*Research article*

ENHANCEMENT OF ANTIOXIDANT ACTIVITY, NUTRITIONAL COMPOSITION, AND CRUDE FIBER CONTENT IN MILKFISH SAUSAGE (*Chanos chanos*) THROUGH ADDITION OF MORINGA LEAF POWDER AND FRESH MORINGA LEAVES

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Abstract

This study investigates the impact of Moringa leaf powder and fresh Moringa leaves (*Moringa oleifera* L.) on the antioxidant activity, chemical composition, and crude fiber content of milkfish (*Chanos chanos*) sausage. Milkfish is valued for its high protein and omega-3 content but poses challenges in processing due to its numerous fine bones. Sausage production offers an effective way to debone and transform milkfish into a convenient and versatile food product. Incorporating Moringa leaves enhances its nutritional profile, as Moringa is rich in essential vitamins, minerals, protein, and antioxidants that provide various health benefits. This study employed a completely randomized design with three treatments: a control (K0), 6% Moringa leaf powder (K1), and 12% fresh Moringa leaves (K2), each replicated three times. Antioxidant activity was measured using the DPPH method, with data analyzed through one-way ANOVA followed by Duncan's Multiple Range Test at a 5% significance level. Results showed that both Moringa leaf powder and fresh leaves significantly increased antioxidant activity, with values of 0.7112% (K0), 16.8424% (K1), and 17.0608% (K2). The proximate composition and crude fiber content also improved significantly, demonstrating the potential of Moringa-fortified milkfish sausage as a functional food.

1. Introduction

Milkfish (*Chanos chanos*) is highly nutritious fish widely cultivated in aquaculture due to its rich in essential nutrition for human health. It contains a significant amount of amino

acids such as leucine (8%), lysine (7,3%), phenyl alanine (6,7%) and histidine (6,1%) (Murthy et al., 2016). Additionally, milkfish is a good source of unsaturated fatty acids (50,74%) of which monounsaturated fatty acids (MUFAs)

like oleic acid, as well as polyunsaturated fatty acids (PUFAs) like palmitic acid and oleic acid (Nopiyanti et al., 2023). It also contains significant amounts of vitamins (A, B1, B12) and minerals such as calcium, magnesium, and potassium, making it a valuable dietary component (Malle et al., 2019). However, the presence of numerous fine bones in milkfish poses a challenge for direct consumption, limiting its culinary applications and consumer appeal. Processing milkfish into sausage offers an innovative solution, transforming it into a convenient, boneless product suitable for diverse culinary uses especially fulfilling consumer desires (Yusuf et al., 2018).

Sausages are versatile and boneless product, functions as an alternative product innovation to expand the application of milkfish in the development of functional foods. Enhancing milkfish sausage functional benefits could be done by incorporating with bioactive ingredients. Moringa leaves are rich in protein (25%), vitamins (A, C, E), minerals (calcium, potassium, iron), and bioactive compounds such as phenolics and flavonoids, which exhibit potent antioxidant activity (González-Burgos et al., 2021). This antioxidant can improve the shelf life by delaying lipid oxidation and enhancing the physicochemical and sensory properties of fish product (Mashau et al., 2021). Given these advantages, moringa leaf is an attractive ingredient for milkfish sausage production, providing health benefits and enhancing product stability.

Moringa leaves are widely recognized for their antioxidant properties that play a critical role in human health by neutralizing free radicals. In Indonesia, moringa leaves were often used as medicinal ingredients for conditions such as colds, improving breast milk production, reducing fever, and treating gout. Additionally, they were utilized as food ingredients in dishes such as clear vegetable soup, stir-fried dishes, and crackers (Khasanah et al., 2023). Antioxidants are essential in reducing oxidative stress, which is linked to chronic diseases such as cancer, diabetes, and cardiovascular disorders. The high levels of polyphenols, flavonoids, and other bioactive

compounds in moringa leaves contribute to their strong antioxidant activity, helping to protect cells and tissues from damage caused by free radicals (Segwatibe et al., 2023). Besides that, phenolic content also can be act as an antimicrobial in food (Swastawati et al., 2019). It was reported that moringa leaves acted as antioxidants that inhibited free radicals, increased glutathione, which served as a radioprotective agent, and inhibited lipid peroxidation induced by radiation (Amalia et al., 2024; Sabrina et al., 2024). The consumption of moringa leaves containing flavonoids was also reported to help reduce worker fatigue levels by increasing glutathione peroxidase levels (Prasetio et al., 2023). Fortified fish products incorporating moringa leaf flour have shown significant improvements in nutritional quality and consumer acceptability. Research indicates that the addition of moringa leaf flour to tilapia nuggets enhances protein content by 0.5-13.18% and increases vitamin A levels (β -carotene) from 1.82 to 20.76% (Sagita et al., 2024). Studies has shown the physicochemical properties of catfish sausage fortified with moringa leaf flour also reflect enhanced nutritional profiles, with protein content reaching 18.09% (Rahayu et al., 2022).

Given the growing demand for natural antioxidants and functional foods, this study aims to evaluate the effect of Moringa leaf powder and fresh Moringa leaves on the antioxidant activity, proximate composition, and crude fiber content of milkfish sausage. By leveraging the bioactive compounds in Moringa leaves, the research seeks to develop a nutrient-dense and health-promoting functional food product that meets consumer preferences and contributes to sustainable food innovation.

2. Materials and methods

2.1. Research Design

The study employs an experimental laboratory approach using a Completely Randomized Design (CRD) to assess the impact of various concentrations and forms of Moringa leaf on milkfish sausage. This design includes three treatments: a control group (K0) without Moringa leaves, a 6% Moringa leaf powder

addition (K1), and a 12% fresh Moringa leaves addition (K2). Each treatment is replicated three times, resulting in nine experimental units. The CRD structure minimizes potential biases, allowing observed variations to be attributed directly to the effects of Moringa leaf treatments rather than to external factors, thereby supporting rigorous statistical analysis.

2.2. Methods

2.2.1. Sample Preparation

Fresh milkfish was obtained from ponds in Tugu District, Semarang, Central Java, Indonesia. Fish transportation was used styrofoam box with ice cube to maintain the freshness. The processing of milkfish begins with fish weeding where the scales and internal organs are removed and separating the meat from the bones, spines, skin and head. The filleted meat is washed using clean water to eliminate any remaining impurities, and the

residual water is discarded. Milkfish fillet was grinded with ice for 10 minutes and stored in a freezer to preserve its quality and freshness.

2.2.2. Moringa Leaf Preparation

Moringa leaf powder supplied by PT. Moringan Organik Indonesia and fresh Moringa leaves sourced locally. The fresh moringa leaves were sorted and removed the stalk. The leaves were cleaned, blanched at 80°C for 3 minutes, and finely ground.

2.2.3. Milkfish Sausage Making

The milkfish paste was mixed with the Moringa leaves as treatment (K1 and K2), along with other ingredients such as tapioca flour, MSG, and spices (Table 1). Each blend was stuffed into sausage casings. The sausages were then immersed in ice water at 11-17°C for 60 minutes and hot water sequentially at 60°C for 10 minutes to solidify the texture and enhance flavor. Boiling process was carried out at 90°C for 10 minutes.

Table 1. Milkfish sausage formulation

Ingredients	Amount
Milkfish fillet	200 g
Tapioca flour	20 g
Sugar	3,6 g
Cooking oil	5 g
Monosodium glutamate	1,5 g
Salt	4 g
Garlic	3 g
Red onion	6 g
Nutmeg	0,25 g
Coriander	1 g
Pepper	1 g
Ginger	1 g
egg	1 pcs
Ice cube	60 g

2.2.4. Proximate Composition

The proximate composition of milkfish sausage was performed by using (AOAC, 1990) method. The analysis including carbohydrate, moisture, protein, fat, crude fibre and ash content, was determined using the following methods: by difference, a hot air oven (105°C), the Kjeldahl method, a Soxhlet apparatus, gravimetric and a furnace (550°C), respectively.

2.2.5. Antioxidant Analysis

The data were analyzed statistically using one-way Analysis of Variance (ANOVA) to detect significant differences between treatments at a 5% significance level. When significant differences were observed, Duncan's Multiple Range Test (DMRT) was applied to further analyses specific pairwise comparisons. The use of these statistical tests ensured that the results were robust and could reveal even subtle

effects of Moringa additions on the dependent variables.

2.3. Data analysis

Results were analyzed by one-way analysis of variance (ANOVA) method and significant differences among means from triplicate analyses at ($p < 0.05$) were determined by Fisher's least significant difference (LSD) procedure using the Statgraphics software (Centurion XV).

3. Results and discussions

3.1. Antioxidant analysis

The antioxidant activity of milkfish sausage (Chanos chanos) was shown in Figure 1. The result was significantly enhanced by the addition of Moringa leaf powder (6%) and fresh Moringa leaves (12%), with average antioxidant activity values of 16.8424% (K1) and 17.0608% (K2), respectively. The control (K0) recorded significantly lower activity at 0.7112%.

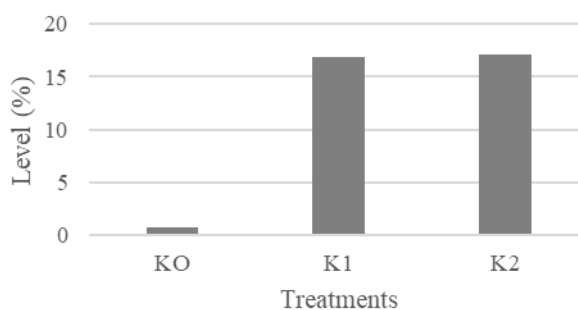


Figure 1. Antioxidant activity of milkfish sausage control (K0), fortified with 6% of Moringa leaf powder (K1), and 12% of fresh Moringa leaves (K2)

In this study, the highest antioxidant activity was recorded in sausages with 12% fresh Moringa leaves (K2), slightly exceeding the activity of sausages with 6% Moringa leaf powder (K1). These results align with prior findings where Moringa was noted for its potent free radical scavenging properties, largely due to phenolic compounds like kaempferol and quercetin (Gao et al., 2022; Swastawati et al., 2019). One potential explanation for the higher activity in K2 compared to K1 could be the preservation of phenolic and flavonoid compounds in fresh Moringa leaves. Processing into powder may cause partial degradation of

Statistical analysis using one-way ANOVA indicated a significant effect of Moringa leaf addition on antioxidant activity ($p < 0.05$). Duncan's Multiple Range Test (DMRT) further confirmed significant differences between all treatments.

The significant enhancement in antioxidant activity observed in milkfish sausages supplemented with Moringa leaf powder and fresh leaves can be attributed to the rich bioactive compound profile of Moringa. These compounds include phenolics, flavonoids, and vitamin C, which act as primary antioxidants by donating hydrogen atoms or electrons to neutralize free radicals like DPPH. Several antibacterial compounds contained in moringa leaves included tannins, triterpenoids, saponins, and flavonoids (Arifan et al., 2021). Moringa leaves were reported to contain several flavonoid compounds, including myricetin, rutin, quercetin, naringenin, kaempferol, and apigenin (Prasetyaningrum et al., 2022).

these sensitive bioactive molecules due to heat and oxidation. However, the overall antioxidant activities in both Moringa-supplemented sausages were markedly superior to the control (K0), which had minimal activity. This underscores the effectiveness of Moringa leaves as a functional additive for enhancing antioxidant properties in processed foods.

Despite the increase in antioxidant activity, the results revealed that the activity in all samples remained below 50%, suggesting moderate efficacy in capturing free radicals. While higher concentrations of Moringa could potentially boost antioxidant activity further,

preliminary sensory analyses revealed diminished acceptability in terms of taste, texture, and aroma at higher Moringa levels. The study from (Trigo et al., 2023) were adding moringa powder in food product and beverage. The adding in food product like nugget can increase phenolic compound led to an increase in the lightness and reduction in the hardness, redness and chewability. on the other side, adding moringa to beverage in higher concentration caused greenish coloration, flavor degradation and imparted a bitter taste due to the presence of catechin. Therefore, the balance between functional benefits and sensory

attributes must be optimized to ensure consumer acceptability (Yusuf et al., 2018). This finding highlights the need for future research into refining the incorporation methods of using Moringa extracts with concentrated bioactives to enhance antioxidant effects without compromising product quality.

3.2. Proximate analysis

The addition of Moringa leaves influenced the proximate composition of the sausages, as shown in Table 2. In general, the effect of moringa leaves fortified milkfish sausage was no significantly different in all treatment.

Table 2. Proximate analysis of milkfish sausage

Parameters	Treatments (%)		
	K0	K1	K2
Moisture	67.0801	67.2492	67.3038
Ash	0.9632	1.1801	1.1082
Fat	4.7135	3.7012	3.9789
Protein	12.0161	12.1694	12.0975
Carbohydrate	15.2272	15.7001	15.4883

The analysis indicated that the moisture content was slightly elevated in the Moringa-supplemented sausages compared to the control. Based on Indonesian National Standard (SNI) of sausage: 01-3820-1995, the result was a bit over with the maximum standard of moisture content 67%. This increase is likely due to the high moisture-retaining capability of Moringa leaves, which may enhance the texture and juiciness of the product (Haruni et al., 2024). Compared to the moisture content of sausages made from mackerel (65%), the samples in this study had higher moisture content (Afifah et al., 2023). The technique to reduce the moisture content in sausages was applied using edible coatings, as in the study by (Rofikoh et al., 2021), which reduced the water content of tilapia sausages from 68% at 0 days of storage to 64% after 21 days of storage. The ash content, indicative of mineral presence, was significantly higher in both Moringa-supplemented treatments (1.18% for K1 and 1.11% for K2) compared to the control (0.96%). This increase supports the notion that Moringa leaves are a valuable source of essential minerals such as calcium and

potassium (Mashau et al., 2021). The observed ash content values were well within the SNI standard of $\leq 3\%$, demonstrating that Moringa leaves contribute positively to the nutritional profile of the sausages without compromising quality standards.

Conversely, fat content decreased in both fortified treatments (3.70% for K1 and 3.98% for K2) compared to the control (4.71%). This reduction may be attributed to the binding effect of Moringa's dietary fibers, which could reduce fat absorption during processing. Additionally, Moringa leaves have a relatively low-fat content, contributing to the overall reduction in sausage fat levels. Compared to the fat content of sausages made from other types of fish, the fat content of milkfish sausages, both with and without the addition of moringa leaves, was still lower than that of sausages made from tilapia fish, which was 5.8% (Maharani et al., 2023). The lower fat content enhances the health appeal of the product while meeting the SNI standard for fat in sausages ($\leq 25\%$).

Protein content showed a slight increase in sausages supplemented with Moringa leaf

powder (12.17%) compared to the control (12.02%), while fresh Moringa leaves resulted in a similar protein level (12.10%). Compared to the protein content of fermented shrimp sausages (9-11%), milkfish sausages with the addition of moringa leaves had higher protein content (Fitriana et al., 2021). However, it was not significantly different from that of barracuda fish sausages (12.58%) (Swastawati et al., 2023). Although these increases are modest, they highlight Moringa's potential as a protein-rich ingredient that complements the fish protein base of the sausage. However, the relatively minor increase suggests that the primary protein contribution still comes from the fish component of the sausage. All treatments exceeded the SNI standard for protein content in sausages ($\geq 13\%$). This occurred similarly to the study by (Swastawati et al., 2021), where nuggets made from milkfish contained protein levels of 13-15%. Boiled or steamed milkfish contained protein levels of 79-83% (Nopiyanti et al., 2023). In the study by (IZMAIL et al., 2022), several proteins detected in fish sausages derived from fish such as tilapia, mullet, and threadfin bream were identified as myosin heavy chain, alpha-actinin, paramyosin, actin, tropomyosin, and myosin light chains.

Carbohydrate content was slightly higher in Moringa-supplemented sausages (K1: 15.70%; K2: 15.49%) than in the control (K0: 15.23%). This increase could be attributed to the polysaccharides and fiber content in Moringa leaves, which are classified as carbohydrates. However, all treatments exceeded the SNI

standard for carbohydrates in sausages (at least 8%), which could be a limiting factor for product certification. Despite this, the higher carbohydrate and fiber content contributes to the product's functional benefits, such as improved digestive health and reduced glycemic load, making it an attractive option for health-conscious consumers. Overall, this study confirms that incorporating Moringa leaves into milkfish sausage not only enhances its antioxidant activity but also improves its nutritional profile across several parameters while maintaining compliance with food safety standards.

3.3. Crude fiber

The addition of Moringa leaves significantly increased the crude fiber content of the sausages: 1.07% (K0), 3.10% (K1), and 3.02% (K2) shown in Figure 2.

This improvement underscores the role of Moringa leaves as a dietary fiber source, which can support digestive health and reduce risks of chronic diseases (Kustiani et al., 2024). This significant enhancement underscores the role of Moringa leaves as an excellent source of dietary fiber. Moringa fiber content, particularly insoluble fiber, contributes to its ability to bind water, improve texture, and potentially promote digestive health by enhancing bowel regularity. These findings align with previous studies that have reported high fiber content in Moringa leaf-based products, emphasizing its potential as a functional food ingredient (Mallillin et al., 2014; Rahmi et al., 2019).

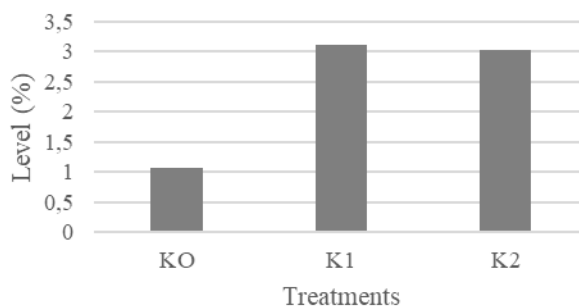


Figure 2. Crude fibre of milkfish sausage control (K0), fortified with 6% of Moringa leaf powder (K1), and 12% of fresh Moringa leaves (K2)

The higher crude fiber content in Moringa-supplemented sausages also offers significant health benefits beyond improving digestive function. Dietary fiber has been associated with reduced risks of cardiovascular diseases, improved lipid profiles, and better glycemic control. The increased fiber levels observed in this study make the Moringa-supplemented sausages a promising functional food option for addressing chronic health issues. These results highlight the potential of Moringa-supplemented milkfish sausages as a novel and nutritionally enhanced alternative to conventional meat products.

4. Conclusions

The addition of Moringa leaves, in both powdered (6%) and fresh (12%) forms, significantly enhanced the antioxidant activity, crude fiber content, and nutritional composition of milkfish sausages. The inclusion of Moringa leaves markedly increased antioxidant activity, crude fiber, protein, and ash contents, contributing to improved health benefits. However, water and carbohydrate contents exceeded SNI standards, suggesting the need for further optimization to meet regulatory requirements. Overall, Moringa-supplemented milkfish sausages offer a promising avenue for producing nutritious, antioxidant-rich food products with potential health benefits, though further research on formulation and sensory quality is recommended to maximize consumer acceptability and compliance.

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