 ± 0.32^{b}
TS1	247.91 ± 34.11^{b}	0.06 ± 0.01^{ab}	5.39 ± 3.45^{b}	0.36 ± 0.25^{b}
TS2	271.66 ± 23.47^{ab}	0.07 ± 0.00^{ab}	7.10 ± 4.87^{ab}	0.77 ± 0.88^{b}
TS3	318.39 ± 29.49^{a}	0.08 ± 0.05^{ab}	9.43 ± 5.06^{ab}	1.63 ± 0.89^{ab}
TC1	167.28 ± 0.97^{cd}	0.06 ± 0.01^{ab}	6.33 ± 3.36^{ab}	0.85 ± 0.33^{b}
TC2	230.33 ± 58.58^{b}	0.08 ± 0.01^{ab}	6.48 ± 1.24^{ab}	1.02 ± 0.81^{b}
TC3	234.81 ± 45.19^{b}	0.20 ± 0.06^{a}	7.00 ± 2.34^{ab}	2.53 ± 1.37^{a}

Remark: Different superscript letters in the same column indicate significant differences ($\alpha = 5\%$).

Hardness in bread texture analysis refers to the maximum force required to compress a sample of bread, which is a key indicator of its structural integrity and overall quality. The implications of increasing or decreasing crumb hardness values in bread are substantial. Decreased hardness is often associated with improved freshness and palatability, favoring consumer preferences. Bread that is softer at the point of production tends to retain its qualities longer than harder bread, which is likely to go stale more quickly (Nkurikiye et al., 2023). Conversely, an increase in hardness can indicate staling or structural changes that may render the bread less enjoyable for consumers; for example, bread fortified with certain ingredients or subjected to prolonged storage showed a noticeable increase in hardness and a corresponding decline in sensory acceptance (Ammar et al., 2016).

In this study, the hardness of sourdough bread made solely with wheat flour was typically lower than that of sourdough bread enriched with green leaf flour. Adding green leaf flour alters the dough's gluten structure, which is crucial for maintaining the integrity and texture of bread. Gluten proteins provide dough elasticity and gas retention properties, leading to softer bread. When wheat flour is partially replaced with other flours lacking gluten, the overall protein network's strength diminishes, often resulting in increased hardness. Research has shown that substituting wheat with flours like chickpea can increase hardness due to the dilution of gluten and a lower gas retention capacity (Pasqualone et al., 2019; Setia et al., 2020; Yaver, 2022). The hardness values of sourdough bread incorporated with green leaf flour that were not significantly different from the control were TM1 and TC1, whose percentage of incorporation of green leaf flour was only 5%. Moreover, the hardness values in each of the other formulations were higher and were significantly different from the control sourdough bread.

The inclusion of green leaf flours generally introduces higher levels of dietary fiber. Fiber has been correlated with increased bread hardness due to increased water absorption capacity and changes in dough rheology. Increased fiber content can lead to tougher bread textures, as observed when composite flours, including oat and barley, were used, resulting in greater firmness than wheat-only counterparts (Malik, 2016). Additionally, the fibrous nature of these green leaf flours can affect moisture retention, potentially leading to drier doughs and harder bread products (Mishra et al., 2021). Sourdough bread incorporated with *Sauropus androgynus* flour had a higher hardness value than the other 2 green leaf flours, which is likely caused by the lower moisture retention in its flour. Studies indicate that higher moisture retention in leaf-flour-enriched breads can soften their texture, enhancing the hydration of the gluten network (Mishra et al., 2021).

Cohesiveness in bread texture analysis refers to how the bread structure holds together during mechanical deformation, reflecting the bread's internal cohesion. Higher cohesiveness implies that the bread will maintain its structural integrity during chewing, resulting in a more pleasant feeling in the mouth as it forms a bolus without crumbling. Conversely, lower cohesiveness suggests a bread product is more likely to fracture, leading to undesirable textural experiences such as crumbling during mastication (Matos & Rosell, 2012). A decrease in cohesiveness typically indicates poorer textural qualities, leading to bread that may feel dry and less satisfying in the mouth. Research shows that significant variations in cohesiveness can arise from ingredient changes, such as fiber addition or alterations in flour composition, which directly affect the bread's ability to maintain its structure (Atudorei et al., 2023; Beltrão Martins et al., 2020). On the other hand, increasing cohesiveness is often perceived positively, as it is associated with better chewing properties and overall sensory acceptance (Han et al., 2024).

The cohesiveness of sourdough bread, whether made solely with wheat flour or by incorporating green leaf flours such as *Moringa oleifera*, *Sauropus androgynous*, and *Centella asiatica*, can yield similar cohesiveness values due to the fundamental properties of the gluten network within the wheat flour. Research has shown that introducing leaf flours may preserve this gluten structure, thereby maintaining comparable cohesiveness when adding these ingredients (Firdausy et al., 2020). When green leaf flour is incorporated, the interaction between the wheat flour gluten and the soluble fibers in the leaf flour can have a noticeable effect. These fibers may contribute to water retention and thus enhance the dough's moisture profile, which is crucial for maintaining cohesiveness (Firdausy et al., 2020; Kokoh et al., 2022). For example, Moringa's nutritional profile includes significant amounts of dietary fiber, which may result in a more pliable dough matrix but does not hinder the fundamental gluten structure provided by the wheat flour (Mishra et al., 2021; Subramani et al., 2024) as for cases with *Sauropus androgynus* and *Centella asiatica*. In addition, green leaf flour adds nutritional benefits without drastically altering the gluten network, allowing the bread's cohesiveness to remain consistent compared with that of 100% wheat flour sourdough bread, except for formulation TC3 with *Centella asiatica* incorporation of 15%, which was significantly different from the control.

With an increase in the percentage of green leaf flour in the formulation, the cohesiveness of the bread can increase due to enhanced water absorption and enriched fiber content. The fibrous nature of leaf flours can lead to a greater capacity for water retention, which supports the development of a better gluten network during fermentation and baking (Firdausy et al., 2020). Furthermore, higher fiber content leads to a denser dough structure that contributes positively to cohesiveness, creating a firmer loaf with improved handling properties and stability (Mishra et al., 2021). Additionally, it has been documented that the physicochemical properties of these green leaves, when processed into flour, exhibit gel-forming capabilities that can contribute to the overall cohesiveness of the sourdough bread. The soluble fibers found in such leaves have been reported to form gels that can aid in moisture retention and texture enhancement (Kokoh et al., 2022). Research suggests that not only the gluten content but also the introduced fiber from green leaf additions works together to produce a cohesive bread product, even as the percentage of incorporation increases.

Springiness is a crucial texture parameter in bread analysis that quantitatively captures the elasticity of the bread crumb after being compressed. Specifically, springiness quantifies the degree to which a bread product will return to its original shape post-deformation, reflecting its ability to recover from being squished or compressed (Matos & Rosell, 2012). This textural characteristic is essential for evaluating the perceived freshness and quality of bread, as high springiness values generally correlate with greater elasticity and a desirable lightness in texture, which consumers often associate with freshly baked products (Puerta et al., 2020). A decrease in springiness can suggest aging or the staling of bread, often leading to increased brittleness and crumbliness during cutting and consumption (Martínez et al., 2013).

In this study, sourdough bread made exclusively from wheat flour typically exhibited higher springiness than sourdough bread made with green leaf flours. One of the primary factors contributing to the higher springiness of wheat flour sourdough is its gluten content. Wheat flour contains a high concentration of gluten proteins, critical for dough elasticity and gas retention during fermentation. Gluten forms a network that effectively traps the carbon dioxide produced by yeast and lactic acid bacteria, leading to a well-aerated structure that can recover after deformation (Hayta & Ertop, 2018; Landis et al., 2021). In contrast, green leaf flours contain lower amounts of gluten and thus can alter this balance. It can disrupt the gluten network, reducing overall gas retention capacity and, consequently, reducing the springiness of the resulting bread. Additionally, the physical interaction between the flour and the added components can impact the rheological properties of the dough. Research showed that the incorporation of non-gluten components can lead to decreased dough elasticity and increased stiffness, which correlates with reduced springiness (Peñalver et al., 2023; Wang et al., 2018). The presence of fibrous materials or unique proteins in green leaf flours may introduce structural challenges that compromise the formation of an adequate gluten matrix needed for optimal springiness.

The metabolic activity of lactic acid bacteria (LAB) during sourdough fermentation plays a significant role in the texture of the bread. LAB can enhance moisture distribution and create by-products that contribute to softer bread characteristics (Sahin et al., 2024; Wolter et al., 2014). When leaf flours are included, the fermentation dynamics can be altered, possibly resulting in variations in acidity and other compounds, affecting the textural profile unfavorably (Clark et al., 2024; Zhao et al., 2015). This explains why this research, with its increase in the percentage of *Moringa oleifera* flours, led to a decrease in the springiness value of the resulting bread. Some volatile compounds produced during fermentation can enhance sensory characteristics but may alter the physical properties, including springiness. Conversely, flours from *Sauropus androgynous* and *Centella asiatica* demonstrate a different behavior when incorporated into sourdough bread formulations. These flours may contain bioactive compounds that interact positively with the gluten network. For instance, it is possible that *Sauropus* and *Centella* flours enhance the viscoelastic properties of the dough due to their moderate protein and carbohydrate content, which could contribute positively to springiness. Research has suggested that the incorporation of alternative flours can improve bread quality, including its springiness, when the right components are balanced in the dough (Xu et al., 2019). Additionally, the unique polysaccharides present in these plants might improve the water retention capacity of the dough, leading to a more elastic structure that promotes better gas retention and overall springiness, as supported by previous studies on mixed sourdough flours (Molfetta et al., 2021).

Chewiness is a critical parameter in bread texture analysis, defined as the energy required to masticate a bread sample into a consumable form. An increase in the chewiness value typically indicates a denser and firmer bread texture, necessitating more energy during mastication (Pasqualoni et al., 2024; Zhang et al., 2023). This increase often correlates with higher hardness levels, where the bread exhibits reduced moisture content and altered structural qualities, such as decreased specific volumes and porosity (Sang et al., 2023; Zhang et al., 2023). Conversely, a decrease in chewiness tends to indicate a softer and potentially less satisfying texture, which may not align with consumer expectations, particularly for certain types of bread where a chewy crust is desirable (Shen et al., 2022). A soft texture might be perceived as less flavorful and less appealing for specific consumer segments who favor a more traditional bread experience (Seyedain-Ardabili & Azizi, 2024; Singh et al., 2014).

Incorporating leaf flours can significantly influence the bread's hydrocolloid characteristics, protein composition, and dietary fiber content. For instance, green leaf is known for its high nutritional value and bioactive compounds, which can alter the gluten network development during fermentation and baking. This may improve the structure and textural quality of the final product, leading to increased chewiness with its increasing percentage in the sourdough bread formulation. However, the reviewed articles did not explicitly support direct evidence linking green leaf flour to enhanced chewiness, particularly concerning gluten network improvement in sourdough (Nouska et al., 2023). Control sourdough bread made with only wheat flour might exhibit lower chewiness due to a relatively less complex protein composition, resulting in a less cohesive structure. Both *Sauropus androgynus* and *Centella asiatica* leaf flours may introduce additional components, like fibers, that influence the rheological properties of the dough. These components can affect moisture retention and the elasticity of the gluten matrix, which may positively impact chewiness, as shown in Table 2. The fibrous nature of these leaf flours likely contributes to the bread's crumb structure and chewiness, enhancing moisture retention (Denkova et al., 2014; Wang et al., 2018).

In the analysis of bread texture, the volume of bread is also a crucial parameter that significantly influences consumer perception and acceptance. Bread volume is often regarded as a primary indicator of flour strength and overall bread quality, as it relates to the ability of the dough to retain gas during fermentation and baking (Butt et al., 2001). Accurately measuring bread volume provides insights into the structural integrity of the gluten network, which is essential for achieving desirable bread characteristics. Higher bread volumes generally indicate a well-developed gluten structure that can trap gases, resulting in a lighter, airier loaf. Conversely, smaller bread volumes may result from poor dough development, which can be attributed to factors such as weak gluten formation or improper fermentation conditions (Różyło & Laskowski, 2011). A decrease in gluten content correlates strongly with reduced gas retention capacity, leading to a loss of loaf volume and compromised bread texture. Table 3 shows the results for bread volume value in this research. It shows that the control sample of sourdough bread had a higher sourdough bread volume development value than the sourdough bread samples incorporated with *Moringa oleifera* flour and *Sauropus androgynus* flour, but that the sourdough bread samples incorporated with *Centella asiatica* flour had a higher bread volume development value than the control, except for the incorporation a percentage at 15%. It also shows that the higher the proportion of green leaf flours incorporated in the sourdough bread formulation, the lower the bread development volume in all samples.

Table 3 Bread volume analysis results

Parameter	Bread volume (mL)
C	181.67 ± 2.89^{b}
TM1	142.67 ± 5.00^{de}
TM2	139.00 ± 1.00^{e}
TM3	119.00 ± 1.00^{f}
TS1	163.67 ± 3.51^{c}
TS2	142.67 ± 2.52^{de}
TS3	139.00 ± 1.00^{e}
TC1	222.67 ± 2.52^{a}
TC2	219.00 ± 1.00^{a}
TC3	145.00 ± 5.00^{d}

Remark: Different superscript letters in the same column indicate significant differences (α = 5%).

The observed variation in bread volume among sourdough bread formulated solely from wheat flour compared to those incorporating green leaf flours can be attributed to several interrelated factors. The gluten content of wheat flour plays a crucial role in gas retention during bread fermentation and baking. High gluten levels facilitate the formation of a stable gluten network, essential for trapping carbon dioxide produced during fermentation, leading to greater bread volume (Messina et al., 2024; Nouska et al., 2023). When green leaf flour is introduced into the formulation, it significantly dilutes the gluten content, thereby impairing the gas retention abilities of the dough (Kokoh et al., 2019). This gluten-strength reduction often results in a decrease in the bread volume compared to the control made solely from wheat flour (Kokoh et al., 2019; Mishra et al., 2021).

The specific properties of *Moringa oleifera* flour, such as its moisture absorption characteristics and fiber content, can also influence the dough's fermentation properties. More fibrous materials typically lead to a denser crumb and can hinder dough expansion (Kokoh et al., 2019). In contrast, *Sauropus androgynous*, while contributing additional fiber, has a modified impact on dough consistency and fermentation dynamics, potentially yielding better gas retention than *Moringa oleifera* flour. However, specific evidence for this claim is sparse (Kokoh et al., 2019). When assessed against *Centella asiatica* flour, which may present different viscoelastic properties facilitating better aeration and gas retention due to its unique protein and carbohydrate composition, one can see why sourdough bread incorporating *Centella asiatica* could yield a higher volume than those with *Moringa oleifera* and *Sauropus androgynus* (Škrobot et al., 2022). The comparative success of *Centella asiatica* in this context underscores the significant role ingredient choice plays in the overall dough characteristics and fermentation performance.

2. Chemical characteristics

Assessing antioxidant activity and pH in sourdough bread, particularly those enhanced with green leaf flours like *Moringa oleifera*, *Sauropus androgynus*, and *Centella asiatica*, is crucial for understanding their chemical characteristics and health benefits. These 2 factors, antioxidant activity and pH, are intricately linked to the fermentation process and the resulting nutritional value of the bread. Table 4 shows the results of antioxidant activity and pH of sourdough bread. It showed that the control sample differed significantly from the other samples in antioxidant activity value. The control sample antioxidant activity was greater than that of the sourdough bread incorporated with *Moringa oleifera* flour at a percentage of 5–10%, *Sauropus androgynus* flour at a percentage of 5%, and *Centella asiatica* flour at a percentage of 5–10%. The other samples all had antioxidant activity values that were higher than the control, as it was observed that all sourdough bread samples containing 15% green leaf flour had higher antioxidant activity levels than the control sample, with the highest antioxidant activity values coming from the sample with *Sauropus androgynus* flour.

The analysis of antioxidant activity in sourdough bread is essential for several reasons, particularly linked to health benefits and consumer preferences. The implications of increasing or decreasing antioxidant activity values in bread are closely tied to its texture and overall consumer acceptance. Increased antioxidant levels, often associated with enhanced hardness and chewiness due to polyphenols and their interaction with the gluten matrix, can lead to a more desirable texture if balanced correctly within sensory thresholds (Homyuen et al., 2023). Conversely, decreased antioxidant activity may correspond with undesirable bread properties, such as staleness, potentially lowering consumer satisfaction (Karimi, 2012; Sik et al., 2025).

The comparison of antioxidant activity in sourdough bread made solely from wheat flour versus those incorporating *Moringa oleifera* flour, *Sauropus androgynus*, and *Centella asiatica* reveals critical insights into the biochemical processes involved in fermentation and the interaction between ingredients. Higher antioxidant activity in traditional wheat sourdough bread can be attributed to the unique nutritional composition and fermentation characteristics of wheat flour and the specific antioxidants released during the sourdough fermentation process. Wheat flour contains significant concentrations of ferulic acid and other phenolic compounds that enhance antioxidant capacity when subjected to sourdough fermentation. Wheat flour contains significant concentrations of ferulic acid and other phenolic compounds that are known to enhance antioxidant capacity when subjected to sourdough fermentation. Studies have indicated that traditional sourdough fermentation elevates the concentration and bio-accessibility of these compounds, particularly through the action of lactic acid bacteria (LAB) (Banu et al., 2010; Coda et al., 2012). The fermentation process catalyzes the breakdown of complex carbohydrates and liberates bound phenolic acids, which contribute to the elevated antioxidant properties of the resulting bread (Dapčević-Hadnađev et al., 2022).

Moreover, as LAB metabolizes the flour, it produces metabolites that exhibit antioxidant effects, further boosting the sourdough bread's overall antioxidant activity (Coda et al., 2012). In contrast, while *Moringa oleifera* flour is recognized for its nutritional benefits, its incorporation and the fermentation process might limit its beneficial effects when blended with wheat flour in a sourdough formulation. Research showed that the introduction of additional non-conventional flours, such as those from *Moringa oleifera* flour, *Sauropus androgynus*, and *Centella asiatica*, may not lead to a commensurate rise in antioxidant levels compared to traditional wheat-based variants. This may be due to the dilution of bioactive compounds when mixed with wheat and due to the specificity of LAB fermentation with certain grain types (Pejcz et al., 2023; Peñalver et al., 2023). Additionally, fermentation parameters play a vital role in determining the final antioxidant profile of sourdough breads. The metabolic pathways activated during the fermentation of wheat flour using LAB generate diverse metabolites that enhance both flavor and nutritional profiles, including the production of bioactive peptides and free radical scavengers that may occur to a lesser extent with the incorporation of the aforementioned flours (Gil-Cardoso et al., 2021; Ertop & Coşkun, 2018).

Table 4 Antioxidant activity and pH results

Parameter	Antioxidant activity (ppm)	pH
C	5.58±0.91^{bc}	5.36±0.02^{f}
TM1	3.91±0.11^{d}	5.53±0.04^{de}
TM2	4.50±0.20^{cd}	5.75±0.03^{c}
TM3	6.98±0.37^{b}	5.81±0.04^{c}
TS1	4.17±0.48^{cd}	5.47±0.02^{ef}
TS2	6.49±0.59^{b}	6.15±0.04^{b}
TS3	9.88±0.15^{a}	6.40±0.05^{a}
TC1	3.77±0.77^{d}	5.51±0.04^{ef}
TC2	4.69±0.07^{d}	5.56±0.02^{de}
TC3	5.79±0.23^{bc}	5.70±0.02^{cd}

Remark: Different superscript letters in the same column indicate significant differences (α = 5%).

The pH analysis in bread-making is crucial because it influences various properties, affecting the quality and acceptability of the final product. The pH level of bread can significantly affect fermentation, texture, flavor, and shelf life. A pH of around 4.5 to 5.5 is often considered ideal for optimizing yeast activity and achieving desirable bread characteristics (Martins et al., 2021). Maintaining a proper pH helps form gluten, which is essential for the texture and volume of bread. Specifically, pH values near the isoelectric point of gluten (approximately 6.4) can enhance gluten's functional properties, thereby impacting the bread's chewiness and overall quality (Ferreyra et al., 2021). Table 4 shows that the entire range of pH values of sourdough bread samples in this study had entered the recommended ideal pH range, except the sourdough bread samples incorporated with *Sauropus androgynus* flour at percentages of 10% and 15%, which differed significantly from the other samples. It can also be seen that higher percentages of green leaf flour incorporated in the sourdough bread formulation caused an increase in the pH value in all samples.

Variations in pH can indicate microbial activity and the metabolic processes occurring during fermentation. A decrease in pH is generally observed as fermentation progresses due to the production of organic acids, such as lactic and acetic acids, by lactic acid bacteria (Martins et al., 2021). This drop in pH enhances the flavor profiles of the bread and increases its antifungal properties, which are vital for prolonging freshness and shelf life (Rizzello et al., 2011). Conversely, an increase in pH can suggest a reduction in fermentation efficiency or microbial spoilage, potentially resulting in poor texture and flavor characteristics (Aplevicz et al., 2013). Higher pH levels can also lead to diminished gluten structure and reduced gas retention, adversely affecting the loaf's volume and crumb texture (Yan et al., 2020). Regarding consumer preferences, most individuals tend to favor bread that has a balanced pH, typically within the acidic range of 4.5 to 5.5. Such pH levels are associated with improved flavor, enhanced aroma, and better preservation of the bread's physical and nutritional qualities (Bender et al., 2018). Notably, recent studies emphasize that the acidity in bread, partly shaped by the pH, can influence satiety responses and subsequent food intake, suggesting a psychological dimension to consumer preferences linked to bread's acidity levels (Sandvik et al., 2016).

3. Sensory characteristics

Sensory analysis is the structured assessment of foods and other products through the human senses, sight, smell, taste, touch, and even hearing. For bread, sensory evaluation is especially important in examining attributes like flavor, texture, aroma, and visual appeal, all of which strongly influence consumer preference. In this study, the sensory evaluation of sourdough bread was conducted with 50 untrained panelists aged between 22 and 35 years. The sensory acceptance outcomes are presented in Table 5, showing significant differences across all tested parameters: appearance, taste, and overall acceptability. Notably, breads containing green leaf flour were rated less favorably compared to control by the panelists across all sensory attributes.

Table 5 Sensory analysis results

Parameter	Appearance	Taste	Overall
C	4.35 ± 0.70^{a}	4.12 ± 0.78^{a}	4.21 ± 0.70^{a}
TM1	3.59 ± 0.80^{b}	2.82 ± 0.31^{bc}	3.00 ± 0.16^{b}
TM2	3.12 ± 0.99^{bcd}	2.47 ± 0.67^{cd}	2.53 ± 0.80^{bc}
TM3	2.65 ± 0.17^{d}	1.94 ± 0.23^{d}	2.06 ± 0.90^{c}
TS1	3.24 ± 0.75^{bcd}	2.76 ± 0.32^{bc}	2.82 ± 0.95^{b}
TS2	3.06 ± 0.43^{bcd}	2.06 ± 0.43^{bc}	2.41 ± 0.62^{bc}
TS3	2.82 ± 0.88^{cd}	1.88 ± 0.27^{d}	2.12 ± 0.15^{c}
TC1	3.65 ± 0.79^{b}	3.88 ± 0.49^{a}	3.76 ± 0.56^{a}
TC2	3.47 ± 0.51^{b}	3.47 ± 0.17^{ab}	3.51 ± 0.67^{b}
TC3	3.29 ± 0.99^{bc}	2.82 ± 0.23^{bc}	3.06 ± 0.28^{b}

Remark: Different superscript letters in the same column indicate significant differences (α = 5%).

Appearance plays a fundamental role in sensory analysis, serving as the first point of interaction between consumers and food products. It encompasses attributes such as color, shape, size, and texture, which significantly influence consumer perceptions and preferences (Pasqualone et al., 2011). Particularly in bread, the appearance can dictate expectations of taste and quality, thus affecting overall acceptance and marketability (Molfetta et al., 2021). For instance, visual characteristics like crust color and crumb structure are indicators of proper baking and ingredient quality, which can indicate texture and flavor (Parenti et al., 2013). The sourdough bread control, which does not incorporate green leaf flours such as *Moringa oleifera*, *Sauropus androgynus*, or *Centella asiatica*, tends to exhibit a higher appearance rating than its sourdough counterparts with these additions.

In this study, the unique protein and pigment characteristics of green leaf flours seem to impart a different coloration that affects the appearance in sourdough bread. Research has shown that breads made from flours with higher carotenoid content, such as those derived from certain wheat varieties, can exhibit more robust coloration, leading to a more visually appealing appearance (Wolter et al., 2014). In contrast, the presence of certain green leaf flours often results in color alterations that may not resonate positively with consumer expectations (Zaidiyah et al., 2022). For example, adding green leaf flour can darken the dough, which might be perceived as less appetizing (Škrobot et al., 2022).

It also can be seen from Table 5 that the higher incorporation percentage of green leaf flour into sourdough bread formulations lead to a decrease in the perceived appearance ratings of the final product, which imparted a greenish hue to the dough. Such color changes are not always viewed favorably by consumers, who may associate uniformity in color with quality (Doğan, 2021). Research indicates that consumer preferences heavily favor aesthetically pleasing products, thus, deviations from traditional bread colors can adversely affect consumer perception, particularly in cultures where bread color is a critical quality attribute (Mishra et al., 2021).

Taste in sensory analysis is a fundamental aspect of evaluating food products, including bread. It encompasses the sensory perception of flavor, which combines the basic tastes of sweet, sour, salty, bitter, and umami along with the olfactory sensations that contribute to flavor perception (Heitmann et al., 2017). In bread production, analyzing taste is particularly important as it affects consumer satisfaction and overall product success. In this study, the comparison between sourdough bread control and sourdough bread incorporating green leaf flours noted that the sourdough bread control, without the incorporation of green leaf flours, achieves a higher rating for taste. The incorporation of green leaf flours tends to introduce additional flavors that may not align with consumer preferences or may dilute the desirable sensory attributes developed during sourdough bread fermentation. Studies have indicated that additions like Moringa oleifera can impact the organoleptic qualities negatively, leading to lower taste ratings due to potential bitterness or off-flavors that are less acceptable to consumers (Kim & Chung, 2017).

It also can be seen from Table 5, that generally, as the percentage of these green flours increases, the taste ratings tend to decline. *Moringa oleifera*, for example, is known for its rich nutrient profile, but its substantial chlorophyll content and the presence of potentially bitter phytochemicals, such as saponins, may contribute to undesirable flavors when used in high concentrations (Arif et al., 2022; Devisetti et al., 2016). Fatima et al., (2024) noted that while lower amounts of Moringa flour maintained favorable sensory evaluation scores for bread, higher concentrations led to negative effects on taste and overall acceptability due to bitterness and an astringent aftertaste, which consumers often find off-putting. Similarly, Devisetti et al., (2016) reported that excessive Moringa addition adversely affected the sensory properties by introducing bitterness, which could overshadow the bread's original flavor.

Sauropus androgynus (commonly known as Katuk) and *Centella asiatica* (also known as Gotu Kola) also have unique flavor profiles that influence bread taste. Studies regarding these flours indicate that they may not impart the same level of bitterness as Moringa, thus possibly enhancing their acceptability when used in moderation. While comparisons between these green flours show that *Centella asiatica* may yield better sensory ratings than *Moringa oleifera* and *Sauropus androgynus*, this can be attributed to its more favorable flavor characteristics, which do not overpower or detract from the overall taste of the bread, making it more appealing to panelists (Khan et al., 2023).

Overall acceptability in sensory analysis refers to consumers' overall impression of a product based on its sensory attributes, including taste, aroma, texture, and visual appeal. It serves as a crucial metric in understanding the likability of food products, including bread. A major finding from related studies indicates that consumer preference is predominantly influenced by taste, which is consistently highlighted as a significant determinant for overall acceptability (Andersen et al., 2019). This study showed that the sourdough bread control with no incorporation of green leaf flours achieves the highest rating for overall acceptability. It can also be seen from Table 5 that as the incorporation of green leaf flour increases, overall acceptability ratings tend to decline due to potential off-flavors or undesirable texture characteristics. This tendency is corroborated by studies indicating that a higher incorporation percentage of green leaf flours can detract from consumer enjoyment (Mishra et al., 2021). Nevertheless, while nutritional improvements can be observed with the inclusion of these flours, they do not always translate to positive sensory evaluations, suggesting that consumer preferences may prioritize sensory attributes over nutritional enhancements.

The incorporation of higher percentages of green leaf flours in sourdough bread formulations tends to lead to a decrease in overall sensory acceptability. Studies indicate that exceeding optimal levels of ingredient substitution can adversely affect bread quality. For instance, Rai et al. 2012 demonstrate that increasing non-wheat flours beyond a certain percentage can detract from sensory quality metrics such as crumb texture and flavor (Rai et al., 2012). Similarly, the addition of Moringa leaf flour has been linked to undesirable sensory characteristics, including bitterness and astringency, which may lead to lower acceptability scores (Devisetti et al., 2016). When comparing specific types of green leaf flour, *Centella asiatica* is often more favored than *Moringa oleifera* and *Sauropus androgynus* in terms of sensory acceptability, as the incorporation of 10% of *Centella asiatica* flour (TC1) had no significant difference with the sourdough bread control. While research indicates that unique aroma compounds generated during fermentation can influence consumer preferences (Rizzello et al., 2019), other studies show that *Moringa* and *Sauropus* may produce harsher flavors, whereas *Centella* contributes milder tastes, potentially leading to higher sensory evaluation scores (Devisetti et al., 2016; Mishra et al., 2021).

Conclusion

The incorporation of green leaf flours in sourdough bread formulations significantly affected their texture, volume, antioxidant activity, and pH. Hardness values differed from the control in most samples, except TM1 and TC1, while cohesiveness and chewiness were generally maintained, except for in TC3. Springiness was reduced in TM2, TM3, and TS1. Bread volume was generally lower with *Moringa oleifera* and *Sauropus androgynus* flours compared to the control, whereas breads containing *Centella asiatica* flour showed equal or higher loaf volume, except at the 15% level. Antioxidant activity increased with higher substitution levels, with the highest values obtained from *Sauropus androgynus*at at 15%. pH analysis indicated that most breads were within the ideal range, except TS2 and TS3, which showed elevated values. Sensory evaluation revealed that the control sample was preferred overall; however, breads with *Centella asiatica* flour were consistently rated higher than those with *Moringa oleifera* or *Sauropus androgynus*. Based on these findings, *Centella asiatica* at lower substitution levels (5–10%) demonstrates the greatest potential for further development, as it balances acceptable bread quality with improved nutritional and antioxidant properties. Further studies should therefore focus on optimizing *Centella asiatica* incorporation, including processing strategies (e.g., pre-treatment or fermentation of the flour) to improve flavor and texture, and expanded sensory evaluations across diverse consumer groups to validate market acceptability.

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Conflicts of interest

The authors declare no conflicts of interest.

Declaration of Generative Artificial Intelligence in the Writing Process

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to improve language clarity and readability. Grammarly was used for grammar and spelling checks. All scientific content, experimental design, data analysis, and conclusions were developed by the authors. The authors reviewed and edited the output and take full responsibility for the accuracy and integrity of the manuscript.

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