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THE EFFECT OF DRYING TEMPERATURE ON THE ANTIOXIDANT ACTIVITY OF BLACK MULBERRY LEAF TEA (*Morus nigra*)

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ABSTRACT

This research is aimed at studying the effect of different drying temperatures on the characteristics of the brewed herbal tea from the mulberry leaves. The benefits of this research is to; provide new information about the processing of mulberry leaves, know the characteristics of brewed herbal tea from mulberry leaves, measure the antioxidant activity of brewed herbal tea from mulberry leaves, develop food diversification regarding herbal tea, increase the economic value of mulberry leaves and it is expected to become a branch of industry of herbal tea made from mulberry leaves. The method used is a randomized block design with a pattern of one factor drying temperature consisting of three (3) levels: at 40° C (s_1), 50° C (s_2), and 60° C (s_3). The results showed that the drying temperature influences the color organoleptic responses and antioxidant activity. The product processed in the drying temperature at 40° C is the best product because it has the value of IC_{50} of 89.43 ppm and belongs to a class of strong antioxidant potential.

Keywords: black mulberry leaves, drying temperature, antioxidant.

INTRODUCTION

Health is a precious thing that needs to be maintained and cared for. The increasing price of modern medicine encourages consumers to try other alternatives to maintain a good health. This problem leads to a new trend in the world of health, namely "back to nature". One of the effects of this trend is seen from the current consumer trend.

The herbal tea is a general term used for a beverage that does not originate from the tea plant, *Camellia sinensis*. The herbal tea is safer for consumption because it does not contain an alkaloid that can harm health as caffeine does. The herbal tea is made from flowers, seeds, leaves or roots of a variety of crops[8]. The herbal tea is consumed as beverage tea or brewed and served as regular tea. *Morus nigra* is the Latin name of the mulberry plant or black mulberry. This plant has a high economic value because the leaves are the main fodder for silkworms. It has been reported that a phenol derivative compounds are the main compounds of the genus *Morus,* namely *stilbene* group, 2-arilbenzofuran and flavonoids[6].

Mulberry leaves are commonly used as the fodder for silkworms. However, due to their rapid growth which is approximately every 30-60 days for the harvest, the mulberry leaves can be used as processed food, namely herbal tea.

In Indonesia, there are about 100 more species or varieties of mulberry, but there are only 6 of them which are well-known, namely *Morus cathayana, Morus alba, Morus multicaulis, Morus nigra, Morus asustralis* and *Morus macruora*. West Java province is one of the producing areas of mulberry plants, both leaves, and fruits.

Herbal tea of mulberry leaf has an antioxidant effect and can address the problem of diabetes mellitus[13]. Mulberry leaf extract also owns and produces a good anti-obesity substance because of its inhibitory effect on melanin bio-synthesis. There are many other phenolic compounds contained and identified in mulberry leaves. Flavonoid (quercetin and kaempferol) and its derivatives, quercetin 3-(6-malonyglucoside), quercetin-3-rutinoside, quercetin 3-glucoside and kaempferol-3-(6-acetylglucoside),

are the phenol contained in mulberry leaves which have an effect on health. Traditionally, mulberry leaves and mulberry leaf tea are used to treat insomnia because they have a therapeutic effect of melatonin.

EXPERIMENTAL

Materials Used

The materials used in the manufacture of herbal tea from mulberry leaves are the fresh mulberry leaves obtained from Maribaya area, Lembang, West Java for the variety *nigra* (V1), and from the Institution of Forest Village Community Sukamanah, Pangalengan for the variety *khunpai* (V2) and *cathayana* (V3).
The materials used for the analysis process of herbal tea making from mulberry leaves are water, 95% ethanol, and Diphenylpicrylhydrazil (DPPH) solution.

The processing methods are carried out in the following stages:

The Stage of raw material preparation

The material preparation was done through trimming, which is disposing of the unused parts of mulberry leaves which had been plucked such as the end of the leaf veins. Mulberry leaves that had been separated from one another were then weighted according to the needs. The laundering is an important stage in the processing of the raw material to remove the impurities on the leaves. The washing of mulberry leaf was carried out with the clean flowing water. After the washing was done, the draining was then performed to dry the leaves. This draining was done for 10 minutes.

Drying phase

The withering is aimed at softening the tissue of the leaves, thus simplifying the process of rolling. The withering was done by arranging mulberry leaves on the tray and putting them in the equipment with controlled humidity (65%). The time for withering was 4 minutes. Next was the rolling process aimed at bruising the leaves and breaking the cell wall so that the liquid comes out on the surface of the leaves evenly. This process was done by rolling the leaves by hand.
The drying was done to reduce the amount of water in the materials and to extend the period of the product when it is stored. The tool used in the drying process is a tunnel dryer for 60 minutes at a drying temperature of 40^{o} C, 50^{o} C, and 60^{o} C.
The products that had been produced were then tested in the organoleptic test to test the characteristics of the herbal tea steeping. To make this steeping is by brewing the 2 grams herbal tea of mulberry leaves with 150 mL of hot water (90° C) for 6 minutes.

RESULTS AND DISCUSSION

The color of Herbal Tea of Mulberry Leaves

The color is an organoleptic attribute that is important for food, both which are not processed and processed foods. Color plays an important role in the reception of food. The color can also give clue about the chemical changes in food, such as browning and caramelizing. The color is the nature of appearance characterized by the distribution of the light spectrum. Therefore, the color can be seen or assessed only if there is light. The color has enormous power in determining opinion on a product, especially food[9].
Based on the analysis of the organoleptic test in terms of the steeping of herbal tea of mulberry leaves, it is known that the drying temperature significantly affected the color of the steeping, while the method of processing and the interaction of both factors had no significant effect. Brewing was done by adding 150 mL of water with a temperature of 90° C in a beaker containing 2 grams of mulberry leaves herbal tea, kept for 6 minutes[18]. Herbal tea steeping colors are usually influenced by the materials or substances contained therein.
The research results showed that black mulberry leaves contain a number of chlorophyll, including the old maturity level of 2.64 mg/g, the medium maturity level of 4.15 mg/g, and the young leaves of 3.32

mg/g[20]. Chlorophyll is volatile and susceptible to the degradation processes to be the derivative molecules[1]. The chlorophyll degradation process can occur because of temperature and oxygen.
The color of steeping of herbal tea mulberry leaves relates to the components contained therein. The research results showed that the extract of dry mulberry leaf nigra varieties contains a total phenol of 24.37 mg/g and total flavonoids of 30 mg/g[16].

Table-1: Effect of Drying Temperature on Color of Mulberry Leaves

Drying Temperatures	average value	Error term 5%
S_1 (40° C)	of 3.45 ± 0:51	a
S_3 (60° C)	of 3.73 ± 0:40	b
S_2 (50° C)	4.02 ± 0:42	c

Description: the same letter in the column shows no significant difference in the level of 5%

Flavonoids are the largest phenolic compounds comprising the C6-C3-C6 and are often found in various kinds of plants in the form of glycosides or cluster of fused sugar in one or more phenolic hydroxyl group[12]. Flavonoids are the secondary metabolites synthesized from the pyruvic acid by amino acid metabolism. Flavonoids are the phenolic compounds so the color changes when they have been added with the base or ammonia. There are about 10 types of flavonoids, namely anthocyanin, proanthocyanidin, flavonols, flavones, glikoflavon, biflavonil, khalkon, Auron, flavanones and isoflavones.[10]
The name of flavonoid derives from the Latin word that refers to the yellow color and most flavonoids are yellow. Flavonoids are often found in the form of co-pigments and pigment. Flavonoids are a class of organic pigments that do not contain molecular nitrogen. The combination of various kinds of pigments forms the pigmentation of the leaves, flowers, fruits and seeds of plants[10].
Flavonoids are the soluble polar compounds in polar solvents. This is evidenced by the dissolution of flavonoid compounds by using methanol. Flavonoids are generally the water soluble component so they can be extracted with a polar solvent and left in the layer of *aqueous*[10].
Phenolic compounds consist of tannins or catechins and flavonols. Catechins are the most important compounds in the tea leaves[19]. The change in the activity of catechins is always associated with the nature of steeping tea, namely the taste, color, and aroma.
Fresh mulberry leaves contain the flavin, tannin, and caffeine[4]. The tannins are an important component in tea in determining the flavor and color of tea steeping[17]. In addition, the mulberry leaf tea contains flavonoids which have an effect on health[13].
In addition to having a health effect, flavonoids in the plant act as a pigment. Flavonoids which are colorless or yellow (antoksantin) are soluble in water and resistant to heat. Antoksantin contains a lot in leaf cells. Antoksantin is different from the yellow or orange (carotenoids) pigment which is soluble in lipids[5].
During the drying process, the water level decreases. The decline in water level can affect the concentration of cell fluid so it attaches to the surface of the leaves. Moreover, with the drying, the process of enzymatic oxidation will be inactive. Enzymes need water be active. The moisture reduction results in the decreasing enzyme activity but the concentration of enzyme and substrate occurs simultaneously[11].
The mulberry leaf tea which is ready for brewing contains the inactive enzymes, coming out of the cell so that when it is brewed the enzymes will be active again and extracted with steeping water. The higher the drying temperature is, the brighter the color of steeping becomes (yellowish green). The product dried at a lower temperature tends to produce a dark steeping color. This is because the enzyme activity which occurs in the product. The mulberry leaf tea dried at a temperature of 40° C produces the high moisture in the material, and can provide a reaction opportunity to the enzyme, one of which is polifenolase. Meanwhile, mulberry tea dried at a higher temperature (50° C and 60° C) produces a product with a lower moisture, so that the enzyme in the material can be inactivated quicker due to the small amount of water.

The brewing process is a process of extraction or separation of one or more components. The brewing is the process of liquid extraction, meaning the separation of solid compounds (flavonoids, tannins) by using water as the solvent. One of the factors that influences the brewing process is the temperature of the water or the brewing condition and brewing period. The higher the water temperature or brewing process is, the higher the ability of water to extract the chemical constituents contained in the tea becomes, and so the brewing period. This would affect the level of dissolved material, color intensity and flavor or aroma.

Aroma Response of Mulberry Leaf Tea

The aroma is one parameter in determining the quality of a food product. A distinctive aroma can be perceived by the sense of smell, depending on the constituent materials and ingredients added to that food. The aroma in food material is caused by volatile components. Besides, the testing of aroma in the food industry is considered important because it can be used as a parameter for the consumer to accept or reject the product. The aroma can be used as an indicator of the product[2].

Table-2: The Effect of Drying Temperature on Aroma of Mulberry Leaf Tea

Drying Temperatures	average value	Error term 5%
S_1 (40° C)	of 3.37 ± 0.42	a
S_3 (60° C)	of 3.71 ± 0.42	a
S_2 (50° C)	3:58 ± 0.30	a

Description: the same letter in the column showed no significant difference in the level of 5%

According to Table-2 above, the steeping aroma of mulberry tea leaf is not significantly different. This is because the intensity of the aroma produced by product is very small. In addition to it, the drying process causes the aroma compound contained in the mulberry tea steeping to have a lower intensity. This situation causes the aroma of mulberry leaf tea produced to be the same between the treatments. When viewed from the average value, the panelists tend to have criteria 'dislike' until 'like'.

The aroma of mulberry leaf tea is produced by volatile compounds contained in foodstuffs. The aroma can occur naturally or because of processing[14]. Salunkhe (1976) stated that the aroma of food is influenced by the type, degree of ripeness, processing, and storage[14].

The fresh mulberry leaves contain theaflavin, tannins, and caffeine[4]. The aroma of the mulberry leaf tea is caused by the aromatic compounds that easily evaporate, the extracting process of chemical components of herbal tea such as carbohydrates, proteins, cluster reduction of sugar when the tea is brewed, and the oxidation of polyphenol compounds and their derivatives such as catechin to be theaflavins and theaburigin which gives a distinctive aroma[3].

The drying with a temperature variation can also eliminate the volatile compounds. The vanished volatile compounds during the process reduce the aroma produced in the brewing process. In addition, the 6 minutes brewing can provide the opportunity for the aroma compounds to evaporate. So the aroma generated on every steeping of mulberry leaf tea is the same, and when viewed in terms of economic value, the products with Indonesia methods with a drying temperature of 40° C are selected.

Antioxidant Activity Test

The analysis of the antioxidant activity of flavonoids contained in herbal tea is from the mulberry leaf. The test of antioxidants with DPPH damping method was further conducted by measuring the extent of the damping reaction on the free radical of DPPH can take place. The measurements were performed by spectrophotometry by measuring the absorbance of each sample that had been treated with a standard solution of DPPH at a wavelength of (λ) 517[21].

The method used to measure the antioxidant activity of mulberry leaf tea is DPPH method. The purpose of this method is to know the concentration parameter which is equivalent giving 50% effect of antioxidant activity (IC_{50}). DPPH is a free radical that can react with compounds that can donate a hydrogen atom and can be useful for testing the antioxidant activity of certain components in an extract. The observation of the DPPH radical capture can be made by observing the decrease in absorbance. This

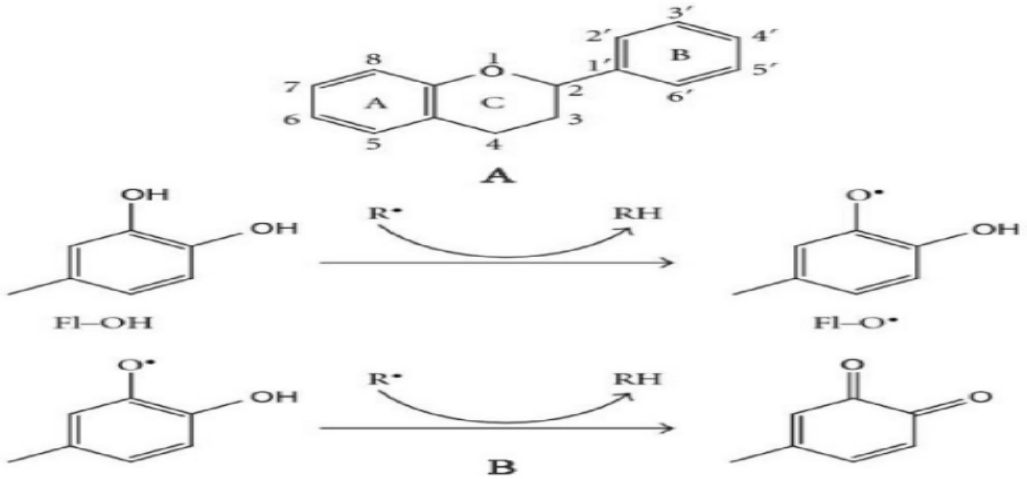
can happen because of the reduction of radicals by antioxidants (AH) or reaction with other radical compounds. To determine the level of color damping as a result of the antioxidant compounds which are capable of reducing the intensity of the purple color of DPPH, the measurement of the color reaction was carried out at a different concentration of extract. The higher the concentration of the extract is, the greater the damping becomes, characterized by the formation of a yellow color. This is because in the high concentration, the compounds contained will increase and cause a greater the antioxidant activity.

Table-3: The Effect of Drying Temperature on Antioxidant Activity of Mulberry Leaf Tea

Drying Temperatures	Average value of IC_{50} (ppm)	Error term 5%
S_2 (40° C)	89.43 ± 37.65	c
S_3 (50° C)	102.26 ± 25.03	a
S_1 (60° C)	395.13 ± 201.80	b

Description: the same letter in the column showed no significant difference in the level of 5%

The activity of free radical damping is usually expressed as inhibition percentage of DPPH, but can also be expressed as the concentration which causes the vanishing DPPH (IC_{50}) activity of 50%. The value of IC_{50} is considered as a good measure of the efficiency of the pure antioxidant compounds or extracts[21].
In the polyphenol compounds, the antioxidant activity is closely related to the structure of the side chains as well as the substitution on the aromatic ring. The ability to react with DPPH free radicals can affect the order of antioxidant strength. The damping activity of free radical compounds on polyphenols is believed to be influenced by the number and position of the phenolic hydrogen in a molecule. Thus, the higher antioxidant activity will be generated in phenolic compounds which have more hydroxyl groups at the core of the flavonoid. These phenolic compounds have the ability to donate hydrogen, so the antioxidant activity of phenolic compounds can be generated in the neutralization of free radicals which initiate the process of oxidation or the reaction termination of the radical chain which occurs[21].
The antioxidant properties of flavonoids derive from the ability to transfer an electron to a free radical compound and also form complexes with metal. Both mechanisms make flavonoids have several effects, including inhibiting the lipid peroxidation, suppressing the tissue damage by free radicals and inhibiting the activity of some enzymes[21].



Scheme-1: Damping of Free Radicals by Flavonoids. (A) Basic Structure of flavonoids. (B) Process of Free Radical Damping by Flavonoids**.**

The drying temperature can affect the value of the IC_{50}. The higher the temperature is, the drier the product produced becomes. The variation of drying temperature used was 40° C, 50° C, and 60° C. At a temperature of 40° C, the herbal tea has a texture that is not too dry, both in Indonesia and Thailand processing methods, so that the water content contained in the material can trigger the mold to grow. It

was estimated that the water content was more than 15%[15]. The mold that grows on the product can utilize the nutrients contained in mulberry leaf tea, resulting in a decrease of the number of nutrients, one of them is polysaccharides or coarse fiber.

The optimum temperature of mold growth is around 25-37°C. The mold has an aerobic property requiring the oxygen to grow. The mold grows in a wide pH range, i.e. from 2 to 8.5. The mold can grow on foods that contain protein, carbohydrates, or fats[15].

A high water content can cause changes in foodstuffs[5]. In addition, the flavonoids contained in the product will be oxidized easily and quickly so when the antioxidant activity is tested, it has a high average value of IC_{50}. Thus, it is classified into the potential that is very weak.

The drying temperature of 50° C on the processing method of Indonesia results in the good texture. In this condition, the herbal tea has enough moisture so in the maceration process of analysis, the main component can be extracted optimally. The average IC_{50} value of this product goes into the strong potential, so it is good to be consumed. While the processing method of Thailand dried at a temperature of 50° C has a fair antioxidant potential.

The last drying temperature is 60° C. The herbal tea processed by the processing method of Indonesia produces a very dry texture. In this condition, the herbal tea that is too dry is hygroscopic which does not have a stable equilibrium moisture content, so that the product will absorb the water from its environment. The absorption of excessive moisture would make the tea wither and wet and have higher water content. In addition, it will cause the tea to be susceptible to mold. The product attacked by the fungi shall affect the value of the $IC_{50,}$ so it is included in the group of fair potential. The processing method of Thailand at a drying temperature of 60° C gives a good result, i.e. has the potential for strong antioxidant activity. When compared with a temperature of 50° C, the resulting texture is drier so it is more resistant to the mold attack.

CONCLUSION

The drying temperature affects the antioxidant activity and response of organoleptic color. Based on the average results of the antioxidant activity, the best products are processed at the drying temperature of 40° C because they have the value of IC50 of 89.43 ± 37.65 ppm and belong to the class of strong antioxidant potential.

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REFERENCES

1. Arrohmah. Studi, Karakteristik Klorofil Pada Daun Sebagai Material Photodetector Organic, Skripsi, Universitas Sebelas Maret Surakarta(2007).
2. B. Kartika, P. Hastuti and W. Supartono. Pedoman Uji Inderawi Bahan Pangan. PAU Pangan dan Gizi Universitas Gajah Mada, Yogyakarta(1988).
3. D. K. Hadi, Proses Pengolahan Teh. http://danang-kurang-kerjaan.blogspot.com/2011/05/proses-pengolahan-teh.html(2011).
4. E. Damayanthi, C.M. Kusharto, R. Suprihatini, and D. Rohdiana, *Media Gizi dan Keluarga*, **32**, 95, (2008).
5. F.G. Winarno, Kimia Pangan dan Gizi,Gramedia Pusaka Utama. Jakarta(1996).
6. Ferlinahayati, Y.M., Syah, L.D. Juliawaty, S.A. Achmad, E.H. Hakim, H.Takayama, I.M. Said and J. Latip, *Z Naturforsch*, **63**, 35 (2008).
7. H. Hadi. Tanaman Murbei, http://id.scribd.com/doc/39834978/TANAMAN-MURBEI#download. (2010).
8. I. G. A. Yudana, Mengenal Ragam dan Manfaat Teh. http://www.indomedia.com/(2004).
9. I. Kartini, Pengaruh Lama pengeringan dan Variasi Perbandingan Formula Terhadap Karakteristik Kerupuk Tiras. Skripsi, Jurusan Teknologi Pangan, Universitas Pasundan, Bandung(2006).

10. J. B. Harborne, Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan. Institut Teknologi Bandung, Bandung(1987).
11. L.H. Afrianti, Teknologi Pengawetan Pangan, Alfabeta, Bandung(2008).
12. M. Sirait, Penuntun Fitokimia dalam Farmasi. Institut Teknologi Bandung, Bandung(2007).
13. P. Pothinuch and S. Tongchitpakdee, *Food Chemistry*, **128**, 415 (2011).
14. R. Saragih, *E-Journal WIDYA Kesehatan Dan Lingkungan*, 1, 46 (2014).
15. S. Fardiaz, Mikrobiologi Pangan,Gramedia Pustaka Utama, Jakarta(1992).
16. S. Iqbal, U. Younas, Sirajuddin, K. W. Chan, R. A. Sarfraz, and M. K. Uddin, *International Journal of Molecular Sciences*, **13**, 6651 (2012).
17. T.R. Muchtadi, F.A. Sugiyono, Ilmu Pengetahuan Bahan Pangan, Alfabeta, Bandung(2010).
18. Thai Agricultural Standard (Tas 3000-2009). Mulberry Tea. The National Bureau of Agricultural Commodity and Food Standards (ACFS) Ministry of Agriculture and Cooperatives. Bangkok. (2009).
19. W. Andamari, Formulasi dan evaluasi mutu minuman fungsional teh hijau jahe selama penyimpanan. Bogor: Fakultas Teknologi Pertanian, Institut Pertanian Bogor. (2005).
20. V. N. Y. Murthy, H.L. Ramesh, G. Lokesh, Munirajappa, B.R.D. Yadav, *International Journal of Pharmaceutical Sciences Review and Research*, **21**, 182 (2013).
21. Yuhernita and Juniarti, *Makara Sains*, **15**, 48 (2011).

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