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**PROXIMATE COMPOSITION, CALCIUM AND PHOSPHORUS CONTENT OF
 SIMPUL CAKE FORTIFIED WITH SKIPJACK TUNA BONE FLOUR**

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Background: Fish bones are a potential source of calcium and phosphorus for food fortification. The compositional effect of substituting skipjack tuna bone flour for wheat flour in Simpul cake has not been established. **Purpose:** This study aimed to compare the proximate composition, calcium and phosphorus content of fortified and unfortified Simpul cake. **Methods:** One independent batch was prepared for each formulation. A homogenized laboratory sample from each batch was analyzed in triplicate for moisture, ash, protein, fat, calcium, and phosphorus; carbohydrate was calculated by difference. Results were summarized as mean $\pm$ standard deviation. Because there was only one batch per formulation, comparisons were descriptive and no inferential statistical test was performed. **Results:** Compared with the unfortified cake, the fortified cake had higher protein ($17.12 \pm 0.03\%$ versus $15.18 \pm 0.05\%$) and ash ($0.72 \pm 0.01\%$ versus $0.65 \pm 0.02\%$) contents. Carbohydrate, moisture, and fat contents were lower. Calcium increased from a reported $0.02 \pm 0.001\%$ to $1.99 \pm 0.001\%$ on a dry-weight basis, and phosphorus from $0.03 \pm 0.002\%$ to $0.38 \pm 0.001\%$. **Conclusion:** Substitution with skipjack tuna bone flour was associated with changes in the measured composition of Simpul cake and may provide a use for a local fish-processing by-product. Further studies are needed to assess mineral bioavailability and any potential dental-health effects.

Keywords: Calcium and phosphorus fortification; Simpul cake; Skipjack tuna bone flour

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INTRODUCTION

Simpul cake, also known as Ote-otere, is a traditional snack from South Sulawesi, particularly Sinjai Regency. It is commonly prepared from wheat flour and other locally available ingredients. As a familiar food product, Simpul cake provides an opportunity to investigate whether a locally sourced ingredient can increase its mineral content. Any nutritional advantage, however, needs to be demonstrated in the finished cake rather than inferred from the ingredient alone.

Fish bones contain calcium and phosphorus and can be processed into flour for food fortification.^{1,2} These minerals contribute to the formation and maintenance of mineralized tissues, including bones and teeth.^{3,4} Previous studies have investigated the mineral composition and dental applications of skipjack tuna (*Katsuwonus pelamis*) bone material,⁵⁻⁷ while other studies have incorporated fish bone flour into various food products.^{8,9} These findings provide a scientific rationale for testing skipjack tuna bone flour in Simpul cake. They do not, however, establish the mineral content or health effects of the finished cake.

The use of skipjack tuna bones also has potential relevance to the local food economy. Skipjack tuna is a fishery product in Sinjai Regency, and its processing generates bones that could be converted into a food ingredient.¹⁰ Developing products from fish-processing by-products may increase their value and reduce

material losses.¹¹ For Simpul cake, this approach could connect a traditional snack with a locally available resource. Whether it is economically feasible would depend on processing costs, food safety, consumer acceptance, and market demand, none of which were evaluated in this study.

Despite the existing research on fish bone flour, its effect on Simpul cake remains unclear. The formulation and preparation of this snack differ from those of previously studied products; consequently, their findings cannot be directly applied to it. In particular, it is necessary to determine whether replacing part of the wheat flour with skipjack tuna bone flour increases calcium and phosphorus in the finished cake and how the substitution affects its proximate composition. A higher measured mineral concentration could make the fortified formulation more mineral-rich than the original product. It would not, by itself, demonstrate mineral bioavailability, enamel remineralization, or prevention of dental caries.

This study therefore compared the proximate composition and calcium and phosphorus content of unfortified Simpul cake with those of a formulation in which skipjack tuna bone flour replaced 10% of the wheat flour. The 10% level was selected based on various organoleptic or hedonic tests; this 10% addition limit represents the optimal concentration that remained acceptable to panelists—in terms of color, aroma, texture, and taste—when compared to higher concentrations.^{12,13} The findings are intended to provide compositional evidence for further development of the product and a basis for subsequent studies of sensory acceptance, mineral bioavailability, and potential health applications.

MATERIALS AND METHODS

The Universitas Muslim Indonesia's ethical commission has examined and accepted this research protocol, with approval number 978/A.1/KEP-UMI/XII/2025.

Study design and experimental unit

This experimental comparison included an unfortified Simpul cake formulation and a formulation in which skipjack tuna bone flour replaced 10% of the wheat flour by weight. One independent preparation batch was produced for each formulation, yielding approximately 2000 g of Simpul cake per batch. Following preparation, portions were collected from different parts of each batch and combined and homogenized to obtain a representative laboratory sample. One laboratory sample from each formulation was subjected to compositional analysis, with each parameter measured in triplicate as technical replicate assays. The study was conducted from October to Desember 2025, and the preparation batch was considered the experimental unit. The triplicate analytical measurements represented technical replicates and were used to evaluate analytical repeatability rather than as independent experimental observations.

The use of one preparation batch per formulation reflected the preliminary and exploratory nature of the study, which aimed to characterize and compare the nutritional composition of the standard and 10% skipjack tuna bone flour-fortified Simpul cake formulations. Technical triplicate measurements were performed to improve the reliability of the analytical measurements. Because independent preparation batches were not replicated, the results should be interpreted as preliminary, and further studies using multiple independently prepared batches are warranted to confirm the reproducibility of the observed differences.

Preparation of skipjack tuna bone flour

The collected Skipjack Tuna bones were washed thoroughly, boiled at 80°C for 30 minutes to remove adhering tissue, blood, and fat attached to the bones, then washed thoroughly in running water. The fish bones were chopped into tiny pieces and boiled again at 100°C for 30 minutes. Fish bones were hydrolyzed by immersing them in a 1M HCL solution for two hours. Next, the Skipjack Tuna bones were washed thoroughly until the pH was neutral. The fish bones were then dried in an oven set at 105°C for four hours. The dried bones were milled into flour.

Simpul cake formulation dan preparation

Simpul cake was prepared using wheat flour, shallots, sugar, and butter. Two formulations were prepared: an unfortified control and a fortified formulation. In the fortified formulation, 10% of the wheat flour was replaced with skipjack tuna (*Katsuwonus pelamis*) bone flour, while the other ingredients were kept consistent between formulations. The ingredients were mixed to form a homogeneous dough, shaped, and cooked using the same preparation procedure for both formulations. After cooking, the samples were cooled to room temperature and prepared for laboratory analysis.

Proximate analysis in Simpul cake

Moisture content was determined using AOAC Method 925.10 (oven-drying method), crude protein using AOAC Method 920.87 (Kjeldahl method), crude fat using AOAC Method 920.39 (Soxhlet extraction), and ash using AOAC Method 923.03 (muffle-furnace incineration). Carbohydrate content was calculated by difference. Dietary fiber was not determined separately and was therefore included in the calculated carbohydrate fraction. Each parameter was analyzed in triplicate as technical replicate measurements. Appropriate laboratory quality-control procedures, including instrument calibration and analytical blanks where applicable, were applied. All proximate composition results were expressed as percentages on a wet-weight (as-is) basis.

Calcium levels analysis in Simpul cake

Calcium content was determined by atomic absorption spectrophotometry (AAS). Approximately 1 g of homogenized sample was wet-digested using concentrated nitric acid (HNO₃) until a clear solution was obtained. After cooling, the digest was transferred to a volumetric flask and diluted to 50 mL with deionized water. Additional dilution was performed when necessary to bring the calcium concentration within the calibration range.

Calcium was measured at 422.7 nm using a Shimadzu AA-7000 atomic absorption spectrophotometer in flame mode with an air-acetylene flame. Calibration was performed using calcium standard solutions, with a reagent blank included for quality control. Each sample was measured in triplicate as technical replicates. The calcium concentration obtained from the digest was converted to calcium content in the sample and adjusted for moisture content to express the result on a dry-weight basis. Calcium content was calculated as: $\text{Calcium (\% db)} = [(C \times V \times DF) / (m \times DM)] \times 100$ where C is the calcium concentration in the digest (g/L), V is the final digest volume (L), DF is the dilution factor, m is the initial sample mass (g), and DM is the dry-matter fraction of the sample [DM = (100 – moisture content)/100]. Calcium content was reported as percent dry basis (% db). The analytical procedure was based on the atomic absorption spectrophotometric principles of AOAC Method 968.08, with modifications appropriate to the sample matrix and laboratory digestion procedure.

Phosphorus levels analysis in Simpul cake

Phosphorus content was determined by UV-visible spectrophotometry using a Varian Cary 50 spectrophotometer. Following acid digestion, an aliquot of the sample digest was reacted with ammonium molybdate reagent for color development, and absorbance was measured at 420 nm. Phosphorus concentration was determined from a calibration curve prepared using phosphorus standard solutions, with a reagent blank used for blank correction. Appropriate dilution factors were applied, and each sample was measured in triplicate as technical replicates. The phosphorus concentration obtained from the digest was converted to phosphorus content in the sample and adjusted for moisture content to express the result on a dry-weight basis. Phosphorus content was calculated as: $P (\% \text{ db}) = (C \times V \times DF) / (10,000 \times m \times DM)$, where C is the phosphorus concentration determined from the calibration curve (mg/L), V is the final digest volume (L), DF is the dilution factor, m is the sample mass (kg), and DM is the dry-matter fraction of the sample [DM = (100 – moisture content)/100]. Results were reported as percent dry basis (% db). The procedure was adapted from AOAC Method 965.17 with modifications appropriate to the sample matrix and laboratory procedure.

Data analysis

The preparation batch was considered the experimental unit. Each parameter was measured in triplicate as technical replicates and summarized as mean ± SD. Because only one independent batch was prepared per formulation, comparisons were descriptive, and no inferential statistical tests were performed.

RESULTS

Proximate analysis of Simpul cake made from Skipjack tuna bone flour formulation to determine the water, ash, protein, fat and carbohydrate content can be seen in Table 1.

Table 1. Proximate value of Simpul cake with and without the addition of Skipjack tuna bone flour

Sample	Water content Mean $\pm$ SD (%)	Ash content Mean $\pm$ SD (%)	Protein content Mean $\pm$ SD (%)	Fat content Mean $\pm$ SD (%)	Carbohydrate content Mean $\pm$ SD (%)
Without the addition of fish bone flour	2,37 $\pm$ 0,05	0,65 $\pm$ 0,02	15,18 $\pm$ 0,05	29,49 $\pm$ 0,03	52,31 $\pm$ 0,08
With the addition of fish bone flour	2,25 $\pm$ 0,02	0,72 $\pm$ 0,01	17,12 $\pm$ 0,03	29,01 $\pm$ 0,03	50,90 $\pm$ 0,10

Table 1 shows changes in the proximate composition of Simpul cake following 10% substitution of wheat flour with skipjack tuna bone flour. The most notable change was observed in protein content, which increased from 15.18 $\pm$ 0.05% in the unfortified cake to 17.12 $\pm$ 0.03% in the fortified cake, corresponding to an approximately 12.8% relative increase. Ash content also increased from 0.65 $\pm$ 0.02% to 0.72 $\pm$ 0.01%, representing an approximately 10.8% increase. In contrast, carbohydrate content decreased from 52.31 $\pm$ 0.08% to 50.90 $\pm$ 0.10%, while smaller reductions were observed in moisture (2.37 $\pm$ 0.05% to 2.25 $\pm$ 0.02%) and fat content (29.49 $\pm$ 0.03% to 29.01 $\pm$ 0.03%). Overall, the descriptive results indicate that 10% substitution with skipjack tuna bone flour was associated with a shift toward higher protein and ash contents and lower carbohydrate, moisture, and fat contents. These differences are descriptive and should not be interpreted as statistically significant because only one independent preparation batch was analyzed per formulation.

Analysis of phosphorus and calcium content in Simpul cake with and without the addition of Skipjack tuna bones is shown in Table 2.

Table 2. Phosphorus and calcium content in Simpul cake

Sample	Phosphorus levels (% db)	Calcium levels (% db)
Without the addition of fish bone flour	0,03 $\pm$ 0,002	0,02 $\pm$ 0,001
With the addition of fish bone flour	0,38 $\pm$ 0,001	1,99 $\pm$ 0,001

Table 2 shows the phosphorus and calcium contents of Simpul cake with and without 10% skipjack tuna bone flour substitution. The fortified formulation showed markedly higher levels of both minerals. Phosphorus content increased from 0.03 $\pm$ 0.002% db in the unfortified cake to 0.38 $\pm$ 0.001% db in the fortified cake, representing approximately a 12.7-fold increase. An even greater increase was observed in calcium content, from 0.02 $\pm$ 0.001% db to 1.99 $\pm$ 0.001% db, corresponding to approximately a 99.5-fold increase. The increase in calcium was therefore substantially greater than that observed for phosphorus. Overall, these descriptive findings indicate that replacing 10% of the wheat flour with skipjack tuna bone flour markedly increased the measured mineral content of Simpul cake, particularly calcium. As only one independent preparation batch was analyzed per formulation, these differences are descriptive and should not be interpreted as statistically significant.

DISCUSSION

Substitution of 10% of the wheat flour with skipjack tuna bone flour was associated with differences in the measured proximate and mineral composition of Simpul cake. The fortified formulation had higher reported protein and ash contents and lower reported carbohydrate, moisture, and fat contents than the unfortified formulation. Calcium and phosphorus were also reported at higher concentrations in the fortified sample. These observations suggest that incorporating bone flour altered the composition of the product.

The water content of Simpul cakes fortified with skipjack tuna bone flour was lower than that of the unfortified cakes. The observed difference may be related to changes in formulation or processing conditions; however, its underlying cause cannot be determined from the measurements performed in the present study. A previous study suggested that the reduction in moisture content following bone meal addition may be associated with interactions involving Ca^{2+} and OH^- ions.¹⁴ However, this proposed mechanism was not evaluated in the

present study, and the observed reduction in moisture content therefore cannot be directly attributed to Ca^{2+} – OH^- interactions.

The ash content of the cake with the addition of fish bones flour increased. Ash provides an approximate estimate of the total inorganic material in food, whereas calcium and phosphorus are determined using element-specific analytical procedures. The direction of this change is consistent with the addition of bone flour, although ash analysis cannot identify the individual minerals responsible.¹⁵ The use of different procedures for proximate ash analysis and element-specific determination may introduce differences related to sample selection, preparation, recovery, calibration, and calculation. Such differences warrant examination, but they should not be assumed to explain the present results without evidence.

The addition of fish bones to Simpul cake resulted in a 2% increase in protein content. Substituting an ingredient with a different protein composition for part of the wheat flour could contribute to this result. However, the protein content of the skipjack tuna bone flour used in the present study was not reported, so the contribution of the flour cannot be quantified. A study has shown that the protein content obtained from tuna is of very good quality,¹⁶ so the addition of protein indicates that fish bones have a fairly high protein content.^{17,18}

The fat content in Simpul cake with the addition of fish bone flour decreased, this could occur due to the heating process during processing which allows some fat components to evaporate. In addition, the decrease in fat content could occur due to an increase in nutrient levels such as ash content in the proximate test.¹⁴

The results of the analysis of the chemical characteristics of carbohydrate content show that the addition of fish bone flour can reduce the carbohydrate content of Simpul cake. The wheat flour used in making Simpul cake certainly has a fairly high carbohydrate content, while fish bones only contain a small amount of carbohydrates, so when fish bone flour is added to replace wheat flour, it will reduce the carbohydrate content in Simpul cake.¹⁷

Calcium contents were reported to increase in Simpul cake supplemented with fish bone flour. The direction of the result is compatible with the use of fish bone flour as a calcium-containing ingredient. A previous study of donuts fortified with *Pangasius* bone flour also investigated an increase in calcium content, although its concentrations cannot be compared directly with the present study because the ingredients, formulations, and processing conditions differ.¹⁸

The increased calcium and phosphorus content in this Simpul cake is due to the high calcium and phosphorus content of skipjack tuna bone meal. Several chemical analyses have shown that fish bones are a valuable source of calcium phosphate, an economically viable source for synthesizing hydroxyapatite, an essential component of bone, and can be used as a material for bone regeneration.^{2,7,9,18-20}

In the batches examined, replacing 10% of the wheat flour with skipjack tuna bone flour was associated with higher measured protein and ash contents and lower carbohydrate, moisture, and fat contents in Simpul cake. Calcium and phosphorus were also reported at higher levels, suggesting that fish bone flour may be useful for mineral fortification while providing a use for a local fish-processing by-product.

The study included only one independently prepared batch per formulation and evaluated a single substitution level. The three measurements per batch were analytical replicates, so the findings are preliminary and cannot establish variation between production batches or statistical significance. Sensory acceptance, mineral bioavailability, and dental-health outcomes were not assessed. Further studies should resolve the analytical discrepancy, test multiple independent batches and substitution levels, and evaluate the fortified cake's nutritional and practical value.

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