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Analysis of Protein Content in Moringa Leaves (*Moringa oleifera*) and Katuk (*Sauropus androgynus*) as an Alternative Plant-Based Protein

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Abstract
Indonesia has many extraordinary biological resources, including flora that have great potential to function as a source of vegetable protein, such as Moringa oleifera and Sauropus androgynus. Humans need protein because it is a macromolecule that does many things for life systems, and is needed for tissue replacement and energy supply. Protein functions as a catalyst, transports molecules such as oxygen, supports the immune system, and transmits nerve impulses. The focus of this study was the protein content in moringa leaves (Moringa oleifera) and katuk leaves (Sauropus androgynus) in the shoots, young, and old parts. The moringa leaf samples examined showed a protein content of 22.24% in the shoots, 2.992% in the young leaves, and 4.272% in the old leaves. Meanwhile, fresh katuk leaves showed a protein content of 3.898% for the shoots, 4.767% for the young leaves, and 5.127% for the old leaves. The method used was the Kjeldahl method, namely the process of destruction, distillation, and titration used to measure protein content. The results of the study showed that moringa leaves, especially the shoots, have a higher protein content compared to katuk leaves, indicating that moringa leaves can function as an important source of vegetable protein. This study aims to measure and compare the protein levels in the top, young and old leaves of the two plants in fresh condition, in order to provide a more accurate picture of the nutritional potential of each part of the leaf and its contribution to the diversification of vegetable protein sources.

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INTRODUCTION

Indonesia has abundant biodiversity, including various plants that have the potential to be sources of plant-based protein. Two of them that have great potential but have not been optimally utilized are the moringa plant (*Moringa oleifera*) and the katuk plant (*Sauropus androgynus*). One member of the Moringaceae family is the *Moringa oleifera* plant, which has a wide distribution in regions including Asia (Guzmán et al., 2020). Both of these plants have long been known to the Indonesian people as vegetables and traditional medicinal plants. Moringa and katuk can grow well in high temperatures, arid conditions, and slightly fertile tropical soils (Devi et al., 2023). Although both are quite popular for consumption as vegetables, many are still unaware of their various nutritional contents and benefits.

Moringa (*Moringa oleifera*) has been widely recognized as one of the plants with great potential as a source of food and health supplements, thanks to its very complete nutritional content and various medicinal properties. This plant, originating from tropical and subtropical regions, has various parts that can be utilized, including leaves, pods, and seeds, all of which are known to have high nutrient concentrations. According to research, moringa leaves contain many essential nutrients, making them highly potential for development as raw materials for processed food products (Guzmán et al., 2020 & Gomes et al., 2023). This plant has small, egg-shaped leaves. The leaflets vary in color from green to brownish-green. Its shape can be described as oval or inverted. The leaves have a rounded base, straight edges, and a blunt tip (Marhaeni, 2022).

Moringa (*Moringa oleifera*) plays a significant role as a source of plant protein, which can help address malnutrition issues. The protein content in this plant shows a remarkable superiority compared to other commonly consumed food sources. Specifically, this plant has up to nine times more protein than yogurt, making it a great choice for those looking to increase their protein intake. These findings underscore the potential of *Moringa oleifera* as an alternative protein source that can support efforts to improve community nutritional status, especially in areas experiencing malnutrition (Gomes et al., 2023 & Islam et al., 2021). According to previous studies, the protein content in fresh moringa leaves is recorded at around 6.7%, which can significantly increase to reach 29.9%, thus showcasing its potential optimization as a protein source that can be considered (Paramita et al., 2021).

Katuk plant (*Sauropus androgynus*), known by various local names such as sweet vegetables, sweet checks, katuk, and sweet grafts, is a promising source of vegetable protein (Anju et al., 2022). Katuk plants are found in areas such as Kalimantan (Petrus, 2013). As one of the main vegetables originating from Kalimantan, the katuk plant (*Sauropus androgynus*) has a distinctive physical characteristic, namely the upright stems and paper -like green leaves. This plant is not only consumed as a fresh vegetable, but also has a high therapeutic value, so it is often used in various traditional medicinal herbs. The edible parts of the plant include young shoots, ends, and leaves, all of which are rich in nutrients and have the potential to improve health. Young shoots and katuk leaves are often used as additional ingredients in cooking, giving a delicious taste as well as nutritional benefits (Anju et al., 2022). Traditionally, this plant is known to have properties in increasing the production of breast milk (ASI) and supports overall health. Fresh katuk leaves contain about 7% protein, 19% crude fiber, as well as various other important minerals (Arsa et al., & Mulyati et al., 2024). Protein content in katuk leaves can vary depending on the condition of growth and the plants analyzed, with several studies showing that protein content can reach up to 29.15% under certain conditions (Senas, 2023).

Along with the increasing public awareness of the importance of vegetable protein intake and functional foods, research on the protein contained in the top, young, and old leaves of the Moringa oleifera and Sauropus androgynus plants has become very relevant. Both plants have been shown to have significant protein content in fresh form, so they have the potential as alternative sources of vegetable protein protein (Gomes et al., 2023 & Anju et al., 2022). This research not only contributes to the understanding of the nutritional composition of both plants, but can also support efforts to provide nutritious food source options in order to meet the nutritional needs of the community. Although several studies have reported the high levels of protein in fresh Moringa and Sauropus leaves, the results obtained show quite large variations, influenced by factors such as leaf age, growth conditions, and the analysis techniques used (Paramita et al., & Sari et al., 2021).

Previous studies on the protein content of Moringa and Sauropus plants were mostly limited to the analysis of young and old leaves, so data on the protein content in the top of the leaves is still very limited, even though this part is also known to contain protein (Sari et al., 2021). Research by Saputri et al. (2019) found that young and old moringa leaves analyzed using the Kjeldahl method had an average protein content of 1.3092% and 11.3473%, respectively (Saputri et al., 2019). On the other hand, Seragih's research in Sartika stated that old and young katuk leaves also contain protein (Sartika, 2019). However, information on protein levels in the shoots of both plants has not been widely explored. Therefore, this study aims to answer the problem formulation: How is the comparison of protein levels in the shoots, young leaves, and old leaves of moringa and katuk plants in fresh condition, and how does each part of the leaf contribute to the potential for diversification of vegetable protein sources? This study is expected to provide a more accurate picture of the nutritional value of each part of the leaf and support efforts to provide more diverse functional food sources.

METHOD

The method applied to analyze protein content in katuk plants (sauropus androgynus) and Moringa oleifera plants is the kjeldahl method which is a standard analytic procedure for determining total nitrogen levels in organic samples. The sample preparation begins with a leaf selection that has green characteristics, free from pest damage, and does not show signs of aging, then a grouping of shoots, young leaves, and old leaves, as well as washing, drying, smoothing, and sifting to obtain flour with uniform particle size. The Kjeldahl analysis procedure consists of three sequential stages, namely the sample destruction using concentrated sulfuric acid and the catalyst tablet in the Kjeldahl flask through heating until the solution changes clear The distillation stage is then carried out by evaporating ammonia gas which is then captured in a boric acid solution to form ammonium boric, and end with the titration stage using a standard hydrochloric acid solution to determine the total number of total nitrogen contained in the sample. The titration results obtained are then used as a basis for the calculation of protein content through conversion using the appropriate nitrogen-protein factor, so that the content of vegetable protein can be determined in both types of leaves as an alternative source of protein.

Equipment and Materials

Included in distillation devices, suction balls, burettes, glass funnels, 100 ml chemical beakers, hotplates, watch glass, 250 ml erlenmeyer flask, 100 ml kjeldahl flask, 100 ml measuring flask, mortar and pestle, oven, dropper, measuring pipette, spatula, stative and clamps.

The ingredients used include distilled water, 2%boric acid, 0.01 N hydrochloric acid, concentrated sulfuric acid, 100 g katuk leaf, 100 g Moringa leaf, 0.1%blue methyl indicator, 0.1%MM indicator, PP indicator, potassium sulfate, sufficient filter paper, 30%sodium hydroxide.

Work procedures

Moringa Leaf Sample Preparation

Fresh Moringa leaf samples are separated from the stem and sampled samples ranging from shoots, young leaves, and old leaves. After that, washed with running water that aims to remove dirt attached to the material. Leaves that have been clean are mashed using pestle mortar until smaller.

Katuk Leaf Sample Preparation

Fresh katuk leaf samples are separated from the stem and sampled samples ranging from shoots, young leaves, and old leaves. After that, washed with running water that aims to remove dirt attached to the material. Leaves that have been clean are mashed using pestle mortar until smaller.

Analysis of Protein Content

Destruction Stage

Weigh 0.5 grams of sample carefully and put it in the 250 ml Kjeldahl flask. Add 7.5 grams of potassium sulfate and 0.35 grams of copper sulfate, then 25 ml of concentrated sulfuric acid.

Then, heat the solution until it boils on an electric heater or bunsen fire, and becomes clear greenish (2 hours). The heater is turned off and left cold. Then, use the pipette to take 5 ml of solution and put it in the distillation device. Add 5 ml of 30% NaOH and PP indicator.

Distillation Stage

Distillation 10 minutes, then take 10 ml of 2% boric acid solution mixed with indicators used as a container. Rinse the cooler with aquades after that. After the last distillate drops become unavorious, the distillation is ended.

Titration Stage

Titration with 0.01 N HCl solution until the solution changes to a constant pink color. Then, do the determination of blanks. Data analysis is carried out using the following calculations:

$$\% \text{ Total protein} = \frac{(V1-V2)\ x\ N\ x\ Fk\ x\ Fp}{W} \text{ x } 100\%$$

Note:
W = Sample weight (gram)
V_1 = HCl volume Titration Sample (mL)
V_2 = HCl volume of blank titration (mL)
N = Normality of HCl (N)
Fk = 6.25 (for *Moringa oliefera* and *Sauropus androgynus*)
Fp = Dilution factor

RESULTS AND DISCUSSION

Table 1. Protein content acquisition

Code	Average Protein Content (%)
KeP	22,24
KeM	2,992
KeT	4,272
KaP	3,898
KaM	4,767
KaT	5,127

Description: KeP (Young moringa leaves); KeM (Young moringa leaves); KeT (Old moringa leaves); KaP (Young katuk leaves); KaM (Young katuk leaves); KaT (Old katuk leaves)

Based on Table 1 the acquisition of protein content using the Kjeldahl method shows a significant difference between the protein content in Moringa plants (moringa oleifera) and katuk plants (sauropus androgynus) of various parts. In Moringa leaves, the shoots have the highest protein content of 22.4%, followed by old leaves by 4.272%, and young leaves by 2.992%. Conversely, in katuk leaves there is a different pattern where the protein content increases along with the age of the leaves, namely the shoots by 3.898%, young leaves by 4.767%, and old leaves by 5.127%. The difference can be seen in Figure 1 and Figure 2.

Based on the graph shown in Figure 1, there is a striking decrease in protein content from the shoots to old leaves, where the highest protein content is found in the shoots of the leaf and the lowest in young leaves. This finding indicates that the top of the leaf has a higher nutritional potential compared to other leaf parts, possibly caused by more intensive metabolic activity in this growth phase. Conversely, Figure 2 shows an increase in attractive protein levels, where the protein content in the shoots of the leaf actually shows a lower percentage compared to the old leaf that records the highest protein content. This can be interpreted as an indication that along with the leaf maturation process, greater accumulation of protein occurs, related to physiological and biochemical changes that occur during leaf development.

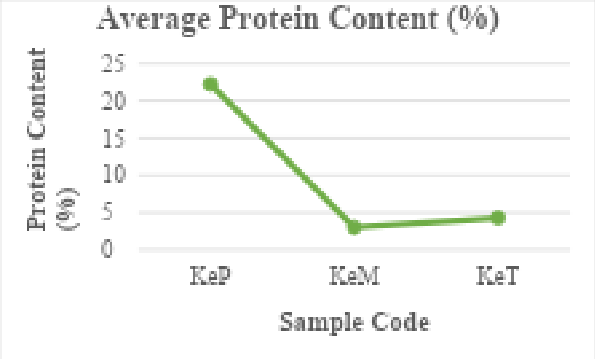

Figure 1. Graph of moringa leaf protein content

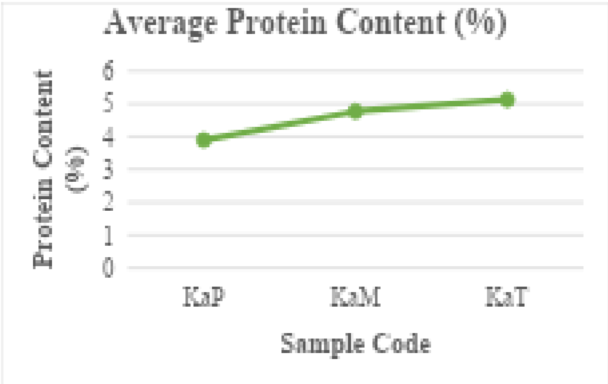

Figure 2. Protein content graph of katuk leaves

This study found a difference in protein content based on parts of each leaf, both from Moringa leaves and katuk leaves. Some factors affect the protein content of this plant, from metabolic activity and accumulation of nitrogen compounds. Differences in the abundance of metabolitic compounds produced are related to growing environmental conditions (Putriani et al., 2022). The protein content in plants varies in each group of leaves, and its measurements are carried out by analyzing nitrogen content. For cell formation, tissue, and plant organs including leaves, and help photosynthesis and chlorophyll synthesis. Therefore, nitrogen is very important and is needed very much, especially during the vegetative growth phase. As a constituent of protein that contributes around 16%, nitrogen also plays a role in chlorophyll synthesis. Along with the process of photosynthesis that takes place, nitrogen concentrations in plants tend to decrease, indicating its crucial role in supporting the growth and function of leaves (Laka & Wanggw, 2018). The findings of the study show that the highest protein concentration is found in shoots, while the lowest levels are in the old leaf group (Irwan, 2020).

In Moringa plants (moringa oleifera), the samples studied include the leaves of the shoots, young, and old. Moringa plants contain the highest amount of protein on the leaves of the shoots. According to research, the concentration of nutrients is higher in the shoots of leaves compared to young and old leaves. The protein content is different in each group of leaf (Irwan, 2020). The findings are similar to Saputri's research which found that in young and old Moringa plants analyzed by the Kjeldahl method, a young Moringa leaf protein content was found an average of 1,3092% and an average old Moringa leaf of 11,3473% (Saputri et al., 2019). As for other studies, find the best content in the leaves of the shoot (Irwan, 2020). Higher metabolic activity in the shoots requires large amounts of protein synthesis to support the process of growth and cell division.

The findings of protein content conducted in this study are in line with previous research which states the protein content in Moringa leaves can reach 29.9% (Paramita et al., 2021). In addition, the nutritional content of Moringa oleifera varies depending on factors such as soil, climate, season, and age of plants, as well as differences in processing and storage procedures. Putriani's research emphasizes that environmental conditions, including soil and climate quality, play an important role in determining the nutritional content of plants, where plants planted in nitrogen -rich soils and in optimal climate conditions show significant increases in protein and other nutrients (Sulistiani et al., 2023). Therefore, a deep understanding of these factors is also important to optimize nutritional potential so that it can be used to improve the nutritional quality of *Moringa oleifera*, and can choose the right part to adjust the protein needs.

In the katuk leaves (*Sauropus androgynus*) also has great potential as an alternative source of vegetable protein. Analysis of protein content in *Moringa oleifera* leaves revealed that the value of protein reaches around 7% protein (Mulyati et al., 2024 & Senas, 2023). Protein content in katuk leaves can vary depending on the condition of growth and the plants analyzed, with several studies showing that protein content can reach up to 29.15% under certain conditions (Nofiyanto & Cahyanti, 2023). This study shows the pattern of increase in protein content from the leaves of the shoots, young parts, and old parts. These results are as shown in Figure 2. Research related to the protein content in the shoot Moringa leaves is still limited, while based on segi research in Sartika states that old katuk leaves contain more protein content that is higher than young katuk leaves (Sartika et al., 2019).

Comparative research shows the difference in nutritional composition between base leaves and the leaves of the end of the sauropus androgynus. This study indicates an increase in the amount of nutrients, such as protein, carbohydrates, calcium, and vitamin C, from the 60th day to day 120, which reflects the difference in the accumulation of nutrients in plant organs according to the stage of development. However, in consumption, there is a possibility of protein and beta-carotene degradation when the leaves are heated, which are influenced by various cooking methods, such as cooking with microwaves, boiling, and raw consumption (Anju et al., 2022).

Seeing the potential content of protein levels in a fairly good sample, allows these two types of leaves to be used in the form of flour to be used as a source of protein, a mixture in functional food products, or as raw material for making concentrate protein (Nofiyanto & Cahyanti, 2023). To maintain the quality of protein, the right processing strategy is very necessary, such as drying at low temperatures to prevent protein denaturation and the use of encapsulation technology to increase bioavailability (Salsabila et al., 2022). By combining the use of the top of oleifera moringa leaves and old *Sauropus androgynus* leaves, optimal synergy can be created in meeting the needs of vegetable protein, especially for populations that have limited access to animal protein.

CONCLUSION

Based on the analysis of the protein content using the Kjeldahl method, a different distribution pattern is obtained in various parts of the leaves of Moringa (*Moringa oleifera*) and katuk plants (*Sauropus androgynus*). The acquisition of the protein content of Moringa plants in some parts of the difference is quite far, where the highest protein content in the shoots is 22.4%, then decreases dramatically in young and old leaves, while the katuk leaves show the protein content increases from the shoots of the shoots by 3.898%, young leaves by 4.767%, and old leaves by 5.127%. These findings indicate that selective strategies must be used to maximize the use of the two plants as a source of vegetable protein by prioritizing the selection of moringa and katuk old leaves.

RECOMMENDATIONS

Based on the findings in this article, it is recommended that Moringa leaves (moringa oleifera) and katuk leaves (Sauropus Androgynus), especially in the shoots and old leaves, are further utilized as a source of potential alternative vegetable protein. Given the high protein content in these parts, its use in the form of concentrate can be a functional solution in the development of nutritious food products. In addition, the selection of appropriate processing methods, such as low -temperature drying or encapsulation technology, is highly recommended to maintain the stability and nutritional value of protein. Further research related to the influence of environmental factors and leaf age on protein content also needs to be done to enrich scientific data that supports the optimization of the use of local plants. Thus, the results of this study are expected to be the basis for the development of high -value local food ingredients and support efforts to increase food resilience and security, especially in regions with limited access to animal protein sources.

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