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# Comparative Analysis of Calcium Content in Milkfish by Varying of Wet and Dry Conditions Using AAS Instrumentation

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**Abstract**

Standard method to determine some metal and metalloid elements using atomic absorption spectrometry (AAS) instrument has been widely applied based on the absorption of radiation by free atoms. Thus, this study applied the standard AAS method to determine calcium (Ca) levels by varying wet and dry milkfish (*Chanos chanos*) in order to observe high Ca content categorized for healthy food. The preparation of milkfish were done by varying the condition of wet and dry meat. Fish meat was washed, crushed, dried, and extracted then filtered to get the filtrate from milkfish. It was analyzed based on qualitative observation that the [$(NH_4)_2CO_3$] and [$(NH_4)_2C_2O_4$] reagents have reacted with the Ca element to form precipitates as $CaC_2O_3$ and $CaC_2O_4$. Furthermore, quantitative data using the AAS instrument to observe the Ca content of wet and dry meat shows that the wet milkfish has Ca content at an average of 0.0152 mg.g$^{-1}$, whereas in dry meat conditions it shows an increase in Ca level to 0.0192 mg.g$^{-1}$. This work has provided an overview of dietary requirements for the determination of Ca content in milkfish under dried meat which is very suitable for developing healthy food.

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## Introduction

Indonesia is an archipelago with abundant natural resources like on land and sea area (Rustiah et al., 2020). It has a strategic position for high profits by developing healthy food sourced from nature (Bazzi et al., 2019). Referring to the new problem of Covic-19 pandemic, new normal conditions have been applied in various countries to increase body immunity from virus disease by eating healthy foods that contain vitamins and minerals (Aizenman et al., 2016; Alpert, 2017; Gagnon et al., 2016; Jayawardena et al., 2020). So in this study, we propose healthy food based on milkfish with the high mineral content of calcium (Ca). Milkfish can be found in freshwater and categorized as a unique type of fish because it can live in a variety of conditions (Chang et al., 2018; Kang et al., 2015). In addition, it is categorized as fish with a slender body to easy for swim quickly with silvery-white fins colour, then it is jagged mouth in order to easy consumption of blue algae that grows on the bottom of the water (herbivores). The several communities in Indonesia really like milkfish because it has fresh meat, sweet-meat, and easy to process. Although there are many bones, it is a unique art in eating for consuming and processing milkfish.

Generally, milkfish is the main source of protein in several Asian countries, especially in Southeast Asia and categorized in the seafood group which has a high nutritional content such as amino acids and polyunsaturated fatty acids (PUFA) (Malle et al., 2019; Murthy et al., 2016). In addition, seafood also contains several types of minerals and vitamin B (Hamed et al., 2015; Khalili et al., 2018). The mineral requirements of fish have the ability to absorb mineral content from the surrounding water (Chanda et al., 2015; Liang et al., 2018). Thus, differences in mineral composition also complicate the assessment of food requirements (Fuller et al., 2015). Only nine minerals have been identified as important in fish food. The nutritional content of milkfish is influenced by several internal factors such as gender, age and reproductive phase, while external factors include the location of cultivation or formulation of habitat food and the quality condition of fish pond waters (Bogard *et al.*, 2015). Especially, the determination Ca-content in milkfish is very important to study animal nutrition and observe differences method in preparation by varying wet and dry milkfish. The Ca-content is one of the essential nutrients to indispensable for various bodily functions like developing bones and teeth, regulating muscle and mineral reactions (Quattrini et al., 2016; Upadhyay, 2017).

One way to identify specific mineral content is to use atomic absorption spectrophotometry (AAS) method combined with qualitative analysis for high Ca content (Sarkar, 2018). Currently, qualitative analysis has forgotten by some researchers that the importance of qualitative analysis. It is due to considered as a conventional method to create a tiring and disposing of chemical compounds. However, it makes easier for us to proceed through instrumentation analysis because the analytical costs are expensive even though the data obtained are quite accurate. Therefore, it is important to review the milkfish nutrient content information for consumers and the fisheries processing industry for storage and processing needs especially Ca-content which is analyzed using qualitative and quantitative analysis (Malle *et al.*, 2019). In this study, we varied the wet and dry milkfish to observe Ca content and basic knowledge to learn simple preparations for analysis with AAS instrumentation.

**Materials and Methods**

Milkfish preparation

The milkfish was cleaned and weighed as much as 10 g then heated on a hot plate until it does not emit smoke at 120°C for 2 hours (Figure 1). Furthermore, it is cooled in a desiccator for 24 h, mixed with 2 mL $HNO_3$ and dried on a heating plate. For high extraction processes, it is burned in a closed condition into the furnace at 500°C for 2 hours to form carbon-free ash (Nazopatul, 2018). Then, it is cooled under ambient temperature and dissolved with 1 mL distilled water, added 10 mL of 2% $HNO_3$, then filtered to obtain the milkfish filtrated and dissolved for 50 mL by distilled water. Finally, the filtrated milkfish has been obtained to analyze the Ca-content by using AAS instrument.

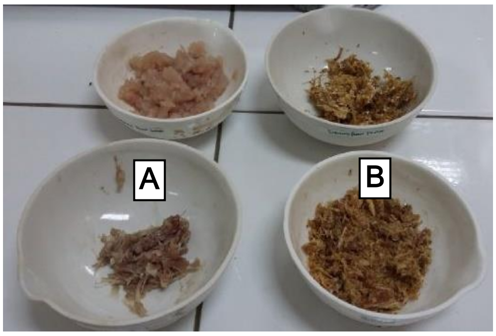


Figure 1. the milkfish sample with wet (A) and dry (B) meats variation

Detection of Ca content by qualitative methods

The initial stage for observing qualitative data was determined to identify Ca content by adding ammonium carbonate [$(NH_4)_2CO_3$] and ammonium oxalate [$(NH_4)_2C_2O_4$] reagents. Identification has been carried out based on the precipitate test, where the sample has been added to a glass tube and mixed with [$(NH_4)_2CO_3$] (tube 1) and [$(NH_4)_2C_2O_4$] (tube 2), if it forms a precipitate that indicates a positive Ca-content (Figure 2).

Figure 2. The qualitative test using glass tube

Determination Ca content by atomic absorption spectroscopy

The Ca content was evaluated by using AAS towards each sample variation like wet and dry milkfish. The determination Ca content was carried out by making the standard solution based on calcium carbonate ($CaCO_3$) by dissolving 250 mg into a mixed solvent (5 mL HCl and 20 mL distilled water). Then, it was dissolved to 100 mL to obtain a stock solution with a concentration of 1000 $mg.L^{-1}$ and it redissolved in various concentrations of 2.0; 4.0; 8.0; 16.0 and 20.0 $mg.L^{-1}$ is used as a determination of standard curve. Thus, it is absorbed using AAS with the cathode lamp Ca by overview a standard curve was created by plotting the absorbance value against the concentration of the solution ($mg.L^{-1}$) and followed by sample measurement against wet and dry milkfish.

**Results and Discussion**

Determination of Ca level in milkfish by varying wet and dry conditions was applied to overview the differences in Ca-content and to study the preparation of materials for analysis using AAS instrumentation. It is an instrument analysis for determining some metal content in low concentration based on the absorption of a specific wavelength that experiences electron excitation from the ground state to a higher electron energy level through the heating process (Sarkar, 2018). This phenomenon occurs very directly when an atom has been emitted, the photon will be fired during the combustion process. Where the liquid sample injected into the AAS and sprayed for the combustion process, Ca-cathode hollow lamp (HCL) is presented as a spectral line and as frequency tuners for specific light-sources directed to the combustion process. Specific atom is detected by absorption of specific wavelength by using Ca-HCL depending on the total concentration of the solution. By measuring the absorption of Ca-atom in a flame, the concentration of Ca-content in the milkfish can be determined. Thus, we can compare with standard solutions to observe the actual concentration in a sample.

Identification of qualitative data

The initial step for determining qualitative data was collected using the two reagent tests under [$(NH_4)_2CO_3$] and [$(NH_4)_2C_2O_4$]. According to Dorozhkin and Hu et al. that the Ca element is easily soluble in water and can be determined based on qualitative data to observe the purpose of the reaction mechanism that occurs by adding reagents and observing the Ca content in the sample (Dorozhkin, 2016; Hu et al., 2017). Extraction techniques in acidic condition show that Ca was spontaneously soluble in water and 2% $HNO_3$ solution (Younis et al., 2015). Furthermore, it is filtered to separate the filtrate and residue, so we can obtain the filtrate to analysis based on initial test for collecting the qualitative data. Proposed precipitate mechanism of Ca reacted with reagents can be seen in Eq. 1 and 2.

$$Ca^{2+}{}_{(filtrated\ milkfish)} + (NH_4)_2C_2O_3{}_{(aq)} \rightarrow CaC_2O_3{}_{(s)}\downarrow + 2NH_4^+{}_{(aq)} \quad (1)$$
$$Ca^{2+}{}_{(filtrated\ milkfish)} + (NH_4)_2C_2O_4{}_{(aq)} \rightarrow CaC_2O_4{}_{(s)}\downarrow + 2NH_4^+{}_{(aq)} \quad (2)$$

The Eq. 1 and 2 shows that $Ca^{2+}$ has actually been bound and formed various types of other chemical compounds such as $CaCl_2$, $Ca(OH)_2$, or $Ca(NO_3)_2$ that occur during the destruction process (Eq. 3 and 4). Based on this phenomenon, we can write the actual mechanism proposed to obtain the destruction process from this research (Fig. 2).

$$Ca(OH)_2{}_{(filtrated\ milkfish)} + (NH_4)_2C_2O_3{}_{(aq)} \rightarrow CaC_2O_3{}_{(s)}\downarrow + 2NH_4OH{}_{(aq)} \quad (3)$$
$$Ca(OH)_2{}_{(filtrated\ milkfish)} + (NH_4)_2C_2O_4{}_{(aq)} \rightarrow CaC_2O_4{}_{(s)}\downarrow + 2NH_4OH{}_{(aq)} \quad (4)$$

Table 1 is a qualitative result test referring to Fig. 2 was characterized by more than 6 glass tubes which intended to accurately test of Ca-content by repetition tests using [$(NH_4)_2CO_3$] and [$(NH_4)_2C_2O_4$] reagents. We observe by triplo techniques to ensure Ca is contained in milkfish and future test using the AAS technique. Based on Table 1, it is shown that milkfish was contained Ca which is identified by the precipitate formed under the glass tube.

Table1. The qualitative test detecting the Ca-content

| Sample variation | Test reagents | Results | Information |
|---|---|---|---|
| Wet milkfish | [$(NH_4)_2CO_3$] | Precipitate | + (positive) |
| | [$(NH_4)_2C_2O_4$] | Precipitate | + (positive) |
| Dry milkfish | [$(NH_4)_2CO_3$] | Precipitate | + (positive) |
| | [$(NH_4)_2C_2O_4$] | Precipitate | + (positive) |

Identification of quantitative data

The application of AAS instrument has been carried out to discover the Ca-content in the milkfish. In addition, it is necessary to establish a standard curve as a reference in calculating accuracy of Ca concentration in the sample (Zeiner et al., 2007). The standard curve is created must contain information about the concentration range of standard sample compared with the real sample (Nurdin et al., 2017; Nurdin et al., 2018). Thus, we can obtain the absorption value by AAS and plot into a standard curve by equation y = ax + b (Mursalim et al., 2017; Wibowo et al., 2017). This is a simple method to easily determining Ca-content based on linear regression. In this study, we obtain a manual absorbance data to form standard curve and then compared with the samples.

Identification of Wavelength Absorption

Before deciding the sample concentration, we determine a calibration curve to obtain the standard solution which is made with $CaCO_3$ dissolved under distilled water containing the HCl aims to high-mineralogy identified in water solution. In this study, the standard concentration was applied by varying of 2.0; 4.0; 8.0; 16.0 and 20.0 $mg.L^{-1}$ based on the Beer-Lambert law that the absorbance is directly proportional to the concentration of the analyte absorbed for the existing set of conditions. Thus, we can plot the concentration ($mg.L^{-1}$) versus absorbance to observe the linear regression for determining the real sample concentration (Hikmawati et al., 2017; Maulidiyah et al., 2017). Based on Figure 3 exhibits that the standard solution was obtained with the linear equation of y = 0.0106x - 0.0045. This condition, we suppose based on the $mg.L^{-1}$ concentration that will be converted into $mg.g^{-1}$ for following up against the weight of milkfish.

Determination of Ca-level in milkfish

Based on Table 2 it appears that the results of Ca-analysis using the AAS instrument shows typically compatible giving good precision at deviation value <1.0. Thus, this method was proportional to determine Ca content using AAS because it is quite valid and the data can be trusted. This result (Table 2 and 3) can be seen that the Ca-content in wet milkfish has quite a significant difference around 0.0040 $mg.g^{-1}$ in 5 g of sample (3 repetitions). Furthermore, the data was converted based on stoichiometry from $mg.L^{-1}$ to $mg.g^{-1}$, we obtain that in 5 grams of milkfish containing 0.0152 mg.g-1 for wet milkfish, while dry milkfish is around 0.0192 $mg.g^{-1}$, respectively.

Table 2. Analysis result based on wet milkfish through AAS identification

| Repeatability | Sample variation | Sample determination | | | | | |
|---|---|---|---|---|---|---|---|
| | | Weight (gram) | Abs. | a | b | Concentration ($mg.L^{-1}$) | Concentration ($mg.g^{-1}$) |
| 1 | Wet milkfish | 5.01 | 0.1557 | 0.0106 | 0.0045 | 15.1132 | 0.0151 |
| 2 | | 5.02 | 0.1569 | 0.0106 | 0.0045 | 15.2264 | 0.0152 |
| 3 | | 5.01 | 0.1557 | 0.0106 | 0.0045 | 15.1132 | 0.0151 |
| **Average** | | | | | | **15.1509** | **0.0152** |
| **Deviation** | | | | | | **0.0654** | **0.0001** |

Table 3. Analysis result based on dry milkfish through AAS identification

| Repeatability | Sample variation | Sample determination | | | | | |
|---|---|---|---|---|---|---|---|
| | | Weight (gram) | Abs. | a | b | Concentration ($mg.L^{-1}$) | Concentration ($mg.g^{-1}$) |
| 1 | Dry milkfish | 5.01 | 0.1983 | 0.0106 | 0.0045 | 19.1321 | 0.0191 |
| 2 | | 5.05 | 0.1998 | 0.0106 | 0.0045 | 19.2736 | 0.0193 |
| 3 | | 5.02 | 0.1975 | 0.0106 | 0.0045 | 19.0566 | 0.0191 |
| | | **Average** | | | | **19.1541** | **0.0192** |
| | | **Deviation** | | | | **0.1102** | **0.0001** |

## Conclusion

Basic knowledge to analyze Ca-content in milkfish based on the AAS instrument has been carried out to study sample preparation for analysis by AAS and provided information on Ca-content by varying the condition of wet and dry milkfish. Qualitative analysis shows that the sample was contained Ca that formed precipitate under the glass tube by adding the [$(NH_4)_2CO_3$] and [$(NH_4)_2C_2O_4$] reagents. Moreover, the basic instrument for quantitative data has been obtained using AAS exhibits the differences in Ca-content of wet and dry milkfish i.e. 0.0152 $mg.g^{-1}$ and 0.0192 $mg.g^{-1}$. Based on this research provides information on techniques for preparing organic samples to analyze by using AAS instrument.

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