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Fishbone Flour Derived from Indian Mackerel (*Rastrelliger Kanagurta*) to Improve the Calcium and Protein Content of Fish Sausage

Tepung Tulang Ikan Kembung (Rastrelliger kanagurta) untuk Meningkatkan Kandungan Kalsium dan Protein dalam Sosis Ikan

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Abstract. Sausages are widely consumed animal-based food products valued for their convenience and desirable sensory properties. Fish sausages are increasingly popular; however, despite being derived from fish, they often have limited calcium content. This study investigated the effect of incorporating fishbone flour derived from Indian mackerel (*Rastrelliger kanagurta*) on the protein and calcium content of fish sausages. Fishbone flour was produced via acid extraction and incorporated into sausage formulations containing 100 g of fish meat, with three levels of addition: 0, 2.5, and 5 g. The optimal formulation was determined based on sensory evaluation, physical characteristics, and nutrient composition analysis. The results indicated that the formulation containing 2.5 g of fishbone flour provided the best overall quality and acceptability. This sausage formulation contained 64.24 g of moisture, 1.97 g of ash, 13.04 g of protein, 3.96 g of fat, 16.80 g of carbohydrates, and 165.19 mg of calcium per 100 g of product. A typical serving size equivalent to two sausages provides approximately 93 kcal. The addition of fishbone flour significantly increased the calcium content without compromising sensory quality. The resulting mackerel sausage formulation can potentially meet the nutritional criteria for labeling as a source of protein and calcium. These findings demonstrate the potential of fishbone flour from Indian mackerel as a functional ingredient to improve the nutritional value of fish-based processed products while promoting the utilization of fish processing by-products.

Keywords: calcium, fish sausage, fishbone flour, Indian mackerel, protein

Abstrak. *Sosis merupakan produk hewani yang banyak diminati dan dikonsumsi oleh masyarakat karena pengolahannya yang cepat dan mudah serta memiliki rasa yang lezat. Sosis ikan kembung yang diperkaya dengan tepung tulang ikan dapat menjadi pilihan lauk alternatif untuk mendukung peningkatan asupan protein dan kalsium pada wanita usia subur. Penelitian ini bertujuan mengembangkan serta menganalisis kandungan gizi produk sosis ikan kembung dengan penambahan tepung tulang ikan. Setiap formula diberi tambahan daging ikan 100 g dan tepung tulang ikan dalam jumlah yang berbeda. Daging ikan tersebut dimasukkan ke dalam tiga formula sosis ikan yang berbeda dengan penambahan tulang ikan yang bervariasi (100 g dengan 0 g; 100 g dengan 2,5 g; 100 g dengan 5 g). Berdasarkan hasil uji organoleptik, karakteristik fisik, dan kandungan gizi, penambahan 2,5 g tulang ikan ditetapkan sebagai formula terpilih. Sosis dengan penambahan 2,5 g tulang ikan mengandung kadar air 64,24 g, kadar abu 1,97 g, protein 13,04 g, lemak 3,96 g, karbohidrat 16,80 g dan kalsium 165,19 mg per 100 g sampel. Satu takaran saji sosis ikan kembung dengan penambahan tepung tulang ikan sama dengan dua buah sosis serta memberikan kontribusi 93 kkal. Sosis ikan kembung dengan penambahan 2,5 g tepung tulang ikan berpotensi memenuhi syarat klaim sebagai produk pangan olahan sumber protein dan kalsium. Penelitian ini menunjukkan bahwa tepung tulang ikan kembung berpotensi sebagai bahan pangan fungsional untuk meningkatkan kandungan gizi produk pangan olahan berbasis ikan.*

Kata kunci: *ikan kembung, kalsium, protein, sosis ikan, tepung tulang ikan*

Practical Application: The present study investigates the production of fishbone flour from Indian mackerel, aiming to provide an alternative source of calcium for the development of various food products. The study's primary focus is on using fishbone flour to produce mackerel-based sausages, demonstrating the successful development of an alternative animal protein product with adequate levels of protein and calcium through a well-formulated recipe.

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INTRODUCTION

According to the Ministry of Health Republic of Indonesia (Kemenkes), malnutrition is an imbalance between the body's nutrient needs and the nutrients it receives. Indonesia faces a double burden of malnutrition, characterized by undernutrition (nutrient intake deficiencies) and overnutrition (Kemenkes 2022). Nutrient deficiencies can occur in both macronutrients and micronutrients. Although the body requires micronutrients in small amounts, these nutrients are essential because the body cannot produce them internally. Micronutrients are categorized into vitamins, minerals, and trace elements.

Calcium is an essential mineral for the human body. The average calcium intake is low in certain regions, particularly in Asia and Africa, where the daily intake typically falls below 500 mg. By contrast, countries in the green zone of calcium intake usually exceed 800 mg daily. As a developing Asian country, Indonesia faces the challenge of calcium deficiency stemming from inadequate intake. The Indonesian context is particularly notable, as Indonesia's calcium intake is categorized in the red zone on the global map of calcium intake, with an average daily calcium consumption of less than 400 mg (Balk *et al.* 2017). This data indicates that a substantial proportion of Indonesians consume insufficient calcium daily.

According to the most recent dietary recommendations, the minimum daily calcium intake for people aged 10 to 80 should be 1000 to 1200 mg. Calcium deficiency can pose significant health risks, as calcium is essential for overall well-being. A global report comparing data from the 2007–2008 period to the 2017–2018 period revealed that while there was no notable increase in osteoporosis among men, the age-adjusted prevalence of osteoporosis in women increased from 14.0 to 19.6% (Sarafrazi *et al*. 2021). According to the Ministry of Health (Kemenkes) of Indonesia, in 2013, the prevalence of osteoporosis among women aged 50–70 years was 23%, and for those aged >70 years, it was 53% (Kemenkes 2023). Osteoporotic fractures, particularly hip fractures, contributed significantly to long-term disability, with various studies reporting that approximately 30–80% of patients experienced permanent functional limitations, including loss of independent walking ability and dependence on assistance in daily living (Magaziner *et al.* 2000; Johnell and Kanis 2006; International Osteoporosis Foundation 2023).

Sausage is a popular food often served as a side dish at meals. Protein-based products are consumed three times daily with main meals (Prasasti and Indrawati 2019). A study involving elementary school-aged children indicated that beef sausage is consumed approximately 7.6 times per week (Masruroh 2016), suggesting potential for developing high-calcium products.

The incorporation of fishbone flour presents a significant opportunity for the development of calcium-rich food products, as evidenced by studies that have demonstrated the efficacy of fishbone flour in enhancing calcium levels in food items. For instance, a study incorporating fishbone flour into egg rolls reported an increase in calcium content from 0.37 to 1.90 g per 100 g of the product (Wang 2020). A similar outcome was observed in a separate study, where incorporating catfishbone flour into nuggets significantly increased calcium levels, from 350 to 1498 mg per 100 g of the product (Nasution *et al.* 2022). Adding striped snakehead fishbone flour has been shown to increase the calcium content of crackers, notably from 151.36 to 550.91 mg per 100 g of product (Putra *et al.* 2015). The fortification of fish meatballs with tuna bone meals has also increased calcium levels from 0.13 to 0.39% (Edam 2016). These findings collectively demonstrate the substantial potential of fish bone in enhancing calcium levels in food products.

Incorporating mackerel bone flour into the mackerel sausage formula is hypothesized to enhance the sausage's protein and calcium content. This study aimed to analyze the effects of adding fishbone flour in the production of fish sausage, focusing on its physical characteristics, nutrient composition, and sensory properties. This innovation is expected to provide a practical solution for increasing the overall calcium intake of the Indonesian population.

MATERIALS AND METHOD

Materials

The materials that were employed in producing fish bone meal included Indian mackerel bones, HCl, and distilled water. The fish used was the Indian mackerel (*Rastrelliger kanagurta*). The mackerel fish was purchased from a local market in Bogor Regency. The materials that were used in the production of mackerel sausage included Indian mackerel fillet and bone meal, tapioca flour, potato starch, milk, chicken eggs, garlic powder, pepper, coriander powder, salt, monosodium glutamate (MSG), and palm oil. Fish bone meal itself was not analyzed; however, the effect of the addition can be observed from the difference between the control sample and the samples treated with fish bone meal. The nutrient analysis of fish sausage included moisture, ash, protein, fat, carbohydrate, and calcium. For proximate and calcium content analysis of the samples, we used H_2SO_4, distilled water, NaOH, boric acid, 0.1 N HCl, Na_2CO_3, $(NH_4)_2SO_4$, selenium mix as a catalyst in the destruction process to break down protein molecules and convert them into free nitrogen, which then forms ammonium sulfate, hexane solvent to extract or isolate fat (lipids) from a food sample and HNO_3 destroys organic compounds in sample.

Production of Indian mackerel bone flour and sausage

The process of making mackerel sausage with the incorporation of mackerel bone flour comprised two main stages: the production of mackerel bone flour and the preparation of the mackerel sausage.

The processing method for mackerel bone flour production consisted of the following steps: washing, boiling, cleaning, pressure processing, acid extraction, removal of acid residues, drying, and grinding, with modifications from Putranto *et al.* (2015) and Darmawangsyah *et al.* (2016). The mackerel bones were washed with running water and boiled to remove any remaining meat. Then, they were thoroughly cleaned. Subsequently, the bones were subjected to high-pressure processing at 121 °C (Eyela, NDO-400, Japan) for one hour to soften them. Subsequently, the bones were subjected to an acid extraction with hydrochloric acid (HCl) at a 1:4 ratio with fish bone, at pH 4, as measured by a pH meter (Ohauss, Starter 3100, China), for 2 hours at 60 °C. Cleaning of the remaining HCl solution by centrifugation at 4000 rpm for 15 minutes and washing with distilled water 10-12 times (fish bones: distilled water is 1:3 per 1 wash). The pH was then adjusted to neutral (6.8 and 7.0) using a pH meter.

This extraction process was conducted while the bones were continuously stirred at 40 rpm using a magnetic stirrer to remove organic components and facilitate mineral release from the bone matrix. Following the extraction process, the residual HCl was removed by centrifugation at 4,000 rpm for 15 minutes (Kokusan, H-26, Japan). Thereafter, the bones were washed with distilled water, and the pH was measured with a pH meter to confirm their neutrality. The bones were then dried in an oven (Eyela, NDO-400, Japan) at 105 °C for three hours. After this, the bones were pulverized and sifted through a standard 70-mesh sieve, yielding the final fishbone flour.

The sausage formula used in this study was based on previous research by Arifin *et al.* (2023) and the results of a preliminary optimization study. Based on the initial research, three mackerel sausage formulas were produced. These formulas were based on the ratio between fish meat and fish bone meal: F0 (100:0), F1 (100:2.5), and F2 (100:5), along with the respective weights of each ingredient used, as outlined in Table 1. The sausage-making process began with preparing mackerel meat, including washing and filleting the fish. After this preparatory step, the fillet was homogenized using a blender with milk, chicken eggs, cooking oil, garlic powder, coriander powder, pepper, salt, and MSG. These seasonings were added at this stage to eliminate fishy odors and ensure even distribution throughout the mixture. Subsequently, the puree was transferred to a container and combined with tapioca starch, potato starch, and fishbone flour. The mixture was then stirred until it attained a uniform consistency. The dough was then loaded into a sausage filler and shaped by inserting it into sausage casings. Finally, the sausages were steamed at 80 °C for 45 minutes. This steaming process induced gelatinization, resulting in sausage expansion and a chewy texture.

Nutrient analysis and nutrition claims

A product could meet the claim of being a source of protein and calcium if it contained 20% NRV per 100 g (in solid form) for protein and 15% NRV per 100 g (in solid form) for vitamins and minerals. The selected formula of mackerel sausage could potentially meet the claim of being a source of protein and calcium. The calculations show that the protein and calcium contributions to the general consumers group were 21.7% and 15.0%, respectively. Additionally, in accordance with Indonesian National Standard (SNI) 3820:2015 on Meat Sausage, the selected sausage formulas were found to meet the SNI requirements.

Calcium and proximate content analysis

The calcium content analysis was conducted using an atomic absorption spectrophotometer (AAS) (Shimadzu 7800, Japan) with a wavelength between 248.3nm through a third-party analytical service. The following tools were used for protein analysis (FOSS, KT 200 Kjeltec, Denmark), fat analysis (FOSS, ST 243 Soxtec, Denmark), and ash analysis (Nabertherm, Muffle Furnace B180, Germany), which were available at the Nutrient Analysis Laboratory of the Department of Community Nutrition, Faculty of Medicine and Nutrition, IPB University.

Sensory analysis

The organoleptic test for mackerel sausage with the addition of fishbone flour consisted of an acceptance test and an attribute-intensity test. The acceptance test aims to gauge panelists' impressions and responses regarding their likes and dislikes of product attributes, including color, aroma, texture, taste, aftertaste, and overall acceptance. The attribute intensity test measures the intensity of the product's characteristics, including brownness, fishy aroma, chewiness, fishy taste, and fishy aftertaste. The acceptance test and attribute intensity test were conducted using a 9-cm line scale, in which panelists were asked to draw a vertical line to assess each attribute. A score of 0 on the line indicated strong dislike, and a score of 9 indicated strong liking. The sensory test panel consisted of 33 people. The panelists read and signed the informed consent form before performing the sensory test.

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Color analysis

Color was measured using a colorimeter (Amtast, AMT511, USA). The sample was cut and ground evenly in a cup or special container suitable for the instrument's port. The instrument displayed the color values for the following parameters: L* (lightness, where 0 = black and 100 = white), a* (where + = red and - = green), and b* (where + = yellow and - = blue). The color data can be processed to obtain average L, a, and b values.

Data processing and analysis

The data were processed using Microsoft Excel and the Statistical Program for Social Sciences (SPSS) version 25.0 for Windows. The data were analyzed using one-way Analysis of Variance (ANOVA) to identify differences among treatments, with a significance level of $p<0.05$. If the p-value was less than 0.05, Duncan's multiple range test (DMRT) was conducted for further analysis.

RESULTS AND DISCUSSION

Physical characteristics of Indian mackerel sausage with the addition of fishbone flour

The physical characteristics assessment entailed a color test and a chewiness test. Table 2 presents the results of physical tests on Indian mackerel sausage formulated with varying percentages of fishbone flour. Color is a crucial factor in the visual perception of foodstuffs. Statistical analysis revealed that incorporating fishbone flour into mackerel sausage significantly altered the color parameters, specifically L* (lightness), a* (redness), and b* (yellowness), with $p<0.05$. Furthermore, the application of the DMRT substantiated a significant difference ($p<0.05$) among the varying mackerel sausage formulas. As shown in Table 2, all three colors increased, indicating brighter coloration due to the high phosphorus and calcium content of fish bone meal, which can increase the whiteness index (Hidayat and Rosidah 2022). The chemical reactions between fish meal protein and food pigments primarily involve interactions between amino groups in the protein and pigment molecules. Food pigments, particularly polyphenols or phenolic compounds, can bind to proteins through oxidation to form quinones, which then react with the amino groups of proteins to form covalent bonds. This changes the protein structure and the product's color properties (Shaviklo 2013). Fish bone meal was generally white to yellowish-white in color due to its high mineral content, particularly calcium and phosphate, which affected the optical properties of the material and increased the lightness (L) value of food products (Kaliky 2022; Alisa *et al.* 2023; Hidayat and Rosidah 2022).

The Maillard reaction enhances the redness of food due to the formation of melanoidins, by-products of this reaction (Kusuma *et al.* 2013). The yellowness of food is influenced by the presence of carotenoids and xanthophylls in egg yolks and by β-carotene in cooking oil, resulting in yellow and orange colors (Argo *et al.* 2013; Martianto *et al.* 2018). Increasing fish bone meal can also increase the product's hardness and density, which changes how light is reflected or absorbed, thereby affecting the color's brightness and intensity.

Table 1. Formulas for fish sausage with the incorporation of mackerel bone flour

Ingredient	Formula (Fish Meat + Mackerel Bone Flour (g))*		
	F0 (100+0)	F1 (100+2.5)	F2 (100+5)
Mackerel	100	100	100
Mackerel bone flour	0	2.5	5
Binder flour	35	35	35
Chicken egg	10	10	10
Milk	50	50	50
Herbs and spices	6	6	6
Cooking oil	2	2	2

Notes: * F0 (100+0): 100 g of fish meat with 0 g of fish bone meal added, F1 (100+2.5): 100 g of fish meat with 2.5 g of fish bone meal added, F2 (100+5): 100 g of fish meat with 5 g of fish bone meal added

Table 2. Color and texture of Indian mackerel sausage added with fishbone flour

Attributes	Formula (Fish Meat + Mackerel Bone Flour (g))*		
	F0 (100+0)	F1 (100+2.5)	F2 (100+5)
Color			
L^*	$62.18±0.34^a$	$62.42±0.27^{ab}$	$62.76±0.20^b$
a^*	$7.36±0.18^a$	$7.53±0.11^a$	$8.91±0.30^b$
b^*	$16.67±0.13^a$	$17.44±0.43^b$	$19.23±0.30^c$
Texture			
Chewiness	$449.8±5.39^b$	$263.9±7.84^a$	$255.8±7.00^a$

Note: * Values followed by similar letters in a row are not significantly different ($p>0.05$)

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The Maillard reaction can also occur when reducing sugars interact with amino acids in proteins, resulting in a brown discoloration of the food product. In this case, the lipid oxidation process that happens in fishmeal also affects pigments and proteins, producing reactive compounds that can react with them and cause color changes. Meanwhile, fish protein tends to undergo structural changes (denaturation) during processing (e.g., heating), which can affect interactions with pigments, increasing the complexity and stability of the product's color (Xiong and Guo 2020).

Incorporating fishbone flour into the sausage formula significantly impacted its chewiness ($p<0.05$). Subsequent analyses revealed a notable difference ($p<0.05$) between the standard formula (control) and the modified formulas (added with 2.5 and 5 g fishbone flour). The gelatinization process emerged as a crucial factor in determining the sausages' chewiness. Additionally, the hygroscopic nature of fishbone flour enabled it to absorb moisture from the environment, thereby increasing the moisture content in the sausages (Njoroge and Lokuruka 2020). This, in turn, resulted in a softer, less chewy texture due to reduced binding of starch and protein to water (Simanjuntak *et al.* 2017). The interaction between minerals in fish bone meal, particularly calcium and phosphate, and other compounds in sausages can affect their physical properties, such as texture, elasticity, and emulsion stability. Calcium, derived from fish bone meal, plays a role in the formation of cross-links in fish meat proteins, which form a gel structure or matrix in sausage products. This can increase gel strength, elasticity, and the capacity to bind water and fat (Rohmah *et al.* 2019).

Characteristics of Indian mackerel sausage added with fishbone flour

An organoleptic test was conducted with a panel of 33 semi-trained individuals, comprising IPB University students and individuals trained in organoleptic testing. Table 3 presents the results of the acceptance test and attribute-intensity tests for the three Indian mackerel sausage formulas produced with varying percentages of fishbone flour.

Adding fishbone flour to mackerel sausage significantly influenced the panelists' overall preference for the sausage's appearance ($p<0.05$). However, no significant difference in the sausage's browning intensity was observed among the treatment levels ($p>0.05$). The results indicated that a decrease in sausage browning intensity was associated with a corresponding reduction in the panelists' preference for its appearance. The panelists' ratings for appearance ranged from 5.36 to 6.60, denoting an average response of "neutral" to "somewhat like."

The findings indicated a significant effect ($p<0.05$) on aroma, suggesting that as the intensity of the fish aroma increased, the panelists' preference for it decreased. This phenomenon is attributable to the decomposition of volatile amine compounds, such as trimethylamine oxide, which produces a distinctive fishy smell (Ratnasari *et al.* 2021). The panelists' ratings for the aroma attribute ranged from 5.02 to 6.34, indicating a preference for a "neutral" or "somewhat like" aroma.

Furthermore, adding fishbone flour to mackerel sausage significantly affected panelists' perceptions of its texture ($p<0.05$). The statistical analysis of the quality test further substantiated a significant relationship between the proportion of fishbone flour to fish meat in the sausage's chewiness ($p<0.05$).

The average results indicated that panelists' preference for sausage texture decreased concomitantly with its elasticity. Fishbone flour exhibits hygroscopic properties, absorbing moisture from its surrounding environment, thereby increasing the moisture content of the food product (Njoroge and Lokuruka 2020). This makes a mushy sausage more prone to solidification and chewier (Prastini and Widjanarko 2015). The panelists evaluated the texture attribute, and the results indicated an average score ranging from 4.74 to 6.31, denoting a preference from "somewhat dislike" to "somewhat like".

Table 3. The mean scores of the sensory tests performed on Indian mackerel sausage added with fishbone flour

Attributes	Formula (Fish Meat + Mackerel Bone Flour (g))*		
	F0 (100+0)	F1 (100+2.5)	F2 (100+5)
Acceptance test			
Appearance	6.60±1.68^{b}	5.45±1.88^{a}	5.36±1.26^{a}
Aroma	6.34±1.72^{b}	5.53±1.99^{ab}	5.02±1.88^{a}
Texture	6.31±1.86^{b}	4.85±1.97^{a}	4.74±1.78^{a}
Taste	6.56±1.87^{b}	5.58±1.39^{a}	5.27±1.03^{a}
Aftertaste	5.67±2.26^{a}	4.74±1.97^{a}	4.82±1.58^{a}
Overall acceptability	6.86±1.49^{b}	5.41±1.51^{a}	5.33±1.21^{a}
Attribute intensity test			
Color (brownish)	4.38±1.83^{a}	3.98±1.67^{a}	3.76±1.81^{a}
Fishy aroma	5.04±1.89^{a}	5.88±1.81^{ab}	6.47±1.50^{b}
Texture (chewiness)	5.45±2.14^{b}	4.58±1.96^{ab}	4.12±1.66^{a}
Fishy flavor	5.82±1.95^{a}	5.94±1.65^{a}	6.44±1.35^{a}
Fishy aftertaste	5.00±1.84^{a}	5.73±1.65^{ab}	6.04±1.68^{b}

Notes: * Values followed by the same letter in the same row are not significantly different ($p>0.05$)

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Incorporating fishbone flour into mackerel sausage significantly affected the perceived flavor of the sausage ($p<0.05$). However, the analysis revealed no significant relationship between the varying proportions of fishbone flour and fish meat in sausage and the sausage's distinct fish flavor ($p>0.05$). The average results suggested that as the typical fish flavor in the sausage increased, the panelists' preference for that flavor decreased.

The addition of fishbone flour has been shown to enhance a product's flavor profile by intensifying the fish flavor (Muna *et al.* 2017). The panelists evaluated the flavor attribute, assigning average scores ranging from 5.27 to 6.56 on a scale in which "neutral" indicated neither liking nor disliking, and "somewhat like" indicated a favorable preference. Statistical tests of the aftertaste revealed that incorporating fishbone flour into mackerel sausage did not elicit a significant response ($p>0.05$) in the panelists' aftertaste preferences. However, the test results indicated that the proportions of fishbone flour and fish meat in the sausage were associated with the typical fish aftertaste ($p<0.05$). The average ratings the panelists gave for the aftertaste attribute ranged from 4.82 to 5.67 on a scale from "somewhat dislike" to "somewhat like". As the aftertaste intensity increased, the overall liking for the sausage decreased. The distinctive taste and aroma of mackerel are attributed to various aromatic compounds in the fish (Susanto *et al.* 2019). The preference tests showed a significant impact ($p<0.05$), indicating that panelists preferred the sausage with the lowest amount of fishbone flour. The panelists evaluated the sausage's overall characteristics with average scores ranging from 5.33 to 6.86 on a scale from "neither like nor dislike" to "somewhat like".

Nutrient composition of Indian mackerel sausage produced with the addition of fishbone flour

The nutritional content analysis was conducted by examining macronutrients, including moisture content, ash, protein, fat, and carbohydrates, as well as a micronutrient analysis focusing on calcium content. Table 4 presents the nutritional composition of Indian mackerel sausage containing fishbone flour.

Adding fishbone flour to mackerel sausage significantly affected the sausage's moisture content ($p<0.05$). The DMRT results demonstrated a notable difference in moisture content between control and the addition of 2,5 g and between control and 5 g fishbone flour ($p<0.05$). As the proportion of fishbone flour increased, the moisture content of the sausages concomitantly increased. This increase in moisture content can be attributed to the hygroscopic nature of bone meals (Fong-in *et al.* 2020). Additionally, incorporating fishbone flour into the mackerel sausage significantly affected the ash content ($p<0.05$). The DMRT results indicated a statistically significant difference in ash content across the three mackerel sausage formulas ($p<0.05$). Fishbone flour is recognized for its high calcium and phosphorus levels, which contribute to increased ash content (Imra *et al.* 2019). Furthermore, incorporating fishbone flour into mackerel sausage also affected the protein content, with statistically significant results observed in the test ($p<0.05$). There was a substantial difference in protein levels between the control sample and the sample with the addition of 2,.5 g and between the control and the sample with the addition of 5 g fishbone flour ($p<0.05$).

Adding bone meals to products such as sausages or processed mackerel products increases calcium and protein levels because fish bone meals contain high levels of both nutrients. Mackerel bone meals contain significant calcium (735 mg per 100 g of bone meal) and protein (9.2 g per 100 g of bone meal). Therefore, when bone meals are added to a product, the calcium and protein levels increase in proportion to the amount of bone meals added.

Furthermore, the protein content of the product is also increased due to the natural protein content of the bones and tissues remaining in the fish bone meal. Calcium from the bones also contributes significantly to the mineral content of the final product. Therefore, adding fish bone meal is an effective way to increase the nutritional value of a product, particularly its calcium and protein levels.

Fishbone flour contains approximately 16.10% protein, which has been shown to increase sausage protein levels (Nemati *et al.* 2017). Additionally, incorporating fishbone flour into mackerel sausage significantly affected the fat content ($p<0.05$). The Duncan's multiple range test (DMRT) revealed significant differences in fat content among the various mackerel sausage formulas ($p<0.05$). Fishbone flour contains 670 mg of sodium per 100 g (Nemati *et al.* 2017), a level well established to accelerate fat oxidation (Mariutti and Bragagnolo 2017). A study has also demonstrated that unsaturated fats accelerate the fat oxidation process (Permatasari *et al.* 2017). This means they are more susceptible to oxidation than saturated fats. Furthermore, mackerel has a high unsaturated fat content of 26.02 mg per 100 g, which contributes to lower fat content in products such as sausages (Alkuraieef *et al.* 2021).

In addition, the incorporation of fishbone flour into the formula of the sausages resulted in a reduction in the percentage of fish meat. Specifically, the rates of fish meat combined with fishbone flour at 2.5 and 5 g were 97.6% and 95.2%, respectively. This reduction may contribute to a decrease in the fat percentage of sausages. Furthermore, the carbohydrate content of the sausages did not show a significant difference ($p>0.05$). Fish meat had a low carbohydrate content (0.56%), suggesting it does not significantly affect the sausage's carbohydrate content (Putra *et al.* 2015).

Table 4. Nutrient composition of Indian mackerel sausage with varying ratios of fish meat to mackerel bone flour

Nutrients (%)	Formula (Fish Meat + Mackerel Bone Flour (g))*		
	F0 (100+0)	F1 (100+2.5)	F2 (100+5)
Moisture (% wb)	60.10±0.23[a]	64.24±0.55[b]	64.87±0.31[b]
Ash (% db)	3.06±0.11[a]	5.50±0.18[b]	7.57±0.68[c]
Protein (% db)	28.33±0.82[a]	36.45±0.63[b]	37.42±0.61[b]
Fat (% db)	21.45±0.34[c]	11.76±0.64[b]	10.12±0.74[a]
Carbohydrate (% db)	47.15±0.71[a]	46.30±1.43[a]	44.89±1.15[a]
Calcium (mg/100 g)	112.93±9.16[a]	462.32±31.43[b]	743.61±62.70[c]

Notes: * Values followed by the same letter in the same row are not significantly different (p>0.05)

Table 5. Percentage of nutrient content of Indian mackerel sausage with the addition of fishbone flour, as compared to the Nutrient Label Reference and its nutrition claims

Nutrients	Indonesian National Standard for Meat Sausage (SNI 3820:2015)	Nutrient Content		NLR for General Consumers	% NLR	Nutrition Claim
		Per 100 g (% wb)	Per 60 g* (% wb)			
Energy	-	155 kcal	93	2150 kcal	7.2	
Moisture	Max. 67	64.2	38.5	-	-	
Ash	Max. 3.0	2.0	1.2	-	-	
Protein	Min. 13	13.0 g	7.8	60 g	21.7	Source of **
Fat	Max. 20	4.0 g	2.4	67 g	5.9	
Carbohydrates	-	16.8 g	10.1	325 g	5.2	
Calcium	-	165.19 mg	99.1	1100 mg	15.0	Source of **

Note: *Serving size of 60 g, **Based on the Regulation of the Indonesian Food and Drug Authority (2016)

Conversely, the statistical test results indicated that adding fishbone flour to mackerel sausage significantly affected its calcium content (p<0.05). The results of the DMRT test revealed a significant difference among the three mackerel sausage formulas (p<0.05). This difference can be attributed to the high calcium content of fishbone flour, which contains 38.16 g of calcium per 100 g (Nemati *et al.* 2017).

Selected treatment

The selection of the best treatment was predicated on the results of organoleptic tests and nutritional content analysis of all the formulas. According to the acceptance test, the control sample, which was sausage without fishbone flour, was the preferred option, with panelists rating all attributes as "neutral" or "somewhat like". However, the nutritional content varied: the sample containing 5 g of fishbone flour had the highest value for all nutrients except fat and carbohydrates. Given these findings, the formula with 2.5 g fishbone flour was selected as the best treatment because it exhibited a nutritional profile that closely resembled that of the 5 g fishbone flour formula and had an acceptance score that most closely matched the control sample.

Nutrition claims

The sausage with 2.5 g fishbone flour has a serving size of 60 g, which is equivalent to two sausages. The potential nutrition claims for mackerel sausage enhanced with fishbone flour are based on the Nutrient Label Reference (NLR) outlined in Regulation No. 9 of 2016 of the The Food and Drug Supervisory Agency of Indonesia (BPOM), which pertains to general group categories. Determining nutrition claims for mackerel sausage products is guided by the BPOM Regulation Number 1 of 2022, which governs the supervision of claims on processed food labels and advertising. Further details are provided in Table 5.

A product is qualified as a source of protein and calcium if it contains a minimum of 20% of the nutrient reference value (NRV) per 100 g (in solid form) for protein and 15% of the NRV per 100 g (in solid form) for vitamins and minerals. Mackerel sausage formulated with 2.5 g fishbone flour may potentially meet these protein and calcium source criteria. Furthermore, the sausage complies with the Indonesian National Standard (SNI) required by the National Standardization Agency of Indonesia (BSN 2015), as outlined in SNI 3820:2015, "Meat Sausage."

CONCLUSION

The addition of fishbone flour significantly increased protein and calcium levels in the samples. The sample produced with the addition of 2.5 g (w/w) of fishbone flour was identified as the preferred option. The selected sample contained 64.24 g of moisture, 1.97 g of ash, 13.04 g of protein, 3.96 g of fat, 16.80 g of carbohydrate, and 165.19 mg of calcium per 100 g. Each 60 g serving is equivalent to two sausages and provides 93 kcal. The mackerel sausage, formulated with 2.5 g of fishbone flour, meets nutritional requirements, particularly as a source of protein and calcium. Further studies are needed to enhance the chewiness and to reduce the strong fishy flavor and aftertaste. Additionally, evaluating the shelf life, microbiological activity, and bioavailability of protein and calcium in the mackerel sausage products could provide valuable insights. The increased amount of fishbone flour in the production of mackerel sausage resulted in samples with a lighter color, a less chewy texture, and a stronger fishy flavor.

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