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Research Article

Effect of fixation methods and various clones of *Camellia sinensis* var. *sinensis* (L) properties and antioxidant activity of Indonesian green tea

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Abstract: Fixation is essential in green tea processing to inactivate the polyphenol oxidase enzyme. In Indonesia, green tea is made from the Assam variety and produced using the panning method. Few studies are reported on green tea made from Indonesian clones of the Sinensis variety. This study aims to identify chemical characteristics, antioxidant activity, and sensory evaluation of green tea from local clones of the Sinensis variety (GMBS 2, GMBS 4, and GMBS 5) with different fixation methods (panning and steaming). The results show that the caffeine content of green tea products ranged from 2.51-2.59% and 2.67-2.74% for panning and steaming methods. The panning method produced green tea with higher total polyphenol and flavonoid content than the steaming method. Green tea with the panning method has an IC_{50} value of 14.45; 14.41; and 17.41 mg/L for GMBS 2, GMBS 4, and GMBS 5, respectively. The panning method resulted in a smaller IC_{50} value than the steaming method for GMBS 2 and GMBS 4 clones. The steaming method produced green tea with a higher taste, aroma, and total score than those the panning method. However, different fixation methods did not significantly affect the appearance, liquor color, and leaf infusion. In conclusion, different fixation methods on GMBS 2, GMB 4, and GMB 5 produced green tea products that met the Indonesian National Standard 3945:2016. Further research is needed to determine the role of the plucking period/season and the characteristics of volatile compounds of green tea from GMBS clones with different fixation methods.

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1. INTRODUCTION

Tea is a refreshing drink containing high bioactive compounds that are beneficial for health. Based on its processing type, tea is divided into white tea, green tea, yellow tea, oolong tea, and black tea (Zhang *et al.,* 2019). Green tea is a favorite drink with many health benefits. Several research report that green tea can act as an inhibitor of herpes virus activity, influenza virus, anticarcinogenic, and cardiovascular disease (CVD) and is antimicrobial as well as suitable for oral health and to prevent colon cancer (de Oliveira *et al.,* 2015; Hajiaghaalipour *et al.,* 2015;

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Reygaert, 2017; Yang *et al.,* 2014). These health benefits are due to bioactive compounds such as catechins found in green tea (Sharangi *et al.,* 2014; Skotnicka *et al.,* 2011).

The main stage in green tea processing is fixation. Fixation aims to inactivate the polyphenol oxidase (PPO) enzyme in fresh tea leaves using high temperatures to prevent polyphenol oxidation. There are two types of fixations used in green tea processing: Japanese style processing using steaming or Chinese style processing using pan-fired (Ozturk *et al.,* 2016). After fixation, the tea leaves are continued to the following processing stage, such as being rolled and dried so that green tea is obtained in curled, tight, or spherical forms (Reygaert, 2017; Zhang *et al.,* 2019).

Tea has been classified into two main varieties based on leaf features like size, pose, and growth habits. The China variety is *Camelia sinensis var. sinensis*, and the Assam variety, *Camellia sinensis* var. *assamica* (Master) Kitamura (Wachira *et al.,* 2013). China and Assam varieties have different growth habits, leaf characteristics, poses, and angles (Ahmed and Stepp, 2013; Wachira *et al.,* 2013; Wong, Sirisena, and Ng, 2022). In Indonesia, green tea is generally made from the Assam variety and produced using the panning method (Prawira-Atmaja *et al.,* 2019). Tea from the Assam variety has a higher polyphenol content than tea from the Sinensis variety (Theppakorn *et al.,* 2014). The panning system has weaknesses, including the fixation temperature that is not controlled and unstable to inactivate the PPO enzyme so that enzymatic oxidation still occurs in the following process. It causes the color liquor of Indonesian green tea to be brownish-yellow compared to Japanese green tea, which tends to be greenish-yellow (Prawira-Atmaja *et al.,* 2019).

However, few studies still report the characteristics of Indonesian green tea made from local clones of the Sinensis variety. A previous study reported on chlorophyll and total polyphenols content on fresh tea leaves and the genetic diversity of the 35 clones of the Sinensis variety in Indonesia (Prawira-Atmaja *et al.,* 2018; Prayoga *et al.,* 2022). This study aimed to identify chemical characteristics, antioxidant activity, and sensory evaluation of green tea with different fixation methods and various GMBS clones from Sinensis Variety. Five local clones from the Sinensis variety were officially released in 2009 by Indonesia Agriculture Ministry. Tea clones are GMBS 1, GMBS 2, GMB 3, GMBS 4, and GMBS 5. This research provides reference to the utilization of Indonesian local tea clones in the processing of green tea to improve the quality of Indonesian green tea.

2. MATERIAL and METHODS

2.1. Chemical and Reagents

Chemicals and reagents used for analysis include 10% hydrochloric acid (J.T. Baker), sulfuric acid (J.T. Baker), magnesium oxide (Merck, Germany), potassium hydroxide (Merck, Germany), chloroform (Emsure, Germany), methanol (Emsure, Germany), Sodium Carbonate (Merck, Germany), Aluminum Chloride (Merck, Germany), Sodium Hydroxide (Merck, Germany), Sodium Nitrite (Merck, Germany), Folin–Ciocalteu (Sigma-Aldrich), Gallic Acid (Sigma-Aldrich, USA), Quercetin (Sigma-Aldrich, USA), 2,2-Diphenyl-1-picrylhydrazyl (Sigma-Aldrich).

2.2. Plants Material

Three local tea clones from the Sinensis variety were used in this research. Tea clones were GMBS 2, GMBS 4, and GMBS 5. The tea shoots (P+3) were plucked manually and further processed. The research was conducted at the Indonesian Research Institute for Tea and Cinchona (IRITC), Gambung, West Jawa.

2.3. Manufacturing of Green Tea

The green tea was processed using the steaming (Japanese style) and panning (Chinese style)

meethods. The Japanese green tea was processed by withering the tea leaves with hot steam (90±5°C) for 2 minutes, then resting with cooling air. The leaves were then rolled manually on the bamboo mat at a temperature of 80±5°C for 15 minutes and followed by cabinet drying (80±5°C for 60 minutes) until a final moisture content of 1-3% was obtained.

In the green tea processing using the panning method, the tea leaves were fixed on a hot clay pan (135±5°C) and continued by rolling the tea leaves for 10 minutes. The total duration of processing was 30 minutes. Tea leaves were dried using a cabinet dryer (80±5°C for 60 minutes) until the final moisture content reached 1-3%.

2.4. Analysis of Moisture Content and Ash Content

The moisture contents in fresh tea leaves and green tea products were determined according to the gravimetric method of ISO 1573. The green tea products were also analyzed for the total ash (ISO 1575), water-soluble ash (ISO 1576), ash alkalinity (ISO 1578), and acid-insoluble ash contents (ISO 1577), as described in a previous study (Prawira-Atmaja *et al.,* 2021).

2.5. Green Tea Extract Preparation

A 500 mg of green tea was added with 40 mL of boiled methanol 70%, continued by heating it for 10 minutes, then followed by maceration (in an oven at 70°C, 120 minutes). The mixture was then sonicated (10 minutes) and filtered using filter paper. The clear filtrate was made up to 50 mL with 70% methanol in a volumetric flask (Maulana *et al.,* 2020). The extract was further used to determine the total polyphenols, total flavonoids, and antioxidant activity.

2.6. Determination of Total Polyphenol Contents of Green Tea

The determination of polyphenols in tea using the Folin-Ciocalteu method refers to ISO 14502-1:2005 (Trinovani *et al.,* 2022). A total of 1 mL of green tea extract solution was pipetted into a 50 mL volumetric flask and diluted with distillation water. 1 mL of diluted tea extract was then pipetted into a tube flask (protect from light) and added 5 mL of Folin 10% reagent (dilution using distilled water), then homogenized using a vortex, let stand for 3 to 8 minutes. After that, 4 mL of 7.5% sodium carbonate solution was added (Na_2CO_3, 37.5 grams added 500 mL of distilled water) and homogenized using a vortex mixer (VM-1000). The samples were stored in a dark room for one hour and continued with absorbance measurements using a UV-Vis spectrophotometer (Varian Cary WinUV) at 740 nm. The standard curve equation determined the total polyphenols content using 10-100 mg/L concentrations of gallic acid.

2.7. Determination of Total Flavonoids Contents of Green Tea

The total flavonoids were determined using aluminum chloride (Zhishen *et al.,* 1999). A total of 1 mL of green tea extract, diluted for 200x, was put into a test tube containing 4 mL of distilled water and reacted with 0.3 mL of 5% $NaNO_2$ solution. After 5 minutes, it was added with 0.3 mL of 10% $AlCl_3$. Then, 2 mL of 1 M NaOH was put at the 6th minute time stamp. Furthermore, the final volume up to 10 mL was determined with distilled water, then homogenized and continued incubation for 15 minutes. The absorbance measurement of the sample used a UV-Vis spectrophotometer (Varian Cary WinUV) at 415 nm. Methanol 70% was used as blank control. The total flavonoid content was measured using quercetin solution with 10-100 mg/L concentrations from the standard curve equation.

2.8. Determination of Caffeine Contents of Green Tea

Determination of caffeine was performed by referring to Alam et al. (2015) with modifications. The tea sample (2.5 gram) was taken in a 500 mL conical flask. Then, 5 g of MgO and 100 mL of distilled water were put into the sample. The mixture was heated in the water bath (40°C for 2 hours), filtered through Whatman-42, and the filtrate was obtained in a 250 mL volumetric flask. The filtrate volume was made up to the mark by adding some distilled water and used as a stock solution.

About 150 mL of the filtrate was taken into a 500 mL conical flask and added with 20 mL diluted H_2SO_4 10%. The mixture was then heated at 90 ±2℃ (maintained in a water bath) to reduce the mixture's volume to about 50 mL. The concentrated mixture was filtered again through Whatman-42 and then collected in a separating funnel. Then, 20 mL of chloroform was added with the filtrate in the separating funnel, shaken well 20 times, and kept undisturbed for 10 minutes. The washed chloroform (from the bottom side of the separating funnel) was collected in a 50 mL conical flask. The same filtrate was washed thoroughly with different volumes (viz, 12.5, 10, 7.5, 5, 5, and 5 mL) of chloroform. The total volume of the collected chloroform was washed with 5mL of KOH 1% in a clean separating funnel. It was contained in a 50 mL oven-dried conical flask (previously weighed) and then kept in the oven at 105℃ until it reached complete dryness and constant weight. The weight of the dried conical flask was calculated using the following formula:

$$\text{Caffeine} = (S\text{-}B)\ \text{mg/g}$$

Where: S= weight of conical flask with caffeine after dryness, and B= weight of conical flask before filtrate collection.

2.9. Determination of The Antioxidant Activity by DPPH

The antioxidant activity of green tea was measured using the DPPH assay (Trinovani *et al.,* 2022). Green tea extract was diluted at different concentrations: 10, 15, 20, and 25 ppm in 70% methanol. Antioxidant activity was carried out by pipetting 2 mL of various concentrations into a test tube, adding 3 mL of 0.1 mM DPPH, homogenizing, and incubating for 30 minutes in a dark room. The absorbance of the sample was then measured by UV-Vis spectrophotometry (Varian Cary WinUV) at 515 nm. The tea extract was replaced with 70% methanol in the blank solution. The absorption value of the DPPH solution was calculated as percent inhibition (% inhibition) with the formula

$$\text{DPPH scavenging activity (\%)} = \frac{A_{\text{Blank}-A_{\text{Sample}}}}{A_{\text{Blank}}}$$

Where: A_{Blank}: blank absorbance; A_{sampel}: sample absorbance. The linear regression equation calculates the sample concentration required to inhibit 50% free radicals (IC50).

2.10. Sensory Evaluation of Green Tea

Three expert panelists from RITC performed a sensory evaluation of green tea. 2.8 g of green tea sample was steeped with boiled water for 10 minutes. The panelist evaluated the samples' shape (appearance), aroma, liquor color, taste, and infused leaves (100 points each) with 25%, 25%, 10%, 30%, and 10%, respectively. The total sensory score was calculated based on the "Methodology of sensory evaluation of tea" of national standards "GB/T 23776-2018" (Wang *et al.,* 2020).

2.11. Data Analysis

All data obtained were analyzed descriptively based on the average value and standard error. The data obtained were analyzed using the two-way analysis of variance method with a significance level of 95%. If there was a significant difference, it was then followed with the Tukey Test. All data were analyzed using XLSTAT 2019 software (Addinsoft, New York, USA) as Add-ins in Microsoft© excel 2019.

3. RESULTS and DISCUSSION

3.1. Moisture Contents and Ash Contents of Green Tea

Moisture content and ash content are the main quality parameter of tea products. The moisture

content of tea products is related to the drying and storage process. In contrast, the ash content shows the value of the inorganic (mineral) content in tea products (Faizasa *et al.,* 2017). The parameter ash content in green tea includes total ash content, water-soluble ash, insoluble acid ash, and ash alkalinity. The moisture content and ash content of green tea with different fixation methods and various clones are shown in Table 1.

The fresh tea leaves of each clone have a moisture content of up to 74%. Different fixation methods produced green tea with moisture content ranging from 4.58% to 5.14%. Meanwhile, the green tea produced with the panning method has a lower moisture content than the steaming method, even though the ANOVA results showed no significant difference ($p > 0.05$). The tea has a moisture content ranging from 1% to 3% after drying. Tea has hygroscopic properties that will absorb moisture from the environment and increase the moisture content of tea products (Diniz *et al.,* 2015; Temple and Van Boxtel, 1999; Teshome, 2019).

The steaming and panning method produced green tea with a total ash content of 5.03 to 5.25% and a water-soluble ash content of 59.15 to 61.87%. Meanwhile, the acid-insoluble ash content ranged from 0.017-0.043%, and the ash alkalinity ranged from 1.49-1.56%. The ANOVA results showed no significant difference between the types of clones, the withering method, and the interaction ($P > 0.05$) in each analysis parameter. Overall, the moisture and ash content of green tea products have met the requirements of the Indonesian National Standard 3945:2016 for green tea.

Table 1. Moisture contents and ash contents of green tea from different fixation and clones.

Parameters	Fixation methods/Clones					
	Panning			Steaming		
	GMBS 2	GMBS 4	GMBS 5	GMBS 2	GMBS 4	GMBS 5
M.C Fresh leaves (%)	74.27±0.62	74.12±1.26	74.47±1.54	74.27±0.62	74.12±1.26	74.47±1.54
M.C of green tea (%)	4.49±0.48	4.90±0.75	4.58±0.55	5.01±0.46	5.14±0.81	4.85±0.59
Total ash (%)	5.03±0.29	5.13±0.18	5.25±0.17	5.21±0.27	5.06±0.17	5.13±0.07
Water-soluble ash (%)	59.15±1.82	58.35±2.57	60.11±1.03	61.87±3.19	61.32±2,74	59.28±1.76
Acid-insoluble ash (%)	0.036±0.010	0.031±0.010	0.037±0.011	0.017±0.004	0.023±0009	0.043±0.016
Ash alkalinity (%)	1.49±0.12	1.49±0.09	1.55±0.10	1.50±0.16	1.54±0.08	1.56±0.08

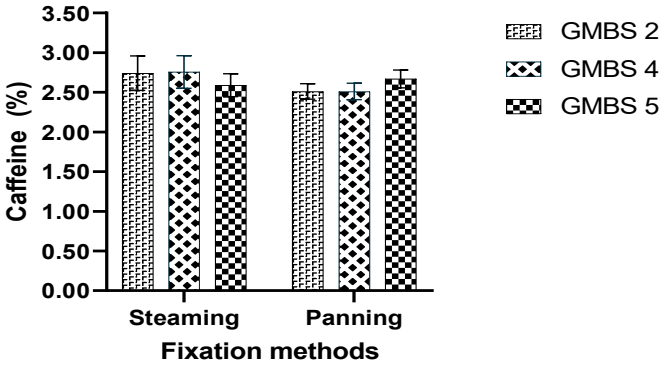
Data ±SE from three replicates. M.C: Moisture content

The high ash content indicates the presence of physical contamination in tea products (Sharma *et al.,* 2011). The low water-soluble ash content indicates that tea processed from tea leaves, which do not meet the plucking requirements, produced low-quality tea (Balasooriya *et al.,* 2019). Ash alkalinity is essential to determine the tea quality. If the ash alkalinity of the tea is higher than the standard requirement, there is an indication of adding infused leaves to the tea product (Balasooriya *et al.,* 2019). Meanwhile, the acid-insoluble ash content indicates the contamination of mineral components such as silica, sand, or soil during the handling of tea leaves or tea processing (Jayawardhane *et al.,* 2016; Suprihatini, 2015).

3.2. The Caffeine Content of Green Tea

Caffeine is a compound that contributes to the brisk taste of tea. The caffeine content of green tea with different fixation methods is presented in Figure 1. The results showed that the caffeine content of green tea products ranged from 2.51-2.59% and 2.67-2.74% for panning and steaming methods, respectively. The green tea with the steaming method had a higher caffeine content than the panning method. ANOVA showed that the type of clone, fixation method, and the interaction were not significantly different ($P > 0.05$) on the caffeine content of green tea.

Figure 1. Caffeine contents of green tea with different fixation methods from GMBS clones.

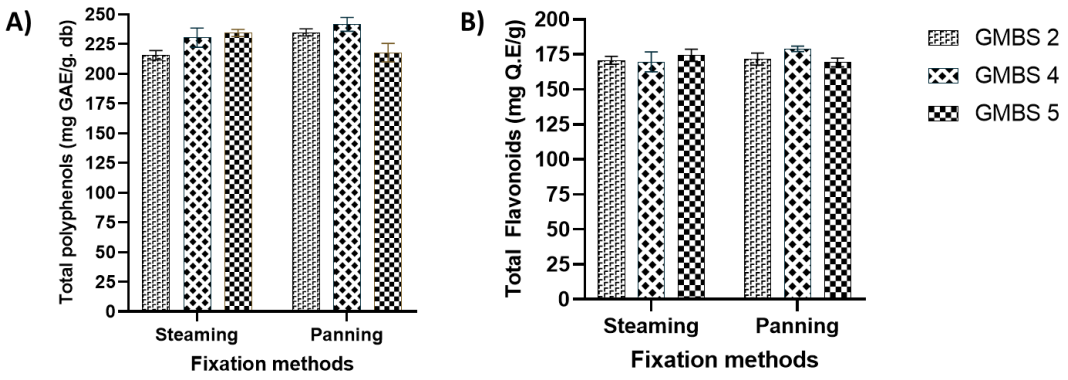


Research conducted by Adnan et al. (2013) on Pakistan's green and black tea products showed different caffeine contents from 3.80-4.24% and 2.34-4.02%. Meanwhile, green tea from various clones in Nigeria has a caffeine content of 1.29 to 2.56% (Aroyeun, 2013). Other studies reported that 35 clones from the Sinesis variety in Indonesia contain 3.0 to 4.53% of caffeine (Prayoga *et al.,* 2022). The tea from young leaves has a higher caffeine content than the old ones (Owuor and Chavanji, 1986). There were also several factors influencing the caffeine content of the tea, such as plucking time (season), clones, climatic conditions of tea plant growth, tea particle size, and brewing time (Hicks *et al.,* 1996; Lin *et al.,* 2003; Paiva *et al.,* 2021). The combination of caffeine with catechins, amino acids, and other compounds is often associated with tea taste. It has a slightly bitter taste, has slightly sweet and sour, and provides an astringent (Martono & Udarno, 2015).

3.3. Total Polyphenols and Total Flavonoids of Green Tea

The panning method resulted in green tea with higher total polyphenol content than the steaming method on GMBS 2 and GMBS 3 clones. Figure 2A and Figure 2B showed green tea's total polyphenol and flavonoid contents from different clones and fixation methods. The polyphenol content of green tea products with varying fixation methods was from 215,54-241,73 mg GAE/g. The total flavonoid content of green tea using the panning method was 171.71; 179.91; and 169.27 mg QE/g for GMBS 2, GMBS 4, and GMBS 5 clones, respectively. The steaming fixation method had a total flavonoid content of 170.78; 169.69; and 174.39 mg QE/g for GMBS 2, GMBS 4, and GMBS 5 clones, respectively. The panning method produced green tea with a higher total of polyphenol and flavonoid contents than the steaming method.

Figure 2. Total polyphenols (A) and total flavonoid (B) contents of green tea with different fixation methods from GMBS clones.



The total polyphenols in tea range from 20-30%, where most of the compounds are from the flavonoid group (Balentine, 2000; Engelhardt, 2010). Anesini et al. (2008) reported that Argentina's green and black tea contained a total of polyphenols at 14.32%-21.02% and 8.42%-17.62%, respectively. Research by Nadiah et al. (2015) showed that the total flavonoid content of green tea ranged from 27.57 mg QE/g to 61.67 mg QE/g. Green tea has a higher total polyphenol content than black tea because polyphenols are oxidized to polymeric tannins from monomeric phenols and reduced flavanol glycosidases. The content of polyphenols and flavonoids in tea is influenced by factors such as geographic area, genetic variability, harvest time, and tea processing conditions (Carloni *et al.,* 2013; Paiva *et al.,* 2021).

Fixation in green tea processing aims to inactivate the polyphenol oxidase enzyme so that oxidase does not occur, which causes the tea to turn brown. Some factors that determine the inactivation of polyphenol oxidase enzymes during fixation are duration and temperature. A higher temperature during fixation can break down cellular constituents affecting an accelerated release of phenolic compounds from the food matrix (Roshanak *et al.,* 2016).

The differences in tea clones also affect the polyphenol content of green tea. It was reported by Yadav et al. (2020) that the Gumti clone of the Sinensis variety had a total polyphenol content of 590 mg GAE/g dry extract, which was higher than the Ambari, Chiniya, Takda-78, and Tinali clones. Meanwhile, in Thailand, green tea from the Sinensis variety ranges from 11.52-17.34 g GAE/100 g D.W., which was lower than green tea from the *C. sinensis* var. *assamica* ranging from 16.63-20.83 g GAE/100 g D.W. (Theppakorn *et al.,* 2014). Another result study by Yadav et al. (2020) showed that green tea from various clones had a total flavonoid content of 200-350 mg QE/g.

3.4. Antioxidant Activity of Green Tea

The antioxidant activity of green tea with different fixation methods was analyzed using DPPH. The DPPH method has been widely used to determine the antioxidant activity of tea extracts. This method is based on the ability of DPPH to act as a hydrogen donor (Chan *et al.,* 2007; Erol *et al.,* 2010).

Figure 3. antioxidant activity of green tea with different fixation methods from various GMBS clones.

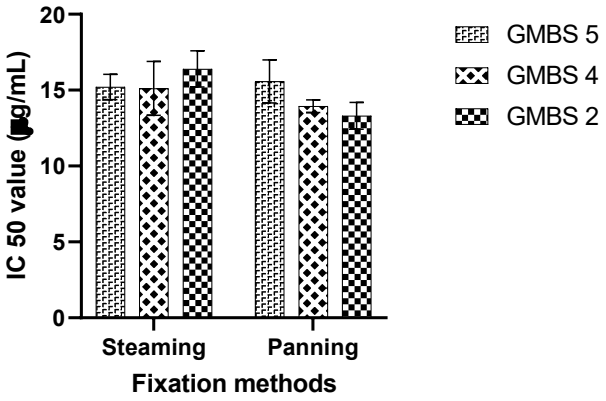


Figure 3 shows that all green tea samples were able to scavenge DPPH radicals. The chelating ability was strengthened with the increasing concentration of extract tea (from 10 ppm to 25 mg/L). The green tea with the panning method has an IC_{50} value of 14.45; 14.41; and 17.41 mg/L for GMBS 2, GMBS 4, and GMBS 5. Meanwhile, the green tea that used the steaming method had an IC_{50} value of 17.16; 16.13; and 15.99 mg/L for GMBS 2, GMBS 4, and GMBS 5, respectively. The panning method resulted in a smaller IC_{50} value than the steaming method for GMBS 2 and GMBS 4 clones. The low IC_{50} value indicates a high antioxidant activity.

The inhibition of radicals DPPH was correlated with green tea's total polyphenols and flavonoid content. The higher the polyphenol and flavonoid compound in tea is, the higher the antioxidant activity will be. Polyphenols and flavonoids have a trihydroxyphenyl B-ring group and a galloyl group that is more active in antioxidant reactions (Zhu *et al.,* 2000). Green tea has the highest antioxidant activity compared to black and oolong tea (Yang & Liu, 2013). It shows that the antioxidant activity of tea is influenced by the type of tea processing, even though it is produced by the same cultivar (Carloni *et al.,* 2013). The tea processing process also influences the antioxidant activity of tea. This study indicated that the enzyme inactivation process in green tea processing affected the antioxidant activity. It is also supported by research by Chan et al. (2007) that green tea with enzyme inactivation using microwave has higher antioxidant activity than green tea with standard processing.

3.5. Sensory Evaluation of Green Tea

Sensory evaluation is essential in determining green tea products' quality, aroma, and taste. The sensory evaluation was determined by attributing parameters such as tea appearance, the color of brewing, aroma, tea taste, and tea leaves infusion. The total score of green tea with different fixation methods is presented in Table 2.

Table 2. Sensory evaluation of green tea with different fixation from various GMBS clones.

Fixation methods	Clones	Appearance	Taste	Color	Aroma	Leaves infusion	Total scores
Panning	GMBS 2	83.33±7.09	76.67±2.89	82.33±7.51	78.67±4.04	84.67±6.51	80.20±2.31
	GMBS 4	81.00±3.46	78.00±3.00	78.00±2.65	79.33±3.79	78.67±10.97	79.15±3.03
	GMBS 5	78.33±7.64	75.67±5.86	77.00±6.24	77.67±2.52	83.67±8.08	77.77±1.01
Steaming	GMBS 2	84.33±3.06	83.00±8.54	82.33±3.21	80.67±0.58	87.00±6.08	83.08±2.62
	GMBS 4	84.33±1.15	79.00±3.61	87.00±2,00	81.67±1.53	89.00±2.65	82.70±0.74
	GMBS 5	80.67±4.04	85.00±6.08	83.00±7.21	81.00±3.61	88.00±3.00	83.02±1.02

Table 2 shows that there was no significant effect on the appearance, liquor color, and leaf infusion. However, they had a significantly different impact on the green tea aroma and taste of all cultivars. The steaming method produced green tea with a higher score of flavor and aroma than the panning method. The steaming method created green tea with 83.08, 82.70, and 83.02 for GMBS2, GMBS 4, and GMBS 5, respectively. While green tea with a panning method, the total score is 80.20; 79.15; and 77.77 for GMBS 2, GMBS 4, and GMBS 5, respectively. Overall, the steaming method of green tea revealed a higher total score than the panning method.

The taste of tea infusion was influenced by soluble sugars, sweet amino acids, MSG-like amino acids, caffeine, ascorbic acid, catechins, and phenols. The green tea with the steaming method gives a slightly bitter taste. The astringent taste of tea is related to caffeine, catechins, phenols, and amino acids (Chaturvedula and Prakash, 2011; Lin *et al.,* 2014). Sensory tea is also influenced by the part of the tea leaf used (Xu *et al.,* 2018). Chlorophyll compounds play a role in the color appearance of green tea products. Meanwhile, the greenish color of the tea is due to the influence of water-insoluble chlorophyll, which dissolves during brewing. The greenish color of steeping green tea is also influenced by flavonoid compounds, the most influential of which is quercetin (Wang *et al.,* 2004).

4. CONCLUSION

This study evaluated the chemical characteristics, antioxidant activity, and sensory evaluation of green tea from GMBS 2, GMBS 4, and GMBS 5 clones produced by different fixation methods. Different fixation methods produce green tea that meets the Indonesian National Standard on green tea (SNI-3945:2016). Different fixation methods produce green tea with

different tastes and aroma characteristics. Further research is needed to determine the role of the plucking period/season and the effect of the fixation method on the characteristics of volatile compounds of green tea from GMBS clones.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

M Iqbal Prawira-Atmaja and **Fadhilatul Ula** have equal contribution to this work. **M Iqbal Prawira-Atmaja:** Investigation, Methodology, Resources, Visualization, Software, Formal Analysis, and Writing Original Draft. **Fadhilatul Ula**: Methodology, investigation, Data Curation, Writing Original Draft. **Hilman Maulana:** Methodology, Validation, Review & Editing Original Draft. **Sugeng Harianto:** Methodology, Writing, Review & Editing, Project Administration. **Shabri:** Conceptualization, Supervision, Project Administration. **Dede Zaenal Arief:** Methodology, Supervision, validation, review & editing original draft

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