Effect of withering time and chopping size on properties of pucuk merah (Syzygium oleana) herbal tea

S S Yuwono and D R Faustina

Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia

E-mail: sdmintos@ub.ac.id

Abstract. Pucuk Merah trees (Syzygium oleana) are ornamental plants whose leaf buds are red. These leaves contain phenol and antioxidant compounds that have potential to be processed into herbal tea. Aim of this research was to find out effect of withering time and size of chopping on the properties and antioxidant activity of pucuk merah herbal tea. The study was conducted using Factorial Randomized Block Design with two factors. Factor I was withering time (4 hours, 8 hours, 12 hours). Factor 2 was the size of chopping (not chopped, 2 cm chopped, 1 cm chopped). The results showed a significant interaction (α = 0.05) between the two factors on water content, antioxidant activity IC50, redness (a*) and color. The best treatment was obtained at 12 hours withering time and 2 cm chopped size. The herbal tea had a water content of 2.67%, antioxidant activity of IC50 48.54 ppm, tannin content of 67.49 mg/g, flavonoid of 84.58 mg/g, total phenol of 134.37 mg / g, lightness (L*) 36.35, redness (* a) 0.99, yellowness (b*) 10.28. Sensory evaluation indicated the product was included in the preferred category by panelist. Compared to green tea from Camelia sinensis, this pucuk merah tea has better antioxidant activity. The antioxidant content of green tea is IC50 is 66.75 ppm.

1. Introduction
Pucuk merah (Syzygium oleana) is one of the ornamental plants that are popular in Indonesia because they have young red leaves which will eventually turn green. Pucuk merah leaves are rich in phenols, flavonoids, antioxidants and betulinic acid [1]. In general the Syzygium genus contains secondary metabolites in the form of flavonoids, alkaloids, tannins, and terpenoids in the leaves [2]. The characteristic of Syzygium leaves is that when squeezed it will produce a fragrance which is like that of Cinnamon [3]. The content of phenol compounds and antioxidants as well as the characteristic of the aroma makes the leaves of pucuk merah potentially processed into herbal tea.

The problem faced in making pucuk merah herbal tea is how to process leaves to get tea with taste and aroma that consumers like but still contain high phenolic compounds and antioxidants. According to [4], some chemical changes that occur during the withering process are inactivation of polyphenol oxidase enzymes, changes in chlorophyll and protein to amino acids, and carotenoid oxidation which can produce aroma volatile substances (unsaturated aldehydes and ketones). In addition, to create the taste and distinctive aroma of tea, it is necessary to treat it after the inhalation in the form of leaf leafling. Cracks can help the formation of a particular flavor and aroma in tea because it can reduce the size of the leaves to expand the evaporation area and so volatile oil is more volatile [5]. This study aims to obtain information about the effect of withering time and cutting on the physical, chemical,
and organoleptic properties of red shoot herbal tea so that the red shoot herbal tea products can be accepted by the community.

In addition, to create the distinctive flavor and aroma of tea, it is necessary to treat the form of chopping and withering the leaves. Chopping can facilitate in forming a certain taste and aroma in tea. Chopping can reduce the size of the leaf to expand the evaporation area so that the essential oil is easier to evaporate [5]. The aim of this study was to analyze the effect of withering time and size of chopping on the physical, chemical and sensory properties of pucuk merah tea, and to obtain the best treatment.

2. Materials and Methods
2.1. Materials
The material used for making pucuk merah herbal tea is the fresh red colored Syzygium oleana leaf obtained in the environment around Universitas Brawijaya. The chemicals used for analysis are all pro analysis (pa).

2.2. Experimental design
The study was conducted using Factorial Randomized Block Design (RAKF) with 2 factors. Factor I is withering time (4 hours, 8 hours, 12 hours). Factor II is chopping size (not chopped, 2 cm chopped, 1 cm chopped). The study was carried out 3 times repetitions so that 27 experimental units were obtained. The data obtained were analyzed using variance analysis (ANOVA) with α = 0.05 using Minitab 17.. If the test results show significant differences, the Least Significant Difference (LSD) or the Duncan Multiple Range Test (DMRT) will be carried out to know the differences. The best treatment is determined using the Zeleny Multiple Attribute method [6].

2.3. Experimental procedures
The study began by sorting and washing the red shoot leaves. After that the leaves are spread in the shade for 4 hours, 6 hours and 12 hours. The pucuk merah leaves that have withered are chopped according to treatment that is not chopped, chopped 2 cm, and chopped 1 cm. Red shoot leaves are dried using a cabinet dryer at 60 °C for 3 hours. Parameters measured included tannin, flavonoids, total phenol, antioxidant activity of IC50, color, taste and odor.

2.4. Methods of analysis
The parameters observed were analyzed using the following methods: color [7], water [8], activity of antioxidant IC50 [9], total Phenol [10], flavonoid levels [11], tannins [12], sensory [13], and best treatment [6].

3. Results and Discussion
3.1. Raw material
Fresh pucuk merah leaves were analyzed by chemical parameters including water content, total phenol, flavonoid levels, tannin content and antioxidant activity. Data from the analysis of the raw material of red shoot leaves is shown in Table 1.

| Table 1. Chemical properties of pucuk merah leaves. |
| Parameters          | Content     | Anggraeni [14] |
|---------------------|-------------|---------------|
| Water (%)           | 74 ± 0.53   | -             |
| Tannin (mg/g)       | 55.98 ± 3.29| -             |
| Flavonoid (mg/g)    | 69.43 ± 3.29| -             |
| Phenol (mg/g)       | 131.32 ± 5.18| 122.1         |
| Antioxidant activity (%) | 69.53 ± 1.26 | 65.65         |
Table 1 shows that the leaves of pucuk merah contain tannins, flavonoids, phenols and have the antioxidant activities. The results indicate that phenol and antioxidants activity are slightly higher than the results from literature. This difference can be made possible due to various internal factors of plants such as age, and variety of plants that are cultivated, and external factors such as soil conditions, weather and others.

3.2. Chemical properties

Table 2 shows that there is a significant interaction between the factor of the whitering time and the size of chopping on the water content and antioxidant activity of the tea. Whereas withering time factor or size of chopping factor has a significant effect on tannins, flavonoids and phenols. The longer the withering time or the smaller the size of chopping, the lower the tannin, flavonoid, and phenol affect on decreasing the antioxidant activity of IC50. This condition is caused by an increased enzymatic oxidation reaction. At the time of withering, the concentration of the solution in the vacuole will rise and trigger the oxidation reaction by catechol oxidase which causes the levels of catechins (tannins) which are part of the flavonoids and the phenol group is reduced [15]. Chopping causes the enzyme catechol oxidase which is located in the cytoplasm of tea leaves to meet and mix with the substrate, namely catechins that are inside the cell vacuole [16]. From the oxidation reaction, the epigalokatekingalat in the tea leaves will be converted to orthoquinone. In the presence of hydrogen ions, orthoquinone is condensed to form bisflavanols and then theaflavins and finally become tearubigins.

Table 2. Chemical properties of pucuk merah herbal tea.

| Whitering time (hours) | Chopping Size | Water (%) | Tanin (mg/g) | Flavonoid (mg/g) | Phenol (mg/g) | Antioxidant Activity IC50 (ppm) |
|-----------------------|---------------|-----------|--------------|------------------|--------------|-------------------------------|
| 4                     | Not chopped   | 4.45 d    | 100.76       | 94.41            | 185.24       | 32.19 a                       |
| 4                     | Chopped 2 cm  | 4.16 d    | 74.36        | 90.80            | 183.42       | 42.48 c                       |
| 4                     | Chopped 1 cm  | 3.36 c    | 51.56        | 87.46            | 167.61       | 51.30 d                       |
| 8                     | Not chopped   | 3.70 c    | 91.75        | 90.49            | 170.76       | 37.18 b                       |
| 8                     | Chopped 2 cm  | 3.42 c    | 67.88        | 86.02            | 166.33       | 47.53 d                       |
| 8                     | Chopped 1 cm  | 2.97 b    | 45.50        | 78.01            | 161.81       | 52.50 de                      |
| 12                    | Not chopped   | 2.87 b    | 86.73        | 89.62            | 141.14       | 45.84 cd                      |
| 12                    | Chopped 2 cm  | 2.67 ab   | 67.49        | 84.59            | 134.37       | 48.54 d                       |
| 12                    | Chopped 1 cm  | 2.41 a    | 44.00        | 75.96            | 125.71       | 57.04 e                       |

Note: means with the same letter are not significantly different according to DMRT at 5% level.

In determining antioxidant activity, the whitering time factor is more dominant in 4 hours compared to 8 and 12 hours. Withering for 4 hours is allegedly not enough to inhibit the oxidation reaction because there are still many polyphenol oxidase enzymes that are active and oxidize phenol so that the catechin level is still relatively high.

3.3. Physical properties

Based on Table 3, it is known that the longer the time of whitering and the smaller the size of the chopping, the lower the level of brightness. It means the color of water steeping tea gets darker and the redness and yellowish will be higher. This phenomenon is due to changes in catechin compounds which are oxidized by the enzyme catechol oxidase. The catechins are converted to teaflavins and condensed to tea-pigments during the oxidation process. Theaflavins play a role in determining the brightness of the color of tea steeping water which is bringing a reddish yellow color. Whereas tearubigin determines brownish red color of tea steeping water. The smaller size in chopping the more theaflavins and tearubigins are formed due to the oxidation process.
Table 3. Colour of steeping pucuk merah tea.

| Whitening time (hours) | Chopping Size | Lightness (L*) | Redness (a*) | Yellowness (b*) |
|------------------------|---------------|----------------|--------------|----------------|
| 4                      | Not chopped   | 55.20          | -1.23 a      | 5.18           |
| 4                      | Chopped 2 cm  | 46.39          | 0.41 d       | 7.71           |
| 4                      | Chopped 1 cm  | 39.67          | 0.94 e       | 10.35          |
| 8                      | Not chopped   | 48.33          | -0.81 b      | 6.83           |
| 8                      | Chopped 2 cm  | 42.57          | 0.52 d       | 9.20           |
| 8                      | Chopped 1 cm  | 36.06          | 1.20 f       | 11.34          |
| 12                     | Not chopped   | 41.74          | 0.26 c       | 7.05           |
| 12                     | Chopped 2 cm  | 36.35          | 0.99 e       | 10.28          |
| 12                     | Chopped 1 cm  | 32.81          | 1.71 g       | 12.96          |

The color of the pucuk merah tea may also be caused by changes in chlorophyll and presence of anthocyanins. Oxidation will change chlorophyll into black feofoitin [17]. The pucuk merah leaves contain anthocyanins by 19.2% [14].

3.4. Sensory properties

Based on Table 4 it can be seen that the most favored pucuk merah tea based on the preference of color, aroma and taste is tea from treatment of whitering time of 12 hours and chopping size of 1 cm. This treatment, enzymatic oxidation may occur at most compared to other treatments. This process results in discoloration, and the release of volatile compounds. The aroma-forming compounds of tea consist mainly of essential oils that are volatile and are easily oxidized to produce tea aroma. Besides that, the oxidation of the catechins that produce orthoquinone will bind to amino acids and form aromatic compounds. The catechin oxidation reaction will also form the aflavin and tearubigin compounds that carry the astringent taste.

Table 4. Sensory properties of steeping pucuk merah tea.

| Whitening time (hours) | Chopping Size | Colour | Aroma | Taste |
|------------------------|---------------|--------|-------|-------|
| 4                      | Not chopped   | 2.48 a | 2.95  | 2.50  |
| 4                      | Chopped 2 cm  | 3.75 de| 3.10  | 2.70  |
| 4                      | Chopped 1 cm  | 3.83 e | 3.21  | 2.93  |
| 8                      | Not chopped   | 2.33 a | 3.01  | 2.78  |
| 8                      | Chopped 2 cm  | 2.97 b | 3.18  | 2.98  |
| 8                      | Chopped 1 cm  | 3.88 e | 3.30  | 3.17  |
| 12                     | Not chopped   | 3.32 c | 3.11  | 2.85  |
| 12                     | Chopped 2 cm  | 3.92 ef| 3.21  | 3.03  |
| 12                     | Chopped 1 cm  | 4.12 f | 3.35  | 3.38  |

Note: Means with the same letter are not significantly different according to DMRT at 5% level

3.5. The best treatment

In determining the best treatment, the parameters which are the minimum values are water content and IC50 antioxidant activity, while the parameters which are the maximum values are tannin levels, flavonoid levels, total phenols, colors, and sensory attributes namely color, aroma and taste. The best treatment was obtained at 12 hours of whitering time and 2 cm of chopping size. The physical properties of tea resulted from the best treatment are brightness (L*) of 36.35, redness (a*) of 0.99, yellowish (b*) of 10.28. While the preferences of color, aroma and taste are 4.12, 3.35 and 3.38 respectively. The chemical properties of pucuk merah tea compared to green tea is showed in Table 5.
significant interactions, Al unstability elucidation and, Hasim Jurusan Teknologi Pertanian. Compared to green tea from, Ghafar stillation by microwave heating. The preferences of, ad a water content of 2.67%, -1070

| Parameters       | Green Tea   | Pucuk merah Tea |
|------------------|-------------|-----------------|
| Tanin            | 43.96 mg/g  | 67.49 mg/g      |
| Flavonoid        | 25.42 mg/g  | 84.58 mg/g      |
| Phenol           | 85.67 mg/g  | 134.37 mg/g     |
| Antioxidant IC<sub>50</sub> | 63.45 ppm  | 48.54 ppm       |

Based on Table 5, it can be seen that the pucuk merah tea contains a better tannin, flavonoid, total phenol and antioxidant activity IC50 than that of green tea from Camelia sinensis. The data shows that pucuk merah tea has great potential to become herbal tea because it has a high content of tannins, flavonoids and phenols. Those compounds have very strong antioxidant activity.

4. Conclusion
Withering time and chopping size had a significant interactions on water content, IC50 antioxidant activity, redness (* a) and sensory parameters of pucuk merah tea. Withering time factor or chopping size factor had a significant effect (α = 0.05) on tannin, flavonoid, total phenol, brightness (L*) and yellowish (* b) the pucuk merah tea. The best treatment was obtained at a treatment of whitening time of 12 hours and chopping size of 2 cm. The pucuk merah tea had a water content of 2.67%, antioxidant activity of IC50 48.54 ppm, tannin content of 67.49 mg/g, flavonoid of 84.58 mg/g, total phenol of 134.37 mg / g, lightness (L*) 36.35, redness (* a) 0.99, yellowness (b*) 10.28. Based on sensory evaluation, the product was included in the preferred category by panelist. The preferences of color, aroma and taste are 4.12, 3.35 and 3.38 respectively. Compared to green tea from Camelia sinensis, this pucuk merah tea has better antioxidant activity.

References
[1] Aisha A, Ismail Z, Abu-Salah K M, Siddiqui J M, Ghafar G, Abdul Majid A M S 2013 Syzygium campanulatum Korth methanolic extract inhibits angiogenesis and tumor growth in nude mice. BMC Complement. Altern. Med. 13 1-11.
[2] Mahmood T, Akhtar N, Khan B A 2010 The morphology, characteristic, and medicinal properties of Camelia sinensis J. Med. Plants Res. 4 2028-2033.
[3] Memon A H, Ismail Z, Aiaha F A, Al-Suade F S R, Hamil M S R, Hasim S, Saeed M A, Laghari M, Majid A M S 2014 Isolation characterization crystal structure elucidation and anticancer study of dimethyl cardamonin isolated from Syzygium campanulatum Korth. Evid.-Based Complement. Altern. Med. 1-11.
[4] Cabrera C, Artacho R, Gimenez R 2006 Beneficial effect of green tea – a review J. Am. Col. Nutrition 25 79-99.
[5] Feriyyanto Y E, Patar J S, Mahfud, Pantjawarni P 2013 Extraction of essential oil of Cymbopogon winterianus using steam and water distillation by microwave heating. Jurusan Teknik Kimia. Institut Teknologi Sepuluh Nopember (ITS). Surabaya.[In Indonesian]
[6] Zeleny M 1982 Multiple Criteria Decision-Making McGraw-Hill New York USA
[7] Yuwono S S, Susanto T 1998 Physical food properties determination Jurusan Teknologi Pertanian. Universitas Brawijaya. Malang. [In Indonesian]
[8] AOAC 1995 Official Methods of Analysis of the Association of Official Analytical Chemist 16th Edition Publisher AOAC Washington DC USA.
[9] Khalaf N A, Shakya A K, Othman A, Agbar Z, Farah H 2008 Antioxidant activity of some common plants Turk. J. Biol. 32 51-55.
[10] Sharma K K, Assefa E Y, Nile S, Lee S H, Park E T 2014 Temperature-dependent studies on the total phenolics, flavonoids, antioxodant activities, and sugar content in six varieties J. Food Drug Anal. 23 243-252.

[11] Li W, Dai R J, Yu Y H, Li L, Wu C M., Luan W W, Meng W W, Zhang X S, Deng Y L 2007 Antithyperglicemic effect of Cephalotaxus sinensis leaves and GLUT4 translocation facilitating activity of its flavonoid constituens Biol. Pharm. Bull. 30 1123-1129.

[12] Pelozo, M I D G, Mara L C C, Joao C P D M 2008 Spectrofotometric determination of tannins and caffeine in preparations from Paullinia cupana var. Sornilis Braz. Archives Biol. Technol. 51 447-451.

[13] Watts B M, Ylikmaki G L, Jeffery L E, Elias L H 1989 Basic Sensory Methods for Food Evaluation International Development Research Centre Ottawa USA.

[14] Anggraeni T 2017 Antioxidant activity of Syzygium olena Pak. J. Nutr. 16 605-611.

[15] Omiadze N T, Mchedlishvili N I, Rodriguez_Lopez J N, Abutidze M O, Sadunishvili T A, Fruidze N G 2014 Biochemical processes at the stage of withering during black tea production Appl. Biochem. Microbiol. 50 394-397.

[16] Wan X, Li D, Zhang Z 2009 Green tea and black tea manufacturing and consumption. In: Ho C T, Lin J K, Shahidi F (eds.) Tea and Product Chemistry and Health-Promoting Properties CRC Press Boca Raton USA.

[17] Towaha B J 2013 Chemical Content of Tea. Warta Penelitian dan Pengembangan Tanaman Industri. [In Indonesia]