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Effect of time and temperature on vitamin c contents and antioxidant activity in the hot water extraction of melinjo peels

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Abstract. Melinjo peel contains ascorbic acid, tocopherol, polyphenols and has the potential as a xanthine oxidase inhibitor. Melinjo peel also has the potential as an antioxidant naturally good for the body. The purpose of this studies to determine effect of variation of time and temperature extractions on antioxidant activity and vitamin C of melinjo peel. This study used a completely randomized design with two factors. firstly, 75 and 100°C for temperature and then secondary factors is different extraction times are 30, 45, and 60 minutes. The highest value of vitamin C content is 313,30 mg/L at 100°C for 60 minutes. Although, the highest value was 30,89% at 100oC for 30 minutes. Consequently, the best treatments at 100 °C and 30 minutes would be appropriate extraction operating conditions.

1.Introduction

Melinjo (*Gnetum gnemon* L.) is a commodity with various benefits. Melinjo fruit is commonly consumed as the additional ingredients in making dishes and the raw material of melinjo crackers (emping) as traditional foods in Indonesia. During the process of making emping, several of peel from melinjo is wasted. Red melinjo peel has bioactive compounds such as lycopene, flavonoid, phenolic, B-carotene, and vitamin C which also can be used as natural antioxidant compounds for human body [1] [2].

Antioxidant is several compounds which provides one or more electron to counter the free radicals. Free radicals itself are reactive compounds which generally have unpaired electrons at its outer layer [3]. Those free radicals could bring negative effect to the body such as destroying the protein, DNA, and cell membrane. This will cause degenerative disease that the body needs a certain compound namely antioxidant to inhibit the free radicals. Vitamin C or ascorbic acid is an essential vitamin for



human body. It is organic substance needed for the body in a small amount to maintain the body metabolism system, repair the body tissue, accelerate collagen formation to heal the wound, decrease cholesterols, and become an aqueous antioxidant which effectively inhibit the free radicals. Vitamin C is able to react to free radicals and oxidants within human body.

This research is aimed to find out the effect of extraction duration and temperature on vitamin C and antioxidant activity of melinjo peel. Normally, extraction process is carried out through maceration method with room temperature. However, the method is not yet considered as the effective one since the less soluble compound will not be perfectly dissolved with room temperature. Therefore, we added variety of temperature and duration to the maceration process to optimize the extraction process.

2. Material and Method

2.1 Tools and Material

Tools used during the melinjo peel extraction process were drying oven (Model R-5A Harvest Saver, USA), hot magnetic stirrer (SH2), thermometer, and spectrophotometer (UV-1800). Ingredient used in this research were orange to red melinjo peel (Magetan Regency), sodium metabisulfite, aquades, methanol 96% (pa, QREAC), DPPH (2,2-diphenyl-1-pikridirazol) (sigma Aldrich), starch indicator, and iodine.

2.2 Experimental Design

This research was carried out through Completely Randomized Design (CRD) with 2 factors and 3 replications.

Factor 1: Extraction Time Duration (Minutes)

T1 = 30

T2 = 45

T3 = 60

Factor 2: Extraction Temperature (°C)

S1 = 75

S2 = 100

From the factors above, we could make some treatment combinations as follows:

S1T1 = Temperature 75°C : Duration 30 minutes

S1T2 = Temperature 75°C : Duration 45 minutes

S1T3 = Temperature 75°C : Duration 60 minutes

S2T1 = Temperature 100°C : Duration 30 minutes

S2T2 = Temperature 100°C : Duration 45 minutes

S2T3 = Temperature 100°C : Duration 60 minutes

2.3 Soaking with Sodium Metabisulfite

Initially, before starting the soaking process, the melinjo peel were sorted and cleaned to remove the dirt on the peel. Next, the peel were immersed in the 0,6% sodium meta-bisulfite solution for 30 minutes. After that, the peel was dried by using dryer oven at 50°C temperature for 12 hours.

2.4 Extraction

The melinjo peel was then extracted by using maceration method with aquadest. We put 5 grams of melinjo peel into the beaker glass 1000 ml and added 500 ml aquadest. Next, we carried out the maceration process for 30, 45, and 60 minutes at the temperature of 75°C and 100°C. Then, we strained the result and obtained the melinjo peel extract with aquadest solvent.

2.5 Determination of Vitamin C Contents

Vitamin C contents on melinjo peel extracts was tested through titration methods from AOAC Official Method 967.21. Sample 5 ml was put into the Erlenmeyer 250 ml, then we added 10 drops of starch indicator 1%, 20 ml aquadest, then titrated with Iodine 0,01 N solution until the color changed from yellow to green and blue. The calculation was applied to the amount of iodine solution spent with the following formula:

$$\text{mg/g} = \frac{\text{Vol iodine titration} \times 0.7832 \times \text{total volume}}{\text{g sample}}$$

Where:

Vol iodine titration : iodine volume of the titration result

Total volume : 500 ml

g sample : weighed sample (in gram)

2.6. Determination of Antioxidant Activity

Antioxidant activity on melinjo peel extract was determined by DPPH methods [4]. 2 ml of DPPH solution mixed with 800 μl ethanol 96%. Then, 200 μl of sample added to DPPH-ethanol 96 % mixture solutions homogenized with vortex for 15 minutes. The mixture solution was incubated at room temperature in the dark for 30 minutes. The sample absorbance was measured at 517 nm wave length. Antioxidant activity was calculated through the following formula:

$$\% \text{ inhibition} = \frac{\text{Blank Absorbency} - \text{Sample Absorbency}}{\text{Blank Absorbency}} \times 100\%$$

2.7. Data Analysis

Observation result data was analyzed through analysis of variance or ANOVA (Analysis of Variance) factorial Completely Randomized Design (CRD) methods followed by tukey's test with a significant level of 5%.

3. Results and Discussion

Melinjo peel contains vitamin C, tocopherol and polyphenol as the antioxidants which could potentially become the xanthine oxidase inhibitor [5]. The ANOVA statistical result of vitamin C content and antioxidant activity within the melinjo peel extracts with variation of time and temperature extractions is significantly ($p < 0.005$) was shown at the Table 1.

Table 1. Result of Vitamin C Content and Antioxidant Activity on Melinjo Peel

Treatment		Vitamin C (mg/100mL)	Antioxidant Activity (%)
Temperature (°C)	Duration (minutes)		
75	30	254,50 ^a	29,32 ^c
	45	250,60 ^a	28,36 ^b
	60	274,10 ^b	27,00 ^a
100	30	293,70 ^c	30,89 ^f
	45	303,50 ^c	30,06 ^d
	60	313,30 ^c	30,48 ^e

The average value of vitamin C content obtained were varied from 254,50 mg/L – 313,30 mg/L. the highest vitamin C contents at 100°C temperature until 60 minutes while the lowest was on the one with 75°C temperature for 30 minutes. Decreasing of vitamin C can inhibit with sodium metabisulphite until drying and extraction operating conditions. This result is similar with [6] where vitamin C and phenolic compounds was found in dried mango slices.

Variation of temperatures and times extraction gave different effect on the antioxidant activity. The highest antioxidant activity was 30,89% at 100°C temperature for 30 minutes while the lowest was 27,00% at 75°C for 60 minutes. Increasing temperatures in a short span of time in line with antioxidant activity with increased. An extraction process with high temperature and short times could accelerate the drawing process of a compound from a material and increase the solubility of the active components on a material since the material cells were damaged by the heating process. Besides, increasing of antioxidant activity in this research was caused the water solvents which had polar properties. The antioxidant properties on the melinjo peels are supposed to have hydrophilic properties which was a compound bound to water due to its polar shape, that it extracted quite much antioxidant bioactive compounds.

According to [7] the effect of using solvent with polar properties would result on a polar extract which indicated an effective antioxidant activity. Meanwhile, [8] stated that temperature had a significant effect on extracted compounds, either the bioactive or anti-nutritional compounds. [9] concluded that antioxidant on several food materials were increased along with the increasing temperature at 45-100°C and it would decrease if the extraction temperature up to 120°C. Then, the effect of higher temperatures and longer exposure times make polyphenol compound degradations [6].

Based on the obtained result of antioxidant activity, the best result was 30,89% with 100°C temperature and 30 minutes extraction times. On the others hand [2] antioxidant activity from red melinjo peel used ultrasonic-assisted extraction with 30 minutes and 20 °C was 37,35%. In contrast, seed extracts using hot water showed the antioxidant activity at 41.86 ± 4.22 % [10].

4. Conclusion

The highest value of vitamin C content is 313,30 mg/L at 100°C for 60 minutes. Although, the highest antioxidant activity was 30,89% at 100°C for 30 minutes. It can be concluded, the optimum treatments at 100 °C and 30 minutes with vitamin C content and antioxidant activity is 293,70 mg/L and 30,89 %.

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