



Analysis of Calcium Content in Tuna Bones Using an Enzymatic Method

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Abstract

Bone waste of mackerel tuna (*Euthynnus affinis*) has great potential as a natural source of calcium. The enzymatic approach using papain offers a safe, environmentally friendly method for green extraction. This study aims to evaluate the effects of variations in papain concentration on the yield and calcium content of cod fish bone hydrolysis. The method used was a one-factor Complete Random Design with four levels of papain concentration (6, 8, 10, and 12 %) and three replicates. Hydrolysis is carried out at 60 °C and pH 6.5 for 4 hours. Calcium levels were analyzed using an Atomic Absorption Spectrophotometer (SSA) at a wavelength of 422 nm. ANOVA showed that papain concentration had a significant effect on yield ($p < 0.05$) but not on calcium content ($p > 0.05$). The highest yield, 105.66%, was obtained at a 6% concentration and was influenced by post-hydrolysis water retention and residual salt retention from the buffer solution. Meanwhile, the highest calcium release was achieved at a 10% concentration of 165.63 mg/g. Hydrolysis with papain has been shown to effectively degrade the collagen matrix, releasing minerals without damaging the bone's hydroxyapatite structure. Implicitly, this study offers an efficient method for the fishery waste treatment industry to produce alternative calcium supplements, while guiding the optimization of enzyme dosage based on production-target priorities.

Keywords: Atomic absorption spectrophotometer (AAS); calcium; enzymatic hydrolysis; mackerel tuna; papain; yields

Introduction

Indonesia is an archipelagic country with a wealth of marine fisheries resources, and one of its important commodities is pelagic fish, such as cod fish (*Euthynnus affinis*). This fish belongs to the family Scombridae and is widely found in the waters of Tomini Bay and along the coast of Gorontalo Province. Mackerel tuna is also one of the capture fisheries commodities with great potential for further processing into value-added food products (Avicena et al., 2024; Patrata & Aluri, 2021). This fish processing activity produces a large amount of bone waste that is often wasted, even though the bones contain essential minerals such as calcium and phosphorus, which play an important role in the formation of the body's hard tissues. Efforts to extract calcium from marine product waste are crucial to support the economic value of the processing industry (Boudreau et al., 2025).

The problem of calcium deficiency remains a global concern, including in Indonesia, where the average daily intake of the population is only around 254 mg, far below the WHO recommendation of 1.000–1.200 mg (Fitri et al., 2025). The use of fish bone waste as a source of organic calcium is a

sustainable and environmentally friendly food fortification strategy (Islam & Solval, 2025). Fish bones are composed of mineral components (55 – 70 %), organic matter (20 – 30 %), and water (10 – 20 %) (Lyssaiaos et al., 2025). Its main mineral component is hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), which is tightly bound to the matrix tissue of type I collagen. In addition to the main element, there are minor minerals such as magnesium (Mg) that maintain the tissue's structural stability. Another form of calcium found in bones is amorphous calcium phosphate (ACP), which acts as a precursor (Yu et al., 2019), as well as tricalcium phosphate (TCP) (Alrebaish & Wilson, 2020). In addition, cod bones are known to contain about 4.2 % calcium by dry mass (Suad & Novalina, 2019); other analyses show levels of up to 8.76 % with certain physical treatment methods (Cahyono et al., 2023).

Various methods have been developed to release calcium from the bone matrix, one of which is the conventional chemical method, which uses strong acidic solutions such as HCl (Mulyani et al., 2021) or organic acid immersion (Wijayanti et al., 2018). However, the method risks causing partial damage to the mineral crystal structure and leaving chemical residues. A safer and physicochemically

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measurable alternative (Nurfadilah et al., 2025; Kasim et al., 2025) is an enzymatic method using papain from papaya fruit (*Carica papaya* L.) (Ischak et al., 2025). These proteolytic enzymes specifically cleave collagen peptide bonds, thereby making the matrix structure more open and facilitating the release of calcium ions without compromising the integrity of hydroxyapatite crystals (Cahyono et al., 2022).

Although several calcium extraction methods have been studied, information on the enzyme saturation limit during specific enzymatic hydrolysis of mackerel tuna bones remains very limited. The novelty of this study lies in optimizing green extraction by varying papain doses to map the stoichiometric equilibrium point that selectively breaks down collagen without altering the phase of the bone's natural hydroxyapatite crystals. Therefore, this study aims to examine the precise effects of variations in papain concentration on the yield and calcium content of the resulting solids, to provide an applied basis for the national fishery waste treatment industry.

Methods

This laboratory's experimental research used a one-factor Complete Randomized Design (CRD), namely, variations in papain enzyme concentrations of 6%, 8%, 10%, and 12% of the sample weight, with three replications each.

Materials

The sample in this study is Mackerel Tuna (*Euthynnus affinis*) from the local fish market in Gorontalo City, and fresh skipjack tuna bones were used. Samples were taken from fish that met specific inclusion criteria (relatively uniform size), namely those with a length of ± 30 –35 cm and a weight of 700–900 grams. The materials used in this study consisted of pure papain enzyme powder, 0.1 M phosphate buffer, and distilled water, Nitric acid (HNO_3 65%), perchloric acid (HClO_4), and 70% ethanol.

The equipment used includes a drying oven, a blender or grinder, an 80-mesh sieve, a glass funnel, a water bath, a desiccator, a measuring cylinder, an Erlenmeyer flask, a pH meter, an analytical balance, a beaker, a volumetric flask, a pipette, a hotplate, and an AAS.

Mackerel tuna bone preparation

A sample of the cod fish's vertebrae is separated from the meat, cleaned, and boiled at 80 °C for 60 minutes to remove any remaining fat. This degreasing process is an important step to ensure that enzymatic activity is not hampered by fat residues (Torres et al., 2024). The bones are then dried in an oven at 60 °C for 12 hours, mashed with a blender, and sifted through an 80-mesh sieve.

Enzymatic hydrolysis process

A total of 10 grams of fish bone powder was dissolved in 50 mL of phosphate buffer

solution at a ratio of 1:5 (b/v) in an Erlenmeyer flask to maintain a pH of 6.5. Papain enzymes are added according to variations in concentration (6, 8, 10, and 12 %). The mixture is incubated at 60 °C for 4 hours to ensure optimal enzymatic hydrolysis (Jensen et al., 2024). Afterwards, the mixture is filtered, and the sediment is repeatedly washed with warm aqueous solution until the filtrate is clear, indicating that the minerals have been well isolated (León-López et al., 2019). The sediment is then dried again in the oven at 60 °C until it reaches a constant weight.

Calcium yield and yield analysis

The yield is calculated from the percentage of the proportion of the weight of the hydrolyzed calcium powder to the initial weight of the bone powder. Calcium level testing is performed using an Atomic Absorption Spectrophotometer (AAS) based on the standard acid dissolution method (Taufik et al., 2018). The yield was calculated by comparing the dry weight of the hydrolyzed product with the initial weight of the fish bones, following the standard biomaterial extraction method (Cahyono et al., 2023).

$$\text{Yield (\%)} = \frac{W_1}{W_0} \times 100 \quad (1)$$

Description:

W_1 : weight of hydrolyzed calcium flour (g)

W_0 : initial weight of fish bone meal (g)

Validation of calcium-level analysis using high-precision instruments is urgently needed (Taufik et al., 2018). Calcium levels were analyzed using the Atomic Absorption Spectrophotometry (AAS) method. Before analysis, 1 g of dry sample from hydrolysis is dissolved in 10 mL of 65 HNO_3 and 5 mL of HClO_4 , then heated on a hotplate (destroyed) until the solution becomes clear. After that, the solution is diluted with 25 mL of distilled water to the measuring limit. Measurements were made at a wavelength of 422 nm using a standard calcium curve (Latimer, 2023).

$$\text{Ca content (mg/g)} = \frac{C \times V \times Fp}{W} \quad (2)$$

Description:

C : concentration of the AAS reading result (mg/L)

V: final volume of the solution (L)

W: weight of the sample (g)

Fp: dilution factor

The test results data were then analyzed using Variegated Fingerprint Analysis (one-way ANOVA).

Result and Discussion

Effect of enzyme concentration on yield

Based on ANOVA results, variations in papain enzyme concentration had a highly significant effect ($p = 0.009 < 0.05$) on the hydrolysis yield. The average yield for each treatment is presented in **Table 1**.

Table 1. Yield value of hydrolysis results

Papain enzyme concentration (%)	Average $\pm$ Standard deviation (%)
6 %	105.66 $\pm$ 3.382b
8 %	97.43 $\pm$ 2.811a
10 %	96.13 $\pm$ 2.196a
12 %	98.33 $\pm$ 1.877a

The yield value exceeding 100% at the initial concentration (6%) was influenced by post-hydrolysis water retention factors and the presence of residual salt deposits from the use of phosphate buffer solutions during incubation. Conversely, the decrease in yield value with increasing papain dose (8% and 10%) is a direct result of over-degradation of the organic matrix. The massive peptide-bond-breaking activity extensively degrades soluble collagen into short peptide fragments or free amino acids, which are highly hydrophilic and thus pass through the filter medium and are lost in the filtrate. The phenomenon of solid mass shrinkage due to degradation of the binding matrix is analogous to the gelatin phase extraction principle in animal tissues (Zhang et al., 2025).

Effect of enzyme concentration on calcium levels

The results of quantitative analysis using the AAS instrument showed that the enzymatic hydrolysis process successfully released a significant amount of calcium (160.73-165.63 mg/g) compared to the control without enzyme treatment (145.45 mg/g). This significant increase proves the effectiveness of proteolytic breakdown in stretching the mechanical bonds of collagen that enclose the mineral components of bone. The average calcium level data are shown in **Table 2**.

Table 2. Calcium levels

Papain Enzyme Concentration (%)	Mean $\pm$ Standard Deviation (mg/g)
6 %	163.29 $\pm$ 1.469a
8 %	160.73 $\pm$ 4.990a
10 %	165.63 $\pm$ 7.458a
12 %	163.79 $\pm$ 2.970a

Although the 10% concentration released the highest calcium levels (165.63 mg/g), ANOVA showed that the variation in papain concentration did not yield a statistically significant difference ($p = 0.675 > 0.05$). Although the data showed that the 10% papain concentration released the highest calcium of 165.63 mg/g, the ANOVA statistical test confirmed that the variation in enzyme concentration did not make a statistically significant difference ($p = 0.675 > 0.05$). This indicates that the biochemical mechanism of hydrolysis has reached enzyme saturation. At the 10% addition point, all

molecules of type I collagen substrate available on the surface of bone particles are fully bound to the active site of papain. As a result, an excess of the enzyme at a concentration of 12% is no longer correlated with accelerated matrix degradation, rendering it ineffective at releasing more Ca^{2+} ions into the product solids (Cahyono et al., 2022).

The non-linear fluctuation pattern that occurs when calcium levels drop slightly at the 8% dose before peaking at 10% is caused by steric constraints at the 8% dose, where overly dense enzyme molecules compete for active sites on the particle surface, thereby disrupting the continuity of hydrolysis. A new equilibrium is achieved at a concentration of 10%, which opens the matrix structure optimally without damaging the minerals. At a dose of 12%, the decline again occurs due to an excess of enzyme triggering further hydrolysis, which dissolves some of the microcalcium into the liquid phase (Sihab et al., 2023).

The papain enzyme breaks the endoproteolytic bonds between collagen peptides, causing the complex helical structure of the organic matrix to become more open and stretched, so that Ca^{2+} ions are more easily detached. The effectiveness of proteolytic enzyme use is supported by findings from other fishery by-products (Sihab et al., 2023). It is known that the activity of the papain enzyme is superior to that of the pepsin enzyme in degrading the collagen matrix of the fish's connective tissue (Nurjanah et al., 2021). The mechanism of breaking the bonds of this amino acid peptide is also in line with that which occurs in the processing of mackerel shells (Rahmawati et al., 2024), hydrolysis of the bones of the benton selar fish (Hadi et al., 2025), as well as the degradation of milkfish bones (Fitri et al., 2022).

The use of this enzymatic method has proven highly selective, as it preserves the quality of calcium minerals and increases their bioavailability without damaging the structure of natural hydroxyapatite crystals (Kusumawati et al., 2022). This result offers a much safer alternative to the chemical extraction method for the base, which risks altering the crystal's composition and structure due to excessive alkalization (Kaliky, 2023). This cob calcium powder can be used as a raw material for fortifying local food products and other functional products (Septiansyah et al., 2020).

Determination of optimum papain enzyme concentration

One of the main goals of this study was to determine the most appropriate papain concentration to maximize calcium levels in mackerel tuna bones (*Euthynnus affinis*). Based on the data obtained, a 10% concentration yields the highest calcium content, so it was chosen as the

optimal concentration for the calcium-level parameters. Meanwhile, the 6% concentration yields the highest yield, making it the optimal concentration for the yield parameters. These two values are consistently obtained from each test repeat.

The 10% concentration was chosen as the optimal condition for calcium levels because it yielded the highest average value of 165.63 mg/g. The calcium level values for the entire treatment were: 10 % (165.63 mg/g), 12 % (163.79 mg/g), 6 % (163.29 mg/g), and 8 % (160.73 mg/g). At a concentration of 10 %, the amount of enzymes added is enough to break down the layer of protein (collagen) that wraps around the bone minerals, so that the calcium in them is more easily released. Enzymes work well without excess, which actually interferes with the process itself. This is what causes the greatest calcium release among the concentrations tested (Cahyono et al., 2022; Fitri et al., 2022).

The stability of calcium release in the range of 160-165 mg/g confirms the highly selective working characteristics of papain, breaking the endoproteolytic bonds of collagen without damaging the hydroxyapatite crystals ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$). This enzymatic method is much safer than strong alkaline-based chemical extraction methods (such as NaOH), which risk triggering structural destabilization and altering the composition of mineral crystals due to over-alkalization, or high-pressure physical methods, which are less selective in separating protein-mineral fractions (Cahyono et al., 2023). These results also strengthen the study on milkfish bone waste processing, which reached saturation at 8% (Fitri et al., 2022). The variation in optimal points among studies was due to differences in density and protein composition specific to the bone species used (Kusumawati et al., 2022).

Apart from the yield side, the selection of 10% also considers the efficiency of using materials. Although the results of the statistical test showed that the difference in calcium levels among the treatments (6, 8, 10, and 12 %) was not statistically significant ($p = 0.675$), the mean value at the 10 % concentration remained the highest on each retest. Adding enzymes up to 12% does not yield higher calcium results; instead, it increases costs. Therefore, from a practical standpoint, a 10% concentration is the most appropriate choice because it yields the highest calcium content without waste (Fitri et al., 2022; Zhang et al., 2025).

The calcium level graph in **Table 2** shows that the value does not always increase with increasing enzyme concentration; instead, it fluctuates. This pattern can be explained through three stages. First, as the concentration increased

from 6% to 8%, calcium levels decreased from 163.29 mg/g to 160.73 mg/g. This is not to say that the enzyme works worse, but rather that at a concentration of 8%, the amount of enzyme added is too much for the collagen available at that time. As a result, enzyme molecules compete for binding sites on proteins and interfere with one another, so the process of protein breakdown becomes less smooth and the calcium released is slightly reduced (Kusumawati et al., 2022; Cahyono et al., 2022).

Secondly, from a concentration of 8 to 10 %, the calcium level rises again from 160.73 mg/g to 165.63 mg/g. This is the best point at which the addition of enzymes has a real impact. The more enzymes are added at this stage, the more parts of the bone protein are broken down, leaving more space in the bone structure. When the bone structure is more open, calcium minerals previously confined within it become easier to release and measure using the AAS tool (Fitri et al., 2022).

Third, from a concentration of 10 to 12 %, calcium levels again drop from 165.63 mg/g to 163.79 mg/g. At this stage, almost all collagen proteins in bones have been broken down by enzymes. An increase in enzyme concentration of up to 12% triggers enzyme saturation, in which excess enzyme molecules in the system are no longer effective because all substrate molecules (collagen) have been degraded. These excess enzymes can interfere with one another. In addition, excessive enzymes may break down previously detached results into very small parts, causing them to dissolve in water and be discarded with the filtrates, thereby slightly reducing the measured calcium (Kusumawati et al., 2022; Zhang et al., 2025).

An up-and-down pattern like this was also found in previous research. Fitri et al. (2022) examined milkfish bones with the papain enzyme. They found that the highest calcium levels were obtained at an enzyme concentration of 8%, and did not increase further at higher concentrations. This reinforces the conclusion that each type of fish bone has its own optimal point, depending on the bone's protein content. The difference in optimal points between this study (10 %) and the study by Fitri et al. (2022) (8 %) is likely due to differences in fish types, bone protein content, and experimental conditions, which, in turn, affect the amount of enzymes required to break down proteins.

Kusumawati et al. (2022) also reported that the enzymatic hydrolysis method in fish bones can increase the bioavailability of calcium because the degradation process of collagen protein takes place without damaging the mineral structure of hydroxyapatite. This condition supports research findings indicating that using papain on cod fish bones could provide a safe, environmentally

friendly, and high-nutrient alternative source of calcium.

Research by Cahyono et al. (2022) on tuna (*Thunnus* sp.) swimming bubbles shows that papain enzymes effectively hydrolyze collagen. The HPLC results showed a predominance of glycine and proline, indicating the breakdown of collagen chains during hydrolysis. The similarity of these mechanisms suggests that the papain enzyme has a good ability to decompose protein tissues in marine fish of the Scombridae family, including cod and skipjack. In addition, research by Cahyono et al. (2023) using bones of mackerel tuna (*Euthynnus affinis*) subjected to physical pressure via the pressure method produced calcium levels of around 8.76%. Although using the same raw materials, the enzymatic method in this study is considered more specific because papain acts directly on collagen degradation, which makes up the bone matrix, thereby enabling more selective and optimal release of calcium minerals.

Kusumawati et al. (2022) also stated that the enzymatic hydrolysis process in fish bones can increase calcium availability because the degradation of collagen proteins takes place more selectively without damaging the structure of the mineral hydroxyapatite. This condition indicates that the enzymatic method can preserve the quality of calcium minerals, thereby potentially producing calcium that is better absorbed by the body. On the other hand, Kaliky (2023) used the alkaline NaOH chemical method to extract fish bone minerals and obtain a relatively high level of calcium. However, the use of strong alkaline solutions risks altering the structure of hydroxyapatite crystals through excessive alkalization. Therefore, the enzymatic hydrolysis method using papain is considered safer because it acts specifically on collagen without damaging the calcium mineral components in fish bones.

Based on a comparison of these studies, it can be concluded that enzymatic hydrolysis with papain is an effective method for releasing calcium from fish bones without damaging their mineral structure. The use of appropriate enzyme concentrations is essential for achieving optimal calcium release while maintaining the quality of the minerals produced.

Based on the papain concentration, 10% is the optimal operating condition because it yields the highest calcium density without incurring excessive chemical costs; further increases to 12% no longer increase yield (cost-inefficient). The industry implications of these findings are strategic; Fishery waste treatment businesses can use this data as a flexible guide to determine enzyme dosage based on production priority targets, either maximizing the yield quantity of mineral flour (at a papain dose of

6%) or pursuing the purity of the highest calcium density (at a papain dose of 10%) as a sustainable functional food fortification material. The results of this study are expected to provide useful guidelines for future research or the production of natural calcium from fish bone waste.

Conclusions

The use of papain enzymes is effective for hydrolyzing cod fish (*Euthynnus affinis*) bones, with concentration variations significantly affecting yield but not calcium levels. These findings have implications for the waste treatment industry's ability to efficiently adjust enzyme dosages based on production target priorities, both to maximize yield quantity and calcium density. Since this study is limited to the optimization of a single variable and has not evaluated the dissolved calcium or its crystal purity, further studies are recommended to examine additional parameters (time, temperature, and pH), measure calcium levels in the liquid phase (hydrolysate), and validate the material structure of the product through XRD and SEM characterization.

Conflict of Interest

All authors stated that they had no financial or commercial conflicts of interest related to this study.

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References

- Alrebaish, A. S., & Wilson, O. C. (2020). Mesocrystal aggregation of biological apatite nanocrystals. *Medical Devices & Sensors*, 4(November), 1–7. <https://doi.org/10.1002/mds3.10155>
- Avicena, R., Syahril, & Purnakarya, I. (2024). Pengembangan biskuit daun kelor (*moringa oleifera*) yang diperkaya tulang ikan tuna (*thunnus* sp) sebagai makanan tambahan tinggi kalsium. *Jurnal Pangan Kesehatan dan Gizi*, 4(2), 1–6. <https://doi.org/10.54771/7w2hkg65>
- Boudreau, S., Lam, E., & Kerton, F. M. (2025). Accessing biominerals from by-products wasted by the seafood processing industry. *RSC Sustainability*, 4(October), 28–60. <https://doi.org/10.1039/D5SU00527B>
- Cahyono, E., Ansar, N. M. S., Tanod, W. A., Sambeka, Y., Batafor, Y. M. J., & Nadia, L. M. H. (2023). Characteristics of the mackerel tuna

- bone flour (*Euthynnus affinis*) produced by pressure hydrolysis method. *Pelagicus: Jurnal IPTEK Terapan Perikanan dan Kelantan*, 4(3), 135–147.
<http://dx.doi.org/10.15578/plgc.v4i3.13169>
- Cahyono, H., Trilaksani, W., & Uju. (2022). Karakteristik fisikokimia papain soluble collagen dari gelembung renang ikan tuna (*thunnus* sp.). *Jurnal Pengolahan Hasil Perikanan Indonesia*, 25(1), 1–17.
<https://doi.org/10.17844/jphpi.v25i1.38213>
- Fitri, M., Tartar, S. U., Nurmiah, S., & Syukroni, I. (2022). Degradation of milkfish bone (*chanos-chanos forsskal*) with pure papain enzyme concentration and heating time. *Asian Food Science Journal*, 21(12), 78–85.
<https://doi.org/10.9734/afsj/2022/v21i12607>
- Fitri, M., Tartar, S. U., & Syukroni, I. (2025). Calcium fortification of bolu cukke (traditional cakes of South Sulawesi, Indonesia) using threadfin bream fish bone. *Journal of Food Science and Technology (Iran)*, 22(164), 16–29.
<https://doi.org/10.22034/FSC.T.22.164.16>
- Hadji, F., Botutihe, D. N., Najmah, N., Bialangi, N., Kunusa, W. R. (2025). Aktivitas antioksidan hidrolisat protein tulang ikan selar bentong (*selar crumenophthalmus*) hasil hidrolisis enzim papain. *Jurnal Crystal: Publikasi Penelitian Kimia Dan Terapannya*, 7(2), 277–285.
<https://doi.org/10.36526/jc.v7i2.6186>
- Ischak, N., Syahrir, M., Solekha, R., Apriyanto, H., Sholiha, I., Minangsih, D. M., Najmah, Dali, N., Botutihe, D. N., Tefa, A. Y., Kalalinggi, S. Y., & Thoriq. (2025). *Biokimia dasar*. Padang Pariaman: Lingkar Edukasi Indonesia.
- Islam, J., & Solval, K. E. M. (2025). Recent advancements in marine collagen: Exploring new sources, processing approaches, and nutritional applications. *Marine Drugs*, 23(5), 1–31. <https://doi.org/10.3390/md23050190>
- Jensen, M. B., Jakobsen, J., Jacobsen, C., Sloth, J. J., Ibarruri, J., Bald, C., Iñarra, B., Bøknæs, N., & Sørensen, A. D. M. (2024). Content and bioaccessibility of minerals and proteins in fish-bone containing side-streams from seafood industries. *Marine Drugs*, 22(4), 1–11.
<https://doi.org/10.3390/md22040162>
- Kaliky, N. (2023). A chemical analysis of red snapper bone (*lutjanus* sp) flour. *Interdisciplinary Social Studies*, 1(7), 809–815.
<https://doi.org/10.55324/iss.v1i7.163>
- Kasim, N. M., Ischak, N. I., Salimi, Y. K., Bialangi, N., Aman, L. O., & Mohamad, E. (2025). Uji organoleptik & karakteristik fisikokimia serta aktivitas antioksidan produk susu berbasis kacang sacha inchi (*plukenetia volubilis* l.). *Pentagon: Jurnal Matematika dan Ilmu Pengetahuan Alam*, 3(1), 112–130.
<https://doi.org/10.62383/pentagon.v3i1.412>
- Kusumawati, P., Triwitono, P., Anggrahini, S., & Pranoto, Y. (2022). Autoclaving and alkaline hydrolysis effects on the particle size and solubility of grouper (*Epinephelus* sp.) nano-calcium powder in in vitro gastrointestinal tract simulation. *JIPK: Jurnal Ilmiah Perikanan dan Kelantan*, 14(2), 176–202.
<https://doi.org/10.20473/jipk.v14i2.36261>
- Latimer, G. W. (2023). *Official Methods of Analysis of AOAC International*, 22nd Edition. New York: AOAC Publications.
- León-López, A., Fuentes-Jiménez, L., Hernández-Fuentes, A. D., Campos-Montiel, R. G., & Aguirre-Álvarez, G. (2019). Hydrolysed collagen from sheepskins as a source of functional peptides with antioxidant activity. *International Journal of Molecular Sciences*, 20(16), 1–17. <https://doi.org/10.3390/ijms20163931>
- Lyssaïos, F. A., González-Coloma, A., Andrés, M. F., & Díaz, C. E. (2025). Biopesticide compounds from an endolichenic fungus *xylaria* sp. isolated from the lichen *hypogymnia tubulosa*. *Molecules*, 30(3), 1–15.
<https://doi.org/10.3390/molecules30030470>
- Mulyani, S., Rohmeita, D., & Legowo, A. M. (2021). Karakteristik kalsium dari tulang ikan bandeng (*chanos chanos*) yang diekstraksi menggunakan larutan HCl. *Journal of Nutrition College*, 10(4), 321–327.
<https://doi.org/10.14710/jnc.v10i4.29960>
- Nurfadilah, M. K., Ino Ischak, N., Salimi, Y. K., Bialangi, N., Aman, L. O., & Mohamad, E. (2025). Organoleptic test & physicochemical characteristics and antioxidant activity of sacha inchi nut-based dairy products (*Plukenetia volubilis* L.). *Pentagon: Journal of Mathematics and Natural Sciences*, 3(1), 112–130.
<https://doi.org/10.62383/pentagon.v3i1.412>
- Nurjanah, Baharuddin, T. I., & Nurhayati, T. (2021). Ekstraksi kolagen kulit ikan tuna sirip kuning (*thunnus albacares*) menggunakan enzim pepsin dan papain. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 24(2), 174–187.
<https://doi.org/10.17844/jphpi.v24i2.35410>
- Patrata, S., & Aluri, J. S. R. (2021). A study on phyto-chemical analysis and antibacterial activity of spices on pathogenic bacteria isolated from commercially important edible marine fishes, *euthynnus affinis* (cantor), *katsuwonus pelamis* l. and *auxis thazard* (lacepede) (family *scombridae*). *Transylvanian Review of Systematical and Ecological Research*, 23(3), 37–72.
<https://doi.org/10.2478/trser-2021-0019>
- Rahmawati, H., Agustini, T. W., Dewi, E. N., & Trianto, A. (2024). Karakteristik hidrolisat kolagen kulit ikan tenggiri kering dengan enzim papain. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 27(12), 1156–1171.
<https://doi.org/10.17844/jphpi.v27i12.55831>
- Septiansyah, E., Putra, O. A., Abshar, K., Jati, D. R., & Apriani, I. (2020). Pemanfaatan limbah tulang ikan tongkol (*euthynnusaffinis* c) dari home industry abon sebagai tepung. *Jurnal*

- Teknologi Lingkungan Laban Basah*, 8(2), 76-82.
<https://doi.org/10.26418/jtlb.v8i2.44169>
- Sihab, M. U., Pranowo, D., & Santoso, I. (2023). Ekstraksi nanokalsium tulang ikan tenggiri (*scomberomorus* sp.) dengan ultrasound assisted extraction. *Jurnal Teknologi Pertanian*, 23(2), 137–148.
<https://doi.org/10.21776/ub.jtp.2023.024.02.7>
- Suad, A., & Novalina, K. (2019). Studi kandungan kalsium pada tepung tulang ikan tongkol (*euthynnus affinis*) dan tenggiri (*scomberomorus commerson*). *Octopus: Jurnal Ilmu Perikanan*, 8(1), 1–4.
<https://doi.org/10.26618/octopus.v8i1.2485>
- Taufik, M., Seveline, & Saputri, E. R. (2018). Validation of the method of analyzing calcium levels in fresh milk by complex titration. *Agritech*, 38(2), 187-193.
<http://doi.org/10.22146/agritech.33147>
- Torres, B., Pérez, A., García, P., Jiménez, P., Abrigo, K., Valencia, P., Ramírez, C., Pinto, M., Almonacid, S., & Ruz, M. (2024). Fish bones as calcium source: Bioavailability of micro and nano particles. *Foods*, 13(12), 1–10.
<https://doi.org/10.3390/foods13121840>
- Wijayanti, I., Rianingsih, L., & Amalia, U. (2018). Karakteristik fisikokimia kalsium dari tulang nila (*oreochromis niloticus*) dengan perendaman belimbing wuluh. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 21(2), 336–344.
<https://doi.org/10.17844/jphpi.v21i2.23089>
- Yu, M., Wang, L., Zhang, W., & Ganss, B. (2019). An evolutionarily conserved subdomain in amelotin promotes amorphous calcium phosphate-to-hydroxyapatite phase transition. *Crystal Growth & Design*, 19(4), 2104–2113.
<https://doi.org/10.1021/acs.cgd.8b01680>
- Zhang, S., Zhao, D., Yin, L., Wang, R., Jin, Z., Xu, H., & Xia, G. (2025). Physicochemical and functional properties of yanbian cattle bone gelatin extracted using acid, alkaline, and enzymatic hydrolysis methods. *Gels*, 11(March), 1–23.
<https://doi.org/10.3390/gels11030186>