Jurnal Pena Sains Vol. 10, No. 1, April, 2023
p-ISSN: 2407-2311
e-ISSN: 2527-7634

DETERMINATION OF ASCORBIC ACID CONCENTRATION IN MYRTACEAE USING THE IODOMETRIC TITRATION METHOD

Nur Syafitri[1], Muhammad Abdurrahman Munir[2], Veriani Aprilia[3], Emelda Emelda[4]

[1,2,3]Department of Pharmacy, Faculty of Health Sciences, Universitas Alma Alta
Bantul, 55183, Yogyakarta, Indonesia
muhammad@almaata.ac.id

[4]Department of Nutrition, Faculty of Health Sciences, Universitas Alma Alta
Bantul, 55183, Yogyakarta, Indonesia

Accepted: April 6, 2023 Published: April 29, 2023

DOI: 10.21107/jps.v10i1.19161

ABSTRACT

Vitamins are essential compounds in food even though the content contained in these foods is small, and this is because vitamins have a vital role in the human body. Vitamin C or ascorbic acid is a secondary antioxidant compound that captures free radical compounds. Vitamin C content is easily found in vegetables and fruits. One of the fruits that contain vitamin C is guava. This study aims to determine and analyze vitamin C concentrations in several guava fruit types with the iodometric titration method. This type of research is a quantitative-qualitative experimental laboratory. The samples used in this study were guava in Myrtaceae family consisting of red guava (Psidium guajava L.), crystal guava (Psidium guajava L.), and red water guava (Syzygium aqueum). The study began with making 0.05 N iodine, iodine formation, then the 3% amylum indicators. The average sample content of red guava, crystal guava, and red water guava was 2.42 mg/100 grams. To conclude, the ascorbic acid concentration of red guava water is higher at 157 mg/100 g compared to the other two guavas, which are 80 mg/100 g and 87 mg/100 g.

Keywords: ***ascorbic acid, determination, guava, titration, iodometry***

[2] Corresponding Author

Determination of Ascorbic Acid Concentration in Myrtaceae Using The Iodometric Titration Method

Introduction

Indonesia is one of the countries in the equatorial region with a tropical climate. This region is undoubtedly exquisite for plants to thrive, and one of them is fruit plants. Fruit plants have many excellent contents, such as macro-nutrients and micro-nutrients, such as minerals and vitamins. A common vitamin found in fruit plants is vitamin C (Putri & Setiawati, 2015).

Ascorbic acid, better known as vitamin C, is a chemical compound that is vital to human growth, but the body cannot store this vitamin. Vitamin C needs can be obtained from daily food and drink and in several packages such as tablets, capsules, and liquids. Based on the Indonesian Pharmacopoeia, vitamin C has several characteristics, such as white crystals that dissolve in water. In the dry state, vitamin C is relatively stable; however, this compound is very easily oxidized when contaminated with light (Dewi, Sumantri, 2017; Septiani, 2017; Nerdy, 2018).

Guava is one of the fruits rich in vitamin C (Rachmaniar et al., 2016). Generally, people in Indonesia consume a lot of guava fruit because this is one way to meet the intake of vitamin C in the body, but it needs to be considered again how to store and extend the storage of fruits containing vitamin C, this is because vitamin C is very easily oxidized and can be accelerated by high temperatures, prolonged exposure to light and the presence of enzymes (Putri et al., 2018).

Guava also contains vitamin C up to 80 mg/100 g of substance. Vitamin C has properties as an antioxidant. Vitamin C contained in guava also has properties for the treatment of ascorbate. Vitamin C has been proven by many studies that have antioxidant activity in lowering total cholesterol concentrations (Dewi, Sumantri, 2017; Way and Favor, 2017; Aprilliani & Hajrah 2021).

Antioxidants have the potential to increase body immunity and protect the body from various conditions that are closely related to free radicals. According to the results of existing research, antioxidants can have the ability to lower cholesterol concentrations in the blood. It has been proven that this compound has helped in the reduction of cholesterol concentrations in the blood. Consuming fruits such as red guava and crystal guava is the most effective way for humans to get vitamin C (Tt et al., 2020; Aprilliani & Hajrah, 2021; Guntarti et al., 2021; Guntarti and Hutami, 2019).

There are several simple techniques for determining vitamin C concentrations in a food and beverage product, such as titration and spectrophotometry, but the simplest and most commonly used method is the iodometric titration method. This method is the most frequently applied because of several factors, such as affordable analysis costs, ease of spread, and the minimal need for sophisticated laboratory equipment. The basic principle of vitamin C analysis with the iodometric titration method is the reduction-oxidation (redox) reaction. Vitamin C acts as a reducing agent (reducing agent) and iodine as an oxidizing agent (oxidizer). Vitamin C in food and beverages will be easily oxidized with an iodine solution, and Amylum will be used as an indicator. Amylum is an indicator that shows the endpoint of the titration, which is characterized by changing color from colorless to fixed blue (Ika, 2009; Ulfa, 2015; Fitriana and Fitri, 2020).

In several previous studies, this method has been used to analyze vitamin C in red guava, but the compound used is 2,6-dichlorophenol. However, the weakness of this compound is that the price is prohibitive, so other compounds are needed in running iodometric methods (Ulfa 2015). Therefore, in this study, vitamin C will be determined by the iodometric method using iodine 0.05 N as an oxidizing agent and 3% amylum as an indicator.

This study aims to determine and analyze the ascorbic acid concentration in Myrtaceae with the iodometric titration method.

Research Methods

Tools and Materials

Tools used in this study include blenders, analytical balances, test tubes, tube racks, Erlenmeyer, glass crackers (Merck), burettes, statics, clamps, measuring flasks (Merck), dark bottles, measuring cups (Merck), water baths, rotary evaporators, measuring pipettes (Merck), pro-pipettes, drip pipettes, watch glass, porcelain dishes, and horn spoons.

The materials used were samples of red guava (*Psidium guajava* L.), crystal guava (*Psidium guajava* L. Merr), red water guava fruit (*Syzygium aquarium*), amylum 3%, iodine solution (I_2) 0.05 N, H_2SO_4, AS_2O_3, NaOH, $NaHCO_3$, HCl 7.3%, methyl orange, aquades and ethanol 70%. The iodometric titration method is the method to be applied in this study.

Procedure

Samples were identified at the Biology Laboratory, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan Yogyakarta, to ensure the plants used were from Myrtaceae. There are three types of guava fruit samples, namely red guava, crystal guava, and red water guava, obtained from the Gamping Market in Yogyakarta, Indonesia.

Before processing the determination of ascorbic acid in Myrtaceae. Identification is conducted first to identify the samples and avoid mistakes during research. Determination of guava plants (*Psidium* sp.) conducted at the Biology Laboratory of the Faculty of Health Sciences, Pharmacy Study Program, Alma Ata University Yogyakarta with results obtained by the book *Flora of Java* that the plant identified as a guava plant (*Psidium* sp.). In the determination that has been done, there are numbers and letters in the form of determination codes that indicate that all the characteristics and anatomical forms of the plant are guava fruit so that it can be ascertained that the plant used in the study is really guava fruit with the scientific name *Mytaceae family L., Mytaceae family L. Meer, Syzyqium aquenum.*

Samples were obtained in the market, cleaned with water, and then cut into small sizes using a knife. Then, the sample is weighed 50 g using an analytical balance. The three types of samples that have been selected are mashed using a blender to become porridge. The maceration process is carried out using 70% ethanol as much as 100 mL in a glass beaker container, and the sample is stirred using a stirring rod for two hours. The bath is left for 48 hours, protected from light to avoid oxidation by wrapping it with aluminum foil. The maceration results are filtered with filter paper to separate the pulp and filtrate, then the pulp obtained will be processed to obtain fiber. The fiber that has been received concentrated with a rotary evaporator at a temperature of 60^{o}C so that a thick extract is formed, then the chemical rendition results are calculated with the following formula:

$$Chemical\ rendition = \frac{weight\ of\ extract}{\text{weight of samples}} \times 100\%$$

Preparation of iodine solution (0.05 N)

8.3 g KI is weighed, then dissolved with aquades little by little until dissolved, add 6.34 g of iodine and 10 mL H_2SO_4 and dissolve with aquades then transfer the solution into a 1000 ml measuring flask, dilute with aquades to the limit mark, shake until homogeneous, the solution is ready to be used as a pin.

Process of making 3% amylum

3 grams of Amylum is weighed, put it in a 100 mL measuring flask, then dissolved with aquades until the limit mark and beat until homogeneous.

Standardization of iodine solution (0.05 N)

150 mg of AS_2O_3 is weighed and dissolve into 20 mL NaOH 2N, dilute using aquades as much as 40 mL and add 2 drops of methyl orange. Then add 7.3% HCl, as much as 16 ml, until there is a color change into pink, then add 2 g of $NaHCO_3$, diluted with 50 mL aquades, add amylum indicators then titrate with iodine solution until a fixed blue color change. One mL of 0.1 N iodine is equivalent to 4.946 mg As_2O_3.

Iodine formation calculation formula:

$$\text{N } I_2 = \frac{\text{mg } As_2O_3 \times \text{Valensi}}{\text{mL iodine} \times \text{BM } As_2O_3}$$

Processing of vitamin C solution in 100 ppm

Vitamin C powder is weighed as much as 10 mg, the powder is put into a 100 ml measuring flask, vitamin C powder is dissolved with aquades to the limit mark, then shaken until homogeneous so that a 100 ppm vitamin C solution is formed.

Preparation of vitamin C concentration series solution

Vitamin C solution is diluted into a 10 mL measuring flask 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. Furthermore, aquades is added in each measuring flask, and beat until homogeneous.

Establishment of vitamin C concentration series calibration curves

Put 10 mL of vitamin C series solution 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 into Erlenmeyer then add 3% amylum indicator as much as 3 drops. Titration with 0.05 N iodine solution, record the amount of iodine volume needed during titration until a color change occurs, then calculate vitamin C concentrations using a linear regression formula (y = bx + a).

Determination of vitamin C concentrations in guava

Each sample is weighed into 1 g, added aquades, stirred until dissolved, then put in a 1000

mL measuring flask and suffice until the limit mark. Each piece was taken 10 ml, put into Erlenmeyer, then 3% amylum indicator added as much as 3 drops. After that, it was titrated with a 0.05 N iodine solution until a color change to blue remained. Record the 0.05N iodine titration endpoint (TAT) volume.

Data Processing and Analysis

Data processing and analysis is conducted using the formula for calculating vitamin C concentrations and Microsoft Excel using solution concentration data that will be compared with the value of vitamin C in iodometric titration treatment with a linear regression equation (y = bx + a).

Result and Discussion

Chemical Rendition

Guava ethanol extract (Myrtaceae) is processed using 70% ethanol solvent and results a chemical rendition of red guava as much as 6.334% (b/b) in pink, crystal guava as much as 10.254% (b/b) in light green, and red water guava as much as 8.773% (b/b) in dark red.

Determination of Vitamin C Concentration in Guava with Iodometric Method

The content results of processing vitamin C concentration in Guava are calculated using the formula (Y = Bx + A), where Y is the end point of titration. A is the concentration to be measured. The results of vitamin C concentrations in guava samples (Myrtaceae) and the color changes that occur can be seen in Figure 1.

(a) (b) (c)

Figure 1. (a) Color change in red guava sample, (b) Color change in crystal guava sample, (c) Color change in red water guava.

Guava fruit is soaked using 70% ethanol solvent using the maceration method. The maceration method aims to separate the chemical compounds in guava fruit using 70% ethanol solvent to prevent the decomposition of vitamin C compounds due to heating (Emelda, 2017). The solvent used in the maceration method is 70% ethanol. Ethanol is one of the polar solvents (Kemit, Widarta, and Nocianitri, 2016); 70% ethanol still contains a lot of water (30%) which helps the extraction process so that some of these compounds are easily attracted to 70% ethanol (Supriningrum, Fatimah, and Purwanti, 2019). The reason for choosing 70% ethanol solvent is because ethanol can attract polar active compounds. Ethanol has a low boiling point of 79oC, requiring less temperature for the extract concentration process using a rotary evaporator. This temperature is also safe to use during the process of vitamin C compounds in the rotary evaporator, and this is because vitamin C will be degraded at temperatures above 100ºC (Nur Hasanah, 2020).

Furthermore, filtering using filter paper can produce filtrate and guava sample residues. The filtrate from the filtrate is concentrated using a rotary evaporator for 2 hours with a temperature of 60oC and a speed of 90 rpm to separate the solvent until a thick extract is formed. The yield results of a sample are used to determine the amount of extract obtained. The vicious section is semi-solid pink, light green, and dark red. In this study, vitamin C was analyzed using the iodometric titration method. Vitamin C reacts with iodine to produce dehydroascorbic acid because iodine can act as an oxidizing agent using amylum indicators. The reaction that occurs can be seen in Figure 2.

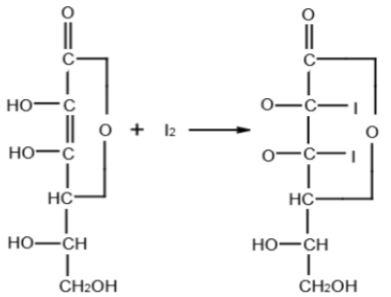

Figure 2. The reaction that occurs between vitamin C in red guava, crystal guava, and red water guava with iodine produces dehydroascorbic acid (Fitriana and Fitri, 2020)

In this study, iodine compounds were used for iodometric methods. The addition of KI compounds in this study aims to reduce the toxicity of iodine, and in general, KI is added with a concentration of 3-4% in a solution of 0.1 N and put into a dark-colored container with the aim of avoiding the decomposition of HIO by sunlight (Underwood, 1993). In the iodometric method or also known as indirect titration, many oxidizing

solid compounds can be analyzed by adding excessive KI and titrating the released iodine. This happens because many oxidizing compounds can trigger an acid solution, in which case ascorbic acid reacts with an iodide solution, then sodium thiosulfate is commonly used as a titrant. Based on some studies, several measures can be taken to avoid the formation of gallic compounds, that is, iodide ions oxidized by oxygen in the air. However, the reaction is prolonged in neutral solutions but very fast in acidic solutions and can be faster in the presence of sunlight. However, after adding KI to an acidic solution, this solution has not been in contact with air for a long time; this can trigger additional iodine, eventually interfering with the end point of titration (Roth, 1988).

Some studies prove that when iodometric titration is performed, the titration must be in a weakly acidic state or close to neutral; this is so that in the alkaline state it will form iodine compounds formed from hypoiodite ions which is the initial reaction between iodine and hydroxide ions. So acidic conditions that are too concentrated when titration is carried out will result in free thiosulfuric acid forming a precipitate (Underwood, 1993).

Basically, the use of starch as an indicator can be avoided because iodine solutions can provide easily observable colors, but some studies show that to get more accurate results, the use of starch is essential; this is because starch with iodine compounds can form complex mixtures that are stable blue and very easy to observe even at deficient concentrations (Harjadi, 1993). According to Sudjaji (2007), this titration process must be done slowly so that iodine can react with vitamin C perfectly; this is because if the titration process is carried out irregularly and very quickly, the final result becomes inaccurate.

Vitamin C Concentration Series Calibration Curve

Vitamin C series solutions with concentrations of 10 ppm, 12 ppm, 14 ppm, 16 ppm, 18 ppm, 20 ppm, 22 ppm, 24 ppm, 26 ppm, and 28 ppm, the results of vitamin C concentrations using the formula for calculating concentrations can be seen in Appendix 6. The standard curve of the vitamin C concentration series can be seen in Figure 3.

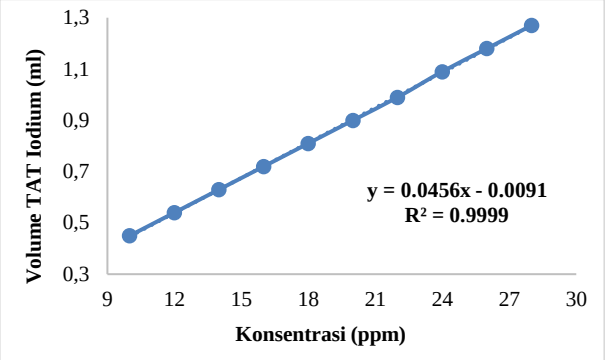

Figure 3. Vitamin C concentration series solution calibration curve

From the data above, it can be concluded that the R^2 value obtained is 0.9999, with a limit detection value is 0.22 ppm and a limit quantification value is 0.68 ppm. This R^2 value indicates that the higher the concentration of vitamin C, the higher the TAT volume.

In this study, the iodometric method was carried out to determine the series of vitamin C concentrations, where vitamin C powder will be dissolved in water free of carbon dioxide. CO_2-free water is used because CO_2 compounds can oxidize vitamin C, so the titration endpoint must be higher and give accurate results. In this study, sulfuric acid was also provided, acting as a catalyst so redox reactions can dash and then titrated with iodine solution. Therefore, to obtain the right titration endpoint, kanji is used in this method to produce a steady blue color. The reaction mechanism that occurs in this study where vitamin C that reacts with I_2 will form excess HI, then iodine that reacts with starch indicators will produce iodine complex bonds with starch causing the starch to release (the blue color is lost) efficiently. However, when vitamin C runs out, iodine will react directly with starch and will produce a fixed blue color.

Vitamin C Concentrations in Guava

The determination of vitamin C concentrations in guava is determined using a calibration curve (y = bx + a) on the vitamin C standard. The results of vitamin C concentrations in guava samples (Myrtaceae) can be seen in Table 1. This data shows that the highest concentrations of vitamin C are in red water guava, followed by crystal guava and red guava. It can also be concluded that the iodometric titration method using iodine as an oxidizing agent can analyze guava samples used in this study.

Table 1. Vitamin C concentrations in guava fruit

No	Sample	Iodine TAT Volume	Vit concentrations. C (ppm)
1	Red guava	0.95±0.01	21.03
2	Crystal guava	1.05±0.02	23.22
3	Red water guava	2.3±0.2	50.63

The basic principle of this method is that iodine compounds combine the double bonds contained in vitamin C on carbon atoms C numbers 2 and 3; the double bonds held by iodine will be broken into single bonds. If all vitamin C has been contaminated by iodine, the next iodine droplet will quickly react with Amylum to form blue iodine-amylum compounds. The formation of this blue color indicates that the titration process has been successfully carried out. As a result, vitamin C compounds have been treated with iodine so that the volume needed during titration is equivalent to the amount of vitamin C. This titration treatment must be done quickly because many factors cause the oxidation of vitamin C, for example, during sample preparation. This is because vitamin C quickly reacts with$_{O2}$ in the air to become dehydroascorbic acid (Nurdin Rahman, 2015; Mulyani, 2018).

Conclusion

This study aims to determine and analyze the ascorbic acid concentration in Myrtaceae with the iodometric titration method. Vitamin C or ascobic acid concentrations in red guava (*Psidium guajava* L.) were obtained by 21.03 mg/100 g. Vitamin C concentrations contained in crystal guava (*Psidium guajava* L.) were obtained at 23.22 mg/100 g. Vitamin C concentrations in red guava (*Syzygium aquenum*) were obtained at 50.63 mg/100 g. From the three data on the results of vitamin C concentrations, it can be seen that the comparison is higher at 50 mg/100 g, compared to red guava 21.03 mg/100 g and crystal guava 23.22 mg/100 g. Where by the theory is that the content of red guava water concentrations is higher at 157 mg/100 g compared to the other two guavas, which are 80 mg/100 g and 87 mg/100 g.

Acknowledgemnt

Great thanks is sent to Alma Ata University for providing facilities and infrastructure in order that this study can be conducted well.

References

Emelda. Potensi Tongkat ALI (Eurycoma longifolia Jack.) Sebagai Anti Inflamasi. JCPS (Journal Curr Pharm Sci [Internet]. 2017;1(Vol 1 No 1 (2017)):25–9.

Fitriana YAN, Fitri AS. Analisis Kadar Vitamin C pada Buah Jeruk Menggunakan Metode Titrasi Iodometri. Sainteks. 2020;17(1):27.

Guntarti A, Ahda M, Nabilla H, Susanti H. The Storage Effect Against Vitamin C Content in Crystal Guava (Psidium guajava L .) Juice. J Sci Islam Repub Iran. 2021;32(1):39–42.

Guntarti A, Hutami EN. Validation and Vitamin C Testing in Crystal Guava (Psidium guajava L .) With Variations of Origin With the HPLC Method (High Performance Liquid Chromatography). Int J Chem. 2019;11(1):52–9.

Harjadi, W. Ilmu Kimia Analitik Dasar. 1993. *Erlangga*. Jakarta.

Ika D. Alat Otomatisasi Pengukur Kadar Vitamin C Dengan Metode Titrasi Asam Basa. J Neutrino. 2009;1(2):163–78.

Kemit N, Widarta IWR, Nocianitri KA. Pengaruh Jenis Pelarut dan Waktu Maserasi Terhadap Kandungan Senyawa Flavonoid dan Aktivitas Antioksidan Ekstrak Daun Alpukat (Persea Americana Mill). J Ilmu Teknol Pangan. 2016;5(2):130–41.

Mulyani E. Perbandingan hasil penetapan kadar vitamin C pada buah kiwi (Actinidia deliciousa) dengan menggunakan metode iodimetri dan spektrofotometri Uv-Vis. Pharmauho. 2018;3(2):14-17.

Nadiyah Amalia Aprilliani, Hajrah HK. Pengaruh Pemberian Jus Daging Buah Jambu Biji (Psidium guajava) Terhadap Penurunan Kolesterol Pada Pasien Hiperkolesterolemia. Proceeding Mulawarman Pharm Conf. 2021;(April 2021):139–45.

Nerdy N. Determination of Vitamin C in Various Colours of Bell Pepper (Capsicum annuum L .) by Titration Method Determination of Vitamin C in Various Colours of Bell Pepper (Capsicum annuum L .) by Titration Method Nerdy Nerdy. Alchemy J Penelit Kim. 2018;14(February):164–77.

Nur Hasanah DRN. Analisis Ekstrak Etanol Buah Labu Kuning (Cucurbita Moschata D.). Jurnall Poltektegall. 2020;9(1):54–9.

Nurdin Rahman MO dan IS. Analysis of Vitamin C concentrations on Gadung Mango (Mangifera sp) and Golek Mango (Mangifera indica L) Based on the Maturity Concentrations Using Iodimetry Method. J Akad Kim. 2015;4(February):33–7.

Putri MH, Septiyani P, Aryani, W. & Abriyani E. Literatur Review: Penetapan kadar vitamin C pada buah jambu biji, jeruk, dan nanas menggunakan metode spektrofotometri Uv-Vis. Jurnal Ilmiah Wahana Pendidikan. 2023; 9(4):333-342.

Putri MP dan Setiawati YH. Analisis kadar vitamin C pada buah nanas segar (Ananas comosus L.) dan buah nanas kaleng dengan metode spektrofotometri UV-Vis. Jurnal Wiyata: Penelitian Sains dan Kesehatan. 2017; 2(1).

Rachmaniar R, Haruman Kartamihardja M. Pemanfaatan sari buah jambu biji merah (psidium guajava linn.) sebagai antioksidan dalam bentuk granul effervescent. Indonesian Journal of Pharmaceutical Science and Tehcnology. 2016;5(1).

Roth, J. & Blaschke, G. Analisis Farmasi. 1988. *UGM Press*. Yogyakarta.

Septiani W. Determination Of Vitamin C Red Guava (Psidium Guajava Linn) Fruit Juice, With Variation Of Beverage Packaging. Int J Adv Sci Eng Technol. 2017;5(2):51–5.

Supriningrum R, Fatimah N, Purwanti E. Karakterisasi Spesifik dan Non Spesifik Ekstrak Etanol Daun Putat (Planchonia Valida). J Al Ulum Sains Teknol. 2019;5(1):6–12.

Tt B, Ar S, Rb K, Ss B. Studies On Physical, Chemical And Mineral Evaluation Of Guava (Psidium guajava L .). Pharma Innov J. 2020;9(3):117–9.

Ulfa AM. Penetapan Kadar Klorin pada Beras Menggunakan Metode Iodometri. J Kesehat Holistik. 2015;9(4):197–200.

Underwood, A.L. & Day, R.A. Analisa Kimia Kuantitatif. 1993. *Erlangga*. Jakarta.

Way M, Favor L. Determination Of Vitamin C In Several Varieties Of Melon Fruits By Titration Method. J Nat. 2017;17(2):118–21.

Yeni Utami Dewi, Sumantri PIU. Pengaruh Lama Penyimpanan Terhadap Penurunan Kandungan Vitamin C pada Jambu Biji (Psidium guajava L.). Pharmacy. 2017;05.