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Exploring Catfish (*Clarias* sp.) Flour and Moringa Leaves as Dual Functional Ingredients in the Development of Protein-Rich Fish-Based Cookies

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ABSTRACT

Functional foods have gained increasing attention due to their ability to provide health benefits beyond basic nutrition. This study aimed to evaluate the nutritional composition, mineral content, total phenolic compounds, and antioxidant activity of cookies enriched with catfish (*Clarias* sp.) flour and moringa (*Moringa oleifera*) leaf flour. A completely randomized design (CRD) was applied with four formulations: control (A), 5% catfish flour + 5% moringa leaf flour (B), 10% catfish flour + 5% moringa leaf flour (C), and 15% catfish flour + 10% moringa leaf flour (D). Proximate analysis followed AOAC methods, while calcium and iron were determined using Atomic Absorption Spectrophotometry. Total phenolics were quantified using the Folin-Ciocalteu method, and antioxidant activity was assessed by DPPH radical scavenging assay. The results showed that protein content increased from 7.5% in the control to 14.3% in the highest substitution, while fat content rose from 15.8 to 17.5%. Carbohydrates decreased from 65.1 to 58.5%, whereas ash content increased from 1.4 to 2.1%. Calcium and iron concentrations improved from 50 and 2.0mg/100g to 120 and 4.2mg/ 100g, respectively. Total phenolic content increased from 1.1 to 5.0mg GAE/g, with antioxidant activity rising from 12.5 to 42.1%. These findings demonstrate that catfish flour and moringa leaves act synergistically as dual functional ingredients, significantly enhancing both nutritional quality and antioxidant capacity of cookies. The novelty of this study lies in combining fish-based protein with plant-derived bioactives, offering a sustainable approach to developing locally sourced functional foods. The product shows potential as a healthy alternative snack to help address protein-energy malnutrition and micronutrient deficiencies.

INTRODUCTION

The functional food sector has experienced remarkable growth in recent years, driven by increasing public awareness of the health benefits that extend beyond basic nutrition **(Intrasook *et al.*, 2024)**. Functional foods are enriched with bioactive compounds such as antioxidants, bioactive peptides, and vitamins that play a crucial role in preventing degenerative diseases and enhancing the human immune system **(Islamy, 2019; Peñalver *et al.*, 2022; Islamy *et al.*, 2024a, b)**.

Cookies, as one of the most popular bakery products, offer an attractive medium for functional food development. By modifying conventional formulations with protein-rich and antioxidant-based ingredients, cookies can be transformed into nutritionally enhanced products with additional health benefits **(Ngah *et al.*, 2024)**.

Catfish (*Clarias* sp.) is a locally available protein source that is rich in high-quality proteins, essential amino acids, and omega-3 fatty acids, all of which support cardiovascular and brain health **(Adi *et al.*, 2025)**. Incorporating catfish flour into bakery products has been shown to improve protein content without compromising taste quality **(Abdel-Mobdy *et al.*, 2021)**. Furthermore, catfish provides vital micronutrients such as B-complex vitamins, calcium, phosphorus, and iron, which are essential for growth and metabolic functions **(Nemati *et al.*, 2024; Adi *et al.*, 2025)**.

Several plants possess natural bioactive compounds and nutritional benefits **(Islamy *et al.*, 2024b, 2025c; Serdiati *et al.*, 2024)**. On the other hand, moringa leaves (*Moringa oleifera*) are recognized as a rich source of bioactive compounds, including flavonoids, polyphenols, vitamin C, and carotenoids, which provide strong antioxidant activity and protection against oxidative stress **(Peñalver *et al.*, 2022; Soto *et al.*, 2025)**. In addition, moringa leaves contain high-quality proteins and easily absorbed micronutrients such as calcium and iron, further complementing their health benefits **(Jin *et al.*, 2025)**. Both *in vitro* and clinical studies have highlighted moringa's role in reducing free radicals and chronic inflammation, strengthening its potential as a functional food ingredient.

The synergistic combination of animal-based proteins and plant-derived antioxidants has gained increasing attention in functional food innovation. Previous studies have shown that this interaction can improve bioavailability, enhance nutritional quality, and strengthen protective effects against oxidative stress **(Devi *et al.*, 2023; Ngah *et al.*, 2024)**. Moreover, enriching cookies with moringa leaf flour or other plant extracts has been reported to increase antioxidant capacity and extend shelf life, further supporting their functional value **(Marak *et al.*, 2019; Pirca-Palomino *et al.*, 2024)**.

The use of local raw materials such as catfish and moringa also aligns with global trends in sustainable functional foods, emphasizing natural ingredients, food security, and environmental sustainability **(Intrasook *et al.*, 2024; Suwarno, 2024; Hati *et al.*, 2025)**. By utilizing domestic resources, such innovations not only create value-added products but also support food security and economic development at the community level.

Therefore, the development of functional cookies enriched with catfish flour and moringa leaves presents an opportunity to address protein-energy malnutrition while delivering antioxidant-rich foods that may help reduce the risk of chronic diseases such as hypertension, diabetes, and cardiovascular disorders **(Peñalver *et al.*, 2022; Yadnya *et al.*, 2025)**. This study was conducted to evaluate the effect of substituting catfish flour and moringa leaf flour on the nutritional composition, antioxidant activity, and chemical characteristics of cookies, with the aim of establishing their potential as a functional food product.

MATERIALS AND METHODS

Experimental design

The study was conducted using a completely randomized design (CRD) with four formulations of cookies based on different substitution levels of catfish flour and moringa leaf flour. The treatments included:

- **A (Control):** 100% wheat flour without substitution
- **B:** 5% catfish flour + 5% moringa leaf flour
- **C:** 10% catfish flour + 5% moringa leaf flour
- **D:** 15% catfish flour + 10% moringa leaf flour

All formulations were evaluated for their proximate composition, mineral content, total phenolic compounds, and antioxidant activity.

Materials

The primary ingredients consisted of catfish (*Clarias* sp.) flour, moringa (*Moringa oleifera*) leaf flour, wheat flour, sugar, margarine, eggs, baking powder, and vanilla flavoring. All reagents used for chemical analyses were of an analytical grade.

Preparation of catfish and moringa leaf flours

Catfish were cleaned, steamed, and dried in a hot-air oven at 60–70°C until the moisture content was below 10%. The dried fish were ground into fine powder and were stored in airtight containers **(Hasanah, 2025)**. Fresh moringa leaves were washed, oven-dried at 40– 50°C, and ground into fine powder, then stored under the same conditions **(Prayitno *et al.*, 2022)**.

Cookie preparation

The cookies were prepared following standard bakery procedures. Dry ingredients (wheat flour, catfish flour, moringa flour, and baking powder) were mixed thoroughly, margarine, sugar, and eggs were blended separately, then combined with the dry mixture. The dough was shaped and baked in a preheated oven at 150°C for 15–20 minutes. The baked cookies were cooled to room temperature and stored in airtight containers for subsequent analyses.

Proximate composition

Moisture, crude protein, fat, carbohydrate, and ash contents were determined following the official methods of **AOAC (2016)**. Carbohydrate content was calculated by difference.

Mineral analysis

Calcium (Ca) and iron (Fe) contents were determined using an Atomic Absorption Spectrophotometer (AAS) following the official protocol **(Okafor *et al.*, 2024)**.

Total phenolic content

Total phenolic compounds were measured using the Folin-Ciocalteu method, with results expressed as mg gallic acid equivalents (GAE) per gram of sample **(Islamy *et al.*, 2024a; Prayitno *et al.*, 2025)**.

Antioxidant activity

Antioxidant activity was assessed using the DPPH radical scavenging assay. Absorbance was measured at 517nm using a UV-Vis spectrophotometer, and results were expressed as percentage inhibition **(Wiji Nurani *et al.*, 2014; Agba *et al.*, 2024)**.

Statistical analysis

All experiments were carried out in triplicate. Data were analyzed using SPSS software version 25. Analysis of variance (ANOVA) was applied to determine significant differences among treatments, followed by Tukey's multiple comparison test at a 5% significance level. Results are presented as mean ± standard deviation.

RESULTS

Protein content

The substitution of wheat flour with catfish and moringa leaf flours significantly increased the protein content of cookies ($P < 0.05$). Protein levels rose from 7.5% in the control to 14.3% in the highest substitution formula (15% catfish flour + 10% moringa leaf flour) (Fig. 1).

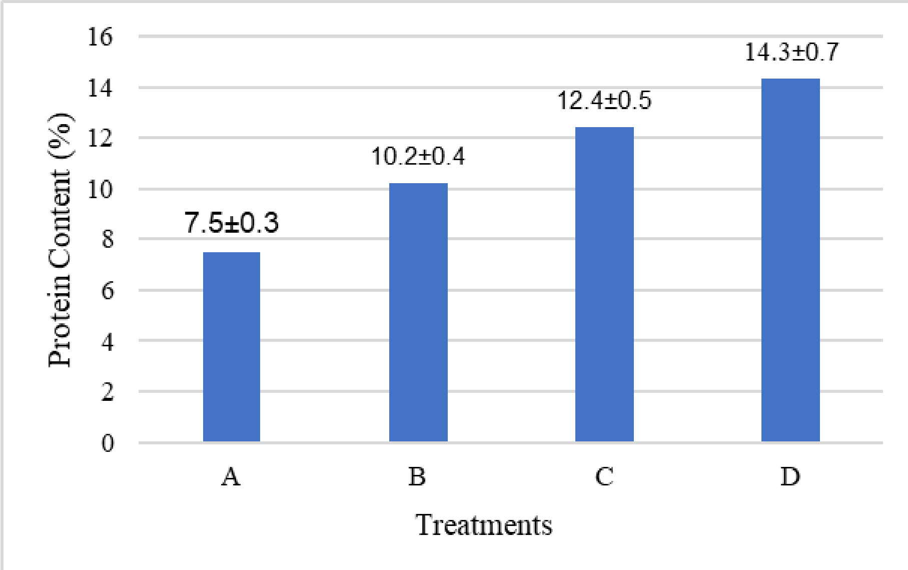

Fig. 1. Protein content of cookies with varying substitution levels of catfish flour and moringa leaf flour

Fat content

Fat content showed a slight but consistent increase with higher levels of catfish flour substitution, ranging from 15.8% in the control to 17.5% in the maximum substitution group (Fig. 2).

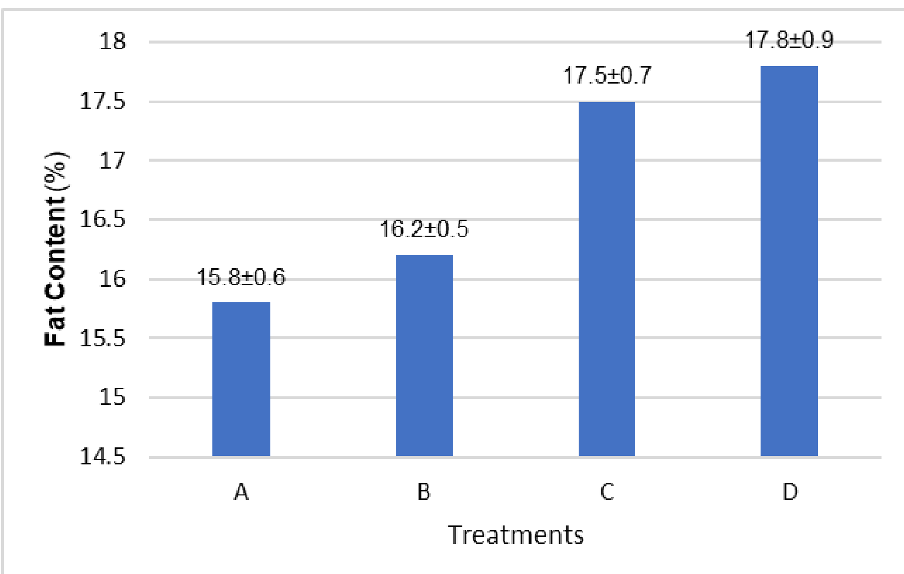

Fig. 2. Fat content of cookies with different substitution formulations

Carbohydrate content

Carbohydrate levels decreased as protein and fiber-rich ingredients were introduced, dropping from 65.1% in the control to 58.5% in the highest substitution treatment (Fig. 3).

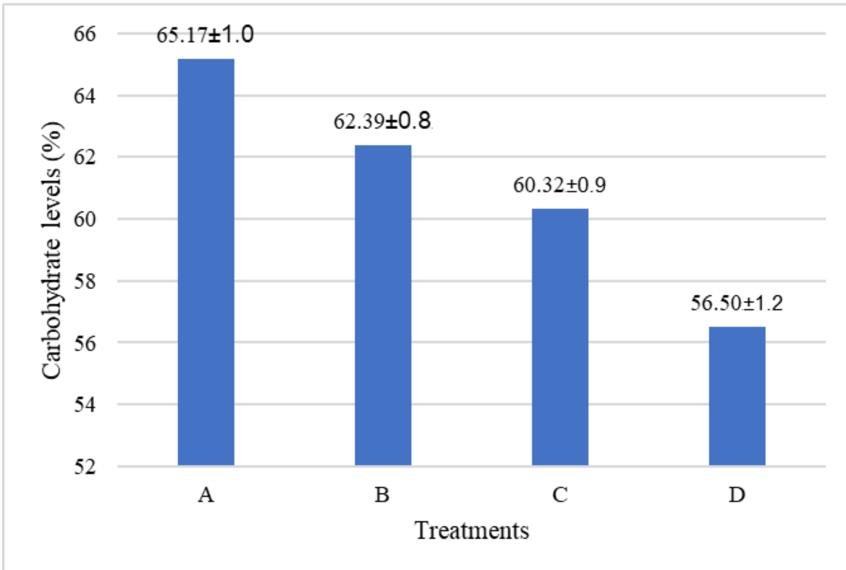

Fig. 3. Carbohydrate content of cookies as affected by substitution with catfish flour and moringa leaf flour

Moisture content

Moisture content remained stable across all treatments, ranging from 4.8 to 5.2%, indicating that substitution did not significantly affect water retention (Fig. 4).

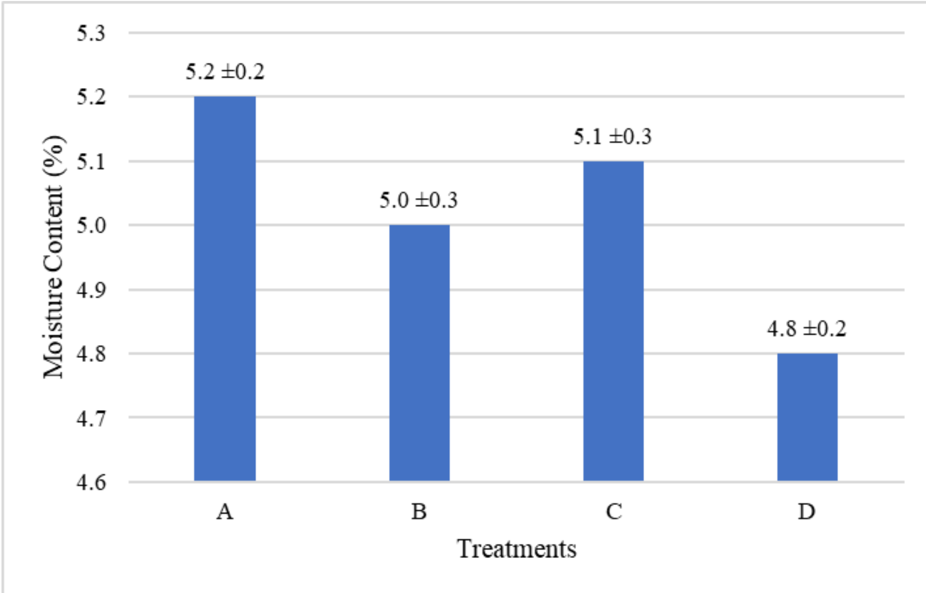

Fig. 4. Moisture content of cookies across different substitution levels

Ash content

Ash content increased significantly with substitution, from 1.4% in the control to 2.1% in the maximum substitution treatment, reflecting a higher mineral content (Fig. 5).

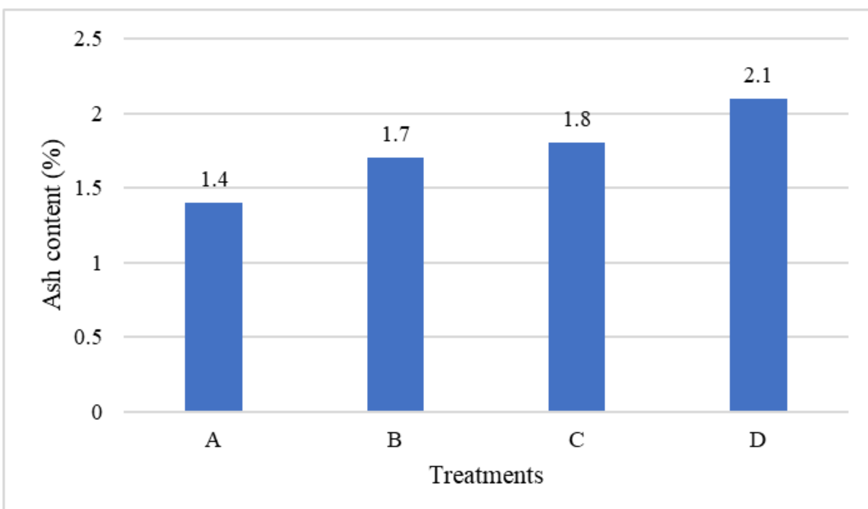

Fig. 5. Ash content of cookies with increasing substitution levels of catfish flour and moringa leaf flour

Mineral content

Calcium and iron contents both increased significantly with substitution. Calcium levels rose from 50 mg/100 g in the control to 120 mg/100 g in the highest substitution group (Fig. 6), while iron increased from 2.0 mg/100 g to 4.2 mg/100 g (Fig. 7).

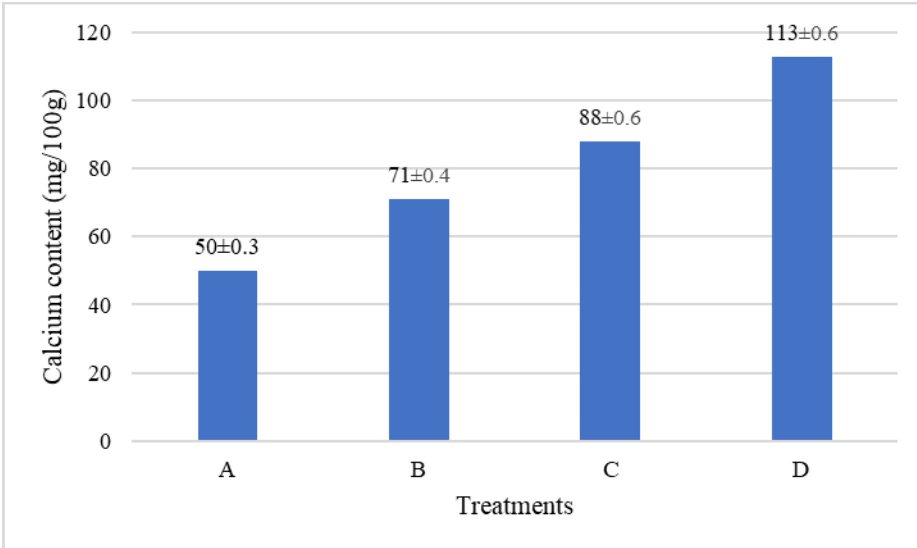

Fig. 6. Calcium content of cookies as influenced by substitution treatment

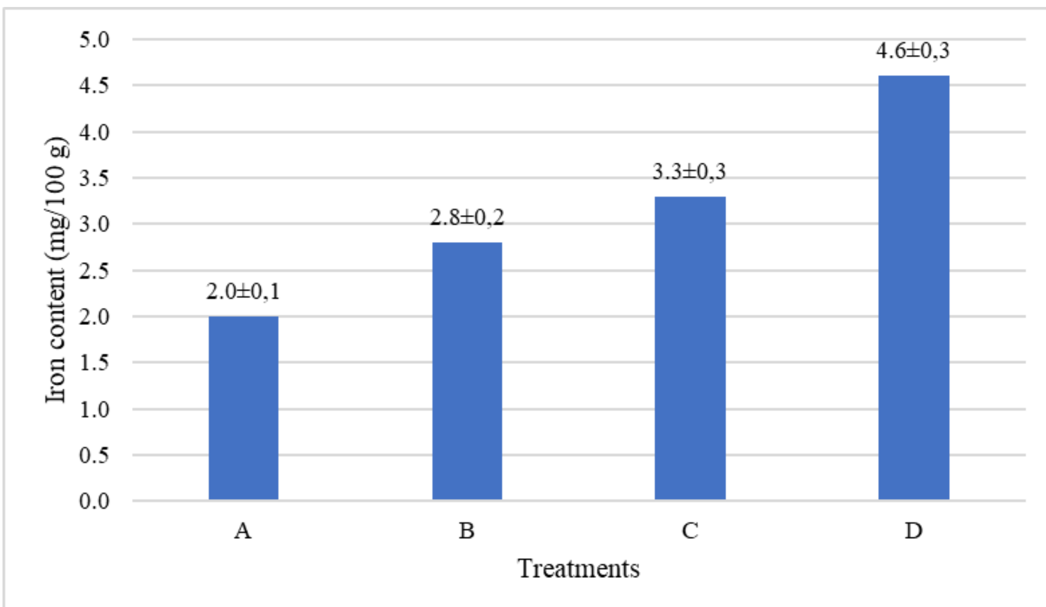

Fig. 7. Iron content of cookies as influenced by substitution treatment

Total phenolic content

Cookies enriched with moringa leaf flour exhibited a significant rise in total phenolic content, from 1.1mg GAE/g in the control to 5.0mg GAE/g in the highest substitution (Fig. 8).

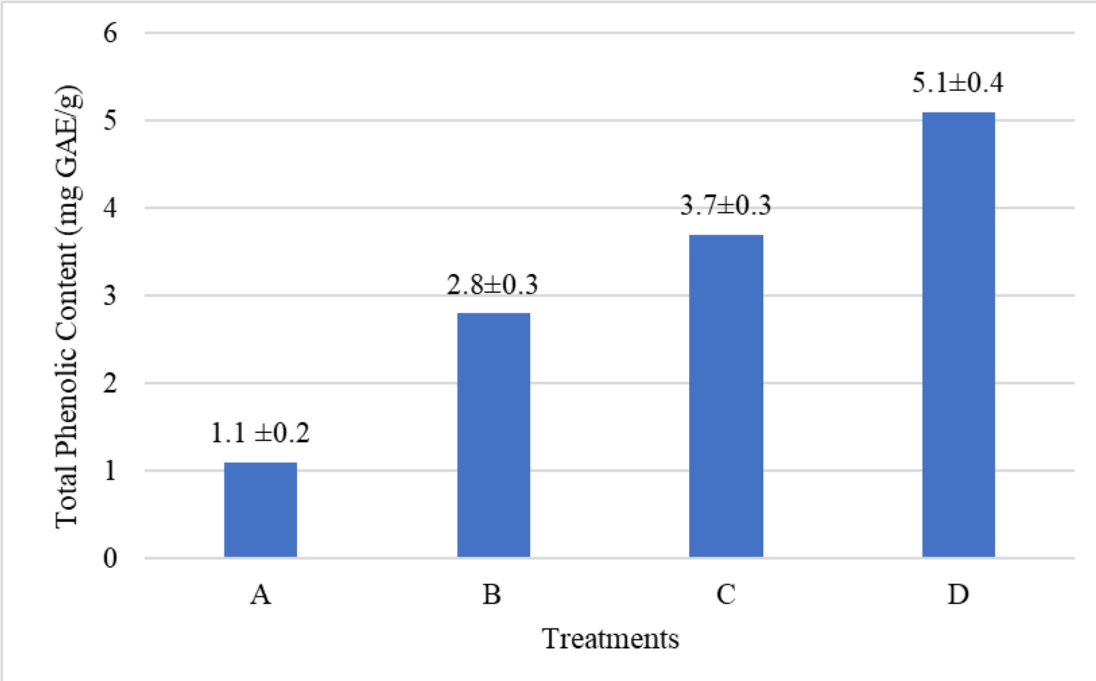

Fig. 8. Total phenolic content of cookies with varying substitution levels of catfish flour and moringa leaf flour

Antioxidant activity

Antioxidant activity, measured by DPPH radical scavenging, increased markedly from 12.5% in the control to 42.1% in the highest substitution formula (Fig. 9). A strong positive correlation was observed between total phenolic content and antioxidant activity.

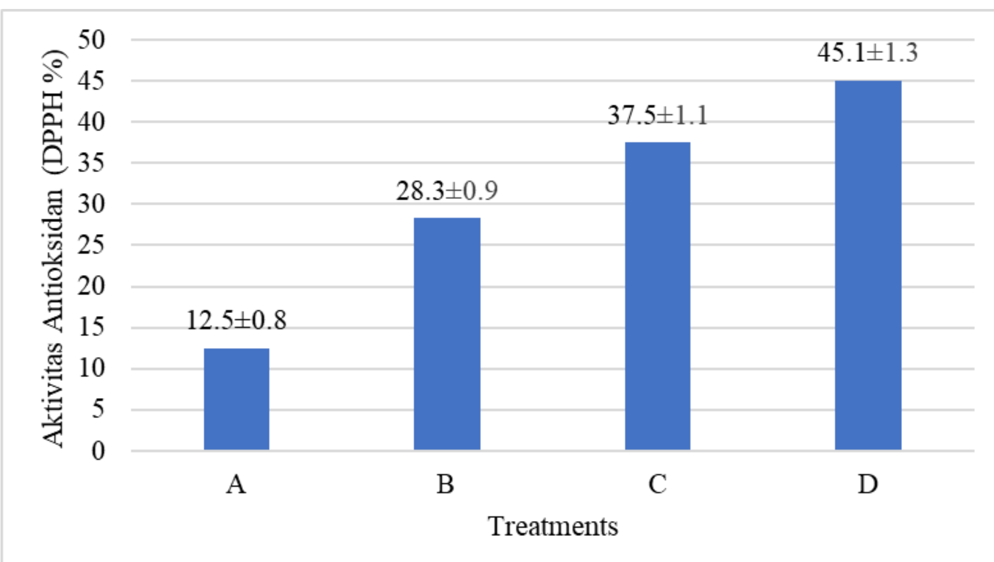

Fig. 9. Antioxidant activity (DPPH radical scavenging) of cookies with different substitution formulations

DISCUSSION

The substitution of wheat flour with catfish flour and moringa leaf flour significantly improved the nutritional and functional properties of cookies. The increase in protein content, from 7.5% in the control to 14.3% in the highest substitution treatment, confirms that catfish flour is a high-quality protein source rich in essential amino acids **(Areta & Adi, 2016; Abdel- Mobdy *et al.*, 2021)**. Similar findings have been reported in bakery products enriched with fish protein, where protein fortification enhanced both nutritional quality and consumer acceptance **(Pratama *et al.*, 2020; Liu *et al.*, 2022)**. This improvement is particularly relevant for addressing protein-energy malnutrition in developing regions, as cookies enriched with catfish flour provide a practical and culturally acceptable source of animal protein.

Fat content also increased slightly with higher substitution levels, reaching 17.5% in the highest treatment. This is consistent with the lipid profile of catfish, which contains beneficial omega-3 and omega-6 fatty acids **(Djuricic & Calder, 2021)**. The presence of healthy unsaturated fats not only contributes to cardiovascular benefits but may also enhance the sensory quality of the cookies by improving texture and mouthfeel **(Guo *et al.*, 2018)**. Importantly, the increase in fat content did not exceed recommended dietary levels, suggesting that the product remains suitable as a healthier alternative snack.

Conversely, carbohydrate content decreased from 65.1% in the control to 58.5% in the maximum substitution treatment. This shift reflects the replacement of carbohydrate-dense wheat flour with protein- and fiber-rich ingredients from catfish and moringa. Lower carbohydrate levels may contribute to a reduced glycemic index, which is beneficial for the dietary management of diabetes and metabolic disorders **(Clemente-Suárez *et al.*, 2022; Kashyap *et al.*, 2022)**. The incorporation of moringa leaves further adds dietary fiber, which slows glucose release and supports metabolic health.

The stability of moisture content (4.8–5.2%) across all treatments indicates that the substitution did not negatively affect water retention during baking. Maintaining low and stable moisture is critical for preventing microbial growth, extending shelf life, and preserving product texture **(Agba *et al.*, 2024; Ngah *et al.*, 2024)**.

Ash content increased significantly, reflecting higher mineral concentrations. Specifically, calcium and iron levels rose from 50 and 2.0mg/ 100g in the control to 120 and 4.2mg/ 100g, respectively, in the highest substitution group. These minerals are essential for bone development, oxygen transport, and prevention of anemia **(Yang *et al.*, 2023)**. The observed improvements align with previous studies on moringa leaves, which are known to be a concentrated source of bioavailable calcium and iron **(Peñalver *et al.*, 2022)**. Hence, fortifying cookies with moringa can contribute to combating micronutrient deficiencies, particularly in vulnerable populations such as children and pregnant women.

Phenolic content and antioxidant activity showed substantial increases with substitution. Total phenolics rose from 1.1mg GAE/g in the control to 5.0mg GAE/g in the highest treatment, while antioxidant activity (DPPH radical scavenging) increased from 12.5 to 42.1%. These results confirm the strong antioxidant potential of moringa leaves, which contain flavonoids, polyphenols, and vitamin C **(Soto *et al.*, 2025)**. Previous research has demonstrated that enriching bakery products with moringa or other plant-based bioactives enhances radical scavenging activity and extends product stability **(Marak *et al.*, 2019; Pirca-Palomino *et al.*, 2024)**. The positive correlation observed between phenolic content and antioxidant activity supports the role of phenolic compounds as major contributors to oxidative stress protection **(Gutiérrez-Del-río *et al.*, 2021)**.

The novelty of this study lies in the synergistic use of catfish flour and moringa leaf flour in cookie formulations. While previous research has investigated either fish protein fortification or moringa enrichment individually, their combination provides a dual functional benefit—animal-derived proteins with essential amino acids and plant-derived antioxidants with bioactive compounds. This integration not only improves nutritional value but also enhances the functional properties of cookies, positioning them as a promising functional food product. Moreover, the use of locally available ingredients supports food security and sustainability initiatives, in line with current global trends in functional food innovation **(Intrasook *et al.*, 2024; Suwarno, 2024; Hati *et al.*, 2025)**.

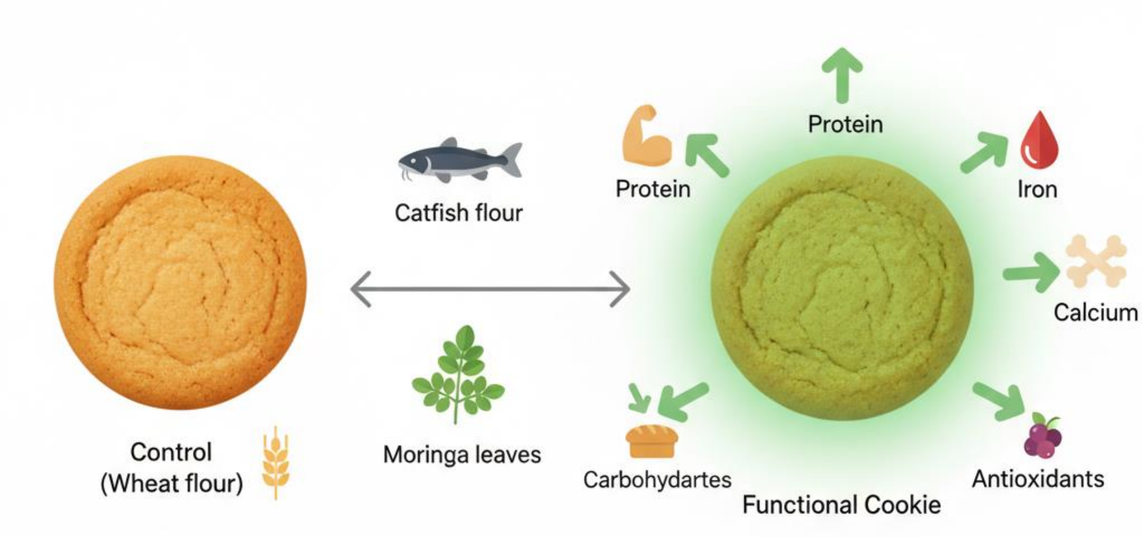

Fig. 10. Integration of dual functional ingredients (Catfish flour and moringa leaves) for food fortification

Although this study demonstrates the nutritional and functional potential of cookies enriched with catfish flour and moringa leaves, further research is needed to explore broader applications and health validations. Future work should include sensory evaluations with diverse consumer groups, shelf-life testing under different storage conditions, and clinical studies to confirm the bioavailability of protein and antioxidant compounds in humans. In addition, integrating amino acid and fatty acid profiling would provide a more comprehensive understanding of the nutritional quality, as demonstrated in wild fish studies **(Islamy & Senas, 2023)**. Investigations into potential environmental contaminants such as microplastics and their effects on food safety are also critical, given the increasing reports of microplastic accumulation in aquatic organisms **(Islamy *et al.*, 2025a, b, d)**. Moreover, the valorization of underutilized and invasive aquatic or plant resources as sustainable functional food ingredients represents a promising direction, in line with recent advances in harnessing agricultural weeds and non-native species for aquaculture and food applications **(Islamy *et al.*, 2024c; Mulyadi *et al.*, 2025)**. Studies on natural bioactive compounds from marine and freshwater organisms have also highlighted their antigenotoxic and antioxidant properties, which may be further applied in functional bakery products. Finally, expanding research into the environmental sustainability of raw materials, including the impact of aquatic plant blooms and ecosystem health, will be essential to ensure the long-term viability of functional food innovations. Overall, the results demonstrate that catfish–moringa cookies offer significant potential as a practical, nutrient-dense, and antioxidant-rich food product. These findings provide a scientific basis for further product development and commercial application, including potential contributions to public health nutrition and local economic empowerment.

CONCLUSION

This study demonstrated that substituting wheat flour with catfish flour and moringa leaf flour significantly improved the nutritional and functional properties of cookies. The enrichment led to increased protein, fat, mineral (calcium and iron), and phenolic contents, accompanied by enhanced antioxidant activity, while maintaining stable moisture levels. These improvements confirm the dual functional role of catfish as a high-quality protein source and moringa leaves as a potent antioxidant contributor.

The novelty of this research lies in the synergistic integration of animal-derived protein and plant-based bioactive compounds in a bakery product, creating a nutrient-dense and antioxidant-rich food with strong potential for functional food development. In addition, the use of locally available resources supports sustainable food innovation, contributing to both public health and food security.

Overall, the findings highlight that catfish–moringa cookies can serve as a practical alternative to conventional snacks, providing added health benefits while addressing issues of protein-energy malnutrition and micronutrient deficiencies. Future work should include sensory evaluation, bioavailability studies, and long-term storage stability to confirm consumer acceptance and functional claims, thereby supporting the commercialization and broader adoption of this product.

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