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Analysis of Levels of Phosphorus and Magnesium in Beef Bones from Kaledo Waste

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Abstract

The Kaili tribe is a tribe with a very distinctive and well-known culinary heritage in Indonesia, one of which is Kaledo (donggala soft leg). The large number of restaurants and restaurants that provide Kaledo menus raises problems, namely the lack of utilization of beef bones from Kaledo waste, causing environmental issues. The community does not know much about the types of minerals contained in the beef bones. Among them are the minerals contained in beef bones, namely phosphorus and magnesium. This study aims to determine the levels of phosphorus and magnesium in beef bones from Kaledo waste taken randomly from Kaledo restaurants in Palu City. Determination of phosphorus and magnesium levels in beef bones from Kaledo waste using a Uv-Vis spectrophotometer and an atomic absorption spectrophotometer (AAS). The results showed that the analysis of the phosphorus content of beef bones from Kaledo waste obtained 95.125 mg / 100 g, and the magnesium content was 102.9 mg / 100 g. The results of this study are expected to be a source of information about the levels of phosphorus and magnesium contained in beef bones from Kaledo waste and can be used further as a substance in the utilization of beef bones from Kaledo waste.

Keywords: Beef bones, calcium, phosphorus, and magnesium

Introduction

Indonesia is the largest archipelagic country in the world, located in Southeast Asia. Kalimantan, Java, Sulawesi, Sumatra, and Papua are the main islands in Indonesia. Apart from being the largest archipelago in the world, Indonesia has 34 provinces with various specialties, both from tribes and cultures, to culinary specialties from each province in Indonesia, one of which is the province of Central Sulawesi. Central Sulawesi has 12 regencies with various tribes and one capital city, namely the city of Palu, with a typical tribe, the Kaili tribe (Satyawati, 2021)

The Kaili tribe is a tribe with a very distinctive and famous culinary heritage in Indonesia, especially in Central Sulawesi. Among them are duo sale, uta kelo, corn rice, palumara, lalampa, labia dange, and the most famous in Central Sulawesi by the Kaili tribe, namely kaledo (donggala lembuh feet). Kaledo is a food made from beef bone with special spices that are good for human growth and development. Kaledo is a food made from beef bones that contains bone marrow, fat, small pieces of beef, and cartilage processed with beef leg bones, raw tamarind, green cayenne pepper, salt, and lime. The main strength of kaledo soup delicacy lies in the taste and freshness of beef meat and bones, which are the main constituent ingredients, in addition to the sour and spicy taste, in just the right proportions, which completes the final impression of the true kaledo taste (Mufida, 2015).

Bone is a waste that can be used as a biomaterial to absorb metals in wastewater, because bones have many pores and contain tricalcium phosphate with a small part of calcium carbonate, which has the potential as an adsorbent (Yadav et al., 2022). The bones of beefs are, in principle, the same as those of any other animal. Has almost the same content, namely in adult beef bones contain 20 % water, 45 % ash, and 35% organic compounds. In 45 % ash, there is 37 % Ca and 18.5 % phosphate (Keene et al., 2004; Gibson et al., 2022).

Phosphorus is one of the elements of bone formation because it is needed in the process of bone mineralization. Low serum phosphate levels will limit bone formation and bone mineralization processes. However, there is little evidence to show that foods with low phosphorus content affect the incidence of low BMD, as well as foods high in

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phosphorus that do not affect the incidence of low bone mineral densitometry (BMD) which is the level of osteoporosis caused by decreased bone mass and increased fractures, as well as foods high in phosphorus that do not directly affect bone health (Palacios, 2006; Serna & Bergwitz, 2020).

Magnesium is the fourth most abundant cation in the body and the second most abundant cation in the intracellular environment after potassium. Magnesium (Mg) has an important role in the structure and function of the human body. The adult human body contains approximately 25 grams of magnesium. Total magnesium in the body of an adult male is estimated to be 1 mole (24 g) (Haruda et al., 2016). Magnesium is indispensable in the body, especially involved in over 300 essential metabolic reactions. It is necessary for energy metabolism, glucose use, protein synthesis, fatty acid synthesis and breakdown, muscle contraction, all ATPase functions, almost all hormonal reactions, and maintaining cellular ionic balance (Pelczyńska et al., 2022)

Beef bones from Kaledo waste have nutritional and mineral content that is good enough for the growth period of toddlers, adolescents, adults, the elderly, and pregnant women. Beef bones from kaledo waste have a low water content which indicates that beef bones from kaledo waste can last for a long time or have good shelf life, the amount of beef bone water content from kaledo waste is 4.59 % and ash content is 61.74 % which indicates that beef bones from kaledo waste have good mineral content for consumption by children, adolescents, adults, the elderly, and also pregnant women. Beef bones from Kaledo waste have calcium levels of 19.68 mg / g. This shows that beef bones from Kaledo waste have high calcium levels in meeting calcium needs for the body in stunting prevention (Satyawati, 2021)

The purpose of this study was to determine the levels of Phosphorus (P) and Magnesium (Mg) in beef bones from Kaledo waste.

Methods

The method used in this study is quantitative. This study is an experimental study in the laboratory conducted to analyze the phosphorus and magnesium content in beef bones from Kaledo waste. The beef bones from Kaledo waste analyzed are in the leg bones taken directly from one of the Kaledo restaurants in the city of Palu, namely the Stereo Kaledo restaurant located in the city of Palu. Each analysis treatment is carried out 3 times (triplicate) with the same treatment to see the accuracy of the final analysis results.

The sample used in this study was beef bones from Kaledo waste in the leg bones in the city of Palu at the Stero Kaledo restaurant located on Jl. Yos Sudarso, Mantikulore sub-district, Palu City. Central Sulawesi and on jl. Diponegoro, west Palu district.

Sampling is carried out specifically, namely on the part of the beef bone that has previously been processed into food with a heating process of about 4-6 hours. The sample used as much as 40 grams specifically analyzes phosphorus levels and magnesium levels.

The tools used in this study were drip pipettes, 10 mL volume pipettes, 100 mL measuring flasks, 50 mL and 100 mL measuring cups, 50 mL and 100 mL beakers, dies and pestles, stirring rods, spatula, evaporation dishes, funnels, digital balances, ovens, sample bottles, 60 mesh sieves, bone breakers (hammers), blenders, aluminum foil, Furnaces, Uv-vis Spectrophotometer and Atomic Absorption Spectrophotometer (AAS).

The materials used in this study were beef bones from Kaledo waste, aquades, tissues, filter paper, concentrated HNO_3 solution, molybdate reagent, and standard solutions of phosphorus and magnesium (Damayanti, 2015)

Sample preparation

1. Beef bones from Kaledo waste are cleaned to remove the remaining meat that is still attached.
2. Drying in a desiccator for ± 30 minutes, then drying in the oven at 110°C for± 6 hours until the moisture content decreases.
3. Cut the bone, then in a blender, mashed using a mortar and pestle, then sift using a 60 mesh sieve.
4. Grinding bone meal using a kiln for ± 6 hours (Satyawati, 2021)

Snippet setup

Samples of beef bone meal ash from Kaledo waste weighed 10 grams each. Then the sample is dissolved with a concentrated HNO_3 solution of 10 mL into each sample and then diluted to 50 mL, then filtered to separate the filtrate and residue. The filtrate obtained is diluted with water in a measuring flask of 100 mL to the limit mark (Satyawati, 2021)

Preparation of standard solutions of phosphorus and magnesium

Preparation of standard Phosphorus Solution 100 ppm

The main raw solution, weighing 1 g of KH_2PO_4 and drying it in the oven for 2 hours at 105 °C, then transferring it quantitatively into a 50 mL measuring flask, adding aquadest as a solvent to the limit mark, then cooling in the refrigerator (Ngibad, 2019)

Preparation of standard Magnesium solution 100 ppm

The standard solution is prepared by inserting 1000 ppm magnesium solution into a 100 mL measuring flask of 10 mL using a volume pipette. Then, it is diluted by adding water until the limit mark.

Phosphorus calibration curve manufacturing

Phosphorus calibration curve solutions are prepared by piping (1, 2, 3, 4, and 5) mL of 100 ppm raw solution, so that the solution has concentrations of (1, 2, 3, 4, and 5) ppm, respectively. Then put into a 100 mL measuring flask and each added 2 mL molybdate reagent, then diluted to the limit mark. Standard solutions of phosphorus are absorbed using a Uv-Vis spectrophotometer with a wavelength of 400 nm (Ngibad, 2019)

Magnesium calibration curve manufacturing

The magnesium calibration curve solution is prepared by piping (0; 5; 10; 15; 20 and 25) mL of 100 ppm raw solution, each of which is inserted into a 100 ml measuring flask and sufficient to the limit mark with aqua demineralisata this solution contains (0, 5, 10, 15, 20 and 25) ppm and measured its absobance at a wavelength of 202.6 nm an acetylene-air flame carries out atomization.

Analysis of phosphorus and magnesium levels

The sample solution that has been made is taken 10 mL and diluted with aquades on a measuring flask of 100 mL to the limit mark. The levels of phosphorus and magnesium in the sample solution are determined by measuring their absorption with a Uv-Vis spectrophotometer and an atomic absorption spectrophotometer.

Data analysis techniques

From the measurement results of the standard solution series, graphs are made for phosphorus and magnesium. The data obtained are analyzed to obtain the concentration of phosphorus and magnesium in the sample using the regression line equation, namely:

$$Y = a + bX \quad (1)$$

Description:
Y = Absorbance value (A)
a = Setting (Constant)
X = Sample solution concentration (mg/L)
b = Line slope

Furthermore, for the determination of P and Mg levels in the sample based on dry weight.

$$Y = \frac{V \cdot X}{m} \quad (2)$$

Description:
Y = Analyte levels in the sample (mg/g)
V = Assignment volume (L)
X = Sample concentration from the tool (mg/L)
m = Dry sample weight (g)
(Mayrink et al., 2022)

Results and Discussion

Result

The results obtained from the analysis of phosphorus (P) and magnesium (Mg) levels in beef bones from Kaledo waste using the Uv-vis method and atomic absorption spectrophotometry (AAS) are presented in the following table:

Standard Solution of Phosphorus

The calibration curve of Phosphorus is obtained from the measurement of the absorbance of a standard solution of metal P. From the measurement of the calibration curve, the regression line equation for phosphorus is obtained, which is y = 0.080x + 0.119. Data on absorbance measurement results of phosphorus standard solutions and the calibration curve of phosphorus standard solutions can be seen in **Figure 1.**

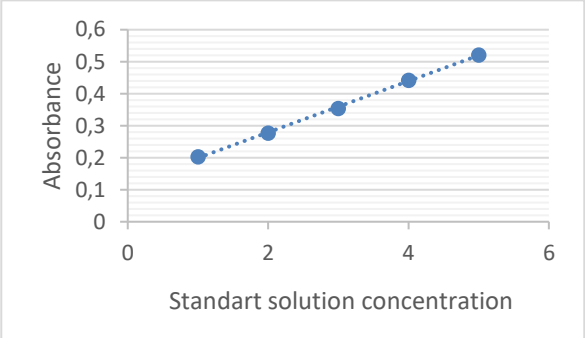

Figure 1. Phosphorus (P) calibration curve

Results of phosphorus analysis

Analysis of phosphorus levels in beef bone samples from Kaledo waste using Uv-Vis is presented in **Table 1.**

Table 1. Data on phosphorus levels of bovine bones from Kaledo waste

sample	Dry sample weight (gram)	phosphorus levels	
		Absorbance (A)	(mg/100g)
Beef Bones From Kaledo Waste	10	0.881	9.525
	10	0.879	9.5
	10	0.880	9.512
	Average		9.512

Table 1 shows the average phosphorus levels contained in beef bones from Kaledo waste. In the calculation results that have been carried out, it was found that phosphorus levels for beef bones from Kaledo waste amounted to 0.951 mg / g.

Standard solution of magnesium

The magnesium calibration curve is obtained from the measurement of the absorbance of a standard solution of metal Mg. From the measurement of the calibration curve, the regression line equation for Magnesium is obtained, which is y = 0.0105x + 0.0005; R^2 = 0.9967. Data on the absorbance measurement results of the magnesium standard solution and the calibration curve of the magnesium standard solution can be seen in **Figure 2.**

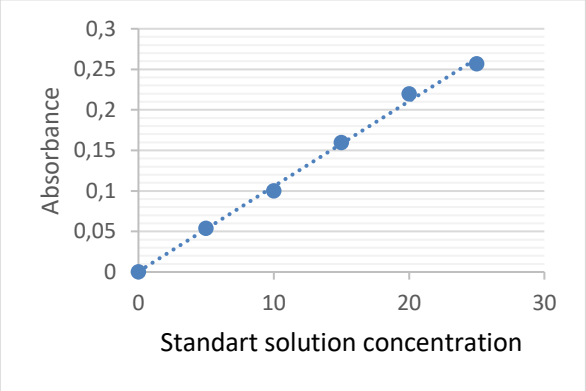

Figure 2. Magnesium (Mg) calibration curve

Magnesium level analysis results

Analysis of magnesium levels in bovine bone samples from Kaledo waste using SSA is presented in **Table 2.**

Table 2. Data on the magnesium levels of bovine bones from Kaledo waste

sample	Dry sample weight (gram)	phosphorus levels	
		Absorbance (A)	(mg/100g)
Beef Bones From Kaledo Waste	10	0.1076	102
	10	0.1093	103.6
	10	0.1087	103
	Average		102.9

Table 2 shows the average levels of magnesium contained in beef bones from Kaledo waste. In the results of calculations that have been carried out, it was found that magnesium levels for beef bones from Kaledo waste amounted to 1.029 mg / g.

Discussion

This study began with sample preparation, or sample preparation carried out to produce beef bone meal from kaledo waste, with the weight of wet beef bone samples used after cleaning as much as 400 grams. After drying, a dry sample weight of 380.35 grams was obtained. Powdered beef bones are obtained as much as 300 grams. The sample used in the study was 80 grams for analysis of phosphorus and magnesium levels in beef bones from Kaledo waste.

Analysis of phosphorus and magnesium levels in beef bones from Kaledo waste

Analysis of phosphorus levels by the UV-Vis spectrophotometry method using molybdate precipitation and magnesium level analysis was carried out by the atomic absorption spectrophotometry method. Both mineral content analyses aim to analyze phosphorus and magnesium levels of beef bones from Kaledo waste. In addition, this study is intended to provide further information related to the substance in the use of beef bones from Kaledo waste. Kaledo is a food made from beef bones that contains bone marrow, fat, pieces of leg bones, and small pieces of beef and cartilage processed with beef leg bones. Bone-based kaledo, in addition to containing fatty acids and vitamins, also contains micronutrients such as iron (Fe) and calcium (Ca) in the bone marrow (Mufida, 2015)

The sample used in the analysis of phosphorus and magnesium levels is beef bone powder from kaledo waste, which has been determined by water content and ash content, namely, beef bone powder from kaledo waste that has been through the sample preparation and ovening stages and has also been used. Based on Satyawati (2021), the water content in beef bones from kaledo waste is 4.59 %, and the water content is 61.74 %. It is stated that the water content in beef bones from kaledo waste is small, and the ash content in beef bones from kaledo waste is a lot, which means the character and shelf life of a good food for a long time, and a lot of mineral content in beef bones from kaledo waste.

Samples of beef bones from Kaledo waste that have been opened later, dissolved with HNO3 1 M with pH = 1, then diluted twice, namely from a 10 mL measuring flask to 50 mL, which is then diluted in a 100 mL measuring flask, so that a dilution factor of 10 times is obtained. The 1 M HNO_3 used in the trailer preparation serves to dissolve the metals present in the sample, as concentrated HNO_3 is a universal metal solvent and can stabilize the metals to be analyzed. The addition of concentrated HNO_3 in the ashing process aims to oxidize all carbon and dissolve the salts contained in the sample.

The process of analyzing phosphorus and magnesium levels is diluted again when they are analyzed, namely, diluting the sample from 10 mL to a 100 mL measuring flask. Furthermore, the dilution factor at the digestion is multiplied by the dilution factor when analyzing phosphorus levels in beef bone samples from Kaledo waste, until a total of 10 times the dilution factor is obtained.

Mineral concentration measurements are made with uv-vis spectrophotometers and atomic absorption spectrophotometers, and the measured snapshot must be a solution. The mineral content in a sample is calculated based on the total weight of beef bone material from Kaledo waste. So that from the results of the study showed an average phosphorus level of 9.512 mg / 100 g and an average magnesium level of 102.9 mg / 100 g.

Phosphorus (P)

In **Table 1** of beef bones from Kaledo waste, it was found that each treatment had a difference that was not much different. Treatment in the analysis of phosphorus levels is carried out by three repetitions (triplicate) to get more accurate results. The results of the analysis of phosphorus levels obtained from each repetition were 9.525 mg / 100 g, 95 mg / 100 g, and 9.512 mg / 100 g. Phosphorus levels in each repetition had a slight difference in

yield because the difference was not much. Phosphorus has very important biochemical and physiological roles and is the most abundant mineral in the body after calcium. About 80 – 85 % is present in bone, and 15 – 20 % is in soft tissue and fluid (Ciosek et al., 2021).

In bone, P is stored in the form of calcium hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, and plays an important role in the process of bone mineralization. Changes in bone composition and structure due to P loss are as important as Ca deficiency. Phosphorus is also a phospholipid component that affects cell permeability, a constituent component of myelin, energy metabolism (adenosine triphosphate), part of genetic material, acid-base regulation, and enzymatic activity (Torres et al., 2021).

The function and metabolism of phosphorus (P) of this element are found as calcium phosphate salts in the hard tissues of the body, namely bones and teeth, giving hard properties to both types of tissues (Hong et al., 2022). Phosphorus minerals, 80% present in hard tissues and 20% present in soft tissues, mainly as phosphoric acid clusters. P levels in the body are about 8% of body weight. Phosphorus is found in hard tissues at lower levels than other minerals, such as calcium (Ca), but in soft tissues, the phosphorus (P) portion is higher than calcium. Many mechanisms of energy transport processes are attributed to phosphate bonds, such as ATP and ADP, keratin phosphate, and phosphoenol pyruvate. Various metabolites that hold important functions contain phosphates, and the metabolism of many nutrients begins with phosphorylation, usually with the participation of ATP (Paath et al., 2014; Nicholls et al., 2023)

In addition to playing a role in bone mineralization, phosphorus, as an organic phosphate, plays an important role in reactions related to the storage or release of energy in the form of Adenine Triphosphate (ATP) (Michigami & Ozono, 2019). The oxidation of metabolic fuels is controlled by the availability of ADP, which is further controlled by the rate at which ATP is used for physical activity and metabolism (Alghannam et al., 2021). Phosphorus participates in the activation of several reactions in all metabolism.

According to Satyawati (2021), the analysis of calcium levels in beef bones from Kaledo waste. The average level of calcium (Ca) contained in beef bones from Kaledo waste is 19.68 mg / g. This shows that beef bones from Kaledo waste have high calcium levels in meeting calcium needs for the body in stunting prevention. Calcium and phosphorus are very important in the process of bone mineralization; a deficiency of these two minerals or an improper ratio can affect bone growth. There is a balanced ratio between calcium and phosphorus that can help calcium absorption, the body will maintain a ratio of calcium: phosphorus 2: 1 or 1: 1, a ratio that is vital for ideal bone growth because inorganic phosphate has many roles in biological processes such as cell metabolism, cell markers, as coenzymes, nucleotide metabolism, energy metabolism, membrane function, and bone mineralization.

Magnesium (Mg)

In **Table 2,** the results obtained from each repetition are 102 mg / 100 g, 103.6 mg / 100 g, and 103 mg / 100 g. The magnesium level in each repetition has a slight difference in results because the difference is not much.

Magnesium has an important role in various processes that affect growth. This element is one that is needed by living things for their metabolic activities. The results of the study showed that magnesium levels in beef bones from Kaledo waste have a large amount. This can help the role of magnesium in the growth process of living things.

Based on similar research that the mineral content in bone samples from Kaledo waste is quite large (Haruda et al., 2016), hydroxyapatite is synthesized from beef bones and phosphoric acid using precipitation methods. Hydroxyapatite is synthesized from bovine bones, which are a source of calcium. Calcium from beef bones comes from calcium oxide (CaO) compounds obtained from the calcination process of beef bones at a temperature of 1000 °C for 6 hours. Calcined beef bones have a weight change from 4 Kg to 2.483 Kg. This change is 62.07 %. Changes in weight and color of beef bones during the calcination process are caused by the decomposition process of organic substances and water loss in beef bones (Hart et al., 2022)

Kaledo (donggala ox foot) is one of the famous culinary specialties in Central Sulawesi. The number of restaurants that provide kaledo menus causes problems because of the lack of utilization of bone waste, which causes problems in the environment. People still do not know that beef bones still contain protein. Protein serves as a builder of new tissue during the growth and development of the body. This study aims to determine the protein content of beef bones from kaledo waste from the city of Palu, Central Sulawesi, which was taken randomly from a kaledo restaurant—determination of protein content in beef bones from kaledo waste using the Kjeldahl method. In the calculation results that have been carried out with three repeats, the protein content in Deuteronomy 1 is 3.85 %, Repetition 2 is 3.59 %, and Replay 3 is 4.2 %. The average protein content of beef bones from Kaledo waste is 3.88 %. The results of this study are expected to be a source of information about the protein content contained in beef bones from kaledo waste and can be further used as material in the utilization of bone waste (Anisa, 2022)

Beef bones from Kaledo waste have a mineral content that is good enough for growth. **Table 3** shows the difference in levels of several mineral contents found in beef bones from Kaledo waste, where beef bones from Kaledo waste have calcium levels of 19.68 mg / g. This shows that beef

bones from Kaledo waste have high calcium levels in meeting calcium needs for the body in stunting prevention. At the same time, the protein content in beef bones from kaledo waste was determined using the Kjeldahl method. In the calculation results that have been carried out with three repeats, the protein content in Deuteronomy 1 is 3.85 %, Repetition 2 is 3.59 %, and Replay 3 is 4.2 %. The average protein content of beef bones from Kaledo waste is 3.88 %.

Table 3. Mineral content

sample	Mineral content	Mineral Levels
Beef Bones From Kaledo Waste	Calcium	19.68 mg / g
	Protein	3.88 %

Based on another study proposed by Sitepu & Fatimah (2022) entitled Hydrolysis of Beef Bone HCl Gelatin Making. The study explained that in principle, beef bones are just like other bones, which contain the same minerals. Based on the results of previous studies that beef bones have magnesium (Mg) levels of 2.09 % in the form of $Mg_3(PO_4)_2$ compounds. The results of this study have a comparison with the research conducted in this study, where in this study used beef bones that have been processed into ready-to-eat food and through a heating processing process and a mixture of flavoring ingredients that cause differences in the results of phosphorus level analysis in unprocessed beef bones and those after being processed into ready-to-eat food.

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