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Research Article

Determination of Vitamin C in *Psidium guajava* Linn Fruit Juice, with Variation of Beverage Packaging

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Abstract

The quantitative analysis of vitamin C content from variations of beverage packaging containing red guava (*Psidium guajava* Linn) fruit juice had been done. In this study, 4 samples were obtained from the shopping center in Garut and Bandung City. Samples were tested quantitatively by 2,6-dichlorophenol indophenol titration method. These results showed different concentration of 4 samples consist of tetrapack packaging, beverage cans, glass bottles, and plastic bottles, such as; 17.99 mg/100 gr, 31.46 mg/100 gr, 13.00 mg/100 gr, and 12.01 mg/100 gr, respectively. These results indicate that packaging variations have an effect on maintaining nutritional value, marked by a decrease, so that the vitamin C content contained in the packaging does not match the results obtained due to a decrease in levels due to the storage process.

Keywords: Red guava, Titration 2,6-dichlorophenol indophenol, Variations of beverage packaging, Vitamin C

Penentuan Vitamin C Jus Buah *Psidium guajava* Linn, Pada Beberapa Kemasan Minuman

Abstrak

Telah dilakukan analisis kuantitatif kandungan vitamin C dari variasi kemasan minuman yang mengandung sari buah jambu biji merah (*Psidium guajava* Linn). Dalam penelitian ini didapatkan 4 sampel dari pusat perbelanjaan di Garut dan Kota Bandung. Sampel diuji secara kuantitatif dengan metode titrasi 2,6-diklorofenol indofenol. Hasil penelitian menunjukkan perbedaan kandungan Vitamin C dari 4 sampel yang telah diteliti, berikut ini hasil yang didapat kemasan tetra pack 17.99 mg/100 gr, kemasan kaleng 31.46 mg/100 gr, kemasan gelas 13.00 mg/100 gr, dan kemasan botol plastik 12.01 mg/100 gr. Hasil ini menunjukkan bahwa variasi kemasan berpengaruh dalam mempertahankan nilai gizi, ditandai dengan penurunan, sehingga kandungan vitamin C yang terdapat pada kemasan tidak sesuai dengan hasil yang didapat disebabkan adanya penurunan kadar akibat proses penyimpanan.

Kata Kunci: Jambu biji merah, Titrasi 2,6- diklorofenol indophenol, Variasi kemasan minuman, Vitamin C

1. Introduction

Vitamin C is one of the nutrients and having function as antioxidants, effectively to protect free radicals that can damage cells. Low intake of fiber can be affect the intake of vitamin, because fiber vegetables, and fruits are also a good source of vitamin C.[1]

One of the food products that are processed in the form of packaging, which is currently circulating in the market is fruit-flavored soft drinks in containers . Fruit drinks packaging is very easy to find in department stores (supermarket).

Generally humans needs vitamin C from outside the body. In fact, people prefer to drink fruit beverage packaging compared to the vitamin C in fresh fruits, which are easily found anytime and their use is relatively more practical.[2]

Vitamain C In a dry state is quite stable, but in a soluble state, vitamin C is easily damaged due to contact with air (oxidation), especially when exposed to heat. Oxidation is accelerated in the presence of copper and iron. Vitamin C is unstable in alkaline solutions, but quite stable in acidic solutions. This research can give the information to public and consumer, about they daily intake of vitamin C and the most stable vitamin C in several variations of guava fruit beverage packaging.[3]

Based on the above researchers wanted to determine levels of vitamin C in some beverage packaging red guava fruit (*Psidium guajava* L) determined by titration methods 2,6-diklorofenol indofenol, which one of the most stable beverage packaging levels of vitamin C in some variations of the packaging used. Beverage sales generally use 4 types of packaging tetrapack, glass, cans, and plastic bottles.[4] The benefits of doing this study to determine the levels of vitamin C and source of information on the levels of vitamin C in some guava fruit beverage packaging.[5]

2. Method

2.1. Tools

The tools used for titration method validation and analysis of 2,6-DFIF vitamin C are an analytical balance, 100 mL flask, desiccator, brown glass bottle, 100 mL flask, stir bar, funnel, blender, filter paper, measuring flask of 50 mL, 250 mL flask, 250 mL glass beaker, micro pipette, pipette, and tissue.

2.2. Materials

Materials used are guava fruits, guava fruit beverage packaging with a variety of containers with an expired date in particular, 2,6-Dichlorophenol Indophenol (2,6 DFIF) (Merck), vitamin C (Merck), glacial acetic acid (Merck), distilled water (Brataco), metaphosphate acetic acid (Merck), concentrated sodium bicarbonate (APS Ajax Finecham).

2.3. Methods

Vitamin C was measured using 2,6-DFIF titration method. The samples studied are some beverage packaging guava (*Psidium guajava* L.) with different packaging are: tetrapack, glass bottles, beverage cans, and plastic bottles.[6]

In this study, the first step to validate methods of analysis that 2,6-DFIF titration method are determines the accuracy, precision and detection limits. The second stage examination vitamin C content in guava fruit beverage packaging using a method that has been validated. Preparing a sample by measuring the sample volume packaging fruit drinks.

2.3.1. Accuracy Test

Accuracy test using the % recovery parameter. The recovery test was carried out by adding standard vitamin C, namely 2.5 mg by adding 25 mg of standard vitamin C to a 100 mL volumetric flask and adding metaphosphoric acetic acid up to the mark line (concentration 0.25 mg/100 mL), then pipetted as much as 10 mL which was added to the carefully weighed sample and carried out six repetitions.

2.3.2. Precision Test

Precision test is a measure that indicates the degree of agreement between individual test results that are applied repeatedly to the sample.

$$\%RSD = \frac{SD}{X} \times 100\%$$

2.3.3. Limit of Detection

The method of determination in analyzes that do not use instruments. The limit is determined by detecting the analyte in the sample at graded dilutions.

From the above experimental results can be calculated amount of vitamin C in every 100 grams of beverage packaging Guava fruits studied.[7]

According to AOAC 2002, the levels of vitamin C can be calculated using the formula:[8]

$$\text{Levels of Vit C}\left(\frac{mg}{g}\right) = \frac{(Vt\text{-}Vb) \times \text{equality} \times Vi}{Vp \times Bs}$$

Description:

Vt = volume titration (mL)
Vb = volume of the blank (mL)
Vi = Volume of flask (mL)
Vp = volume pipetting (mL)
Bs = weight of sample (g)

2.3.4. Sample Collection and Determination

Samples used is red guava fruit (*Psidium guajava* L.) obtained from the area Tarogong Garut district. While the sample studied for quantitative analysis of vitamin C are a couple of drinks packaging guava (*Psidium guajava* L.) containing vitamin C to be studied with different packaging are tetrapack, glass bottles, beverage cans, and plastic.[9]

2.3.5. Preparation of Samples

Samples are cleaned, weighed about 100 grams and then cut into small pieces put into a blender and then added about 20 mL of metaphosphate-acetic put in a blender, then blended, then weighed 10 g then added to 100 mL volumetric flask and add sour metaphosphate-acetate until the line mark. Homogenized, and then filtered.

2.3.6. Preparation of Reagent solution:

A solution of 2,6-diklorophenol indophenol (DFIF)

Dissolve 50 mg of sodium 2,6-DFIF that has been stored in a desiccator, then added 50 mL of water containing 40 mg of Na bicarbonate concentrated when it is dissolved to 200 mL of water added. Then filtered into a brown glass bottle. 2,6-DFIF standard solution used within 3 days and standardized before use. Procedure for standardized, weighed 50 mg of ascorbic acid, put into a 100 mL flask, then dissolved with metaphosphoric-acetic acid solution, added up to the mark line. Pipette 1 mL, put into an Erlenmeyer and add 6 mL of metaphosphoric-acetic acid solution. Titrate immediately with 2,6-DFIF solution until a steady pink color is not less than 5 seconds. Perform a blank titration using 7 mL of metaphosphoric-acetic acid and titrate with 2,6-DFIF solution until a strong pink color.[10]

2.3.7. Quantitative Test on Samples

A total of 4 samples were taken each 5 mL in 100 mL Erlenmeyer each added 2 mL of strong acid and shaken metaphosphate then a solution of 2,6-DFIF titration and titration was stopped until the pink solution.

3. Result

Vitamin C does not provide energy to the body but it is essential and necessary nutrients for the growth of the body and also associated with the enzyme function in the body.[11] In this study, the determination of vitamin C levels in samples of beverage packaging such as plastic bottles, tetrapack, beverage cans and glass

Table 1. Results of Accuracy Test for Determination of Vitamin C Levels in Fruit Juice

No.	Sample Weight (g)	Volume 2,6 DFIF (mL)	Level (mg/100 g)	Avarage Level (mg/100 g)
1.	10.0900	2.3	73.40	75.040
2.	10.1200	2.3	73.25	
3.	10.3400	2.5	78.52	
4.	10.3600	2.5	78.36	
5.	10.0561	2.3	73.71	
6.	10.1542	2.3	73.00	

Table 2. Determination of Vitamin C Levels in Fruit Juice + 25 mg Vitamin C

No.	Vitamin C added (mg)	Sample Weight (g)	Volume 2,6 DFIF (mL)	Level (mg/100 g)	Avarage Level (mg/100 g)
1.	2.5	10.0900	3.0	97.95	97.585
2.	2.5	10.1200	3.0	97.66	
3.	2.5	10.3400	3.1	98.99	
4.	2.5	10.3600	3.1	98.81	
5.	2.5	10.0561	2.9	94.77	
6.	2.5	10.1542	3.0	97.33	

bottles. This was done to determine the most stable vitamin C levels between the packaging variations. In this study, the first phase of the analytical method validation 2,6-DFIF titration method to determine in advance the accuracy, precision and limit of detection.[12] Determination of vitamin C content of guava juice was done to validate 2,6-DFIF titration method. The accuracy of test results was done by adding a standard solution of vitamin C.[13] The results can be showed at table 1 and 2.

A good analytical method has an accuracy range of average recovery test for analyte concentration of 0.001%-0.01% in the samples tested was 90%-107%. Percent recovery obtained was 91.96%. These results indicate a closeness between the results of the analysis with the real values of 2,6-DFIF titration method used in this study fit for use and provide valid.[14] The results can be showed at table 3.

Precision test done to prove the accuracy of the practitioner or a work based on the level of accuracy of the results of the analysis indicated standard deviation (SD) and the relative standard deviation.[15] The percent value of RSD in this study was 1.7394%. The accuracy of the tool was good if the RSD value is less than 2%. The RSD value is less than 2% which means that the 2,6-DFIF titration method used has good accuracy, and suitable for use in vitamin C analysis.

The limit of detection was defined as the lowest analyte concentration that was detected by the method specified confidence level.[15] This is the result of limit detection, showed at table 4. Detection limits obtained by the concentration of 2 ppm.

The result analysis quantitative sample of guava fruits juice with 4 varian packing can be showed at table 5.

4. Discussion

One of the vitamins found in fruits that is needed by the body is Vitamin C.[7] The human body does not naturally provide Vitamin C. Vitamin C weighs 176.1 grams and has a molar mass of 68 grams per mole. Its density measures around 1.694 grams per cubic centimeter with a melting point of 190ºC. Vitamin C boils at 553ºC. Vitamin C is a white crystalline water-soluble.[16] In the dry state of

Table 3. Results of %Recovery Test for Determination of Vitamin C Levels in Fruit Juice

No.	Sample Weight (g)	Vit C Standard (mg/ mL)	Theority Score (%)	Recovery (%)
1.	10.0900	0.25	99.54	99.91
2.	10.1200	0.25	99.39	98.94
3.	10.3400	0.25	95.62	84.76
4.	10.3600	0.25	95.59	84.85
5.	10.0561	0.25	96.00	84.81
6.	10.1542	0.25	99.31	98.94

Table 4. Limit of Detection

No.	Vitamin C Concentration (ppm)	Volume 2,6 DFIF (mL)
1.	100	10
2.	50	1
3.	10	0.8
4.	8	0.6
5.	6	0.4
6.	4	0.2
7.	2	0

Table 5. Result Quantitative Analysis of Sample

Sample Packaging	Titration	Weight Sample (gram)	Volume 2.6-DCPIP as Volume Titration (mL)	Level (mg/100gr)	Avarage Level (mg/100 g)	Level Packaging (mg/100gr)
	1	10.015	0.6	13.12		
Plastic Bottle	2	10.032	0.5	9.82	12.01	48
	3	10.026	0.6	13.10		
	1	10.362	0.6	19.02		
Tetrapack	2	10.445	0.5	18.87	17.99	27.6
	3	10.217	0.6	16.08		
	1	10.030	1.2	32.76		
Tin Can	2	10.221	1.2	32.14	31.46	65.46
	3	10.027	1.1	29.49		
	1	10.050	0.6	13.07		
Glasses Bottle	2	10.040	0.6	13.09	13.00	36.36
	3	10.220	0.6	12.00		

vitamin C is quite stable, easily destroyed by exposure to air (oxidation), especially when exposed to heat.[17] Nutrient loss in fruit juices is of considerable importance since consumers mostly consumed juices to derive their health beneficial effects.[18]

The next stage is the determination of the levels of vitamin C in the sample. The principle of the vitamin C content analysis of 2,6-DFIF titration method is to set the levels of vitamin C in food by titration with 2,6-DFIF where there is a reduction reaction of 2,6-DFIF the presence of vitamin C in an acid solution. Ascorbic acid to reduce the 2,6-DFIF in a solution that is colorless. Function of reagent as an indicator of electron-accepting color and vitamin C which will change the dye from blue to red. End point marked with a color change to pink in acidic conditions. Samples metaphosphate-acetic acid is added to prevent oxidation of vitamin C in the sample as long as the vitamin C will be a lot of exposure to oxygen, heat, and others. Therefore, to prevent excessive oxidation then added metaphosphate-acetic acid. In addition, metaphosphate-acetic acid solution also serves to separate the vitamin C that is bound to protein.[13] The reactions occurring 2,6-DFIF and vitamin C can be seen in Figure 1.

Assay of vitamin C in this study using the titration method in triplo. This method uses a dye solution as titer and sample solution as titrant. The sample used in this study beverage packaging, namely red guava fruit drink plastic bottles, tetrapack, beverage cans, and glass bottles. Then compare it with nutritional value information contained on the packaging label. Levels of vitamin C which is indicated on the packaging label expressed in percent nutrition adequacy figure (NAF). Here, the information content of vitamin C contained on the packaging label per serving the drink plastic bottles (120%) in 150 mL,

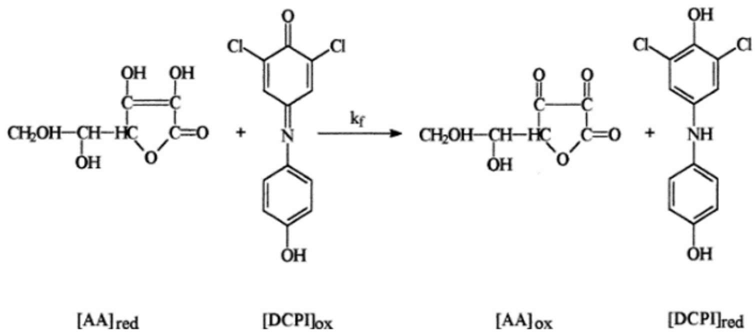

Figure 1. Oxidation Vitamin C with 2,6-DFIF

tetra pack (115%) in 250 mL beverage cans (260%) in 238 mL glass bottles (20%) in 33 mL. To obtain the sequence of levels of vitamin C based label do the calculation in the same volume that is 100 grams for all products. The calculations are as follows:

$$\text{Mass Vitamin C} = \frac{\text{mass of vitamin C}}{100} \times \text{ NAF Vit C}$$

$$= \frac{115}{100} \times 60 \text{ mg}$$

$$= 69 \text{ mg}$$

$$\text{Mass Vitamin C in 100 mg} = \frac{100 \text{ mg}}{250 \text{ mg}} \times 69 \text{ mg}$$

$$= 27.6 \text{ mg}$$

When compared with information from the packaging label, all products have vitamin C levels decline. The order of the vitamin C content in percent of nutrition adequacy figure per 100 grams by the label are as follows:

1. Beverage cans (65.54 mg/100 g)
2. Beverage Packaging tetrapack (27.6 mg/100 g)
3. Drink bottles plastic (48 mg/100 g)
4. Drink a glass bottle packaging (36.36 mg/100 g)

Sample juice in plastic bottles which have advantages and disadvantages as follows; advantages: the nature of gas and water vapor permeability of plastic packaging materials low, causing a longer shelf life of the product. While the drawbacks that the monomer substances and small molecule contained in plastic that can migrate into the packaged foodstuffs. Plastic is a high polymer compound that is printed in sheets that have different thicknesses. The main ingredient for making plastic is resin, both natural and synthetic.[19] Packaging plastic bottles has a vitamin C content of 12.01 mg/100 g, which amount is measured 7 months ahead of expiration. While on the packaging label for 48 mg/100 g. This shows that the levels of vitamin C in beverages plastic bottles experienced a drastic decline, it is because in addition to processing, the expiration date is also the transmission of light into the packaging, sometimes required in order to view the contents of the packaging.

Sample juice in tetrapack packaging. The process of making the packaging consists of six layers arranged from the outermost layer to the innermost layer:

1. Polyethylene (LDPE) is the layer that provides protection from humidity environmental outside of the package. One of the packaging that can maintain the quality of the product is plastic LDPE.[7]
2. Paperboard serves as the guardian of the stability of the form and give the strength of the various pressures.
3. Polyethylene (LDPE) in the third layer serves as an adhesive.
4. Aluminum foil is useful to keep the liquid from light, oxygen, and off-flavors as well as maintaining the stability of taste. aluminum foil packaging is better in maintaining the stability of the product brightness compared to other packaging.[20]
5. Adhesive polymer in the fifth layer serves as an adhesive.
6. M-polyethylene is the inner most layer that serves as a sealing.

This packaging has advantages and disadvantages. Those advantages are created by and UHT aseptic packaging. Aseptic packaging is packaging material in a container that meets the four conditions, in the sterile container products or place and environment in product filling and packing containers used must be sealed to prevent re-contamination during storage. While the UHT (Ultra High Temperature) that is heating to a high temperature (135ºC-150ºC) for 2-5 seconds. Tetra pack packaging beverages have vitamin C content of 17.99 mg/100 g which amount was measured 5 months before expiration. While on the packaging. label of 27.6 mg/100 g This shows that the levels of vitamin C in tetrapack packaging beverages has decreased, it is caused during processing and storage.

Samples of juice in beverage cans which have advantages and disadvantages as follows; advantages that can reduce the concentration of oxygen, so as to reduce the possibility of changes due to oxidation reactions such as oxidation of vitamins, fats, discoloration and corrosion processes. If the storage conditions allowing the microbes to grow, then the microbes would multiply and spoil the food in the beverage cans. Measurement results on beverage cans had higher levels of vitamin C that is 31.46 mg/100 g, which amounts are measured 6 months before expiry. While on the packaging label of 65.54 mg/100 g This shows that the levels of vitamin C in beverage cans experienced a drastic decline, it is because in addition to processing, expiration date beverage cans also have a corrosive nature caused much residual oxygen in foodstuffs, especially in air spaces, temperature, and storage time.

Sample juice in a bottle glass has advantages and disadvantages as follows the excess glass packaging does not react with the product packed. The measurement results on the beverage packing glass bottles have the vitamin C content of 13.00 mg /100 g, which amount is measured 7 months ahead of expiration. While on the packaging label of 36.36 mg/100 g. This shows that the levels of vitamin C in the packaged beverage glass bottles decreased. This is due in addition to the processing and storage glass bottle packaging also has the properties of invisibility is less favorable for vitamin C is sensitive to light.

7. Conclusion

The results of quantitative assay of vitamin C in some variation of beverage packaging using 2,6-DFIF obtained vitamin C content of red guava fruit drinks packaging tetra pack measured at 5 months prior to the expiry of 17.99 mg/100 g whereas on the beverages has decreased, it is caused during processing and storage.

Samples of juice in beverage cans which have advantages and disadvantages as follows; advantages that can reduce the concentration of oxygen, so as to reduce the possibility of changes due to oxidation reactions such as oxidation of vitamins, fats, discoloration and corrosion processes. If the storage conditions allowing the microbes to grow, then the microbes would multiply and spoil the food in the beverage cans. Measurement results on beverage cans had higher levels of vitamin C that is 31.46 mg/100 g, which amounts are measured 6 months before expiry. While on the packaging label of 65.54 mg/100 g This shows that the levels of vitamin C in beverage cans experienced a drastic decline, it is because in addition to processing, expiration date beverage cans also have a corrosive nature caused much residual oxygen in foodstuffs, especially in air spaces, temperature, and storage time.

Sample juice in a bottle glass has advantages and disadvantages as follows the excess glass packaging does not react with the product packed. The measurement results on the beverage packing glass bottles have the vitamin C content of 13.00 mg/100 g, which amount is measured 7 months ahead of expiration. While on the packaging label of 36.36 mg/100 g. This shows that the levels of vitamin C in the packaged beverage glass bottles decreased. This is due in addition to the processing and storage glass bottle packaging also has the properties of invisibility is less favorable for vitamin C is sensitive to light.

This indicates that the packaging variations affect levels of vitamin C were characterized by a decrease in the levels of vitamin C, so that the levels of vitamin C research results do not match the information on the packaging of nutritional value, due to the effect of storage.

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