METHOD OF PROCESSING GREEN, YELLOW AND BLACK GAHARU TEA

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Abstract-

Aquilaria tea also known as Gaharu tea in Malaysia is made from the leaves of aquilaria a protected tree species of the tropicla forest. The popular plantation species are Aquilaria crassna, A. malaccensis and A.sinensis. Currently, there is no formal method in processing gaharu tea in the market. Compared to tea derived from the leaves of Camillia sinensis the standard tea in the market, there are three type of tea that can be produced through different processing method mainly by the degree of fermentation from each type of tea which result in green tea, yellow tea and black tea. The objective of this study is to perform processing method of green tea, yellow tea and black tea on gaharu leaves and to analyse the chemical constituents which is caffeine and antioxidant. To analyse caffeine, elemental analyzer was used to determine the composition of nitrogen because molecular structure of caffeine is composed mainly of nitrogen atoms. Based on the result obtained, the nitrogen composition is the highest in black gaharu tea compared to yellow and green gaharu tea. This shows that fermentation process increased the amount of caffeine in gaharu tea leaves. Compared to market tea derived from leaves of Camillia sinensis, caffeine content in gaharu leaves is lower. Furthermore, the analysis of antioxidant was done using DPPH method. The result shows that antioxidant content is higher in fermented black and yellow gaharu tea compared to green gaharu tea. This shows that fermentation processed increased the antioxidant content in tea leaves. When comparing to standard commercial tea, gaharu tea has lower antioxidant content.

I. INTRODUCTION

Tea is a non-alcoholic and the second most consumed beverage after water. Tea is produced from the plant Camellia Sinensis where it consisted of two or more leaves. The plant Camillia Sinensis is a genus from a flowering plants from the family Theaceae where the main varieties are Camellia sinensis var.sinensis and Camellia sinensis var.assamica [1]. The reason tea is popular in most countries around the world is because local surrounding environment is favorable for the growth of the plant tea. Tea can be divided into three main categories based on the level of fermentation that differ from each other. Green tea is classified as unfermented tea [2] and the processing of green tea always includes three steps: (1) Steaming or firing to deactivate the activities of enzyme in freshly plucked tea leaves; (2) Rolling of tea leaves to release the cell juice onto the leaf surface; (3) Drying to reduce the moisture content and to ensure final product is dry and high quality. In addition, yellow tea is classified as semi fermented tea or known as oolong tea. Yellow tea is identical to green tea where initial processing step of both teas is similar but production yellow tea requires additional step called ‘sealed yellowing’ where it lower oxidation level [3] while the process of black tea is similar to yellow tea except longer duration of fermentation. Black tea processing method is known as crush, tear and curl (CTC) method and orthodox method [1].

Each type of tea contained different concentrations of bioactive compounds due to different in processing method. The three main components in green tea are catechins, amino acids and caffeine. For example, it is found that high content of polyphenol was detected in green tea which beneficial in preventing cancer and radiation-scavenging free radicals. Green tea comparing to black tea contained greater amount of phenolic or tannins substances (5-27%) consisting of gallic acid and catechin (flavanol).

Furthermore, in yellow tea, polyphenol content is greater than in green tea and black tea. The major group of polyphenols are catechins, flavone glycosides, flavone, anthocyanidin, phenolic acids and leucoanthocyanidin [4]. Apart from that, yellow tea is also rich in catechins. There are seven main catechins were quantitatively studied in yellow tea which is (+)-gallocatechin (GC), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (+)-catechin (C), (-)-epigallocatechin-3-O-gallate (EGCG), (-)-gallocate chin-3-O-gallate (GCG) and (-)-epicatechi n-3-O-gallate (ECG). For example, the concentrations of GCG and C are higher in yellow tea compared to green tea [3]. The content of EGCG is lesser in yellow tea compared to green tea. Moreover, it is reported that yellow tea has the greatest concentrations of methylxanthines while theophylline and theobromine were detected at lower concentrations. In yellow tea, there are 18 amino acids, the compounds contribute for the fresh taste of yellow tea are glycine, theanine and glutamic which were found relatively at high concentrations [3].

Moreover, withering process plays an important role in processing black tea. Withering process increase the level of caffeine content, amino acids, sugar and polyphenol oxidase of black tea [5].There are two types of withering which is physical and chemical withering. Physical withering is defined as moisture removal from the tea leaf while chemical withering begins instantly once the tea leaves are plucked. The biochemical changes during chemical withering is formation of amino acids by roughly 1.2% breakdown of proteins. The breakdown of protein take place due to present of peptidase which increase the levels of free amino acid such as aspartic acid, valine, glutamic acid, serine, thromine, alanine and phenylalanine [5]. In addition, during withering carbohydrates are converted into simple sugars. Content of carbohydrates slowly decreases while simple sugars content increases. Besides, sufficient withering will result in greater amount of caffeine in black tea.

Due to high demand for tea from people to exploit the health benefits, tea is now also being processed from other types of plant materials besides Camillia Sinensis such as leaves of Mentha

piperita (peppermint), Psidium guajava (guava), Momodica charantia (bitter gourd), Hibiscus sabdariffa (roselle), Cymbopogon citratus (lemon grass) and Orthosiphon aristatis (‘misai kucing’). For the past ten years, tea derived from a new source of plant has emerged which is from Aquilaria leaves due to popularity of Agarwood. Plant genus Aquilaria from the family called Thymelaeaceae is an evergreen tropical woody tree that is famous for its fragrant resin called agarwood. Agarwood are commonly used in fragrances, aromatherapy, incense and religious ceremonies [6]. Agarwood was first introduced as one of the Chinese traditional medicines during the 5th century. In addition, agarwood are used as sedative, relieve gastric problems, high fever and rheumatism [7]. Wood derived from Agarwood is the most expensive in the world which is an occasional product of a few genera of Gyrinops and Aquilaria in the plant family Thymelaeaceae [6]. As demand of agarwood is high in the market, natural Aquilaria in the wild are greatly reduced in population due to illegal harvesting. Declining in population of Aquilaria trees in natural forest has acquired it the status endangered. The genus is presently listed in Appendix II of the convention on international trade in Endangered Species of the wild of Flora and Fauna [8]. Countless efforts have been made to preserve the population of Aquilaria species and expand agarwood by intentionally injuring the cultivated trees to produce agarwood [9]. To produce a matured agarwood, the tree must first grow at a certain size and age commonly between five to seven years before they undergo a process called induction. To induce the agarwood formation, the tree is purposely wounded with different methods. Due to long duration of time invested in tree induction and growth, numerous farmers decide to support their living by exploring into alternatives such as processing tea derived from the abundant Aquilaria leaves in the plantation. In Malay language, agarwood tea is known as ‘teh gaharu’. There are few reports on the benefits of Aquilaria leaves to human health. To treat trauma-related illnesses such as bruises and fractures, leaves of A. sinensis are applied as medicine. Besides, leaves of A. crassna are beneficial as a supplement to counter multiple health conditions for example constipation, high blood pressure and diabetes [8]. In order to exploit the health benefits from the plant, gaharu leaves are being processed into tea. Currently there is no formal record on when gaharu tea became a commercial product. For this research study, gaharu leaves will be processed into three type of tea which is green, yellow and black gaharu tea by different processing method. The bioactive compounds which is caffeine and antioxidant were analysed from each type of processed gaharu tea.

II. METHODOLOGY

A. Materials

Leaves of species Aquilaria Malaccensis is chosen for this research study. Freshly young leaves are plucked from the tree excluding deadwood or diseased bud obtained from Jalan Kebun Shah Alam. The fresh leaves are cleaned by rinsing with tap water to remove any dirt. The total leaves are divided into three portions equally and stored in sealed container in a refrigerator prior to chemical analysis. For comparison chemical analysis, BOH green tea and BOH black tea are selected and obtained from the market.

B. Equipment

Equipment used to dry the sample gaharu leaves during processing step is conventional oven with brand model ‘Zanussi Freestanding electric oven ZOE552W’. For nitrogen element analysis, elemental analyzer brand model Thermo Fisher Scientific Flash EA1112 was used. Grinder was used to grind each gaharu tea leaves.

C. Sample preparation

Preparation of Gaharu tea

Preparation step of green gaharu tea

The preparation of green gaharu tea was based on processing step by [10] with some modifications. Cleaned gaharu leaves undergo drying process using a conventional oven with the temperature of 80ºC for 15 minutes. Next, the leaves were rolled by hand for 13 minutes. The rolled leaves undergo second drying process at temperature of 90ºC for 18 minutes. The leaves then undergo second rolling process for 7 minutes. The leaves undergo final drying process with at temperature 115ºC for 24 minutes. Finally, the leaves were grinded until final product in powder form achieved using grinder.

Preparation step of yellow gaharu tea

The processing step yellow gaharu tea is the same as green gaharu tea with few additional processes [3]. Cleaned gaharu leaves undergo withering process at room temperature for 6 hours. After the completion of withering process, the leaves undergo fixing process using a conventional oven at temperature 100ºC for 15 minutes. Next, the leaves are rolled by hand for 10 minutes. The rolled leaves then undergo yellowing process. The leaves were left for 1 day to ensure color change to yellow. The leaves undergo final drying process of temperature 80ºC for 20 minutes. Finally, the leaves are grinded to achieve final form of powder using a grinder.

Preparation of black gaharu tea

Method of processing black tea is same as orthodox tea based on [10] and [1] with some modifications. Cleaned leaves then undergo withering process for 16 hours. The leaves then hand rolled for 20 minutes. Next, the rolled leaves undergo fermentation. The leaves are left for 3 days until sufficient color and aroma developed. The leaves undergo drying process at temperature 110ºC for 20 minutes. Finally, the leaves are grinded to achieve final powder form using a grinder.

Elemental analysis of nitrogen

This analysis was done to determine the composition in percentage of nitrogen from each sample of grinded tea leaves. Each sample of tea leaves were measured at 2.0mg using a high precision weight balance and was stored in a capsule prior elemental analysis.

DPPH assay

Antioxidant activity in the leaves extract was studied by using 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) as established by Blois (1958). The antioxidant scavenging activity was determined by procedures done by [11] and [12] with some modifications.

Preparation of DPPH solution

Preparation of DPPH was based procedure done by Nik Wil [11] with some modifications. 19.7 mg of DPPH powder manufactured from Sigma was weighted by a high precision balance in a 250ml beaker. Methanol was poured into the beaker and glass rod was used to stir the mixture until complete dissolution of DPPH powder. The mixture was then transferred to 250ml flask. The beaker was rinsed several times with methanol to ensure all the remaining solution was rinsed and transferred to the flask. Methanol was added into the flask up until 250 ml mark. Lastly, the flask is covered with aluminium foil to kept it form light and stored in a fridge at 4ºC prior to antioxidant activity determination.

Preparation of aliquot of concentration 400,800,1200,1600 and 2000 µg/ml prior to antioxidant analysis

Grinded green gaharu tea leaves were measured 1.5g using high

precision balance. The tea leaves then were infused with freshly boiled ultra-pure water of 200ml for five minutes. The tea infusion was then filtered using Whatman filter paper (No.1) to separate the leaves from the tea infusion. The concentration of the tea produced was 7500µg/ml. The equation used to make dilutions of 400, 800, 1200, 1600 and 2000 µg/ml was;

$$M_1V_1 = M_2V_2$$

The total volume of the dilution was 10 ml. The dilution was summarized in the table 1.0 below. The preparation of aliquot dilution was repeated for every tea leaves sample.

Table 1.0: Aliquot of different concentration.

Concentration, µg/ml	Volume of tea, m^3 (7500µg/ml)	Volume of ultra-pure water, m^3
400	0.53	9.470
800	1.067	8.933
1200	1.600	8.400
1600	2.133	7.868
2000	2.667	7.333

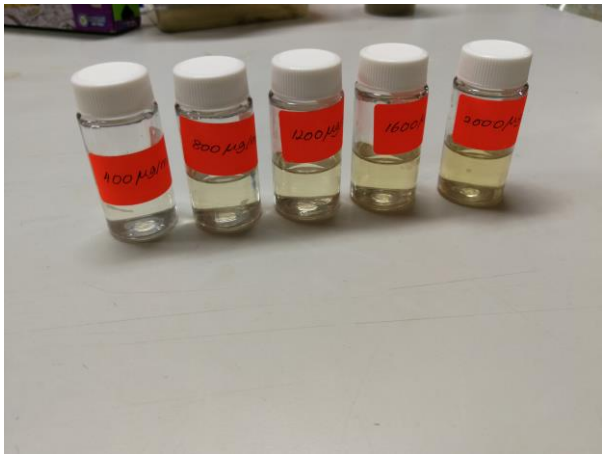

Figure 1.0: The concentration 400, 800, 1200, 1600 and 2000 µg/ml of black gaharu tea from left to right

Antioxidant activity determination

3 ml of each extract dilutions were allowed to react with 3 ml of 0.2mM DPPH in methanol. The solution was then incubated in dark at room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm wavelength using a spectrophotometer against water as blank and recorded as A (extract). The same procedure was repeated for each different dilution and was performed in triplicates. The scavenging activity was calculated as a percentage of DPPH decolouration relative to a negative control using the equation:

$$Free\ radical\ scavenging\ activity = \frac{A\ (blank) - A\ (extract)}{A\ (blank)}\ x\ 100\%$$

IC_{50} is defined as half concentration of inhibition. It is concentration of antioxidant that inhibits DPPH radical. IC_{50} of each extract was determined via plotted graph of percentage inhibition versus concentration and expressed as the antioxidant activity of extract.

III. RESULTS AND DISCUSSION

Elemental composition

Table 2.0: Nitrogen composition for each tea leaves

Sample	Element %
	Nitrogen
1	1.828906
2	1.749637
3	2.511481
4	3.178114
5	3.897035

Green gaharu tea (1), Yellow gaharu tea (2), Black gaharu tea (3), BOH green tea (4), BOH black tea (5)

Based on the table 2.0 there are significant changes in trend occurred for nitrogen composition based on the fermented teas. From this analysis, it was found that green and black tea leaves derived from Camellia sinensis has greater nitrogen composition compared to green, yellow and black gaharu tea. Unfermented tea which was green gaharu tea has the lowest nitrogen composition of 1.83% when compared to yellow 1.75% and black gaharu tea 2.51%. The result showed the same trend in commercial tea where BOH black tea has greater nitrogen composition of 3.90% than BOH green tea of 3.18%. It was observed that higher degree of fermentation on tea leaves will result in higher nitrogen composition [13]. From the analysis, the concentration of caffeine has increased significantly as the tea leaves undergo fermentation processed. This is because nitrogen element dominates the overall chemical composition of caffeine. Apart from caffeine, amino acids also could be the contribution of nitrogen composition. Amino acids also composed of nitrogen element. This analysis is also in agreement with other studies done by [14].

Antioxidant activity

IC_{50} of each sample was used to represent the amount of sample needed to scavenge 50% free radicals of DPPH. Lower value of IC_{50} indicates greater antioxidant activities. Table 2.0 shows the antioxidant activities of the samples.

Table 2.0: Antioxidant activities of every tea leaves sample

Percentage antioxidant activities %						
Sample	Concentration, µg/ml					IC_{50}, g/ml
	400	800	1200	1600	2000	
1	93.36±0.06	90.07±0.15	88.60±0.07	87.86±0.12	87.32±0.15	36.050
2	94.53±0.12	94.13±0.03	93.49±0.12	92.89±0.12	92.32±0.09	0.0322
3	93.49±0.09	93.09±0.07	92.79±0.07	92.12±0.03	91.75±0.06	0.0390
4	94.47±0.06	94.23±0.03	93.26±0.06	92.42±0.03	91.92±0.03	0.0250
5	92.45±0.06	91.99±0.03	90.68±0.09	89.91±0.03	88.46±0.03	0.0077

(1) Green gaharu tea, (2) yellow gaharu tea, (3) black gaharu tea, (4) green tea (BOH), (5) black tea (BOH). Each value in the table above were performed in triplicates and represented as mean ± SEM (n=3).

Based on the table 2.0 the result clearly showed a positive outcome that fermentation affect the antioxidant activity in plant-based food. Black gaharu tea a fully fermented has greater antioxidant than in green gaharu tea. The result also showed the same trend in commercial BOH tea where black tea with 0.0077 g/ml has greater antioxidant than in green tea, 0.0250 g/ml [15] .This is because fermentation process enhanced antioxidant activity by releasing greater amount of flavonoids [16]. Besides flavonoids, total phenols also increased significantly after fermentation. Phenolic compounds able to act as hydrogen donors, singlet oxygen quenchers and reducing agents. The observed antioxidant activity might be due to increased total phenolic compounds [16]. IC_{50} of yellow gaharu tea was 0.0322 g/ml slightly less than black gaharu tea of 0.039 g/ml. The possibility of the result could be that degree of fermentation of black gaharu tea is same as yellow gaharu tea despite the duration of fermentation was different. However, from this research, it was found that tea leaves derived from Camellia sinensis has higher antioxidant compared to gaharu tea leaves.

IV. Conclusion

In conclusion for this study, tea derived from gaharu leaves has lower content of caffeine compared to tea derived from plant Camellia Sinensis. This shows that gaharu tea is suitable for people who are looking for herbal drink with lower content of caffeine compared to commercial tea in the market as high doses of caffeine can cause increase in blood pressure. A recent study found that acute intake of dietary does of caffeine ranging 200-250 mg raised the systolic (SBP) by 3-14 mm Hg and diastolic (DBP) by 4-13 mm Hg [17]. From this research, it is found that the lowest caffeine content is green gaharu tea compared to yellow and black gaharu tea. It can be said that fermentation process increase the caffeine content in gaharu leaves. In terms of caffeine intake, green gaharu tea is the most to be consumed. Furthermore, the antioxidant content in black and yellow gaharu tea is the highest compared to green gaharu tea. However, comparing to commercial tea in the market, antioxidant content in BOH green tea and black tea is greater than in gaharu tea. Forms of antioxidants in tea may be flavonoids and polyphenols. Health benefit of antioxidant is that it protects the cells against the effect of free radicals. Free radicals may play a role in heart disease, cancer and other diseases [18]. Therefore, in terms of antioxidants content, commercial tea BOH green and black tea derived from Camellia sinensis is preferable compared to gaharu tea. From this research, it is found that fermented gaharu tea has greater antioxidant content compared to unfermented gaharu tea.

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