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

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Abstract
Fishery waste, especially tuna bones, which are abundant in Gorontalo, is one of the causes of environmental pollution because it has not been optimally utilized. This research aims to utilize tuna bone waste as an environmentally friendly and high nutritional value calcium source. One of the innovations is using Belimbing Wuluh as a substitute for chemicals in the tuna bone processing. The research method includes boiling tuna bones using Averrhoa bilimbi solution to remove collagen, drying to reduce water content, pulverizing with a blender, and sifting to produce fine bone meal. The calcium content in the bone meal was tested using Atomic Absorption Spectroscopy (SSA). The results showed that the tuna bone meal produced was white in color 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 the production of canned processing, fillets, and surimi products (FAO, 2021). Data Kementerian Kelautan dan Perikanan (KKP) in 2020 showed that tuna production was 236,373 tons. Tuna in Tomini Bay is so abundant that Gorontalo is included in the largest tuna-exporting province within and outside the country. Some of the destination countries are Malaysia, Japan, Vietnam, Singapore, America and others. Based on data from the data dari Dinas Kelautan dan Perikanan Provinsi Gorontalo, in 2022, tuna exports amounted to 31.8 tons. According to the Badan Pusat Statistika (BPS), exports through ports/airports as of June 2024 amounted to $ 2,124,989, and tuna commodities are one of the largest. Various types of domestic and foreign export forms, namely fresh, chilled, and frozen tuna fish, as well as fillets (Infopublik 2023; BPS Provinsi Gorontalo, 2024). This data also reflects the potential of tuna fish waste in Gorontalo that can be utilized, one of which is tuna fish bones. A skeleton or bone is a sturdy and strong tissue that functions to provide structure to the body. Bone is composed of a hard organic matrix reinforced by calcium and other minerals. Due to its hard 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 odors and piles of bones that spoil the aesthetics. One of the processing methods that can be applied is to process bones into flour (Husna et al., 2020). The tuna fish used in this study is *Thunnus albacares*. The nutritional content of tuna fish bones is rich in minerals; precisely this calcium offers an alternative daily calcium intake that can be obtained in the daily diet; in bone flour, the most dominant element is calcium (Savlak et al., 2020; Cui et al., 2021; Novianty et al., 2024).

Calcium has many benefits, including bone and tooth formation, coagulation, nerve cell signal transmission, muscle contraction, and bone density (Raya et al.,2023). Calcium preparations are used in various fields, namely the livestock and fisheries sector as feed, agriculture as fertilizer, health as supplements and food fortification, toothpaste making, and other industrial fields. Calcium extraction generally uses chemicals, but its safety still needs further research, so this study uses natural ingredients, namely Averrhoa bilimbi. According to Carangal et al. (1961), it has a high content of organic acids. There are several organic acids in *Averrhoa bilimbi* wealth: acetic acid, citric acid, formic acid, lactic acid, and lactic acid. Acid solutions can dissolve proteins and fats accompanied by heating (Sri et al.,2021). In research (Wijayanti et al., 2018), Averrhoa bilimbi can be utilized in the calcium extraction process, which is an alternative to acid substitutes, with variations in concentration and length of soaking time can significantly affect calcium extraction levels in nila bones.

Our study is designed to make a significant contribution to the fisheries sector by utilizing fishery waste, particularly tuna bones. These bones will be processed into flour preparations, extracted with *Averrhoa bilimbi*, and then analyzed for calcium levels using SSA. This innovative approach not only addresses the issue of fishery waste but also offers a potential solution for enhancing the calcium content in food and feed products.

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 80oC, and then the bones are drained and dried in the sun for 1 hour and then boiled again using 100 gr of Averrhoa bilimbi with a temperature of 100 oC (Decolagenation). Next, it was drained and dried at 60 oC for 6 hours. The stage of making tuna bone flour is done by blending and sieving so that tuna bone flour is obtained.

Weigh 1 gram of tuna bone meal, then add 2 mL of aquabides and 5 mL of nitric acid (HNO3) 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 (HClO4) 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 (Musdalifah et al., 2016; Dewi et al., 2021; SNI 06-6989.56-2005). 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

Tuna bone waste was obtained from Gorontalo auction market weighing 0.5 kg. Tuna fish bones were prepared by removing the remaining meat and marrow by boiling them at 100 0C. Then, washing the meat and marrow attached to the bone was done using running water. 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* as a source of high citric acid as a substitute for acid solvents (Carangal et al., 1961). Of the five acids, remove collagen while extracting calcium in tuna fish bones. Heat denaturation can separate or break down collagen (Gomez et al., 2009). According to research (Wijayanti et al., 2018). Averrhoa bilimbi can extract calcium from nila bones. The choice of extraction method dramatically affects the breakdown of the inner bone matrix, causing calcium release (Sumartono et al., 2021). Figure 1 shows the preparation of tuna bone 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 color of the flour obtained is close to white; in research (Novianty et al., 2024), the color 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 color of the flour. In this study, the protein removal process using Averrhoa bilimbi may be more optimal, giving a lighter color or more to the white color and indicating that the protein removal in tuna bones is more optimal. The utilization 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 nila seed feed for 42 days can increase body weight and nila growth rate as well as several variations with the addition of 1%, 3%, and

5% nila 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 significantly affected absolute weight, absolute length, specific length, and specific weight growth of cultured name shrimp. Fortification of 3% and 6% tuna bone meal significantly affects the calcium content in canned tuna (Baba et al., 2021; Laitupa & Husen, 2021).

In addition, nine essential amino acids for the human body, lysine, valine, leucine, isoleucine, methionine, threonine, histidine, phenylalanine, and tryptophan, were detected in tuna bones. The number of amino acids associated with collagen, such as glycine, proline, and hydroxyproline, was high, and the amount of glutamic acid, arginine, alanine, aspartic acid, and serine was relatively higher. 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. Calcium content in tuna skeleton was 24.56% with alkaline solvent (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%. This means that the minerals contained in tuna bones have potential as mineral preparations. In Table 1, the absorbance of standard and sample solutions is presented. Table 1 shows the absorbance values of standard and sample solutions, which 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.

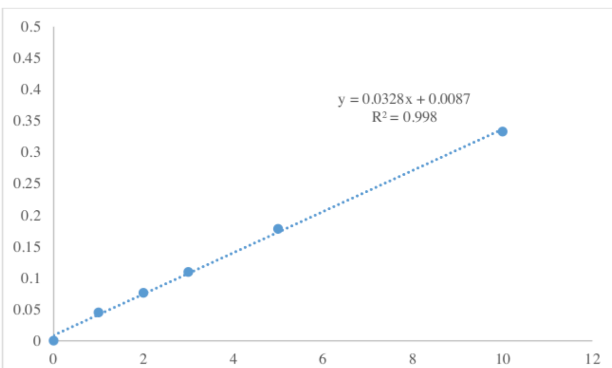

Figure 2. Grafik standar kalsium

Figure 1 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 percentage 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).

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%.

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