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The Effect of Various Tea Processing Methods on Antioxidant Activity of Guava (*Psidium Guajava* L. Var *Pomifera*) Leaves Tea in East Java Indonesia

Ni Luh Putu Sherly Yuniartini[1], Joni Kusnadi[2*], Elok Zubaidah[2]

[1]Postgraduate Program of Agricultural Product Technology, Brawijaya University, Jl. Veteran, Malang, Indonesia 65145
[2]Department of Food Science and Technology, Faculty of Agricultural Technology, Brawijaya University, Jl. Veteran, Malang, Indonesia 65145

Abstract: This study was aimed to get tea processing method that can produce guava leaf tea products with the best physical and chemical properties based on its antioxidant activity, to identify bioactive antioxidant compounds that contained in guava leaves tea and to produce guava leaf tea with the best functional ability. Variations processing used is white tea, green tea, green tea-low caffeine and black tea of guava leaves. The results showed that processing green tea with low caffeine (L3) is the best treatment method that produces tea with the ability to capture free radicals DPPH by 70.54%; total phenol 12.95 mg /g; total flavonoids 5.34 mg /g; and 0.15 mg caffein /g.
Keywords: Antioxidant Activity, Caffeine, Green Tea, Guava Leaves.

Introduction

Tea is a beverage infusion of leaf plant *Camellia sinensis* L. which has a specific taste and aroma. Tea leaves can be processed into various types of tea such as black tea (fermented), oolong tea (semi-fermented), green tea and white tea (unfermented)[1, 2].

Epidemiological studies recognized that by consuming tea regularly can reduce the risk of heart disease (cardiovascular), diabetes, arthritis, osteoporosis and dental caries because antioxidant properties of polyphenolic compounds contained in tea, particularly the catechin derivatives: epicatechin, epigallocatechin gallate, epigallocatechin, epicatechin gallate and gallocatechin. Tea leaves also contain important compounds which very important for human health like methylxanthine (caffeine, theobromine and theophilline), amino acids (theanine), and minerals (potassium, magnesium, calcium, nickel and zinc)[3].

Various types of tea is processed with the aim to change the chemical composition in fresh tea leaves and to produce a product that can be consumed by generating the desired properties in the infused water such as, colour, flavour and aroma[4].

Today, it had been developed tea beverages which not only made from leaves of *Camellia sinensis* L., but also used guava plant leaves. Guava is multifunctional medicinal plants from Mexico and is a member of Myrtaceae family. This plant especially the leaves were most widely processed traditionally for curing various diseases.

In general, guava plant was classified into two varieties which are red guava (*Psidium guajava* L. *var pomifera*) and white guava (*Psidium guajava* L. *var pyrifera*)[5]. Guava leaves contained quercetin, ferulic acid, protocatehuic acid, guavin B, β-carotene and asiatic acid that has been known to have antioxidant activity[6,7].

Processing guava leaves are needed to be developed because it has higher price than fresh or dried form. Improvement of guava leaves into guava leaves tea has not been done yet, whereas in terms of chemical content it's suitable to be used as an alternative raw material in the manufacture of tea and serve as a health beverage that can be accepted by consumers.

Infusion of guava leaves in Taiwan has been consumed as an herbal tea for health due to a high antioxidant activity (gallic acid and ferulic acid)[8]. In Japan, guava leaves tea is also used as a healthy drink because it can control blood sugar levels in people with diabetes and have been medically proven[9].

Various types of tea are processed by different processing methods; so it is interesting to know the type of tea which provides more benefits because of the increase of antioxidant activity. Many factors during production can affect the tea components which caused differences on the antioxidant activity of each type of tea[2].

Therefore, this research was conducted to determine the effect of various tea processing methods to the antioxidant activity of white, green and black tea produced from the leaves of red guava (*Psidium guajava* L. *var pomifera*).

Materials and methods

Materials

The main material used in this study is leaves of red fruit cultivars guava (*Psidium guajava* L. *var pomifera*). The shoots and leaves of four to seven years old guava plants were collected from Junrejo village, District Junrejo, Batu, Malang, Indonesia. Chemicals used were: DPPH (*1,1-diphenyl-2-picrylhydrazyl*), methanol, ascorbic acid, Folin-Ciocalteu, Na_2CO_3 7.5%, gallic acid, quercetin, sodium nitrite, $AlCl_3$, NaOH , caffeine, Na_2CO_3, chloroform and aquadest.

Methods

Tea Processing

There were four methods of processing tea used in this experiment[2,7].

1. **White Tea Processing Method**

   Guava leaves withered in four days by putting in the shade on natural daylight and then dried using a vacuum dryer at 60°C for 1 h.

2. **Green Tea Processing Method**

   Guava leaves were steamed at temperature of 90°C for 1 minute, and then cooled and withered using dryer cabinet at 40°C for 2 h, then immediately rolled for 15 minute and then dried using a vacuum dryer at 60°C for 1 h.

3. **Green Tea-Low Caffeine Processing Method**

   Guava leaves were immersed in water at the temperature of 90°C for 15 second (water and material ratio of 20:1 ml/g), then immediately cooled in water with the temperature of 18^{o}C for 5 second and drained. Furthermore, the drained leaves were withered with dryer cabinet at 40^{o}C for 2 h and rolled for 15 minutes then dried using a vacuum dryer at 60°C for 1 h.

4. **Black Tea Processing Method**

   Guava leaves were withered with dryer cabinet at 40^{o}C for 4 h, and then crushed with a dry blender for 10 second, and fermented with conditions of fermentation temperature is 29^{o}C-31^{o}C, 27^{o}C-28^{o}C for 100 minutes. The fermented tea then dried using a vacuum dryer at 60°C for 1 h.

Preparation of Crude Plant Extract

The dried plant materials were powdered using a grinder. About 100 g of dried, ground plant materials were soaked in methanol (1 L of 98%) for 5-7 days at room temperature. The soaked material was stirred every 18 h using a sterilized glass rod. The final extracts were passed through Whatman filter paper No.1 (Whatman Ltd., England). The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40^{o}C and

stored at 4°C for further use. The stock solution of crude extracts (5 mg/ml) was prepared by dissolving a known amount of dry extract in 98% methanol. The working solutions (1, 2, 4, 6, 8, 10, 15, 25, 50, 75, 100, 250, 500 and 750 µg/ml) of the extracts were prepared from the stock solution using appropriate dilution[10].

Antioxidant Activity (DPPH Free Radical Scavenging Activity) of Methanolic Extract

Assessment of antioxidant activity was done using 1-100 µg/ml ascorbic acid solution as standard. The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method[10]. The diluted working solutions of the test extracts were prepared in methanol. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 minutes and the optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and the percentage of inhibition was calculated using the formula given below:

Percent (%) inhibition of DPPH activity = $\frac{A-B}{A} \times 100$

Where A = optical density of the blank and B = optical density of the sample.

Total Phenol Content

Total phenol content of the different tea samples was determined using the Folin–Ciocalteu reagent[11]. Briefly,0.025 ml of each tea sample previously diluted, 1.5 ml of water were added, followed by 0.125 ml of Folin–Ciocalteu reagent and mixed. After 1 minute, 0.375 ml of 20% Na_2CO_3 and 0.475 ml of water were added to the mixture to reach a final volume of 2.5 ml. After mixing, the samples were left for 2 h at room temperature in the dark. The absorbance was then read at 760 nm against blank containing water instead of tea. For determining the TPC, standard concentrations of gallic acid (0–80 µM) were used for constructing the calibration curve (mM gallic acid vs. absorbance). Gallic acid stock solution was prepared in ethanol at a concentration of 26.5 mM. The results are expressed as mM gallic acid equivalents (GAE).

Total Flavonoid Content

The total flavonoid content in the tea was measured using a spectrophotometry assay according to the modified method of Carloni[2]. Briefly, 0.05 ml of tea infusion or appropriately diluted (+)-catechin standard ethanol solution, or water as blank, were added to 1.35 ml of distilled water. After mixing, 0.05 ml of 5% $NaNO_2$ followed by 10% $AlCl_3$ (0.05 ml) were added and mixed and samples were left for 10 min at room temperature in the dark. Absorbance was read at 510 nm and the results were expressed as mM Catechin Equivalents (CE) using the linear regression value obtained from the catechin calibration curve.

Analysis of Caffeine

Caffeine content in the tea was measured using a spectrophotometry assay according to the method of Sabrina with some modifications[12]. 100 g of tea sample were added 2-3 g of Na_2CO_3 followed by 250 ml aquadest and mixed was left for 15 min at room temperature in the dark. After mixing, the samples were added with 25 ml chloroform and then distilled in the temperature of 30°C. The absorbance was then read at 273 nm against a blank containing water instead of tea.

Statistical Analysis

The data obtained were analyzed using analysis of variance (ANOVA), if the results of the analysis show that the treatment are significantly different then continued with further test using Least Significant Difference test (LSD) at 5% significance level ($p \leq 0.05$).

Results and discussion

Capacity of Free Radical Scavenging Activity (DPPH)

The free radical trapping activity of guava leaf tea produced by various processing methods showed a significant difference (Table 1).

Table 1. Free Radical Scavenging Activity (%) of Guava Leaf Tea produced by Various Processing Method

Sample	DPPH
White Tea (L_1)	67,848±0,191[b]
Green Tea (L_2)	71,946±0,099[d]
Green Tea-Low Caffeine (L_3)	70,539±0,381[cd]
Black Tea (L_4)	66,352±0,189[a]

Description: The average number followed by similar letter in the same column are not significantly different at α = 0.05 LSD.

The free radical trapping activity in green tea of guava leaves which processed with steaming was higher (71.94%) compared with other researcher finding[10]. It is due to much of polyphenol content of guava leaves are protected, especially catechin and epicatechin [8], whereas in the processing method of green tea, fresh leaves were immediately steammed at high temperature and then dried to inactivate polyphenol oxidase enzyme and prevent oxidation and maintain polyphenols in monomer form[13]. However, the process of fermentation in black tea processing will cause the amount of catechins reduced because it has been converted into theaflavins and thearubigin. The amount of catechin content positively correlated with antioxidant activity[2].

A study on the antioxidant activity of white tea, green tea and black tea derived from the same cultivar, showed that green tea processed with steaming for 1 minute contains higher catechins especially EGC (epigallocatechin) and EGCG (epigallocatechingallate) than other teas, and showed a high antioxidant activity[2]. The effects of high antioxidant in the two catechins was associated with the higher degree of hydroxylation of the B ring and/or location of the hydroxyl group at C-3 on the basis of catechins structure that enhances its ability to capture free radicals. Heat treatment maybe can damage the cell walls of guava leaves and release the active components in large numbers and resulting in a stronger free radical catcher components than in the fresh form[14].

Analysis of Total Phenol

Phenol compounds are including a variety of compounds from plants that have same characteristics of aromatic ring and containing one or two hydroxyl groups. Phenol compounds tend to dissolve in water because often bind with sugars as glycosides and usually present in the vacuole cell[15].

The phenolic compund of guava leaves tea produced by various processing methods showed a significant differences (Table 2).

Table 2. Total Phenol (mg/g) of Guava Leaf Tea produced by Various Tea Processing Method

Sample	Total Phenol
White Tea (L_1)	12,456±0,081[b]
Green Tea (L_2)	13,317±0,020[d]
Green Tea-Low Caffeine (L_3)	12,954±0,048[cd]
Black Tea (L_4)	12,115±0,017[a]

Description: The average number followed by the same letter in the same column are not significantly different at α = 0.05 LSD.

Total phenol in green tea from guava leaves which was processed by hot steam treatment was higher than other guava leaves tea. This is caused by the difference in the treatment process resulting differences in levels of phenol in each types of tea. Heat treatment leads the degradation of tannins to be a simple phenolic compounds, whereas steaming treatment will cause hydrolysis of tannic acid into galloyl like gallotanin so the levels of total phenols increase[16]. Total phenol make a contribution to the antioxidant activity[17].

The processing of fresh guava leaves to green tea were made without fermentation. To prevent the fermentation process, the inactivation of the polyphenol oxidase enzyme was done by steaming the leaves which caused the enzymatic oxidation toward catechins can be prevented. On the other hand, the process of enzymatic oxidation to catechin compounds in fresh leaves was utilized in processing of black tea. Processing of black tea involves the oxidation of catechins with the help of catechol oxidase and resulting in the production of o-quinone[18]. Therefore phenol levels in green tea were higher than phenol content in black tea and other teas.

Guava leaves contain phenolic compounds like procatechiuc acid, chlorogenic acid, caffeic acid, kaempferol, ferulic acid, morin, quercetin-3-0-glucopyranoside[7], gallic acid, catechin, epicatechin , routine, naringenin and kaempferol[8]. Catechin, epicatechin and chlorogenic acid is a specific substrate for polyphenol oxidase enzyme which can lead to the formation of a dark colour because they were converted into quinone compounds[19,20].

Analysis of Total Flavonoids

The processing of guava leaves tea using various processing methods showed a significant differences on the content of flavonoid compounds in guava leaves tea (Table 3).

Table 3. Total Flavonoids (mg/g) of Guava Leaf Tea produced by Various Tea Processing Method

Sample	Total Flavonoids
White Tea (L_1)	4,808±0,013^{b}
Green Tea (L_2)	5,340±0,034^{d}
Green Tea-Low Caffeine (L_3)	5,112±0,025^{cd}
Black Tea (L_4)	4,552±0,008^{a}

Description: The average number followed by the same letter in the same column are not significantly different at $\alpha = 0.05$ LSD.

The total flavonoids of green tea that was made by steaming to the entire surface of leaves is higher than the other guava leaves tea. This was due to the hydrolysis in glycosides into aglycones. Flavonoid inside the cells in the form of glycosides can be damaged because of the present of enzyme, acid and heat treatment and to form aglycone and sugar. Aglycones are known to have a potential as antioxidantscompared to glycosides, so its presentcan increase antioxidant activity after getting heat treatment[14].

The steaming process of fresh guava leaves during the manufacturing of green tea will inactive polyphenol oxidase enzyme, control the level of flavonoids compound of flavanol group or known by catechins (contributed 20-30% of the weight of dry tea leaves) and prevented from enzymatic oxidation process to catechins to maintain polyphenols in monomer form[7,13]. Guava leaves contains many flavonoid compounds available as quercetin, catechin, epicatechin, morin, leucocyanidin, myricetin, kaempferol, quercetin-3-α-L-arabinofuranoside, guaijaverin, avicularin and mecocyanin[21].

The existence of flavonoid have a positive correlation with the antioxidant activity, because of catechin compounds in tea leaves, especially EGC (epigallocatechin) and EGCG (epigallocatechingallate) can be prevented from the enzymatic oxidation process. Catechin molecular structure consisting of two phenol groups (rings A and B) and one group dihydropyran (ring C), which is the antioxidant effects of EGC (epigallocatechin) and EGCG (epigallocatechingallate) connected by a degree of hydroxylation in ring B that higher and/or location of hydroxyl group at C-3 on the basis of catechins structure can improve the ability to capture free radicals[13].

Caffeine Content Analysis

The caffeine compound in guava leaves tea processed using various processing methods showed a significant differences (Table 4).

Table 4. Caffeine content (mg/g) of Guava Leaf Tea produced by Various Processing Method

Sampel	Caffeine
White Tea (L_1)	0,168±0,001^{c}
Green Tea (L_2)	0,163±0,003^{b}
Green Tea-Low Caffeine (L_3)	0,146±0,002^{a}
Black Tea (L_4)	0,170±0,002^{c}

Description: The average number followed by the same letter in the same column are not significantly different at $\alpha = 0.05$ LSD.

The caffeine content in green tea-low caffeine of guava leaves which was made by immersion, lower than white tea, green tea and black tea.

Caffeine content in green tea-low caffeine of guava leaves in this study was lower (0.146 mg /g) then the results of research on elimination of caffeine in fresh tea leaves (*Camellia sinensis* L.) in green tea[7]. They used tea and water ratio of 1:20 (w/v) at 100°C for 3 minutes. The treatment using hot water can reduce caffeine content from 23.7 mg/g to 4.0 mg/g. Tea can be categorized into low-caffeine tea products if the caffeine content less than 4 mg of caffeine per gram of tea leaves[22].

A decrease in the amount of caffeine in processing of green tea-low caffeine due to the location of the caffeine inside vacuole of guava leaves so that when processing green tea-low caffeine using immersion treatment, caffeine will diffuse into membrane cell and dissolved into the water[7]. The solubility properties and molecular weight of caffeine makes it easier to dissolve in water. Solubility of caffeine is 67.0 g/100 ml (100°C) while the molecular weight of caffeine is 194.19 g/mol. When fresh guava leaves immersed in hot water, caffeine will diffuses and easily dissolved in hot water through the formation of hydrogen bonds involving atom with a free pair of electron N and O.

Conclusion

Various processing methods to produce white, green, green tea-low caffeine and black tea in guava leaves produce free radicals scavenging activity of more than 60% due to the rich content of polyphenols in leaves of guava. Green tea processing method produces the highest total phenols, tannins, and total flavonoid content compared to other processing methods, while low levels of caffeine found in processing method of green tea-low caffeine. Based on the result of various application methods of processing tea on guava leaves studied in this research, it can be concluded that guava leaves have potential to producing as herbal tea beverage that provide health benefits and contains natural antioxidant compounds and have a higher price than in fresh form or dry (bulbs).

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