

## The Storage Effect against Vitamin C Content in crystal guava (*Psidium guajava* L.) Juice

A. Guntarti<sup>1\*</sup>, M. Ahda<sup>1</sup>, H. Nabilla<sup>1</sup>, H. Susanti<sup>1</sup>

<sup>1</sup> Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

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### Abstract

Guava fruit (*Psidium guajava* L.) has many varieties, one of which is crystal guava. Crystal guava contains vitamin C which is easily oxidized by temperature. Therefore, this study aimed to determine the levels of vitamin C in crystal guava juice at different storage. Crystal guava samples were obtained from Bogor and then stored at different places and temperatures. Hence, guava fruits were placed in the freezer, refrigerator, and room temperature for 3 days, and all samples were juiced. Based on visual evaluation, guava fruit color is different. Moreover, the quantification method of vitamin C levels in guava juice by HPLC can be carried out with a flow rate of 1 mL/minute using the mobile phase ratio (methanol/water: 5/95, v/v),  $\lambda_{max}$ : 265 nm and C18 as a stationary phase. Retention time of vitamin C in guava is around 2.765 minutes. The effect of storage of vitamin C content in guava juice from fresh samples, and guava stored in the freezer, in the refrigerator, and at room temperature are 3.28 mg/mL, 3.20 mg/mL, 1.65 mg/mL, and 0.82 mg/mL. Therefore, it can be concluded that the best storage of guava fruit is in the freezer because vitamin C levels in crystal guava do not have a significant difference after treatment.

**Keywords:** Guava crystal; Storage condition; Vitamin C.

### Introduction

Guava is a tropical plant in the scientific language of *Psidium guajava*. Guava fruit has its uniqueness, such as the crystal guava. Crystal guava is a result of a mutation from Bangkok guava, which was first discovered in 1991, in Taiwan. The fruit has a crispy texture, a sweet taste, and only has very few seeds. The portion of the fruit in which consumable is larger. Therefore, it is an interesting fruit among planters and consumers [1]. Guava fruit contains fairly high nutrients; such as protein, lipids, carbohydrates,

mineral salts (P, Fe, K), water, and vitamins (A, B1, and C) [2]. Besides, it is also used as an antioxidant source.

Vitamin C is one of the active compounds used as an antioxidant agent. Vitamin C is a soluble compound but it is an organic acid that is easily damaged by oxidation [3]. Some other factors that can cause oxidation of vitamin C include heat, light, alkali, enzymes as well as copper and iron catalysts. Vitamin C also has chromophores that are sensitive to light stimuli [4]. Hence, the oxidation process will be accelerated by high temperature, heating too long,

\* Corresponding author: Tel: +6285868696923; Fax: +274564604; Email: any\_guntarti@yahoo.co.id

drying, and storage [5]. The fruit storage at a certain temperature significantly affects the content of vitamin C. The higher the storage temperature will cause the content of vitamin C becomes low. Meanwhile, storage time did not significantly affect the content of vitamin C in fruit. However, if the storage time is long; the vitamin C content tends to decrease [6].

The determination of the vitamin C content in guava fruit has been done through various methods, including High-Performance Thin Layer Chromatography (HPTLC) [7] and HPLC [8; 9]. Analysis of the vitamin C content in this study was carried out using HPLC due to it is simple and has a high sensitivity [9]. HPLC is a separation system with high speed and efficiency. It is a non-destructive method that can be used to analyze various samples qualitatively and quantitatively, both in single and mixed components [10].

## Materials and Methods

### Materials

Materials used in this study were crystal guava (*Psidium guajava* L.) obtained from Nusagrow Farm, vitamin C 99% standard (E-Merck), methanol grade HPLC (E- Merck). HPLC instrument is High-Performance Liquid Chromatography (Shimadzu LC-20 AT UV Detector SPD-20A) with Shimadzu C18 stationary phase.

### Storage process of Guava fruit

The treatment was carried out for 3 consecutive days. The sample was stored in a freezer, refrigerator, and closed room. The comparison sample wasn't given storage treatment. After 3 days, each treatment is put in juicer [11]. Crystal guava from each treatment picked 5 fruits to be analyzed. The fruit flesh that has made into

slurry weighed for 10 gr. The result from the juicer was then centrifuged at 5,000 rpm for 30 minutes. The obtained supernatant then then filtered with filter paper (Whatman no. 42) [12].

### Guava Juice Producing

The Crystal guava samples were washed and then drained. To produce guava juice, the flesh of the guava fruits was taken and put in the juicer [11].

### Determination of Vitamin C from the Storage Guava fruits

Determination of Vitamin C in guava juice was analyzed using HPLC. Vitamin C standard ranges from 1-9  $\mu\text{g/mL}$  are made 1  $\mu\text{g/mL}$ , 3  $\mu\text{g/mL}$ , 5  $\mu\text{g/mL}$ , 7  $\mu\text{g/mL}$  and 9  $\mu\text{g/mL}$ . Guava juice samples were pipetted as much as 100  $\mu\text{L}$  and then dissolved with 10 mL aquabidest. After that, taken back 1 mL and diluted with aquabidest up to 10 mL. all standards and samples were filtered with 0.22  $\mu\text{m}$  millipore before injected into the HPLC [13]. The mobile phase used was a mixture of methanol and aqua bidest in the ratio of 5:95 v/v [14] and flowrate was set 1 ml/min and the stationary phase was C<sub>18</sub>, with a column length of 25 cm.

### Data Analysis

The data analysis was analyzed using SPSS at a 95% confidence interval. The normality test was conducted using the Kolmogorov-Smirnov test, while the homogeneity test was conducted using the Levene's Test and Mann-Whitney Test to find out 2 averages.

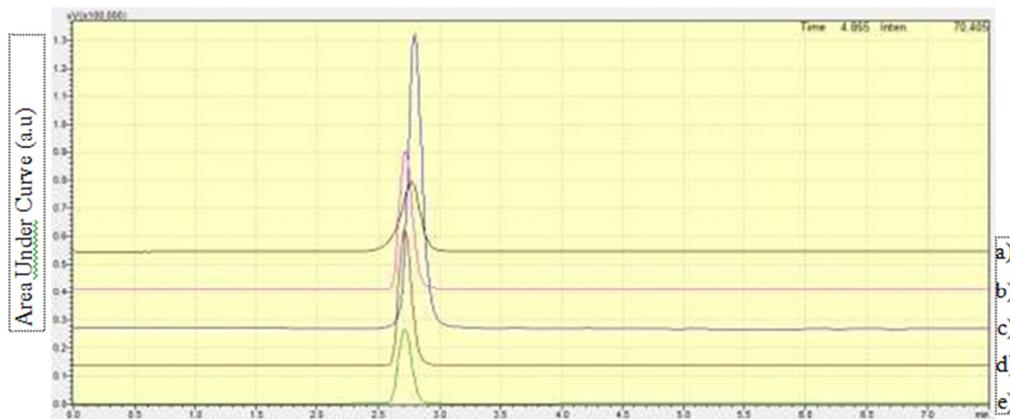
## Results and Discussion

### Organoleptic evaluation of Guava fruit in the Storage Conditions

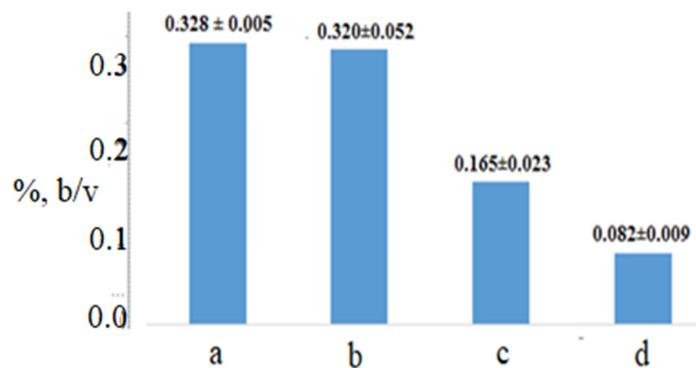
In the three samples of crystal guava which have

**Table 1.** Organoleptic Test Result of Crystal Guava Based On Its Storage

Storage	Without storage	After treatment	Result
Freezer			The surface of the flesh is not brownish and the fruit looks fresh
Refrigerator			Brownish on some surfaces of the flesh, but the fruit still looks fresh
Closed Room			Brownish on the entire surface of the flesh and the fruit is juicy



**Figure 1.** Retention Time of vitamin C from Fruit Juice Analyzed by HPLC: mobile phase is methanol: aquabidest (5:95) v / v, the stationary phase is C18,  $\lambda$  265 nm, run time 7.5 minutes, flow rate 1mL / minute. Remarks: a) Standard vitamin C, b) Fresh fruit sample, c) Fruit sample with freezer Storage, d) Fruit sample with refrigerator storage, e) Fruit sample with room temperature storage.



**Figure 2.** Comparison Diagram of the Level of Vitamin C at different storages for 3 days: a) Fresh fruit, b) freezer (-18 °C), c) refrigerator (4 °C), and d) room temperature (27-30 °C)

been treated in several storages and stored for 3 days; there are differences in the color of the surface of their flesh. This is due to the oxidation process, which occurred at the time of storage with a temperature that is different from the storage of the three samples. The process of color change showed that the fruit contained vitamin C. Table 1 presents the result of the organoleptic testing.

#### ***Determination of Vitamin C in Guava Juice from the Stored Guava fruits***

Guava juice samples contain vitamin C that has been proven by the retention time between the standard vitamin C and the slurry sample of the crystal guava is not significantly different; which are 2.765 minutes for the vitamin C standard and 2.708 minutes for the sample of the guava crystal (Figure 1).

Furthermore, the quantitative analysis of vitamin C

levels in guava juice is conducted at 2.765 min. The equation of linear regression resulted is  $y = 105257.6x + 40161,6$  with the correlation coefficient ( $r$ ) = 0.9908. A comparison of vitamin C in each group showed that the fresh fruit sample has higher vitamin C content than other fruit samples. The vitamin C level of the fresh fruit, the stored fruit in the freezer, in the refrigerator, and room temperature is 0.328% b/v, 0.320% b/v, 0.165% b/v, and 0.082% b/v respectively (Figure 2). Whereas, fresh fruit samples and the stored fruit sample in the freezer contain similar vitamin C content based on the statistics testing with a 95% confidence level. Based on the reported study from [15] showed that the increasing the temperature would reduce the vitamin C levels in Citrus fruits and also highly dependant highly on the storage condition. Hence, the oxidation process of vitamin C is influenced by the temperature difference in fruit storage [16]. This

study is also supported by another research from [17] reported that the level of vitamin C at a storage temperature of 10° C for 5 days is higher than at and 29° C for 15 days which is around 43.5 mg/100 mL (0.044% w/v) and 23.6 mg/100 mL (0.024% w/v), respectively.

### Conclusion

Based on this study, the storage condition will influence the level of vitamin C in the crystal guava, especially in guava juice. To control the oxidation process, we suggest putting guava fruit in the freezer (-18 °C) for 3 days to before use because the oxidation process of vitamin C was not effect significantly. Hence, the temperature will decrease the vitamin C level through the oxidation process if the storage and time conditions is not control.

### References

1. Abanto-Rodriguez C, Pinedo-Panduro M, Alves-Chagas E, Cardoso-Chagas P, Tadashi-Sakazaki P, de Menezes PHS, et al. Relation between the mineral nutrients and the vitamin C content in camu-camu plants (*Myrciaria dubia*) cultivated on high soils and flood soils of Ucayali, Peru. *Scientia Agropecuaria*. 2016;7(3):297-304
2. Lubis N, Prasetiawati R, Septiani W. Determination of vitamin C red guava (*Psidium Guajava* Linn) fruit juice with variation of beverage packaging, *International Journal of Advances in Science Engineering and Technology*. 2017;5(4):51-55
3. You C, Fang B, Zhang W. Research on the stability of vitamin C in non-aqueous carrier. *Journal of Biomedical Engineering*. 2012;29(5):929- 932
4. Lee SK. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*. 2015;20:207-220
5. Tareen H, Mengal F, Masood Z, Mengal R, Ahmed S, Bibi S, et al. Determination of vitamin C content in citrus fruits and non-citrus fruits by titrimetric method, with special reference to their nutritional importance in human diet. *Biological Forum*. 2015;7(2):367-369
6. Kaleem A, Nazir H, Pervaiz S, Iqtedar M, Abdullah R, Aftab M, et al. Investigation of the effect of temperature on vitamin C in fresh and packed fruit juices. *FUFAST J Biol*. 2016;6(1):117-120
7. Chandra R, Sharma KD. Quantitative determination of paracetamol and caffeine from formulated tablet by reversed phase-HPLC separation technique. *International Journal of Chromatographic Science*. 2013:31-34
8. Meyer FR. *Practical High-Performance Liquid Chromatography*, 4th Ed. New York: John Wiley & Sons. 2004.
9. 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). *International Journal of Chemistry*. 2019;11(1):52-59
10. Sugihartini N, Fudholi A, Pramono S. Validation method of quantitative analysis of epigallocatechin gallate by high performance. *Pharmaciana*. 2014;4(2):111-115
11. Shafiqatullah HA, Khaliqurrehman A, Asadullah J. A simple and rapid HPLC method for analysis of vitamin C in local packed juices of Pakistan. *Journal of Scientific Research*. 2012;12(8):1085-1091
12. Teepoo S, Chumsaeng P, Jongjinakool S, Chantu K, Nolykad W. A new simple and rapid colorimetric screening test for semi-qualitative analysis of vitamin C in fruit juices based on prussian blue. *Journal of Applied Sciences*. 2012;12(6):568-574
13. Kumar KR, Kumar PP, Mallikarjuna RN. Development and validation of RP-HPLC method for the estimation of ascorbic acid in health drink. *Journal of Chemical and Pharmaceutical Research*. 2011;363-374
14. Schulzová V. Determination of vitamins, caffeine, and preservatives (method: liquid chromatography with UV detection). *Analysis of Food and Natural Products Laboratory Exercise*. 2012.
15. Njoku PC, Ayuk AA, Okoye CV. Temperature Effects on Vitamin C Content in Citrus Fruits, *Pakistan Journal of Nutrition*. 2011;10(12):1168-1169
16. Zhang J, Han H, Xia J, Gao M. Degradation kinetics of vitamin C in orange and orange juice during storage. *Advance Journal of Food Science and Technology*. 2016;12(10):555-561
17. Rahmawati Rahman S, Wati A, Herman H, Arsyad F. Test of antioxidant activity leaves of *Scaevola tacadda* (Gaertn) Roxb. using DPPH (1-1-Difenil-2-Pikrilhidrazil). *International Research Journal of Pharmacy*. 2014;5(3):159-162.