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Identification of Ascorbic Acid Content in *Carica papaya* L. Using Iodimetry and UV-Vis Spectrophotometry

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Abstract

Ascorbic acid mostly comes from vegetables and fruits, especially fresh fruits. Vitamin C is a vitamin that can be formed by several types of plants. One of them is papaya which has various contents including vitamin C that can increase endurance, help skin rejuvenation and repair body tissues. The purpose of this study was to determine the levels of vitamin C contained in papaya using iodimetry and UV-Vis spectrophotometry. The sample used in this study was papaya fruit. Determination of vitamin C levels in papaya samples using the titration method bas done by adding sample filtrate with starch indicator then titrated with titrant I_2 until the endpoint color of blue titration is formed. Other hands, the determination of vitamin C levels in papaya samples using the UV-Vis spectrophotometry method bas conducted by making an ascorbic acid calibration curve then the filtrated sample was added with H_2SO_4 5% and ammonium molybdate reagent, then the absorbance of the sample was measured at 494 nm wavelength. The results of the determination of vitamin C levels using the iodimetry was 0.0147% and the results of the determination of vitamin C levels using the UV-Vis spectrophotometry method was 0.1313%. In conclusion, vitamin C levels analyzed by using UV-Vis spectrophotometry methods were greater than vitamin C levels analyzed by using the iodimetry method.

Keywords

Ascorbic acid, papaya, iodimetry, UV-Vis spectrophotometry

INTRODUCTION

Papaya is a fruit that has good nutritional value. It can be used in the form of fresh fruit and processed products. Papaya contains 1.0-1.5% of protein, 1.0-1.5% of vitamin A, and 69-71 mg (100 g)$^{-1}$ of vitamin C. Minerals that are contained in papaya including 11-31 mg (100 g)$^{-1}$ of calcium, and 39-337 mg (100 g)$^{-1}$ of potassium. The other vitamin content in papaya fruit is fat which is 0.1%, 7-13% of carbohydrate. Meanhwile, the enrgy content in papaya is 200 kJ and 85-

90% of water. The vitamin C can increase the body resistance and help in body skin and tissue rejuvenation (1).

Deficiency of vitamin C can can affect bone strength, chronic energy deficiency, weakness, depression and increase risk of various disorders (2). Vitamin C is often present together with substances or other vitamins in food. Foodstuffs contained vitamin C are mainly fruits and vegetables (3).

One of the fruit that contains of many antioxidant compounds such as carotenoids, is papaya fruit. Ripe papaya has beta-carotene which is 276 micrograms per 100 gram, 761 micrograms per 100 gram of betacryptoxanthin, and 75 micrograms per 100 g lutein and zeaxanthin. The beta-carotene is provitamin A and also act as an effective antioxidant to ward off free radical attacks (4).

There are several methods developed for The determination of vitamin C level including the UV-Vis spectrophotometry method (5) and the iodimetry method (6). The spectrophotometric method can be used to determine the mixture content with an overlapping spectrum without prior separation, because the software is easy to use for analysis and is microcomputer instrumentation. Spectrophotometry is widely used in various fields of chemical analysis, especially pharmaceuticals. While, the iodimetry method was a simple method, which is easy to be applied in a research and can give good result in the measurement of analyte content (7).

The reaction of the iodimetry method between vitamin C and amilum was the double bond that is added by iodine that will break into a single bond. If all of the vitamin C has been added to iodine, the iodine which drops during titration will react with the starch indicator solution to form blue iod-amilum. The formation of blue color indicated that the titration process has been completed, because all of vitamin C has been added to iodine so that the volume of iodine needed during titration was equivalent to the amount of vitamin C. The titration treatment must be done quickly because there are many factors that cause oxidation of vitamin C, for example, it occurs when sample preparation. This is because vitamin C easily reacts with O_2 in the air to become dehydroascorbic acid (8).

MATERIALS AND METHODS

Material

The materials included california papaya, aquadest, starch solution, iodine 0.01 N solution, KI solution, H_2SO_4 solution, sodium thiosulfate, ascorbic acid, oxalic acid, and ammonium molybdate.

Equipment

The equipments included glass equipments, Erlenmeyer flask, volumetric

flask, burette, statif, clamp, analytic balance and UV-Vis spectofotometry.

Titrimetric Method

Standardization of 0.01 N sodium thiosulfate

As much as 10 mL of KIO_3 was taken with volume pipette, then it was poured into 250 mL Erlenmeyer. Then, it was added with 10 mL of 10% KI and 2 mL of 2 N H_2SO_4. Then, it was titrated with sodium thiosulfate solution until the solution was formed, indicated by light yellow. Then it was added with 1 mL of starch until the right blue color disappears.

Standardization of 0.01 N I_2

As much as 10 mL of sodium thiosulfate was taken. Then, it was added with 1 mL of starch. Then, it was titrated with I_2 solution until blue color.

Determination of Vitamin C Levels

As much as 25 grams of sample was weighed and dissolved with distilled water. Then, it was put into 100 mL volumetric flask and was added with distilled water to mark boundaries and was homogenized. As much of 10 mL of filtrate sample was taken and put it into erlenmeyer. Then, the solution was added with 1 mL of starch and 10 mL of distilled water. The titration process using 0.01 N 1_2 standard solutions until blue color endpoint was formed.

UV-Vis Spectrophotometry Method

Preparation of 1000 ppm ascorbic acid stock solution

As much as 25 mg of standard ascorbic acid was weighed carefully and bas put into 25 mL volumetric flask and was diluted with 0.4% oxalic acid until 25 mL.

Determination of maximum wavelength

As much as 1 mL of 10 ppm ascorbic acid solution was pipetted and bas put into 25 mL volumetric flask. Then, the solution was added with 10 mL of H_4SO_4 and 5% ammonium molybdate until the mark limit and Then it was homogenized.

Preparation of standard curve

As much as 100 ppm of ascorbic acid solution was pipetted for 7 times, 0.5; 0.75; 1; 1.25; 1,5; 1.75 and 2 mL. Each of the solution was put into 25 mL volumetric flask and then it was added with 5 mL of 10% H_2SO_4 and the volume was sufficient with 5% ammonium molybdate until the mark limit and then it was homogenized. From this procedure, concentration of 0.2 will be obtained; 0.3; 0.4; 0.5; 0.6; 0.7 and 0.8 ppm. Then, incubation process for 30 minutes was done. The absorption was measured by UV-Visible spectrophotometry at wavelength of 494 nm and linear regression equation was determined.

Determination of sample content using UV-Vis spectrophotometry method

Papaya fruit was peeled and the seed was removed and it was cut into small pieces and then it was crushed in blender until resemble

slurry (juice). Samples that have been blended was weighed as much as 10 gram and was put into a100 mL beaker glass. Then, it was added with distilled water and filtered by using filter cloth to separate residue and filtrate. The filtrate was added to 100 mL volumetric flask and was added with distilled water to the mark mark. The filtrate obtained was ready to be used as samples in this research. Then, it was pipetted 2,5 mL of filtrate and was put into 25 mL volumetric flask. The solution was added with 4 mL of 5% H_2SO_4 and the volume was sufficient with 5% ammonium molybdate to the limit and then incubated for 30 minutes and measured using spectrophotometry at wavelength of 494 nm (9).

RESULTS

The results of determination of vitamin C levels in *Carica papaya* L. samples using titrimetric method can be seen in Table 1.

Table 1. Result of Determination of Vitamin C Levels Using Titrimetric Method

Sample	Volume (mL)	Volume average (mL)	Vitamin C levels (%)	SD	%RSD
n_1	0.6	0.6	0.0147	0	0
n_2	0.6				
n_3	0.6				

Description: n_1 = 1st test, n_2 = 2nd test, n_3 = 3rd test

The calibration curve of vitamin C standard solutions of various concentrations in determination of vitamin C levels in *Carica papaya* L. samples using UV-Vis Spectrophotometry Method can be shown in Figure 1.

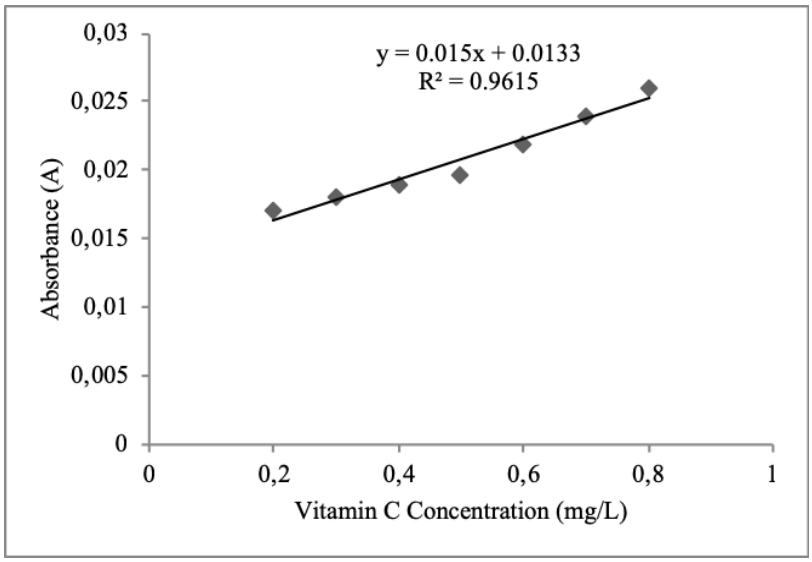

Fig 1. The Calibration Curve of Vitamin C Standard Solutions of Various Concentrations

Table 2 show the result of determination of ascorbic acid level in *Carica papaya* L. samples using the UV-Vis spectrophotometry method.

Table 2. Results of Ascorbic Acid Using the UV-Vis Spectrophotometry Method

Sample	Absorbance (A)	Vitamin C Levels in 10 g of Sampel (%)	Average (%)	SD	%RSD
n_1	0.062	0.130	0.1313	0.001	0.0088
n_2	0.062	0.132			
n_3	0.062	0.132			

Description: n_1 = 1st test, n_2 = 2nd test, n_3 = 3rd test

DISCUSSION

In previous research, determination of vitamin C levels was done by using iodimetry method with amilum as an indicator and iodine as standard Solutions (6). The results can be seen in Table 1. The principle of this measurement is vitamin C (ascorbic acid) as a strong reducing agent and can simply be titrated with iodine standard solution. In the results of the study using the iodimetry method, iodine standardization was replicated three times. When standardization process of iodine, there was an error during the making of the solution so that the remodeling of the iodine solution was made with the results obtained as desired by 0.0071 N with a change in yellow and will turn to blue color immediately so that it can be used for titration.

In the study, vitamin C that analyzed using the iodimetry method produced vitamin C levels of 0.0147 % with addition of sulfuric acid as an acidic giver. This titration treatment must be done immediately because many factors will cause oxidation of vitamin C, for example oxidation by air, especially when exposed to heat. The longer the fruit was stored in the open place (outdoor), the higher the chace of deacreasing in the vitamin C levels. Vitamin C is easily reacts with O_2 in the air to become dehydroascorbic acid (10) so that the vitamin C level will be lower.

Furthermore, the analysis of vitamin C on papaya was done by using UV-Vis spectrophotometry. This method was used to test the amount of light absorbed at wavelength in the ultraviolet region so that the light of ray will be splited directly through transparent cells containing solvents, then the light will pass through compounds in the analyte to be absorbed and recorded as absorbance.

Analysis of vitamin C levels using UV-Vis spectrophotometry method begins by determining the maximum wavelength and was obtained in the wavelength of 494 nm. When the sample preparation was done, oxalic acid was added to keep vitamin C in the sample to remain stable and as an inhibitor of the oxidation process. Addition

of ammonium molybdate reagents to form color so that the shifting wavelengths that occur will be easily observed at visible wavelengths. Based on data (Figure 1) from the measurement using UV-Vis spectrophotometry method of a standard solution of vitamin C, it can be made the curve of relationship between absorbance and concentration vitamin C with linearity equation of y = 0.015x + 0.0133.

Based on Table 2, it can be seen that the results of the analysis of vitamin C content in California papaya fruit using UV-Vis spectrophotometry method was 0.1313% in 10 grams of fruit. Incubation samples were analyzed for optimum reaction between vitamin C and ammonium molybdate to form a stable blue complex. Based on this research, there are differences between the results of the determination of Vitamin C using the iodimetry method and the UV-Vis spectrophotometry method. The vitamin C content in papaya which measured using the iodimetry method was 0.0147% while the vitamin C content in papaya which measured using the UV-Vis spectrophotometry method was 0.1313%. The result of UV-Vis spectrophotometry method was higher than the result of UV-Vis spectrophotometry method. Based on the result, there are significant differences between the vitamin C level contained in papaya using the iodimetry method and UV-Vis spectrophotometry method. The result was in line with the result of identification of vitamin C content in kiwifruit (*Actinidia deliciousa*) by using iodimetry and UV-Vis spectrophotometry method (11). It was because the UV-Vis spectrophotometry method provided the simple way to measured very small quantity of substances and the number which read directly was recorded by the detector (6).

CONCLUSIONS

Based on the results obtained, it can be concluded that the vitamin C levels using the spectrophotometric method was 0.1313% in 10 gram samples which is nine times greater than the results of the determination of vitamin C using the iodimetry method (0.0147%).

CONFLICT OF INTEREST

There are no conflicts of interest.

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