Determination Of Vitamin C And Antioxidant Activity In Fresh Red Guava (*Psidium Guajava* L.) And Red Guava Commercial Fruit Juices

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Abstract.
Red guava (Psidium guajava L.) has various benefits such as overcoming digestive disorders, thrush and dengue fever and can be used for anticancer herbal therapy. Red guava fruit is a fruit with the highest levels of vitamin C compared to other fruits. It contains carotenoid compounds and phenolic compounds that act as antioxidants. Red guava fruit is often processed into fruit juice drinks that are packaged in various types of packaging and distributed commercially so that people can consume red guava fruit more efficiently. Determination of vitamin C levels using the UV-Visible spectrophotometric method and the results obtained that the vitamin C content of red guava commercial fruit juice is higher than fresh red guava juice. Measurement of antioxidant activity of red guava fruit and its commercial fruit juice using ABTS methods. The results obtained in the ABTS method, fresh red guava juice and its commercial fruit juice have very strong antioxidant activity (IC_{50} < 50 µg/mL).

Keywords: *Red guava fruit, Vitamin C, Antioxidant, ABTS.*

I. INTRODUCTION

At this time, people are more interested in consuming commercial fruit juices instead of fresh fruit. Both fresh fruit and the commercial fruit contain very beneficial ingredients that can help prolong life by protecting and regenerating tissue and organ cells such as carbohydrates, proteins, minerals (Ca, K, Zn and Fe) and also Vitamins (A, B1, B2, C, D and E) [3][14].Vitamin C is an essential micronutrient which means it is needed for normal metabolic functions of the body. Vitamin C acts as an antioxidant. Antioxidants are compounds that are able to donate protons to free radical compounds, so that there are no further reactions that cause degenerative diseases. It is known that free radicals can come from the surrounding environment such as cigarette acid, pollution and vehicle fumes [15]. Antioxidant components are most abundant in fruits [7][12].Red guava (*Psidium guajava* L.) can be used as a good antioxidant because it contains carotenoid antioxidant compounds and phenolic compounds such as caffeic acid, vitamin C, guavin, gallic acid, quercetin and ferulic acid [5][9]. IC_{50} value in the study of antioxidant activity of red guava extract using DPPH method was 56.93 µg/mL [13]. Vitamin C levels in red guava fruit was found around 87mg/100g.Antioxidant activity testing using the ABTS method has various advantages such as very high ABTS sensitivity, ABTS is very good for testing antioxidant activity in food products and ABTS works over a wide pH range [2][16].

II. METHOD

a. Sample

Red guava (*Psidium guajava* L.) fruits were obtained from Simalingkar B, Medan Tuntungan, North Sumatera. Which is the common plantation area of red guava fruits in Medan city and the commercial red guava fruit juice (plastic, glass and tetrapack packaging) were purchased from a wholesale store in Medan city.

b. Preparation of Fresh Red Guava Fruit Juice

Washed red guava fruits with water and drained it. Cut red guava into small pieces, blend red guava fruits with water (3:1), filtered to separate the filtrate and seeds, then pasteurized the filtrate at 70°C for 15 minutes, put the fresh red guava fruit juice into a bottle [8]

c. Determination of Vitamin C

Weighed 10 grams of sample fresh red guava fruit juice and the commercial red guava fruit juice (plastic, glass and tetrapack packaging), put into a 100 mL flask, added aquadest up to the mark the homogenized, then filtered each flask of the samples using Whatmann filtered paper No.1, pipetted 5 mL of the filtrate then put it into a 50 mL flask, added aquadest up to the mark then measure the absorbance using UV-Visible spectrophotometer at 266 nm wavelength [10].

d. Antioxidant Activity by ABTS Method

5 grams of each samples put into a 100 mL flask, add methanol pro analysis up to the mark, left for 3 days, then filtered each flask using Whatmann filtered paper (Standar Solution I 50.000 µg/mL). Pipetted 1 mL from each samples standart solution I, put into a 25 mL flask, add methanol pro analysis up to the mark (Standart Solution II 2.000 µg/mL).2 mL ABTS solution put into test tubes, add 0.1 mL of each samples with concentration 10, 20, 30 and 40 µg/mL, incubated in the dark for 6 minutes (operating time). The absorption of ABTS against each samples were read at 734 nm wavelength. The % antioxidant power of each samples were calculated against ABTS free radicals [2]

Antioxidant Power (%) = $\frac{Ao-As}{Ao}$ x 100%

Description :

Ao : Absorbance of Control

As : Absorbance of Samples after adding ABTS

III. RESULT

3.1 Determination of Vitamin C

Determination of Vitamin C in fresh red guava fruit juice and red guava commercial fruit juices (plastic, glass and tetrapack packaging) were done using UV-Visible spectrophotometer because chromophore and auxochrome group were found in vitamin C which can be read in UV-Visible spectrophotometer [4]. The maximum wavelength in this research is 266 nm. Then measure the calibration curve to get linear regression equation. The results of the measurement of the standard calibration curve of vitamin C can be seen in Table 1 and standard curve linear regression equation of vitamin C can bee seen in Figure 1.

Table 1. Standart Calibration Curve of Vitamin C

Concentration (µg/mL)	Absorbance	Regression Equation
0.000	0.000	Y = 0.07587x + 0.01810 R = 0.9960
3.000	0.252	
5.000	0.429	
7.000	0.544	
9.000	0.713	
11.000	0.837	

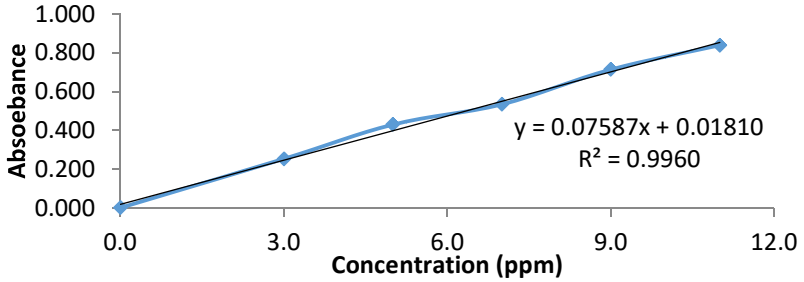

Fig 1. Standard Curve Linear Regression Equation of Vitamin C

The result of Vitamin C levels in red guava fresh fruit juice and its commercial fruit juices (plastic, glass and tetrapack packaging) can be seen in Table 2.

Table 2. Vitamin C Levels

Samples	Vitamin C (mg /100 g)
Red guava fresh fruit juice	49.31 ±0.8450*

Red guava commercial fruit juice (plastic packaging)	77.02 ± 0.6910*
Red guava commercial fruit juice (glass packaging)	94.46 ± 0.5255*
Red guava commercial fruit juice (tetrapack)	89.71 ± 0.2801*

* = Average of 6 replications

The results of the determination of vitamin C levels using UV-Visible spectrophotometer in red guava fresh fruit juice and it commercial fruit juices (plastic, bottle and tetrapack packaging) were found that red guava commercial fruit juice (plastic, glass and tetrapack packaging) have higher levels of vitamin c compared to red guava fresh fruit juice. This can be happened because the use of preservatives like benzoic acid, sulfur dioxide, sorbic acid and ascorbic acid were added into the red guava commercial fruit juices. And also the manufacturing process like red guava commercial fruit juice glass packaging is pasteurized. Pasteurization is carried out in the manufacturing process of some commercial fruit juice in order to exted the shelf life of the commercial fruit juice so the stability of nutrition and vitamin can lasts longer before the packaging is opened and can be more safety to consume [1] [17] . In red guava fresh fruit juice, the vitamin C level is can be lower than the commercial fruit juice because the effect of the environment in the area where the fruit grows, climate factor such as altitude, soil pH, humidity, soil moisture, weather, rain intensity and light intensity [11].

3.2 Antioxidant Activity by ABTS Method

The maximum wavelength was carried out by measuring 1 mL of ABTS in PBS pH 7.4 in the range wavelength of 400-800 nm. The results of ABTS maximum wavelength is 734 nm and the spectrum can be seen in Figure 2.

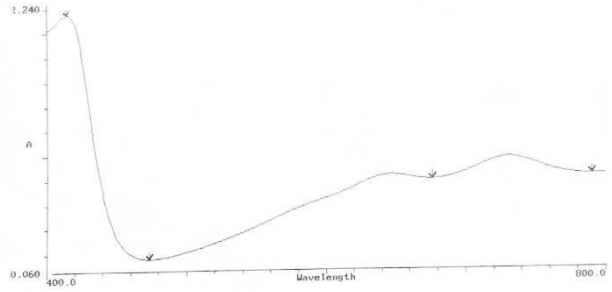

Fig 2. Spectrum of ABTS Maximum Wavelength

Mechanism of free radicals scavenging using ABTS method can be seen in Figure 3.

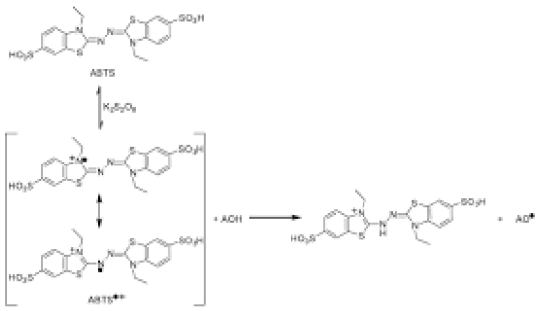

Fig . Mechanism of Free Radicals Scavenging using ABTS Method [2]

Based of Figure 3, can be seen that ABTS was oxidized by potassium persulfate to form the cation radical ABTS •+. Cation radical ABTS can be stabilized by antioxidant compound by donating electrons to the free radicals so the cation radical ABTS is stable. The result of measurement of red guava fresh fruit juice and red guava commercial fruit juices (plastic, glass and tetrapack packaging) can be seen in Table 3.

Table 3. Results of Antioxidant Activity by ABTS Method

Samples	IC_{50} (µg/mL)
Red guava fresh fruit juice	39.87
Red guava commercial fruit juice (plastic packaging)	19.00
Red guava commercial fruit juice (glass packaging)	16.87
Red guava commercial fruit juice (tetrapack)	18.25
Vitamin C	7.03

Based on IC_{50} value of red guava fresh fruit juice and red guava commercial fruit juices (plastic, glass and tetrapack packaging) showed that have antioxidant activity. All the IC_{50} value were under 50 µg/mL so can be concluded that all samples have antioxidant activity on very strong category (IC_{50} < 50 µg/mL). A compound can be said to be a very strong antioxidant if having IC_{50} value < 50 µg/mL [6]. Whereas, vitamin C also has a strong antioxidant activity because its IC_{50} value was also under 50 µg/mL.

IV. CONCLUSION

The vitamin C levels in red guava commercial fruit juice is higher than red guava fresh fruit juice. The measurement of antioxidant activity by ABTS method showed that fresh red guava juice and red guava commercial fruit juice have very strong antioxidant activity (IC_{50} < 50 µg/mL).

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