DETERMINATION OF VITAMIN C IN SPINACH (*Amaranthus* sp.) USING TITRATION METHOD

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ABSTRACT

Vitamin C is a compound that is included in the group of water-soluble vitamins. The human body really needs vitamin C intake because it can act as an antioxidant which is closely related to collagen formation and iron absorption. Vitamin C is an organic compound that the human body needs in small amounts to maintain the body metabolic functions. Vitamin C is also one of the most important vitamins in increasing the body immunity This compound is very easy to find in fruits and vegetables, one of which is spinach. Spinach is a vegetable which generally contain vitamin C with an average of 158.54 mg/100 g. The research aims to analyze vitamin C levels in spinach using a titration method or also known as iodometric titration. This research method is included in an experimental study. The sample used in this research was obtained from Kotagede Market, Yogyakarta. The titration results for each spinach will be analyzed using Microsoft Excel, where the results have shown that the levels of vitamin C contained in snapper spinach (18.95 mg/100 g), picked spinach (46.39 mg/100 g), thorn spinach (14.52 mg /100 g) and red spinach (15.42 mg/100 g). Therefore, it can be concluded that this titration method can be used to accurately measure the vitamin C content in spinach. The iodometric titration method was chosen to analyze vitamin C levels because this application is very simple when compared to other methods. Ascorbic acid or vitamin C is known as a strong reducing agent and can simply be titrated using iodine solution.

Keywords: Determination, Method, Spinach, Titration, Vitamin C

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Introduction

Vitamin C is an organic compound that the human body needs in small amounts to maintain the body metabolic functions (Hasanah, 2018). Vitamin C is one of the most important vitamins in increasing the body's immunity (Evana & Barek, 2021). Vitamin C, or what is known as ascorbic acid, is included in the group of water-soluble vitamins (Hadi Pranoto, 2021). The human body is unable to produce vitamin C, so to reach the body need for vitamin C, this can only be done by consuming fruit and vegetables (Rahayuningsih et al., 2022).

Vitamin C is a compound that acts as an antioxidant which plays an important role in the formation of collagen to improve the formation of bones and teeth and the absorption of iron (Hasanah, 2018), helps heal wounds, is involved in repairing body tissue, metabolic processes with oxidation-reduction processes, hydroxylation processes of adrenal cortex hormones, reduces susceptibility to infection and is able to reduce cholesterol levels in human blood (Cunha-Santos et al.. 2019),(Abror et al., 2020),(Cresna et al., 2014). The recommended daily intake of vitamin C is 90 mg for adult men and 75 mg for adult women (Simonson, 2020).

From the statement above, it can be concluded that there are many advantages to consuming vitamin C, therefore there will be many deficiencies that occur if vitamin C levels are very low in the human body, including causing gums and skin to bleed easily, mouth ulcers, a long healing process. as well as pain in the joints (Hasanah, 2018). Several studies also show the risks of vitamin C deficiency, such as skin becoming rough and scaly, hair becoming dry, anemia, nosebleeds (epistaxis) and teeth becoming brittle and easily falling out of the gums (Hasanah, 2018).

Each person's need for vitamin C is certainly different, depending on age, gender, and the body ability to process metabolism, absorption and excretion. Vitamin C is easily found in various types of fruit and vegetables such as broccoli, peppers, tomatoes and spinach(Rahayuningsih et al., 2022),(Lessy & Pratiwi, 2020),(Nita, 2019). Spinach is a fastgrowing plant that is widespread throughout the world, including in Indonesia, where it generally comes from the Amaranthaceae family (Sarker et al., 2020). This plant is also one of the types of vegetables most consumed by Indonesian people, making it an alternative to vitamin C besides fruit. Spinach plants are divided into types and varieties, spinach includes cultivated plants and wild plants. In Indonesia there are two types of spinach, namely green spinach and red spinach (Rahmayadi & Ariska, 2022). Based on several previous studies, spinach contains macronutrients and micronutrients, including vitamins, minerals and antioxidants (Rashid et al., 2020).

There are many approaches that have been used to analyze vitamin C levels in food and drinks, including titration methods, spectrophotometry and high performance liquid (Fitriana chromatography & Fitri, 2020),(Sharaa & Mussa, 2019).(Jubahar, 2015),(Satria et al., 2021). However, in this study, the iodometric titration method was chosen to analyze vitamin C levels because this application is very simple when compared to other methods. Therefore, the aim of this research is to analyze the vitamin C content contained in several types of spinach that are commonly consumed by people in Indonesia using the iodometric titration method.

Research Methods

This research is included in a quantitative study, using an experiment design. Tools that were used in this study were blender, glass beaker, Erlenmeyer, burette, stative, test tube, rack, measuring pipette 5 mL and 10 mL, dropper pipette, measuring flask 1000 mL and 10 mL, measuring cup, analytical balance, horn spoon, watch glass, water bath, rotary evaporator and stirring rod. In addition, the materials used in this research were snapper spinach leaves, picked spinach leaves, red spinach leaves, thorn spinach leaves, 0.1 N iodine, 2% starch, distilled water, As2O3, methyl orange, NaOH, NaHCO₃, 70% ethanol and H₂SO₄ 2 N.

The research was conducted in several stages. Fresh samples of snapper spinach leaves (*Amaranthus hybridus*), picked spinach leaves (*Amaranthus tricolor* L.), spinach leaves (*Amaranthus spinosus*), and red spinach leaves (*Amaranthus tricolor* L.) were obtained from Kotagede Market, Yogyakarta. The sample was washed with clean water, then weighed and ground using a blender, then the sample was put into a glass beaker to be macerated using 100 mL of 70% ethanol solvent. Then leave it for 2 days, covered with aluminum foil, to avoid

looking for light. After 2 days, each spinach leaf sample was filtered through flannel cloth, then concentrated using a rotary evaporator, until a thick extract was obtained.

The next stage is preparing 0.1 N Iodine Solution by weighing 18 grams of KI, dissolving it little by little with distilled water until it dissolves. Then, 12.69 grams of iodine was added, dissolved in distilled water, 10 mL of 2% H₂SO₄ was added and stirred until homogeneous. The solution was put into a 1000 ml measuring flask, and distilled water was added to the mark.

The next stage is processing 2% starch by weighing 2 grams of starch, adding distilled water until dissolved. Then, it was heated until the solution becomes clear. The solution was put into a 100 mL measuring flask and distilled water was added to the mark. Furthermore, standardization of 0.1 N iodine solution was completed by weighing 150 mg of arsentioxide (As₂O₃) dissolved in 1 N sodium hydroxide (NaOH), add 40 mL of distilled water, then add 2 drops of methyl orange, add 0.1 N HCl until the color changes, from yellow to pink. Then added 2 grams of NaHCO₃ which was diluted with 50 mL of distilled water. Then titrated with iodine solution using 2% starch indicator until the color changes to blackish blue.

Furthermore, it was continued by preparing 100 ppm vitamin C solution. It was weighed 10 mg of vitamin C powder, t put it in an Erlenmeyer, added distilled water, and homogenized. Then, put it in a 100 ml measuring flask, add distilled water to the mark. Next stage is completing preparation of Vitamin C concentration series solutions. A vitamin C series solution was made with a concentration of 10 ppm; 12 ppm; 14 ppm; 16 ppm; 18 ppm; 20 ppm; 22 ppm; 24 ppm; 26 ppm; and 28 ppm. Each concentration was made up to 10 ml. Put it in a test tube and label it according to the concentration of each solution.

Next stage is conducting in the determination of calibration curves for vitamin C series solutions by processing pipet 10 mL of vitamin C series solutions with concentrations of 10 ppm, 12 ppm, 14 ppm, 16 ppm, 18 ppm, 20 ppm, 22 ppm, 24 ppm and 28 ppm into an Erlenmeyer, add 3 drops of 2% starch indicator. Titrate with 0.1 N iodine solution. Note the volume of iodine needed during the titration until a color change occurs. The last stage is determination of vitamin C levels in spinach by

processing pipette 10 ml of each spinach leaf extract which has been diluted, put it into an Erlenmeyer, add 3 drops of 2% starch. Then titrated with 0.1 N iodine solution. Replicate 3 times.

The titration results obtained will be analyzed using Microsoft Excel. Data processing and analysis to determine vitamin levels in spinach leaves (Amaranthaceae family) was obtained from iodometric titration, then vitamin C levels were calculated using a linear regression equation (y = bx + a).

Results and Discussion

The plants used for research have been determined in the Biology Laboratory, Faculty of Applied Science and Technology, Ahmad Dahlan University using the book Flora of Java (1965). Determination is carried out to ensure the correctness of the plants and prevent errors in the plants used in research. The results of the determination showed that the plants used in the research were snapper spinach, picked spinach, thorn spinach and red spinach.

Extraction of snapper spinach leaves, picked spinach, thorn spinach and red spinach was carried out using the maceration method. The selection of spinach leaf samples also greatly influenced the results of this research, therefore fresh spinach leaves were chosen in this research, and the crushing process also used a blender. Each sample was weighed as much as 50 g. The solvent used was 100 mL of 70% ethanol and remaceration was carried out once.

In this research, the maceration process was carried out repeatedly using the same type and concentration of solvent. According to several studies, the basic purpose of the extraction process is to withdraw the chemical components contained in the sample. Maceration was also chosen in this research because it is the simplest method without requiring a heating process, thereby minimizing damage to chemical compounds in the sample (Hasanah et al., 2020). The results of this research can be seen in Table 1.

Table 1. Resu	ults of Spina	hch Leaf Extract
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No	Sample	Rendement
1	Snapper spinach leaves	7.218%
2	Pick spinach leaves	36.598%
3	Spinach leaves	13.476%
4	Red spinach leaves	13.352%

In this study, iodine standardization (0.1 N) was carried out 3 times. The purpose of the iodine standardization process is to equalize the solution to be used for titration with the standard solution. The average result of the titration was 22.8 ml with normality obtained at 0.1 N with a color change from yellow to pink to a stable blue color. The reactions that occur during the standardization process of the iodine solution can be seen in Figure 2 below:

 $As_2O_3 + 6 NaOH \longrightarrow 2 Na_3AsO_3$

 $Na_3AsO_3 + I_2 + 2 NaHCO_3 \longrightarrow Na_3AsO_4 + NaI + 2 CO_2 + H_2Opreservative is generally added. Commonly used preservatives are boric acid, formic acid or mercury (II) iodide. The concentration of the$

The basis of the iodometry method is by reducing vitamin C. Ascorbic acid or vitamin C is known as a strong reducing agent and can simply be titrated using iodine solution. In this research, the iodometric method was used to analyze the vitamin C contained in spinach. This method is also guaranteed to have good precision and accuracy because it produces almost the same number of titrants in each series of measurements (Rohman, 2007). In this method, the oxidation-reduction reaction occurs due to the use of iodine solution, that is, the standard iodine solution is an oxidizing solution that is added with potassium iodide in excess, resulting in the release of iodine (equivalent to the amount of oxidizer) titrated with a standard solution of sodium thiosulfate (Rivai, 1998).

Oxidation refers to any chemical change in which an increase in the oxidation number occurs, while the term reduction can be used for a decrease in the oxidation number. This means that the oxidation-reduction process that occurs in this research is due to a molecule losing electrons and gaining electrons (Khopkar, 2003). Several studies have shown that the use of starch or starch as an indicator is not really needed in this iodometric method. This is because the iodine solution has a very specific color and can disappear at the end point of the titration until the end point is reached. However, observing the end point of the titration will be easier to observe if the starch solution is used as an indicator, this is because the starch will form a complex compound with iodine which will produce a stable blue color.

The use of starch as an indicator must be given when the titration approaches the end

point. This aims to prevent the starch from enveloping the iodine, which makes it difficult to release again, and this can result in the blue color being difficult to remove, so that the end point of the titration is not clearly visible (Wunas, 1986).

The use of starch as an indicator is very commonly used in the implementation of the iodometric method, however, several studies have shown that starch indicators must be made fresh before use, this is because starch is easily degraded by the presence of microorganisms so that in the process of using indicators, a

Opreservative is generally added. Commonly used preservatives are boric acid, formic acid or mercury (II) iodide. The concentration of the indicator solution can also be influenced by high temperatures and the use of other organic compounds such as methyl and ethyl alcohol (Underwood, 1993).

Analysis of standard series solutions of vitamin C at concentrations of 10-28 ppm was carried out using the iodometric titration method using 2% starch indicator and titrated with 0.1 N iodine solution. This titration process resulted in a color change to blackish blue. The series determination of vitamin C levels can be seen in Table 2.

Table 2. Determination of Vitamin C ConcentrationSeries Using the Iodometric Titration Method

Concentration (ppm)	TAT Iodine Volume (ml)
10	0.11
12	0.13
14	0.15
16	0.18
18	0.20
20	0.22
22	0.24
24	0.27
26	0.29
28	0.31

Determination of the calibration curve was carried out by pipetting 10 mL of a series of vitamin C solutions, adding 3 drops of 2% starch, then titrating with 0.1 N iodine solution which then produced a color change to blackish blue, then from the results of the titration the data was processed to produce a calibration curve. which can be seen in Figure 3. In this study, a vitamin C calibration curve was also plotted, where a linear regression y = 0.0113x -0.0042 was obtained, with an R₂ value = 0.9984. From this data, the detection limit (Limit of Detection) was obtained, 0.84 ppm and the limit of quantitation (Limit of Quantification), 2.54 ppm.



Figure 3. Vitamin C Calibration Curve

The research carried out used four types of spinach leaves, namely snapper spinach leaves, picked spinach leaves, thorn spinach leaves and red spinach leaves obtained from Kotagede Market, Yogyakarta. Spinach leaf extract is made from ground fresh simplicia. Spinach leaf extract is made using the maceration method by mixing crushed spinach leaves with 70% ethanol solvent.

The levels of vitamin C contained in the sample solution for each spinach leaf can be seen in Table 3 below. Qualitative determination of vitamin C can be done using the iodometric method, because the solvent used in this method is capable of oxidizing vitamin C. Vitamin C is a reducing agent and will undergo a simple oxidation process by titrating with an iodine solution which acts as an oxidizing agent.(Fitriana & Fitri, 2020).

 Table 3. Determination of Vitamin C Levels in

 Spinach

No	Sample	TAT Iodine Volume (ml)	Vit levels. C (mg/100g)
1	Picked Spinach	0.52 ± 0.01	46.39 ±0.89
2	Snapper Spinach	0.20 ± 0.01	18.95 ±0.89
3	Red Spinach	0.17 ± 0.01	15.42 ±0.89
4	Thorn Spinach	0.16 ± 0.01	14.52 ±0.89

Determination of vitamin C levels in samples using the iodometric titration method, using 0.1 N iodine as titrant and starch as indicator (Evana & Barek, 2021). The results of the research show that the average level of vitamin C in picked spinach leaves is higher than other spinach, which can be seen in Table 3. In this study, a 0.1 N iodine solution and starch with a concentration of 2% were used. The principle of the iodometric titration method is that the iodine solution will oxidize vitamin C in an acidic environment, produces dehydroascorbic acid as in the reaction that can be seen in Figure 4.

$$C_6H_8O_6 + I_2 \longrightarrow C_6H_6O_6 + 2I^- + 2H^+$$

Figure 4. Reaction of Vitamin C with Iodine

Titration using iodine aims to reduce acidic compounds such as vitamin C. The results of the titration test with iodine solution produce a change in the color of the solution to blue. The blue color that forms is a sign that the titration process has reached the end point (Ngginak et al., 2019). The results of vitamin C levels obtained in this study were different when compared to theoretical levels of vitamin C in spinach leaf samples.

In the theory, the vitamin C level for snapper spinach leaves is 9.54 mg/100 g. In picked spinach leaves 158.54 mg/100 g. In spinach leaves, 8.10 mg/100 g and in red spinach leaves 44.07 mg/100 g. The difference in vitamin C levels can be caused by sample preparation which may be exposed to light so that the vitamin C in the sample can be oxidized(Purnama et al., 2021). In several studies using the titration method also showed the vitamin C content found in several vegetables and fruit, such as Ngginak et al. (2019) reported that the vitamin C content in figs was 4.13 mg/100 g while in passion fruit it was 1,904 mg/100 g (Lobefaro et al., 2021).

Meanwhile, vitamin C in tangerines has also been successfully analyzed using the iodometric method as much as 8.8 mg/ 100 g (Ngginak et al., 2019). The vitamin C content in spinach has also been successfully analyzed using this method with a yield of 9.68 mg/ 100g (Rahayuningsih et al., 2022),(RI Ministry of Health, 2020),(Fitriana & Fitri, 2020),(Sharaa & Mussa, 2019). Therefore, it can be concluded that the iodometric method can be used as a technique for measuring the vitamin C content in vegetables and fruit.

Conclusion

The aim of this research is to analyze the vitamin C content contained in several types of spinach that are commonly consumed by people in Indonesia using the iodometric titration method. The iodometric titration method used

to determine vitamin C levels in spinach has been successfully carried out. Based on the results of research that has been carried out, it can be concluded that the level of vitamin C in picked spinach leaves is 46.39 mg/100 g (w/w) ± 0.89 . Vitamin C levels in snapper spinach are 18.95 mg/100 g (w/w) ± 0.89 . Vitamin C levels in red spinach are 15.41 mg/100 g (w/w) ± 0.89 . Vitamin C levels in spinach are 14.52 mg/100 g (w/w) ± 0.89 . Based on the findings, the further research also can be conducted to determine the levels of vitamin C in spinach leaves using different methods.

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