The changes in the antioxidant activities, total phenol, curcumin and hedonic quality of first and second brewing spiced drinks

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Abstract. This study is aimed to determine changes in antioxidant activity, total phenol, curcumin and hedonic quality of first and second brewing spiced drinks as functional drinks. This study used two treatments which were the first brewing and the second brewing. The basic material used was spiced drink consisted of ginger, turmeric, white turmeric, ginger sand, temulawak, temu mangga, lime, secang, rock sugar, brown sugar and water. The method was done by making spiced drinks with spices in a beaker glass which was brewed for 30 minutes with 1000 ml of boiled aquabidestilate water (100˚C), then filtered to get the first brewing spiced drink (S1). Furthermore, the brewing process was done with the same spices to get the second brewing spiced drink (S2), then testing the parameter of the spiced drink. The parameter tested included antioxidant activity with the DPPH method, total phenol using the Folin-Ciocalteu method and the level of curcumin with High Performance Liquid Chromatography (HPLC). Hedonic quality tested included taste, aroma, color and overall preference. The results of this study show that the first brewing spiced drinks has antioxidant activity 78.642%, total phenol 33.156 ppm and curcumin 46.916 ppm, while the second brewing spiced drinks has antioxidant activity 61.905%, total phenol 17.378 ppm and curcumin 16.184 ppm. Based on these data, it appears that antioxidant activity decreases by 16.737%, total phenol decreases by 15.77 ppm and curcumin decreases by 30.732 ppm. The color, aroma and overall preference of the first brewing spiced drink are more preferably by panelists whereas the taste of the second brewing spiced drink is more preferably by panelists compared to the taste of the first brewing spiced drink.

1. Introduction
Spiced drink is a ginger juice which has been modified by adding turmeric, white turmeric, ginger sand, temulawak, temu mangga, lime, secang, rock sugar, brown sugar which is brewed and served either warm or hot. The spice that is used in that drink contains bioactive compounds that are good for body. Ginger (Zingiber officinale) contains some major bioactive compounds which have high antioxidant activity; for instance, gingerol, shogaol and gingeron [1]. Temulawak (Curcuma xanthorrhiza), turmeric (Curcuma domestica), temu mangga (Curcuma mangga), white turmeric, (Curcuma zedoaria) are some examples of species which are categorized as genus Curcuma [2]. Curcuma Rhizome contains active compounds of flavonoids, terpenoids, alkaloids, essential oils and others. Meanwhile, Curcumin, demethoxy curcumin and bis-demethoxy-curcumin are some examples
of compounds which are most widely in the genus Curcuma. The compounds which is contained in Curcuma rhizome can act as antibacterial, antifungal, antiviral, anti-inflammatory, and anti-cancer [3].

Ginger sand (Kaempferia galanga L.) contains flavonoids, polyphenols, tannins, quinones and sesquiterpenes. Flavonoid acts as antioxidants and it is considered as the secondary metabolites which is mostly contained in Ginger sand rhizome. Moreover, Ginger sand can be used to treat hypertension, rheumatism and asthma [4]. Meanwhile, lime has some useful chemical compounds; such as, citric acid, amino acids, essential oils, resin, glycosides, citric acid, fat, calcium, phosphorus, iron, sulfur, vitamins B1 and C and flavonoids which can act as antibacterial [5]. Since palm sugar has high sucrose and mineral content, palm sugar and sugar are used as sweeteners for ginger spices. In addition, Secang wood rich in antioxidant.

Spiced drink has been known by society for a long time; besides, it is believed to provide more benefits for body. Many researchers have been conducted to find out the bioactive components contained in each spice or in certain spiced drink. However, bioactive components contained in spiced drink (ginger with the addition of turmeric, white turmeric, ginger sand, temulawak, temu mango, lime, secang wood, rock sugar and brown sugar) have not been studied in depth.

Society has various pattern of spiced drinks consumption. Half of people like spiced drink with strong taste, whereas the rest of people like spiced drinks with mild taste. The strong and mild taste are obtained from brewing process, so that the brewing process can affect the level of consumer acceptance of a beverage product [6]. Moreover, the brewing process in making spiced drinks can affect chemical compounds which is extracted from brewed ingredients; such as, antioxidants in tea leaves [7]. Therefore, the first and second brewing are used as treatment in making spiced drinks. This study is aimed to determine changes in antioxidant activity, total phenol, curcumin and hedonic quality of first and second brewing spiced drinks. The significance of this study is to increase people's interest in consuming spiced drinks as functional drink.

2. Material and method

The study was conducted in December 2018 at Laboratory of Food and Agricultural Products Engineering, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang and Laboratory of Food Science, Soegijapranata Catholic University, Semarang.

2.1. Material

The materials which are used in this study was "Mbah Jo" spiced drink which can be obtained at Jl. Menteri Supeno, Semarang City (behind the Governor's Office of Central Java), water, acetonitrile, 2% acetic acid, deionized water, ethyl acetate, standard curcumin solution, methanol, sodium carbonate, Folin-Ciocalteu reagent, gallic acid calibration standard. Furthermore, the tools used are erlenmeyer, beaker glass, thermometer, water bath, measuring cup, filter, High Performance Liquid Chromatography (HPLC), pipette, cup, spectrophotometer, label paper, and questionnaire sheet.

2.2. Method

This study used exploratory method by applying one factor that was the difference of level of brewing. The treatment which was given in this study was the levels of brewing namely first brewing and second brewing.

The process of making spiced drink was done by making spiced drinks with spices in a beaker glass which was brewed for 30 minutes with 1000 ml of boiled aquabidestilate water (100 °C), then filtered to get the first brewing spiced drink (S1). Furthermore, the brewing process was done with the same spices to get the second brewing spiced drink (S2), then testing the parameter of the spiced drink.

2.2.1. Antioxidant activity. The antioxidant activity test was conducted using DPPH solution [8]. DPPH solution was prepared by dissolving 24 mg DPPH in 100 ml of methanol, and stored in the refrigerator until it is used further. Moreover, the test tube that contained 3 ml of DPPH solution was mixed with 0.1 ml of spiced drink or standard solution. Control contained 100 μl of methanol (0.1 ml).
Absorbance was measured after 30 minutes at a wavelength of 517 nm. Percent of antioxidant activity or radical capture was calculated using the following equation:

\[
\% \text{ Antioxidant Activity} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of the sample}}{\text{Absorbance of control}} \right) \times 100
\]

2.2.2. **Total phenol.** The analysis of the total phenol level of spiced drink was conducted by using Folin-Ciocalteu reagents with analytic gallic acid as a standard [9]. This analysis was done using 1 ml of spiced drink with the standard used is gallic acid (0–500 mg/l) which is added to deionized water (10 ml) and phenol Focal-Ciocalteu reagent (1.0 ml). After 5 minutes, 20% sodium carbonate (2.0 ml) was added to the mixture. Furthermore, it was incubated for 1 hour in the dark or no light conditions, then absorbance was measured at a 750 nm wavelength using a spectrophotometer. The total amount of phenol was calculated using the gallic acid calibration curve. Moreover, the result was expressed as gallic acid equivalent (GAE) ppm.

2.2.3. **Curcumin.** The analysis of the levels of curcumin compounds in spiced drink was conducted using the Shimadzu High Performance Liquid Chromatography (HPLC) [10–12]. The preparation of HPLC injection was done by preparing a standard solution of curcuminoid in methanol with several concentrations of 25, 50 and 100 ppm. Moreover, the calibration curve was created by connecting standard of curcuminoid concentrations with area. Then, sample is injected. The elution system was done with gradient phase which had a flow rate of 0.5 ml/minute, the temperature was maintained at room temperature and injection volume was 20 μl. In addition, the mobile phase used was acetonitrile and 2% vinegar acid (40:60) with a flow rate of 0.5 ml per minute. The column which was used for separation in this test was column C-18 with a length of 200 x 4.6 mm and the detector used was UV at a wavelength of 370 nm. The curcuminoid level of each spiced drink was determined based on the area of the sample compared to the standard of curcuminoid area. The Calculation was done using peak areas at specific retention times and standard curves.

2.2.4. **Hedonic quality test.** According to Novita et al [13], the analysis of hedonic quality is conducted with each 5–10 ml sample from the first and second brewing which have placed in a cup, then they are served to 25 semi-trained panelists. Furthermore, panelists are asked to give score of preference for each sample based on a numerical scale (1–5), starting from very dislike (1) to very like (5) on the organoleptic test sheet. The scoring includes preferences for color, taste, aroma and overall.

3. **Statistical analysis**

The quantitative data of antioxidant activity, total phenol and curcumin which had obtained were tabulated with Microsoft Excel 2016. Moreover, the data of hedonic quality was processed using the Independent t-test, then it was presented in the form of data in tables or illustrations and explained descriptively. Data analysis was calculated using the SPSS 24.0 for Windows program.

4. **Finding and discussion**

The results of testing the antioxidant activity, total phenol and curcumin in first and second brewing spiced drinks can be shown in table 1.
Table 1. Antioxidant activity, total phenol and curcumin of spiced drink.

| Test Parameters       | Unit | Treatment | Reduction |
|-----------------------|------|-----------|-----------|
|                       |      | S1        | S2        |
| Antioxidant Activity  | %    | 78.642    | 61.905    | 16.737    |
| Total Phenol          | ppm  | 33.156    | 17.378    | 15.778    |
| Curcumin              | ppm  | 46.916    | 16.184    | 30.732    |

S1 The First Brewing; S2 The Second Brewing

4.1. Antioxidant activity

We can learn from table 1 that the antioxidant activity of the first brewing spiced drink is 78.65%, while the antioxidant activity of the second brewing spiced drink is 61.905%. The first and second brewing spiced drinks have antioxidant activity which fits to the standard; besides, it is considered as high. This condition corresponds with what Wulansari and Chairul [14] had stated that if the capture of free radical using the DPPH method has results above 50%, it is indicated as high antioxidant activity; 20–50% shows moderate antioxidant activity; the result below 20% indicates low antioxidant activity. The antioxidant activity of the first brewing spiced drink is higher than the antioxidant activity of the second brewing spiced drink. The antioxidant activity of spiced drinks is decreased by 16.737% (21.3%), as the result of repetition of brewing process. This condition corresponds with what Pal et al [15] had stated that the process of preparing spiced drink involves brewing successively which can affect the decrease in total phenol of brewing spiced drink significantly; besides, the decrease in the antioxidant activity.

The antioxidant activity of the first and second brewing spiced drinks is higher than the same type of spiced drinks since they have seven kinds of spices. This condition corresponds with what Septian et al [16] had stated that the addition of four types of spices to spiced drinks which made from one spice; such as ginger, turmeric or beras kencur can increase antioxidant activity from 40% to 50–60%. Moreover, the process of brewing spiced drink which is categorized into the process of blanching helps to increase antioxidant activity by changing less active compounds to active. It corresponds with what Pujimulyani et al [17] had stated that antioxidant activity will increase during the process of blanching. It may be occurred since there is changing of compound from less active to active. The spices that are contained in spiced drink; such as, ginger, turmeric, temulawak, white turmeric, ginger sand, and temu mangga are spices that rich in antioxidant activity. It corresponds with what Melannisa et al [18] had stated that the curcuma rhizome is classified to the family of zingiberaceae which is widely used as a traditional medicine; besides, it contains many antioxidants. It corresponds with what Listiana and Herlina [19] had stated that spice plants contain many phenolic compounds; such as, curcumin, gingerol, tannin, saponins and other compounds that have antioxidant characteristics.

4.2. Total phenol

From table 1 we can learn that the first brewing spiced drink has total phenol of 33.156 ppm, while the second brewing spiced drink has total phenol of 17.378 ppm. Furthermore, the total phenol greatly influences antioxidant activity of spiced drink since phenol compound has hydrogen donors so that it has antioxidant characteristic. It corresponds with what Ibrahim et al [20] had stated that phenol compounds contribute greatly to antioxidant activity; besides, a positive correlation between total phenol and antioxidant activity occurs because of the presence of hydrogen donors in phenol compounds which are effective so that they are antioxidant. Until now there is no limit on the value of high and low total phenol levels in spiced drink. However, the first and second brewing spiced drink fulfill the standard of total phenol levels since spiced drinks have antioxidant activities which fit to the standard; besides, it is considered as high. It corresponds with what Sumitro et al [21] had stated that the total phenol level proportional to antioxidant activity. Therefore, the higher the antioxidant activity, the higher the total phenol level and vice versa.
The first brewing spiced drink has total phenol level higher than the second brewing spiced drink. The total phenol level of the second brewing spiced drink has decreased by 15.778 ppm (47.6%), as the result of successive brewing processes. It means that the process of re-brewing spiced drink can decrease the total phenol. It corresponds with what Pal et al [15] had stated that total phenol is strongly influenced by the process of brewing since successive brewing process can decrease the result of total phenol of brewing significantly. Furthermore, the total phenol level of both the first and second spiced drink is low compared to the same type of spices drink which is also made from various spices. It corresponds with the research which was conducted by Nirmagustina et al [22] which had stated that spice drink made from secang wood, red ginger, lemongrass, clove, cinnamon, cardamom, and nutmeg have total phenol level of 186.056 mg/l. The total phenol which is contained in spiced drink comes from spices used in spiced drink; such as, ginger, turmeric, white turmeric, ginger, temu mangga and ginger sand. It corresponds with what Septiana et al [16] had stated that ginger, turmeric, temulawak and ginger sand contain a lot of total phenol.

The process of brewing spices is classified to the process of blanching. This process is commonly used to inactivate enzymes and soften tissues, so that it is less effective to extract total phenol of spices. It corresponds with what Ananiingsih et al [23] had stated that the purpose of blanching is to reduce microbial contaminants, soften tissues and inactivate enzymes. Water solvent which is used in making spiced drink has low ability to extract phenol contained in spiced drink, so that the total level of phenol which is detected in spiced drink is very little. The best solvent which is used to extract phenol is solvent which has high polarity; such as, methanol. It is corresponds with what Stankovic et al [24] had stated that the use of high polarity solvent can produce high phenol level. Moreover, it is supported by the opinion of Romadanu et al [25] which stated that methanol is a polar solvent that can dissolve phenol compounds well. Moreover, ethanol can be used as a solvent to extract phenol since it can produce high total amount of phenol. It is supported with what Setyowati and Suryani [26] had stated that the extraction with 80% ethanol at a ratio of 1:9 powder-ethanol and temulawak instant drink contain a total of phenol up to 2736 ppm, whereas turmeric instant drink contains total phenol up to 3705 ppm.

4.3. Curcumin
From table 1 we can learn that the level of curcumin in the first brewing spiced drink is 46.916 ppm, whereas the level of curcumin in the second brewing spiced drink is 16.184 ppm. The result shows that the second brewing process can reduce curcumin level by 30.732 ppm (65.5%). The level of curcumin contained in the first and second spiced drinks are still in the maximum limit of the level of curcumin in spiced drink. It corresponds with standard that had set by Codex Alimentarius [27] which stated that the level of curcumin in beverage that contains milk is 150 mg/kg. The level of curcumin which is contained in the first and second brewing spiced drinks are relatively low compared to turmeric and temulawak drinks. This condition occurs because the dominant spices used in spiced drink are ginger and temulawak. It corresponds with what Setyowati and Suryani [26] had stated that temulawak drink has curcumin levels up to 323 ppm, whereas turmeric drink has curcumin levels up to 2440 ppm. Moreover, it is supported by Iijima and Joh [28] who had stated that the level of curcumin in ginger is very small which is 2.2–20 mg / 100 g of fresh ginger.

The brewing process of spiced drink categorized as the process of blanching, heat in this process may affect the decreasing of curcumin. It corresponds with what Gan et al [29] had stated that the blanching process can cause thermal degradation in curcumin. Moreover, water which is used as a solvent can be one factor that causes low levels of curcumin in the first and second brewing spiced drinks. It corresponds with Putri’s [30] opinion which had stated that curcumin compounds are soluble in glacial acetic acid, acetone, alkali hydroxide and alcohol; On the other hand, they are less soluble in diethyl ether and water. In addition, it is supported by the opinion of Wahyuningtyas et al [31] which had stated that ethanol is better solvent in dissolving curcumin than other hydrocarbon solvents.

The low level of curcumin may because of the use of fresh spices. Fresh curcumin rhizome spices tend to be uneven or even centered so that solvents are harder to extract curcumin from rhizomes.
corresponds with what Cahyono et al [32] had stated that dried rhizomes tend to have greater levels of curcumin than fresh rhizomes since curcumin and essential oils of fresh rhizomes are in the oleoresin which is located unevenly and tends to centered; besides, the drying treatment can even the distribution of curcumin in rhizomes so that curcumin is easier to extract. The level of curcumin contained in spice drink comes from the spices of the genus Curcuma which is used as basic material for making spiced drink; such as, temulawak, turmeric, white turmeric and temu mangga. It corresponds with the opinion of Dosoky and Setzer [2] which had stated that the main bioactive component of the genus curcuma spices consists of curcumin compounds, demethoxycurcumin, bisdemethoxycurcumin and essential oils.

4.4. Hedonic quality
The hedonic quality of the taste, aroma, color and overall preference of the first and second brewing spiced drink can be seen in table 2.

| Sensory Attribute     | Treatment  |      |      |
|-----------------------|------------|------|------|
|                       |            | S1   | S2   |
| Taste                 | 3.48 ± 0.963 | 3.52 ± 1.005 |      |
| Aroma                 | 4.08 ± 0.862 | 3.64 ± 0.638 |      |
| Color                 | 4.04 ± 0.889 | 3.60 ± 0.866 |      |
| Overall Preference    | 3.72 ± 0.891 | 3.68 ± 0.748 |      |

**Table 2. Hedonic quality of spiced drink.**

4.4.1. Taste. From table 2 we can learn that the taste of the second brewing spiced drink is more preferably by panelists compared to the taste of the first brewing spiced drink. This condition occurs because the first brewing spiced drink has bitter taste and too strong spicy taste so that the panelists less interest. This condition corresponds with what Ibrahim et al [20] had stated that panelists tend to prefer slightly spicy drink than strong spicy drink. The taste that comes from a spice drink consists of sour, sweet, spicy and bitter taste. The sour taste in spice drink comes from the lime which contains organic acids; such as, citrate and malate so that the drink becomes fresher. It corresponds with what Hamidi et al [33] had stated that lime helps to provide a sour taste, refresh, add aroma and maintain the color of ingredients. Furthermore, sweet taste in spiced drink comes from palm sugar and rock sugar. It corresponds with what Siagian et al [34] had stated that sugar is added to spiced drink to give a sweet taste, while the spicy and bitter taste of spiced drink can be caused by the presence of ginger which contains oleoresin. It corresponds with the opinion of Yuliani et al [35] which had stated that ginger contains oleoresin in the form of gingerol, shogaol and resin which can cause spicy and bitter taste.

4.4.2. Aroma. We can learn from table 2 that the aroma of the first brewing spiced drink is more preferably by panelists compared to the aroma of the second brewing spiced drink. This condition occurs since it can cause disappearing of many volatile compounds, so that the aroma produced by the first brewing spiced drink is sharper than the second brewing spiced drink. It corresponds with what Setiawan et al [36] had stated that volatile compounds are easy to vapor, so that long process can reduce volatile compounds. Moreover, typical aroma of spices which is used as basic material can increase consumer's preference for spiced drink. It corresponds with what Mardhatilah [37] had stated that spices have typical aroma which can satisfy consumer's taste, refresh and warm. In addition, the aroma of spiced drink comes from the content of essential oils. It corresponds with what Rialita et al
[38] had stated that the spices contain essential oil which is volatile; besides, they are often used as a scent.

4.4.3. Color. We can learn from table 2 that the color of the first brewing spiced drink is more preferably by panelists compared to the color of the second brewing spiced drink. The spices, specifically Curcuma contains curcumin compounds that can give yellow color to spiced drink. It corresponds with what Tensiska et al [39] had stated that the curcumin pigment produces yellow color which is a pigment that is stable in heat and acid, but the yellow color will darken when it is exposed to light. Furthermore, secang wood contains brazilin compounds which give a yellow color at pH below 5. It corresponds with what Sari and Suhartati [40] had stated that secang wood has brazilin compound which will produce the yellow-orange color at pH below 5; besides, it will produce red-purple color along with increasing pH. In addition, palm sugar influences the color of spiced drink. It corresponds with what Siagian et al [34] had stated that palm sugar is also used to improve the color of spiced drink.

4.4.4. Overall preference. From table 2 we can learn that the overall preference of the first brewing spiced drink is more preferably by panelists compared to the overall preference of the second brewing spiced drink. It means that panelists like and intend to consume spiced drinks as an alternative to maintain their health. It corresponds with the study which had conducted by Sari et al [41] which stated that consumers attitudes towards traditional herbal medicine are consumers who want to accept and consider traditional herbal medicine as an alternative to maintain their health. Furthermore, the process of brewing spiced drink can influence the color, aroma, taste and overall preference of consumers towards spiced drink. It corresponds with what Mulyani et al [42] had stated that we need to test the color, taste, aroma and acceptance of overall product to find out consumer's preference for spiced drink. Panelists accept the first and second brewing spiced drink since they have typical taste of spiced drink; for instance, they have good taste, they are made from various spices, they have typical aroma, and they are made from natural ingredients. It corresponds with what Djamaludin et al [43] had stated that a good taste of herbal medicine contains a variety of herbs and natural ingredients; besides, their benefits will increase consumers satisfaction towards spiced drinks.

5. Conclusion
From the result above, we can conclude that the first and second brewing spiced drinks have antioxidant activity, total phenol and curcumin which fit to the standard. However, the second brewing spiced drink decreases the levels of curcumin, total phenol and antioxidant activity. Furthermore, the first brewing spiced drink is better than the second brewing spiced drink. The color, aroma and overall preference of the first brewing spiced drink are more preferably by panelists, whereas the taste of the second brewing spiced drink is more preferably by panelists compared to the taste of the first brewing spiced drink.

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