RESEARCH ARTICLE

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The detection of ascorbic acid in orange (*Rutaceae Sp.*) using titration technique

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Abstract

Vitamin C is a vitamin that belongs to the group of water-soluble vitamins. Some sources of vitamin C are obtained from fresh vegetables and fruits. Vitamin C has several functions in the body, including helping to replenish the body's supply of twins and optimizing functionality. This study aims to determine the levels of vitamin C in lime, kaffir lime, tangerine, and lemon by the iodometric titration method. The sample used in this study was obtained from the Brosot market, Kec. Galur, Kab. Kulon Progo, Yogyakarta. The vitamin C levels obtained from lime, kaffir lime, tangerine, and lemon were 0.004%, 0.004%, 0.002%, and 0.005%.

Keywords: *vitamin-c, lemon, yogyakarta*

Introduction

Orange is a fruit that contains vitamin C, which is beneficial for the human body. In addition, the vitamin C content in citrus fruits can bind free radicals to prevent and reduce the risk of diseases such as skin cancer, liver cancer, and high cholesterol and maintain heart health [1].

Vitamin C is a supply of vitamins that are included in the group of water-soluble vitamins. The recommended nutritional vitamin C intake for adults is about 30-100 mg daily. This vitamin is essential in activating the enzyme prolyl hydroxylase to support hydroxylase in forming hydroxyproline, an integral component of collagen [2].

Vitamin C has low stability compared to other vitamins because vitamin C is easily damaged during processing and storage; besides that, vitamin C is easily damaged when exposed to heat and air; the temperature can also affect vitamin C because as the temperature rises, the vitamin C content will get lower. Therefore vitamin C must be considered in storage where vitamin C must be stored in a room with low temperatures to slow down the speed of the oxidation reaction [3].

Vitamin C is a catalyst in chemical reactions in the human body. Therefore, if Vitamin C is not present in the body, the normal function of the body can be disrupted. The body can meet vitamin C intake by consuming fruit and vegetables because the human body cannot produce Vitamin C, so fruit and vegetables are needed for food intake [4].

The reason for using citrus fruits as research is because citrus fruits are easy to find and the price is affordable. In addition, citrus fruits contain more organic acids (citric acid) than other fruits [3]. The researcher chose the iodometric titration method because this method is often used, practical, and does not require a long time for vitamin C analysis.

The principle of the iodometric titration method is that the color changes in the sample after the titration process is carried out. This test was carried out based on a transparent color change to a blue color from the starch sample titrated using an iodine solution. This titration used iodine as an oxidizing agent, which will oxidize vitamin C, and used starch as an indicator [5].

Previous studies that have been studied, namely the determination of vitamin C levels of lemon pulp (Citrus limon L.) juice using visible spectrophotometry, showed that lemon pulp juice contains a vitamin C compound with a level of 0.66 mg/g sample [6]. Meanwhile, studies on vitamin C levels in lemon juice using the iodometric titration method have not been conducted. Therefore, researchers are interested in determining vitamin C levels in jerk fruit using the iodometric titration method using iodine with a concentration of 0.01 N.

Research Methodology

Materials and Method

The tools to be used in this study were watch glass, beaker glass, 1000 ml measuring flask, 100 ml measuring flask, 250 ml Erlenmeye, funnels, states, burettes, clamps, 10 ml measuring pipette, 100 ml measuring flasks, 10 ml volume pipette, proppipet, analytical balance, water bath, rotary evaporator, stir bar, blender, knife, and filter paper. Materials applied in this study were citrus fruit samples, vitamin C, 1% starch, 0.01N iodine solution, H_2SO_4 solution, KI, AS_2O_3, NaOH, $NaHCO_3$, 7.3% HCl, methyl orange, 70% ethanol solution, and distilled water.

Procedure

Sample preparation

The samples used in this study were limes, lemons, tangerines, and kaffir limes obtained from Brosot market, Kec. Galur, Kab. Kulon Progo, Yogyakarta. Samples of peeled orange fruit were weighed as much as 50 g, then blended. Then the extract was macerated using 70% ethanol, as much as 250 ml, after which the extract was stirred until homogeneous. The extract was macerated for 2×24 hours and protected from direct sunlight. Then the maceration results were filtered using filter paper until the desired maserate is obtained; the maserate was concentrated using a rotary evaporator at 90°C to form a thick citrus fruit extract [7-8].

Preparation of 0.01 N Iodine Solution

Dissolve 1.66 g of KI in a 1000 ml volumetric flask with distilled water little by little until dissolved, then add 1.27 g of iodine and H_2SO_4 solution, then dissolve with distilled water up to the mark, shake until homogeneous.

Preparation of vitamin C concentration series solutions

Vitamin C solution was diluted into a 10 ml measuring flask with a concentration of 1 ml of 10 ppm, 12 ppm of 1.2 ml, 14 ppm of 1.4 ml, 16 ppm of 1.6 ml, 18 ppm of 1.8 ml, 2 ml of 20 ppm, 2.2 ml of 22 ppm, 2.4 ml of 24 ppm, 2.6 ml of 26 ppm, and 2.8 ml of 28 ppm then add distilled water in each measuring flask, shake until homogeneous.

Making 1% Starch

1 g of starch was weighed and put into a 100 ml measuring flask. After that, it dissolved with distilled water up to the mark, then shaken until homogeneous.

Preparation of 10% H_2SO_4 solution

Pipetted 1 ml of H_2SO_4 solution, then dissolved it in a 100 ml measuring flask using distilled water up to the mark, shaken until homogeneous.

Standardization of 0.01N iodine solution

AS_2O_3 weighed as much as 150 mg and then dissolved with 20 ml of 2 N NaOH; after that, it was diluted using 40 ml of distilled water, then added two drops of methyl orange, after that, added 16 ml of 7.3% HCl until a pink color changed, then added 2 g of $NaHCO_3$ it is re-diluted using 50 ml of distilled water, then added 1% starch indicator, then titrated using iodine solution until changed to a permanent blue color.

Determination of the vitamin C calibration curve in serial solutions

Each standard vitamin C concentration series solution was taken 25 ml, then put into a 250 ml Erlenmeyer, then three drops of 1% starch were added, then titrated using 0.01 N iodine standard solution until the color changed to purple-black [9].

Determination of vitamin C levels in the sample

Each orange sample filtrate was added with 1% starch indicator three drops. Then the sample was titrated with 0.01 N iodine standard solution until a purple-black color was formed.

Processing and analysis of data

After all the data had been collected, then the data was presented in tabular form using Microsoft Excel to find out the levels of vitamin C contained in oranges (Rutaceae family) obtained from iodometric titrations, which are then compared with the value of vitamin C in iodometric titrations by calculating the formula for vitamin C levels and the linear equation regression (y=bx+a) so that it can be seen the results of the percentage error of the vitamin C level measuring device obtained from the comparison of the data.

Results and Discussion

The research was conducted using four types of citrus fruit samples, namely limes, lemons, tangerines, and kaffir limes which were obtained directly from the Brosot market, Galur District, Kulon Progo Regency, Yogyakarta, which were then peeled from each orange and then weighed with the weight of 50 g then the sample was mashed using a blender after the fine sample was then dissolved using 70% ethanol solvent as much as 100 ml.

The research entitled Determination of Vitamin C Levels in Citrus Fruits (*Rutaceae Sp.*) uses the iodometric titration method. Iodometric titration was included in the experimental titration method. Iodometric titration was a titration of iodine released in a chemical reaction with a starch indicator. The starch indicator is used to determine the endpoint of the titration based on the color change of starch with iodine from a clear color to a fixed blue color. Making Kandi indicators is carried out on a stove or water bath so the starch solution can dissolve completely to produce maximum titration.

Table 1. Calculation results of Vitamin C levels

Sample	Replicates (n)	Vitamin C levels
Lime	3	0,005%
Tangerine	3	0,002%
Kaffir lime	3	0,004%
Lemon	3	0,004%

This study aimed to determine the levels of vitamin C in citrus fruit samples using the iodometric titration method. The results of determining the levels of vitamin C in citrus fruit samples can be seen in Table 1.

Citrus fruit weighed as much as 50 g; it was then blended until smooth. The delicate orange flesh was then put into a glass bottle to be macerated using 250 ml of 70% ethanol for 2×24 hours.

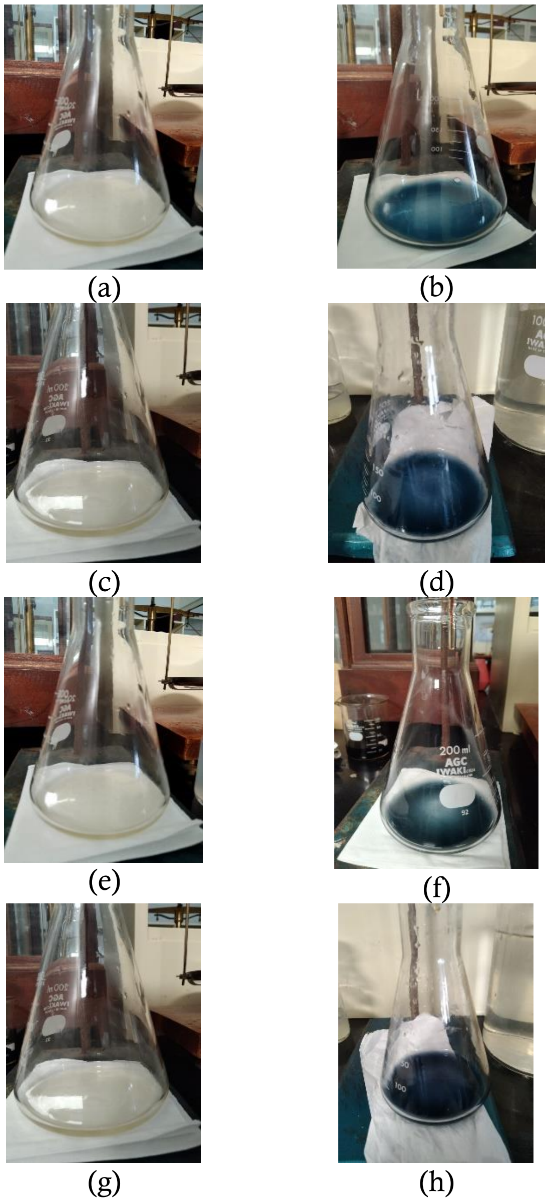

Figure 1. (a) Lime fruit samples before titration, (b) Lime fruit samples after titration, (c) Tangerine fruit samples before titration (d) Tangerine fruit samples after titration, (e) Kaffir lime fruit samples before titration, (f) Kaffir lime fruit sample after titration, (g) Lemon fruit sample before titration, (h) Lemon fruit sample after titration.

The reason for using 70% ethanol was because 70% ethanol is a polar solvent compared to 96% ethanol, and 70% ethanol is more non-polar than 50% ethanol. The maceration method is an extraction method using the process of soaking the material in a solvent suitable for extracting the active compound. Factors affecting maceration extraction are time, temperature, type of solvent, material and solvent ratio, and particle size. The advantage of this maceration extraction method is that the active substance extracted will not be damaged.

Table 2. Results of Calculation of Vitamin C Standard Solution Levels (replicate, n=3)

Vit. C cons. (ppm)	Average titration end point volume (ml)	Vit. C level (%)
10 ppm	11,3	99,5078%
12 ppm	13,6	99,8013%
14 ppm	15,8	99,382 %
16 ppm	18,1	99,6178%
18 ppm	20,4	99,8013%
20 ppm	22,7	99,9481%
22 ppm	24,9	99,6679%
24 ppm	27,2	99,8013%
26 ppm	29,5	99,9142%
28 ppm	31,7	99,6965%

Preparation of a vitamin C calibration curve was carried out by presenting data in tabular form using Microsoft Excel to determine the levels of vitamin C contained in citrus fruit samples obtained from iodometric titrations, which were then compared with the value of vitamin C in iodometric titrations by calculating the formula for vitamin C levels in the regression equation linear (y=bx+a). The results of calculating the formula for vitamin C levels can be seen in Table 2. The linear regression equation y = 1.1433x – 0.2333 is obtained with a relation coefficient = 0.9998 (Figure 2).

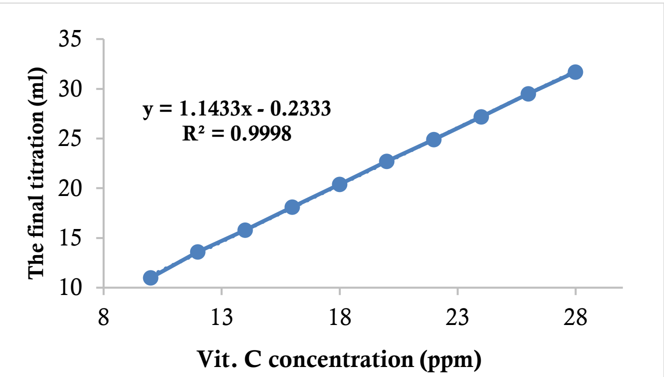

Figure 2. Calibration Curve for Vitamin C Concentration Series Solutions

The results of the analysis of vitamin C levels with lime, tangerine, kaffir lime, and lemon fruit samples were 0.005%, 0.002%, 0.004%, and 0.004%. According to Figure 2, from these results, it can be concluded that the greater the ml of iodine used, the greater the concentration of the sample solution used. The equation of the linear regression value of the standard solution was obtained, the equation y = 1.1433x-0.2333, with a relation coefficient (r) of 0.9998. As well as the results obtained from the extracts of lime, tangerine, kaffir lime, and lemon fruit samples of 6.24%, 6.26%, 13.88%, and 10.24%. The data can be seen in Table 3.

Table 3. Yield yield of citrus fruit samples

Sample	Material weight (simplicia)	Extract weight	% extract yield
Lime	50 g	3,12 g	6,24%
Tangerine	50 g	6,94 g	13,88%
Kaffir lime	50 g	3,13 g	6,26%
Lemon	50 g	5,12 g	10,24%

Conclusion

Based on the results of the research that has been done, it can be concluded that the levels of vitamin C obtained in each sample of citrus fruit were 0.005%, 0.002%, 0.004%, and 0.004%. Also, the yield of extracts from lime, tangerine, kaffir lime, and lemon jerk samples was 6.24%, 6.26%, 13.88%, and 10.24%.

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