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Calcium Assay of Tuna Bone Waste with Atomic Absorption Spectrophotometer (AAS)

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Abstract
Yellowfin tuna in Gorontalo is very abundant and is often exported outside the region and even abroad as fillets, so yellowfin tuna bones are also very plentiful. The community frequently processes yellowfin tuna bones into kuah asam or namely ilahe. One of the sources of ilahe is *Averrhoa bilimbi* L., but the leftover tuna bones are only a source of pollution. This research focuses on processing the waste into a product of high nutritional value and highlights the novelty of a more environmentally friendly fish bone calcium extraction method. One of the main novelties of this research is the use of natural materials, namely star fruit, as an alternative for calcium extraction, which reduces dependence on harmful chemicals and supports the principle of sustainability. The extraction method used heat, followed by drying to reduce moisture content, pulverizing (blender) to reduce particle size and sieving. The calcium content in tuna bone meal was then analyzed using the Atomic Absorption Spectroscopy (SSA) method. The results showed that the tuna bone meal was white in colour, and the calcium content was 184.4890 mg/g or 18.45%. This product has the potential to be applied in various fields, such as food, health, animal feed, pellets, and organic fertilizer, as well as a solution to reduce fishery waste and support environmental sustainability.

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INTRODUCTION

Tuna fish in Indonesia is very abundant, and so is the production of canned processing, fillets, and surimi products (Food And Agriculture Organization, 2021). Data Kementerian Kelautan dan Perikanan (KKP) in 2020 showed that tuna production was 236,373 tons (Kementerian Kelautan dan Perikanan, 2020). According to the Badan Pusat Statistik (BPS), the export of tuna commodities in May 2024 recorded an increase in export volume of 17.4% from 2023, and the export value was US $ 374.69 million as of May 2024 (Badan Pusat Statistik Provinsi Gorontalo, 2024). Various export forms within and outside the country are fresh, chilled and frozen tuna fish and fillets. This data also reflects the potential of tuna fish waste in Gorontalo that can be utilized in the form of tuna fish bones. A skeleton or bone is a sturdy and strong tissue that provides structure to the body. Bone is composed of a complex organic matrix reinforced by calcium and other minerals (Husna et al., 2020).

Due to its complex nature, bone is difficult for decomposers to decompose, so it often becomes solid waste that is considered to have no economic value. As a result, bone waste management is necessary to prevent environmental pollution, which is usually characterized by pungent odours and piles of bones that spoil the aesthetics. One processing method can be applied is to process bones into flour (Husna et al., 2020). The nutritional content of tuna fish bones is rich in minerals; this calcium offers an alternative daily calcium intake that can be obtained in the daily diet. In bone meal, the most dominant element is calcium (Cui et al., 2021; Novianty et al., 2024; Savlak et al., 2020).

Calcium has many benefits, including bone and tooth formation, coagulation, nerve cell signal transmission, muscle contraction, and bone density (Muhali et al., 2023; Raya et al., 2023). The use of calcium preparations in various fields, namely the livestock and fisheries sector as feed, as well as in agriculture as a fertilizer and the health sector as a food supplement or fortification, making toothpaste and other industrial fields. Calcium extraction generally uses chemicals, but its safety still needs further research, so this study uses natural ingredients, namely fruit *Averrhoa bilimbi* L. *Averrhoa bilimbi* L. contains various organic acids, giving it a characteristic sour taste. One of the most dominant acids in *Averrhoa bilimbi* L. is oxalic acid, the highest organic acid found in this fruit (Muzaifa, 2018). In addition, *Averrhoa bilimbi* L also contains citric acid and acetic acid, which contribute to its sour taste and potential use in various culinary and health applications (Hatta et al., 2022). Research shows that the acetic acid content in *Averrhoa bilimbi* L reaches 1,9%, while citric acid can reach 44.6% (Hatta et al., 2022). Acid solutions can dissolve proteins and fats accompanied by heating (Sri et al., 2021). In research (Wijayanti et al., 2018), *Averrhoa bilimbi* L. can be utilized in the calcium extraction process, which is an alternative to acid, with variations in concentration and length of soaking time can significantly affect calcium extraction levels in tilapia bones.

Calcium is an essential mineral with numerous physiological and industrial applications. It plays a crucial role in bone and tooth formation, blood coagulation, nerve signal transmission, muscle contraction, and overall bone density maintenance (Muhali et al., 2023; Raya et al., 2023). The industrial applications of calcium span across livestock and fisheries for feed production, agriculture as a fertilizer, the health sector for supplements and fortification, and various manufacturing processes including toothpaste production. Traditional calcium extraction techniques largely rely on chemical processes, often involving strong acids and bases. However, concerns about the safety, environmental impact, and sustainability of such methods have led researchers to explore alternative, natural extraction techniques. One such approach is the use of Averrhoa bilimbi L., a fruit rich in organic acids such as oxalic, citric, and acetic acid, which has shown potential in calcium extraction processes (Muzaifa, 2018; Hatta et al., 2022).

Averrhoa bilimbi L. is characterized by its high acidity, attributed to its composition of oxalic acid, citric acid, and acetic acid. The dominant organic acid in the fruit is oxalic acid, which has a high chelating ability for calcium, forming insoluble calcium oxalate. This interaction is crucial in calcium extraction as it facilitates the release of calcium ions from complex biological matrices (Wijayanti et al., 2018). Oxalic Acid the major organic acid, responsible for calcium chelation and precipitation. Citric Acid functions as a mild acid that can enhance calcium solubilization. Acetic Acid assists in breaking down proteins and fats that may encase calcium-rich structures (Hatta et al., 2022;Muhali et al., 2023).

Acid solutions, particularly when combined with heat, can dissolve proteins and fats that encase calcium in biological materials (Sri et al., 2021). This makes Averrhoa bilimbi L. a suitable natural agent for extracting calcium from sources such as fish bones. Conventional calcium extraction methods typically use hydrochloric acid (HCl) or sulfuric acid (H2SO4). These methods, while efficient, pose several risks. Chemical residues in extracted calcium may cause health concerns. Environmental Impact disposal of acidic waste can contribute to soil and water pollution. Nutritional Integrity harsh chemical processing may degrade the nutritional quality of calcium compounds.

Using Averrhoa bilimbi L. provides a safer, eco-friendly alternative with reduced environmental impact and the potential to preserve calcium's bioavailability in supplements and food fortification. Recent research by Wijayanti et al. (2018) demonstrated that calcium extraction efficiency is significantly influenced by acid concentration and soaking duration. Key factors to consider include. Higher concentrations of organic acids enhance calcium solubilization but may lead to excessive degradation of bone structures. Longer exposure times improve calcium release but may also result in excessive leaching of other minerals. Heating can accelerate the breakdown of proteins and fats, improving calcium extraction efficiency. Despite its advantages, the use of *Averrhoa bilimbi* L. for calcium extraction presents some challenges. The high oxalic acid content may lead to calcium precipitation as calcium oxalate, which is insoluble and can reduce calcium bioavailability.

The efficiency of large-scale extraction *using Averrhoa bilimbi* L. needs further evaluation to ensure feasibility for industrial applications. Variability of fruit composition due to factors like maturity, environmental conditions, and storage methods may affect extraction consistency. Averrhoa bilimbi L. presents a promising natural alternative for calcium extraction, offering benefits in terms of safety, sustainability, and environmental impact. While challenges such as calcium oxalate formation and process standardization need to be addressed, further research could pave the way for its integration into commercial applications. The exploration of hybrid techniques and optimized processing conditions will be key in maximizing the potential of this natural extraction method.

The purpose of this research is to process the abundant yellowfin tuna bone waste in Gorontalo into a product of high nutritional value, especially calcium flour, by using an environmentally friendly calcium extraction method. This method uses *Averrhoa bilimbi* L, which uses natural ingredients that can reduce dependence on hazardous chemicals. Then, calcium levels will be analyzed using Atomic Absorption Spectroscopy (AAS).

METHOD

Materials

The materials used consist of were yellowfin tuna bone waste (Thunnus albacares), Averrhoa bilimbi, aluminum foil, nitric acid (HNO_3) p.a., perchloric acid ($HClO_4$) p.a, aquabides (H_2O), aquadest, whatman papers no. 42, solution calsium (Ca) 1000 mg/L.

Equipment and Instruments

The equipment used includes general laboratory glassware, Atomic Absorption Spectrophotometer (AAS) Varian AA240ES, hot plate, analytical balance, pH meter, blender, thermometer, and filter, hot plate.

Work Procedures

Preparing tuna bone waste followed the modified procedure of Musdalifah et al. (2016). Fish bones are boiled and cleaned to remove the remaining meat still attached. After cleaning, the degreasing process is carried out by cooking for 30 minutes at 80^{o}C, and then the bones are drained and dried in the sun for 1 hour and then boiled again using 100 gr of *Averrhoa bilimbi* L. with a temperature of 100 ^{o}C (Decolagenation). Next, it was drained and dried at 60 ^{o}C for 6 hours. The stage of making tuna bone flour is done by blending and sieving so that tuna bone flour is obtained (Musdalifah et al., 2016).

Weigh 1 gram of tuna bone meal, then add 2 mL of aquabides and 5 mL of nitric acid (HNO_3) p.a., then add aquabides as much as 50 mL. The sample was heated on a hotplate until the volume of the solution was 40 mL. After the volume reaches 40 mL and white smoke appears, add perchloric acid ($HClO_4$) p.a. as much as 1 mL. Heat the solution to 20 mL, and the solution is clear yellow. The deconstructed solution is filtered into a 100 mL flask with Whatman paper no. 42, then the volume is adjusted to the limit mark with aquabides and homogenized. Store in a dark bottle. Doing in Duplo (Badan Standarisasi Nasional, 2005; Dewi et al., 2021; Musdalifah et al., 2016). Furthermore, calcium content using SSA determines that the tuna bone solution is measured for absorbance with an Atomic Absorption Spectrophotometer at a wavelength of 422.7 nm (Dewi et al., 2021).

RESULTS AND DISCUSSION

Gorontalo is a speciality food processed from fish bones; one of the sources is tuna fish bones, which are then known as kuah asam or ilahe. Interestingly, this sour soup is processed with the addition of belimbing wuluh as a source of acid. This local wisdom illustrates how people know how to utilize fish bones that have little protein left with the right natural ingredients. However, the remaining bones that cannot be consumed are only wasted as waste. The utilization of star fruit as an acid substitute is a natural way to remove protein or in other words, decolagenation and the remaining minerals. Tuna bone waste was obtained from the Gorontalo auction market weighing 0.5 kg.

Tuna bones were prepared by removing the remaining meat and marrow by boiling them at 100 ^{o}C and followed by washing using running water to remove meat and marrow attached to the bone. At this stage, it is necessary to pay attention because fat and meat residues become undesirable ingredients. The next stage of hot extraction is using *Averrhoa bilimbi* L as a source of acid; acetic acid in *Averrhoa bilimbi* L reaches 1,9%, while citric acid can reach 44.6% (Hatta et al., 2022). Acetic acid dissolves both non-crosslinked and crosslinked collagen, thus increasing extraction efficiency (Astiana et al., 2016). Acid solutions can dissolve proteins and fats accompanied by heating (Sri et al., 2021). According to research (Wijayanti et al., 2018), *Averrhoa bilimbi* L can extract calcium from tilapia bones. The choice of extraction method dramatically affects the breakdown of the inner bone matrix, causing calcium release (Sumarto et al., 2021). Figure 1 shows the preparation of tuna bones into flour.

a b c

Figure 1. (a) tuna bone, (b) crude tuna bone powder and (c) tuna bone fine powder

The results of tuna bone meal preparation after preparation and extraction obtained 91,8 g of tuna bone meal; the colour of the flour obtained is close to white; in research (Novianty et al., 2024), the colour of yellowfin tuna flour is darker. The higher protein content and the possibility of stimulation of the Maillard reaction in the drying process results in the colour of the flour. In this study, the protein removal process using Belimbing Wuluh may be more optimal because it gives a lighter colour or more to the white colour and indicates that the protein removal in tuna bones is more optimal. The use of tengiri bone flour as toothpaste (Hernawan et al., 2021) and in the tuna bone itself, there have been those who have used calcium hydroxyapatite tuna bone (Hariyanto & Antasionasti, 2023). Tuna bone preparations have utilization, including research conducted (Syazili et al., 2021). Adding 3% tuna to tilapia seed feed for 42 days can increase body weight and tilapia growth rate as well as several variations with the addition of 1%, 3%, and 5% tilapia survival rates are not significantly different. This means that tuna bone meal preparations can be used as a very potential feed in the future.

The use of tuna bone meal as feed was also carried out by (Umam et al., 2024), namely in name shrimp culture; the results obtained had a significant effect on absolute weight, absolute length, specific length and specific weight growth of cultured name vannamei shrimp. Fortification of 3% and 6% tuna bone meal highly affects the calcium content in canned tuna fish (Baba, 2021; Laitupa & Husen, 2021). In addition, tuna bone powder extracted using an alkaline solution was identified to contain amino acids and saturated and unsaturated fatty acids. The amino acids identified were lysine, valine, leucine, isoleucine, methionine, threonine, histidine, phenylalanine, and tryptophan.

The amount of amino acids associated with collagen, such as glycine, proline and hydroxyproline, is high, and the amount of glutamic acid, arginine, alanine, aspartic acid and serine is relatively more. The amounts of oleic acid, palmitic acid and gondoic acid are higher, and the amounts of hexadecanoic acid, γ-linolenic acid and dihomo-γ-linolenic acid are lower. The amounts of myristic acid, stearic acid, eicosapentaenoic acid (AEP) and docosahexaenoic acid (ADH) were also relatively higher. The calcium content in tuna fish bone meal is 24.56%, and with alkaline solvents, it is 38.16% (Nemati et al., 2017). Istiqlal (2017) reported that the ash content in bluefin tuna bones was 54.79% and that in fresh tuna bones, it was 55.14% (Istiqlal, 2017). This means that the minerals contained in tuna bones have potential as mineral preparations. The tuna bone meal extract was then analyzed for calcium content using SSA. The absorbance of the calcium standard solution and sample is shown in Table 1. The absorbance values of standard solutions and samples can be seen in the table below.

Table 1. The absorbance of calcium standard solution and sample

Contentration (ppm)	Absorbtion
0	0
1	0,0449
2	0,0761
3	0,1095
5	0,1781
10	0,3329
Sample	0,2515

The graph in Figure 1 is obtained based on the measurement results of the calcium standard solution using AAS.

Figure 2 shows the graph of the calcium standard solution and then calculates the calcium content in tuna bone meal. The calcium content obtained was 184.4890 mg/g. This means that every gram of tuna fish contains 184.4890 mg of calcium. This level was converted to a percent of calcium obtained of 18.45%. Calcium is a micronutrient that the body needs and is very important; the amount of calcium intake for adolescents and adults is 1000-1300 mg/day (Hardiansyah & Supariasa, 2016). Several other studies have shown that yellowfin tuna bones contain significant calcium levels, which can benefit human and animal health. For example, research by Talib and Noh showed that yellowfin tuna bones extracted using an alkaline solution had a high calcium value, with the total mineral in the treated tuna bones reaching 287.08 mg/g (Talib & Noh, 2024). In addition, Haniastuti et al. also noted that tuna bones are high in calcium and phosphorus, which can be synthesized into hydroxyapatite, a form of calcium important for dental and bone health (Haniastuti et al., 2020).

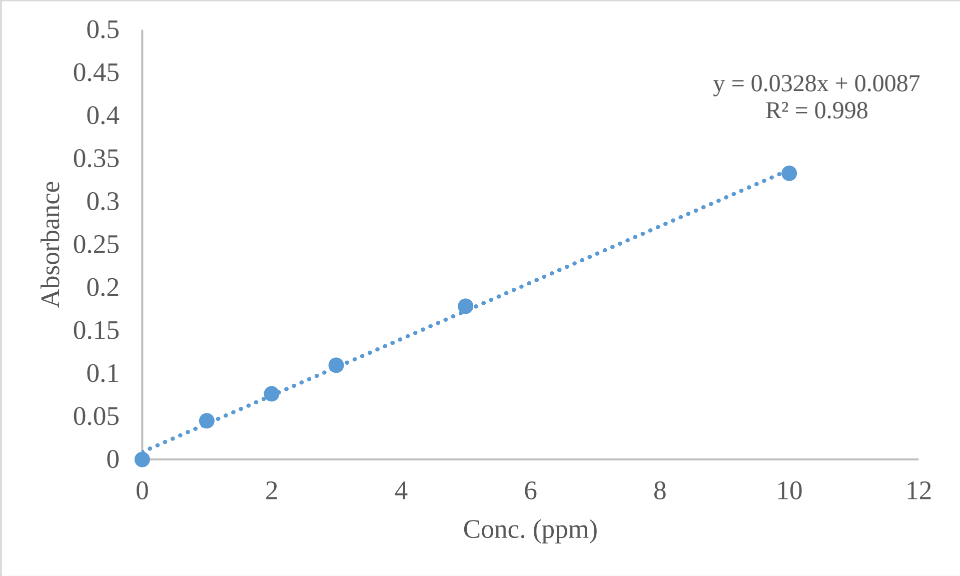

Figure 2. Linearity plot of calcium standard solution by atomic absorption spectroscopic

Yuliani et al. showed that cork fish bone meal, similar to tuna bone, can be used as a calcium fortifier in cracker ingredients. This study revealed that the substitution of cork fish bone meal improved the sensory and physico-chemical properties of crackers, thus making them more nutritious (Yuliani et al., 2018). In addition, research by Edam emphasized that fish bone meal can be used to fortify meatballs, a popular food in Indonesia. This study showed that adding fish bone meal to meatballs can increase the calcium content, providing health benefits for various age groups (Edam, 2018). Furthermore, research by Daeng showed that skipjack bone meal can be used in biscuit making, which can also be applied to tuna bone meal. This study emphasized that adding skipjack bone meal not only increased the nutritional value of biscuits but also improved the sensory quality of the final product (Daeng, 2019). This suggests that tuna bone meal can function similarly to other processed products.

The utilization of tuna fish bone meal by Fitrasyah et al. showed that tuna bone meal can be used in making biscuits, which contributes significantly to the calcium content in the product. This study revealed that tuna fish bone meal-based biscuits increased nutritional content and had good acceptability among consumers (Fitrasyah et al., 2023). This suggests that tuna bone meal can be a good alternative to increase calcium intake in the daily diet for both humans and animal feed.

CONCLUSION

Based on the study's results, it can be concluded that the extraction of tuna bone waste with the addition of Averrhoa bilimbi produces a white flour preparation with a calcium content of 184.4890 mg, equivalent to 18.45%..

RECOMMENDATIONS

Future research can focus on developing calcium-rich tuna bone meal-based products, such as food supplements or animal feed, to increase the added value of this fishery waste and support environmental sustainability.

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