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Submission date: 19-Oct-2020 11:39AM (UTC+0700)

Submission ID: 1419449373

File name: iliyanti_2018_IOP_Conf._Ser.__Earth_Environ._Sci._207_012044.pdf (1.06M)

Word count: 3633

Character count: 18502

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To cite this article: M W Apriliyanti *et al* 2018 *IOP Conf. Ser.: Earth Environ. Sci.* **207** 012044

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4 Antioxidant Activity, Total Phenol, and Sensory Properties of Melinjo Peel Tea with Pre-Treatment

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Abstract. Melinjo (*Gnetum gnemon*) is a melinjo fruit producing plant that has many benefits. Melinjo peel contains ascorbic acid, tocopherol, and polyphenols which have activity as antioxidant and potential as xanthine oxidase inhibitors. Melinjo peel has the potential as an herbal tea because of its antioxidant properties. This study aims to determine antioxidant activity, total phenol, and sensory properties of melinjo peel tea with pre-treatment variation (concentrations of citric acid 0%, 0.05%, 0.1% and the blanching process for 5 and 10 minutes). The total phenol content and antioxidant activity were analyzed to support the potential of melinjo tea as an herbal tea. The results showed that melinjo peel herbal tea can be accepted by panelis and the most preferred one is the tea made from the blanching treatment duration of 5 minutes and 0% citric acid concentration. The steeping water of melinjo peel herbal tea has a clear yellow color, a rather distinctive taste and a slightly typical aroma of melinjo peel. Melinjo peel tea contains total phenol between 2.02 - 2.52 mg/ g and antioxidant activity 32.47 - 48.47%.

1. Introduction

The seeds of melinjo (*Gnetum gnemon*) have been known to have various benefits. It is very well known throughout Indonesia because almost all parts of the melinjo tree are very useful, e.g. as foods or food ingredients (the flowers, seeds, and leaves) and as household appliances (the stem). The old melinjo trees can produce melinjo seeds which have high economic value, i.e. as the main ingredient for making melinjo crackers [1].

Melinjo trees are commonly planted for their leaves, seeds, and flowers. One of the products of melinjo which has been widely known is *emping melinjo* (a type of cracker). This product is typical Indonesian food which is usually consumed as a snack or can be eaten together with rice. To make the crackers, the peel of melinjo seeds is often separated from the fruits and discarded as agricultural waste.

Melinjo peel contains ascorbic acid, tocopherols, and polyphenols which have antioxidant activity as well as the potential to serve as xanthine oxidase inhibitors. Xanthine oxidase plays an important role in the process of formation of uric acid by successively catalyzing hypoxanthine into xanthine and then uric acid [2]. According to Cos [3], some antioxidant compounds have the potential to serve as xanthine oxidase inhibitors because they are able to capture electrons. Flavonoids in



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flavones and flavonols have higher inhibitory power than other flavonoids because the position of the hydroxyl group is easier to capture electrons from the active side of xanthine oxidase. Other compounds such as polyphenols and saponins also have the potential to serve as xanthine oxidase inhibitors because they have hydroxyl groups serving as electron acceptors from xanthine oxidase.

Based on the studies conducted by Ardiyansyah & Apriliyanti [4] and Khoiriyah [5], melinjo peel tea with oxidation and non-oxidation treatment as well as withering duration produced a total phenol of 25.30-79.67% (through the titration method) and antioxidant activity of 48.23-53.16%. Nevertheless, the resulting melinjo peel tea has a deficiency, i.e. its organoleptic properties have not been well received by consumers. Furthermore, Pujimulyani's research [6] suggested that white turmeric blanched in 0.05% citric acid medium, 100°C for 5 minutes had total phenol content, total flavonoids, condensed tannins, DPPH values, and FRAP significantly higher than fresh white turmeric extracted with 6 types of solvents. In addition, the increased phenolic content of white turmeric correlated significantly with the increased antioxidant activity of white turmeric after undergoing blanching than that in a fresh condition.

This study, therefore, was aimed at determining the antioxidant activity, total phenol, and sensory properties of melinjo peel tea with pretreatment. Melinjo peel tea with pretreatment performed using the concentration of citric acid and the blanching process was expected to increase the antioxidant activity and total phenol, as well as the sensory properties accepted by consumers. Melinjo peel tea processing is an effort to utilize waste from an Indonesian local plant that has the potential to contain bioactive compounds. This study is also expected to get a pretty patent regarding the processing of melinjo peel tea which is beneficial for health.

2. Material and Methode

2.1 Materials and Tools

The raw materials needed were peel of melinjo (*Gnetum gnemon*), distilled water, folin ciocalteu, Na₂CO₃, Gallic acid, 1,1-diphenyl-2-picrylhydrazil (DPPH), and methanol. The tools used were a blender, basins, glass cups, Erlenmeyer, measuring flask, measuring cup, glass bottles, cups, gauze, filter paper, analytic balance, oven, hot plate, volume pipette, micropipette, and spectrophotometer.

2.2 Processing of Melinjo Peel Tea

The selected melinjo peel was old/ripe, dark orange to red, and still fresh. In addition, the peel must be clean and not damaged. Melinjo peel was separated from dirt and washed with running water. Melinjo peel was weighed according to the necessary treatments. The pretreatment was done by blanching the peel using citric acid media and setting the duration of the blanching process. The concentration used was 0; 0.05%; and 0.1% and the duration was 5 and 10 minutes. The drying process was performed at a temperature of 105 °C for 30 minutes in an electric oven so that the drying can be spread evenly and quickly. After being dried, the peel was ground with a blender until turning into powder. Afterward, the powder was packaged using a tea bag. Parameter analysis was performed on each treatment of the produced melinjo peel tea. The parameters observed in the tea included the analysis of bioactive compounds, i.e. the content of total phenol, antioxidant activity, and functional groups, as well as sensory properties.

2.3 Research Design

This study employed a factorial completely randomized design which consists of two factors with 3 replications. Factor 1 with 3 levels included the concentration of citric acid media which is 0; 0.05; and 0.1%, while factor 2 with 2 levels included the duration of the blanching process which is 5 and 10 minutes.

2.4. Analysis Parameters

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2.4.1 Determination of antioxidant activity.

The antioxidant activity testing⁶ was performed using a sample of 0.1 ml and then added with 5 ml of methanol, vortexed, and taken as much as 4 ml. A 1 ml of 20 ppm DPPH was then added. The solution of 1,1-diphenyl-2-picrylhydrazil (DPPH) in methanol was vortexed and then incubated in a dark space for 30 min⁴ and absorbed at a wavelength of 517 nm (modified from Brand-Williams [7]). The antioxidant activity was calculated by the following formula:

$$[(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] * 100$$

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where:

Abs control: the absorbance of DPPH before reacting with the sample

Abs sample: the absorbance of DPPH after reacting with the sample

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2.4.2 Determination of total phenol content.

The analysis of the total polyphenol content was performed spectrophotometrically using the modified folin ciocalteu method [8]. The sample extract with a certain volume was put into a test tube, then added with distilled water to a volume of 5 mL. Afterward, a 0.5 mL of folin ciocalteu was added into the test tube, then vortexed, and allowed to stand for 5 minutes. Then added Na₂CO₃ (7% as n¹³ as 1 mL., Distorted, and allowed to stand for 60 minutes in a dark space. The absorbance value was measured using the spectrophotometer at a wavelength of 765 nm. The total polyphenol content in the extracted sample was calculated using a standard curve made from Gallic acid (GA) in some concentrations. The total polyphenol content in the material was expressed as mg GAE/g of sample.

2.4.3 Sensory test.

The sensory or organoleptic test was performed by presenting the samples and giving an organoleptic questionnaire to each panelist. The method of serving samples was done by brewing melinjo peel tea in a tea bag into hot water. One tea bag contains two grams of melinjo peel tea powder. The brewing was done with 150 mL⁶ of hot water for 10 minutes. This sensory test was performed by 20 trained panelists. The hedonic test was performed to determine the level of panelists' preference for the tested products, which were three formulation treatments without and using foam and bubble effects and the best formulation. The test was done by giving a score on each sample according to each panelist's favorite level. The rating scale used for the hedonic test ranged from 1 - 5 (dislike - extremely like).

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2.5 Data Analysis

The observation data was analyzed using the Analysis of Variance (ANOVA) method. If the test results were different, the analysis was then proceeded with the Tukey HSD (Honestly Significant Difference) test with 5% and 1% to see the difference between treatments.

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3. Results and Discussion

3.1 Antioxidant Acti¹⁹

The DPPH radical (1,1-diphenyl-2-picrylhydrazyl) is a stable nitrogen organic radical, which has a purple effect. The antioxidant activity test using DPPH method is based on the measurement of the reduction ability of DPPH radicals. The measurement can be done by measuring the decrease in absorbance [9]. The purple DPPH solution is a collection of free radicals and will be bound by H ions from antioxidant²¹ compounds so that the purple intensity will decrease. The decrease in purple intensity can be measured at a wavelength of 517 nm [7]. The capacity of radical capture is usually measured by using a DPPH (1,1-diphenyl-2-picrylhydrazyl) radical compound, which is stable and able to receive electrons or hydrogen radicals to become a diamagnetically stable

compound (Soares *et al.*, 1997). Furthermore, Duh *et al.*, (1999) stated that the ability of DPPH radicals can be reduced or stabilized by antioxidants at a wavelength of 517 nm. Therefore, DPPH is commonly used to assess radical capture capacity.

The variance analysis results showed that the duration of blanching had no significant effect, while the concentration of citric acid had a very significant effect. This is because the duration of blanching has a short time span, i.e. only 5 minutes. The higher the concentration of citric acid, the higher the antioxidant activity for citric acid is a preservative which can be used to increase antioxidant activity. The interaction of the two treatments was very significantly different. The average test results of antioxidant activity of melinjo peel herbal tea can be seen in Table 1.

Table 1. Antioxidant Activity

Citric Acid Concentration (%)	Blanching Duration (minutes)	Antioxidant activity (%)
0	5	35.32 ^a
	10	32.37 ^{bc}
0.05	5	38.95 ^{ab}
	10	33.70 ^c
0.1	5	40.14 ^d
	10	48.47 ^d

Note: Figures followed by the same letters indicate 'not significantly different' with Duncan's test 5%.

3.2 Total Phenol

Phenol is a polar compound with the highest solubility in polar solvents. Phenolic compounds have antioxidant activity because they are able to donate H atoms from hydroxyl groups to radical compounds. Phenolic compounds include a variety of compounds derived from plants that have the same characteristic, i.e. an aromatic ring containing one or two OH- groups. Phenolic compounds in nature are very wide, diverse in structures, and easily found in all plants, leaves, flowers, and fruits. Thousands of natural phenolic compounds which have been known in terms of their structures include flavonoids, simple monocyclic phenols, phenyl propanoids, polyphenols (lignin, melanin, tannin), and phenolic quinones (Rohyami, 2008).

The results of variance analysis showed that the duration of blanching had a significant effect and the concentration of citric acid had a significant effect with a downward trend. The interaction of the two treatments had a significant effect. The total phenol value of melinjo peel herbal tea can be seen in Table 2. The highest total phenol was obtained from blanching treatment for 5 minutes and a citric acid concentration of 0% (2.51 mg/g).

Table 2. Total Phenol (mg of gallic acid/ 100g)

Citric Acid Concentration (%)	Blanching Duration (minutes)	Total Phenol (mg/ 100 g)
0	5	2.51 ^a
	10	2.41 ^d
0.05	5	2.19 ^{ab}
	10	2.15 ^c
0.1	5	2.08 ^{bc}
	10	2.02 ^{cd}

Note: Figures followed by the same letters indicate 'not significantly different' with Duncan's test 5%.

3.3 Sensory Test

The sensory test of melinjo peel herbal tea was performed using the hedonic method and the test of color quality, taste and aroma parameters. The analysis results of quality parameter sensory test towards the color of melinjo peel tea can be seen in Table 3. The results of variance (ANOVA) analysis indicated that the variation of blanching duration significantly affected the color of melinjo peel tea while the treatment of citric acid concentration had no significant effect. The interaction between the two treatments had a significant effect on the color quality of melinjo peel tea. The color of brewed melinjo peel tea was recorded from 2.07 to 3.97 quality scale (i.e. from clear yellow to almost solid yellow). Blanching is a preliminary process of food for further processing and can improve the color and texture of food ingredients for it can activate the polyphenol oxidase enzyme.

The blanching process is not only intended for preservation methods but as a preliminary treatment which is generally conducted between raw material preparation and final treatment such as heat sterilization, drying, and cooling. Blanching method is performed by soaking a material in hot water (boiling) or in steam (steam blanching). Factors that influence the duration of blanching include the type of fruit and vegetables, size and unit, blanching temperature, and heating method (Winarno, 1991).

Table 3. Parameters of Melinjo Peel Tea Color Quality

Citric Acid Concentration (%)	Blanching Duration (minutes)	Color Quality
0	5	2.07 ^{ab}
	10	3.97 ^{ef}
0.05	5	2.97 ^{bc}
	10	3.97 ^{de}
0.1	5	3.40 ^{bcd}
	10	3.28 ^a

Note: Figures followed by the same letters indicate 'not significantly different' with Duncan's test 5%.

The mean sensory test results of the quality parameters for the taste of melinjo peel tea can be seen in Table 4. The results of variance (ANOVA) analysis showed that the variation of blanching duration significantly affected the taste of melinjo peel herbal tea while the treatment of citric acid concentration had no significant effect. The interaction of the two treatments significantly affected the quality of melinjo peel tea taste. The blanching process can improve the quality of melinjo peel tea taste compared to the previous studies that suggested the taste of melinjo peel tea is still very typical of melinjo peel. Thus, the results of this study can reduce the typical taste of melinjo peel with a quality value of 2 to 3, which means the taste of the tea ranges from not typical to slightly typical melinjo peel.

Table 4. Parameters of Melinjo Peel Tea Taste Quality

Citric Acid Concentration (%)	Blanching Duration (minutes)	Taste Quality
0	5	2.98 ^{ab}
	10	2.12 ^{ef}
0.05	5	2.78 ^{bc}
	10	2.42 ^{de}
0.1	5	2.78 ^{bcd}
	10	3.02 ^a

Note: Figures followed by the same letters indicate 'not significantly different' with Duncan's test 5%.

The mean sensory test results of the quality parameters for the melinjo peel herbal tea aroma can be seen in Table 5. The results of variance (ANOVA) analysis showed that the variation of blanching duration significantly affected the aroma of melinjo peel herbal tea while the treatment of citric acid concentration had no significant effect. The interaction of the two treatments had no significant effect on the aroma quality of melinjo peel tea. The aroma of the melinjo peel tea was recorded on a quality scale of 2.60 to 3.43, which means the aroma ranges from not typical to slightly typical melinjo peel. Fatah [14] explained that blanching aims to remove unpleasant odors, help remove tannin compounds, strengthen tissue so that the shape or texture of the fruit remains stable despite various processing processes, and activate enzymes in the fruit.

Table 5. Parameters of Melinjo Peel Tea Aroma Quality

Citric Acid Concentration (%)	Blanching Duration (minutes)	Aroma Quality
0	5	3.43 ^{ab}
	10	2.60 ^{def}
0.05	5	2.92 ^{bc}
	10	2.68 ^{bcde}
0.1	5	2.90 ^{bcd}
	10	2.73 ^a

Note: Figures followed by the same letters indicate 'not significantly different' with Duncan's test 5%.

The sensory test results of melinjo peel herbal tea with the hedonic method can be seen in Figure 1. Based on the data from Figure 1, the most liked color, taste, and aroma of brewed melinjo peel tea are those made by blanching the peel for 5 minutes with a citric acid concentration of 0%. When being correlated with the test results of the brewed melinjo peel tea aroma quality attributes, the best treatment to produce tea with an aroma not typical of melinjo peel is blanching the peel for 5 minutes with a citric acid concentration of 0% with a quality value of 2.67. The study of melinjo peel herbal tea with blanching treatment and citric acid concentration can reduce the typical aroma of melinjo peel, which is a quality scale of 3.43, and enhance the taste of melinjo peel herbal tea with a slightly typical taste of melinjo peel with a quality scale of 2.02 to 3.02.

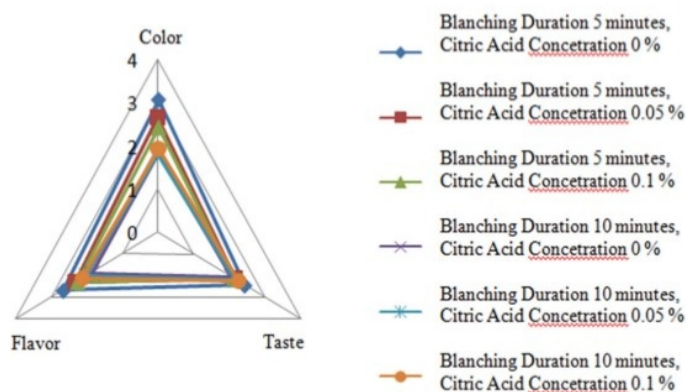


Figure 1. Results of Melinjo Peel Tea Hedonic Test

4. Conclusions

The melinjo peel tea has the potential to serve as herbal tea due to its antioxidant activity and bioactive compounds, i.e. total phenol. Melinjo peel herbal tea contains a total phenol of 2.02 - 2.52 mg/ 100g with the antioxidant activity of 32.47 - 48.47%. The brewed water of melinjo peel tea which is liked and accepted by panelists is that made by blanching the peel for 5 minutes with a citric acid concentration of 0%. The most-liked brewed melinjo peel herbal tea has the following characteristics: clear yellow, slight taste of melinjo peel, and slight aroma of the typical melinjo peel.

Acknowledgments

We would like to express our gratitude to the State Polytechnic of Jember, in particular, the Center for Research and Community Service (P3M) that has organized and funded this study through the PNBPFund Sources Research Program in 2018.

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