

Quantitative and Qualitative Analysis of Protein Content in Moringa Leaves (*Moringa oleifera* L.)

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Abstract

Protein is one of the macronutrients that have an important role in the formation of biomolecules. Protein can be found in various plants, one of which is Moringa leaves. Moringa leaves (*Moringa oleifera* L.) are proven to be effective in treating various diseases, including diabetes, hepatitis, heart disease, and high cholesterol. The purpose of this study was to see if there was protein content in Moringa leaves (*Moringa oleifera* L.) and how much protein content in Moringa leaves. The parameters used are qualitative tests and quantitative tests, qualitative tests are carried out using the biuret method to determine the presence or absence of protein content in Moringa leaves, while quantitative tests are carried out using spectrophotometric methods to determine protein levels contained in Moringa leaves. From the research results, it is known that Moringa (*Moringa oleifera* L.) leaves contain protein with a protein content of 2,783; 2,657; 2,547 mg/50g.

Keywords: Moringa Leaf, Protein, Spektrofotometri

Received: 17 June 2022. Accepted: 27 July 2022

Introduction

Indonesia has the potential for abundant natural resources, both from animals and plants, which can be used as a source of food or medicine. One of the plants that can be used as food and medicine is Moringa (*Moringa oleifera* L.). Moringa is a plant that is rich in nutrients because it contains many vitamins, minerals, antioxidants and essential amino acids (Krisnadi, 2013).

Proteins are macromolecules that make up more than half of the cell. Protein determines the size and structure of cells, the main component of enzymes, namely biocatalysts for various metabolic reactions in the body (Mustika, 2012). Qualitative analysis of protein content can be carried out by various methods, one of which is the biuret reaction,

this test is to show compounds containing an acid amide group that is with other amide groups. This test gives a positive reaction which is indicated by the appearance of a red violet or blue violet color (Apriandi, 2011).

While quantitative analysis can be carried out by various methods, such as: the Dumas method, the Lowry method, UV spectrophotometry, turbidimetry or turbidity, the painting method, Formol titration and the Kjeldahl method. Spectrophotometry is an analytical method based on measuring the absorption of monochromatic light by a strip of colored solution at a specific wavelength using a prism monochromator or a diffraction grating with a phototube detector (Winarno, 2004).



From the results of this study, it is hoped that it can be applied and applied in everyday life, knowing and proving how much protein content is being studied and the possibility that it can be used as an alternative protein substitute which is equivalent to protein in 2 yogurts (Mahmood et al., 2011).

Method

This research is a descriptive study that analyzed qualitatively and quantitatively which was conducted at the Integrated Chemistry Laboratory, University of Muhammadiyah Gresik. The sample used was old Moringa leaves (*Moringa oleifera* L.).

Determination of Protein Levels in Moringa Leaves (*Moringa oleifera* L.)

1. Protein Qualitative Biuret Method

The protein solution to be tested was put into different test tubes, each as much as 2 ml, added with 5 ml of Biuret reagent. 5 drops of CuSO₄ solution were added to each protein solution. Also added 2 ml of NaOH solution into each protein solution. The solution is shaken slowly and observe the color change that occurs.

2. Protein Quantitative Spectrophotometry

The mashed sample was weighed 50 grams, added 5 ml of 1 M NaOH and distilled water up to 25 ml. Then heated at 90°C for 10 minutes. After that, the solution was cooled and centrifuged for 10 minutes. Then 5 ml of the supernatant was taken and 5 ml of Biuret reagent was added. The mixture was homogenized and incubated for 20 minutes at room temperature. Then the absorbance of the sample was measured with a spectrophotometer at a wavelength of 540 nm. The results of the absorbance of the sample solution are interpolated in the equation $y = bx + a$, so that the protein concentration of the sample solution is obtained.

$$y = bx + a, \text{ where}$$

a=Constant; b=Slope

The values of a and b are calculated using the equation:

$$a = \frac{\sum y - b (\sum x)}{n}$$

$$b = \frac{n(\sum xy) - (\sum x)(\sum y)}{n(\sum x^2) - (\sum x)^2}$$

Results and Discussions

From the research that has been done, the following results were obtained:

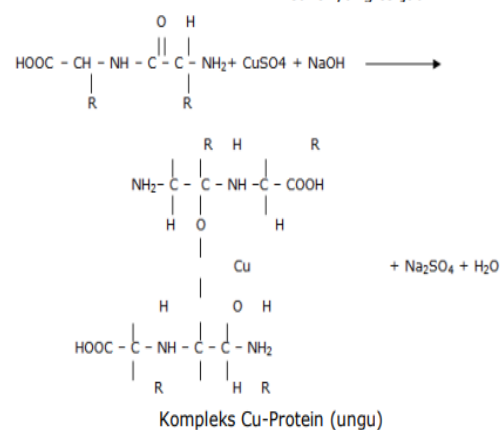
1. Protein Qualitative Biuret Method

Biuret protein testing was carried out to determine the presence of peptide bonds which were marked by the appearance of a blue-violet color in the test solution. For better biuret test results, a positive control was used as a comparison, the positive control used was egg white.

Table 1. Results of Biuret Protein Identification

No	Sample	Result	Description
1	Egg white (positif control)	+ (positif)	violet
2	Moringa leaf (<i>Moringa oleifera</i> L.).	+ (positif)	violet

From table 1, it is known that all samples gave a positive reaction with a violet color formed. Thus the biuret test is not only for protein but other substances such as biuret or malonamide also gives a positive reaction, which is marked by the appearance of a red-violet or blue-violet color (Sylvia, 2021).



Cu-Protein Complex (Violet)

Figure 1. Protein Reaction with Biuret (Sudarmadji, 2007)

The biuret method is based on the principle that substances containing two or more peptide bonds



can form purple complexes with Cu salts in alkaline solutions (Saputri, 2019).

2. Protein Quantitative Spectrophotometry

Analysis of protein content in moringa leaves (*Moringa oleifera* L.) was carried out using spectrophotometry. Spectrophotometry is a tool used to measure absorbance by passing light with a certain wavelength on a glass or quartz object called a cuvette. The wavelength used in this study was 540 nm. This is in line with the research of Andarwulan, et., al. (2011).

Moringa leaves (*Moringa oleifera* L.), because this plant is included in a protein source that can complement poor forage feeds such as elephant grass and is very good at replacing commercial concentrate constituents in ruminants (Mendieta-Araica et al. ., 2011).

Conclusion

Based on the results of the research that has been done, it can be concluded that based on the qualitative test using the biuret method, Moringa leaves (*Moringa oleifera* L) are positive for protein. Based on quantitative tests using spectrophotometry, the protein content of Moringa (*Moringa oleifera* L) leaves was 2,783 mg/50g; 2,657 mg/50g; 2,547 mg/50g.

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Table 2. Results of Analysis of Protein Levels in Moringa Leaves (*Moringa oleifera* L.) Using Spectrophotometry

Replication	Absorbance (Y)	Protein content (mg/50g)
1	0.183	2.783
2	0.175	2.657
3	0.168	2.547

Based on the plot of the relationship between each concentration and absorption of the protein standard solution, namely $Y = 0.00624 + 0.0636x$, the protein content was obtained in each ml of the sample solution. From table 2, the measurement of protein content in Moringa leaves (*Moringa oleifera* L.) using Spectrophotometry at a wavelength of 540nm, for measurements of the absorption of the average protein content in moringa leaves (*Moringa oleifera* L.) with 3 replications obtained 2,783 mg /50g; 2,657 mg/50g; 2,547 mg/50g. Based on previous research, it was confirmed that Moringa leaves (*Moringa oleifera* L) have varying protein content, namely 18.6%, 37.2%, 9.4% (Barakat & Ghazal, 2016; Saini et al., 2016; Hariyanto et al., 2022). From the calculation results obtained in this study, it is not in accordance with the literature, this is because there is a factor of tannin compounds contained in Moringa leaves (*Moringa oleifera* L). The presence of the effect of tannin compounds of more than 5% in Moringa leaves (*Moringa oleifera* L.) can affect the level of protein absorbed (Rossida et al., 2019). In addition, it may be due to environmental factors that affect protein levels in Moringa leaves (*Moringa oleifera* L.). However, this does not affect the benefits of



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