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Variation of Fermentation Time of Crystal Guava Leaf Tea (*Psidium guajava* L.) on Antioxidant Activity and Sensory Profile with *Rate-All-That-Apply* Method

Rosida Rosida[1,2*], Anugerah Dany Prianto[1,2], Hadi Munarko[1,2], Muhammad Alfid Kurnianto[1,2]

[1]Department of Food Technology, Faculty of Engineering,
University of Pembangunan Nasional Veteran Jawa Timur, East Java, Indonesia
[2]Innovation Center of Appropriate Food Technology for Lowland and Coastal Area, East Java, Indonesia
Corres Author Email: rosidaupnjatim@gmail.com

ABSTRACT

The making of crystal guava leaf tea had been studied based on fermentation time treatment. This study aims to determine the effect of fermentation time on antioxidant activity in vitro and the sensory profile of the product using the Rate-All-That-Apply (RATA) method. This research used completely randomized design with one factor, such as fermentation time (0, 5, 10, 15, 20 hours) and 3 replicatins and the products were analyzed for antioxidant activity, preference score and sensory profile.The results of this study indicate that the duration of fermentation affects the level of antioxidant activity, preference score, and sensory profile of CGL tea. The tea from the 15-hour fermentation treatment had the best antioxidant levels (94.65%) and a relatively high preference score, with taste preference score ranging from 2.60. The chemical profile of crystal guava leaf tea also generally meets the quality parameters of SNI 1902-2016, such as water content below 7% and total ash ranging from 4 – 8%.

Keywords*: antoxidant activity; crystal guava leaf; tea; sensory profile*

INTRODUCTION

The changing of life style of modern society caused the increase of non-communicable disease or degenerative disease, which was the biggest cause of death worldwide (WHO, 2017). It caused comsumption patterns of instant food, which is high in sugar, salt, and fat (SSF), became high. Whereas continuous consumption of instant food high in SSF could trigger oxidative stress. Oxidative stress was a condition where the number of free radicals exceeded the number of antioxidants (Puspawati & Rungkat, 2012). This condition caused cell damage, pathological conditions, and cell death in the body which was triggered by molecular oxidation (Suarsana et al., 2013). Oxidative stress can be neutralized, one of which was through compounds that have antioxidant properties (Rosahdi et al., 2015).

Antioxidants are compounds that can reduce or counteract free radicals(Martono et al., 2016). Antioxidants are easily oxidized, so free radicals will oxidize antioxidants first and protect other molecules in cells from damage due to oxidation (Wehasari, 2014). Naturally, the body can produce antioxidant enzymes that can degrade free radicals. However, in conditions of excess free radicals, the body cannot handle the entire burden of oxidative stress (Suarsana et al., 2013). Therefore, antioxidants are needed that come from outside the body, one of which comes from food. A study by Prangdimurti et al., (2006) showed that increased food consumption with phytochemical compounds with antioxidant activity can reduce the incidence of degenerative diseases / non-communicable diseases. One food ingredient known to contain antioxidants is crystal guava fruit (*Psidium guajava* L.).

Crystal guava is one of the leading horticultural commodities in Indonesia. As a leading commodity, one of the efforts to optimize crop yields is pruning tree branches. This process

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causes the leaves of the crystal guava plant to be wasted and end up as waste. To overcome this, efforts are needed to utilize guava leaves, one of which is processed into tea. Generally, tea is made from tea leaves (*Camelia sinensis*). However, currently, there are various processed teas from other plants, such as roselle (Mohtar et al., 2019), mangosteen peel(Asri & Martina, 2018), and cascara or coffee skin tea (Yuwanti et al., 2018). Crystal guava leaves (CGL) contained a variety of flavonoid phytochemical compounds such as quercetin, avicularin, apigenin, guaijaverin, kaempferol, hyperin, myricetin, gallic acid, catechins, epicatechins, chlorogenic acid, epigallocatechin gallate, and caffeic acid (Kumar et al., 2021). The presence of these various flavonoid compounds, especially quercetin, can provide strong antioxidant activity (Taha et al., 2019).

Apart determined by the content of phytochemical compounds, antioxidant activity is also known to be influenced by the method or process of processing or production. Guava leaves can be processed into various types of tea, such as green tea (unfermented), oolong tea (semi-fermented), and black tea (fully fermented). Therefore, this study aims to determine the effect of fermentation time on antioxidant activity in vitro and the sensory profile of the product using the Rate-All-That-Apply (RATA) method.

METHODS

Material

Crystal guava leaves were obtained from plantations on the Prambon outbound basecamp, the leaves then processed into green, oolong and black tea. The chemical reagen used were methanol p.a, DPPH, acetic acid, and chloroform, potassium iodide, 1% starch, sodium thiosulphate.

Tool

The tools used in this study were UV-Vis spectrophotometer, dropping pipette, measuring cup, pH meter, micropipette, and analytical balance, incubator, oven, desiccator and furnace.

Research Design

The study was conducted using a completely randomized design with 1 factor (fermentation time). In the process of making tea, guava leaves will be fermented for 0, 5, 10, 15, and 20 hours. Chemical profile, pH, and antioxidant activity (DPPH and FRAP) tests were carried out for each factor, and organoleptic tests. Each test per treatment was repeated three times.

Test Parameters

Chemical profile analysis

Analysis of the chemical profile of the samples, including ash content, protein content, fat, and carbohydrate content, was carried out based on the AOAC method (AOAC, 2012). Meanwhile, a pH analysis was carried out using a calibrated pH meter (Kurnianto & Munarko, 2022). Water content analysis was carried out based on AOAC (AOAC 2012), which began with drying the cup in the oven at 105^{o}C until it reached a stable weight, cooled, then weighed. The sample is weighed, put into a cup, and dried in an oven at 105^{o}C until it has a constant weight. The formula determines the water content of the sample:

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$$\text{Moisture content} = \frac{M2 - (M3 - M1)}{M2} \times 100$$

Note: M1 = disk weight, M2= sample weight, M3= disk weight + sample weight

Antioxidant potential analysis using DPPH method

Antioxidant activity assay using the DPPH (2,2-difenil-1-pikrilhidrazil) method (Kurnianto et al., 2021) was carried out by reacting the sample and 0.6 mmol/L DPPH solution in ethanol. The mixture was incubated in the dark for 30 minutes in room temperature. After 30 minutes, the absorbance value of the mixed solution was measured by a spectrophotometer at a wavelength of 517 nm. The absorbance value obtained is calculated by the percentage of inhibition with the formula:

$$\text{Inhibition (\%)} = \frac{(abs\ control - abs\ sample)}{abs\ control} \times 100\%$$

Antioxidant potential analysis using FRAP method

The antioxidant activity test using the FRAP (Ferric Reduction Antioxidant Power) (Safitri et al., 2022) method was initiated by preparing a stock solution, namely acetate buffer pH 3.6, 10 mmM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_2.6H_2O$. The stock solution was then mixed with acetate buffer, TPTZ, and $FeCl_3.6H_2O$ ratio of 10:1:1 (v/v). The mixed solution was then incubated so that the temperature increased to 37°C. Furthermore, the mixed solution was reacted with the sample in a test tube in a dark room at 37°C. The absorbance value of the mixture was measured using a spectrophotometer at a wavelength of 593 nm.

Sensory analysis

Sensory analysis was conducted by involved 70 panelists. Guava leaf tea was brewed by using hot water with the proportion of 1:100 (w/v) and allowed to cool before served. Panelists were asked to taste each sample and assessed how much the attribute appeared. The sensory attributes tested consist of taste (bitter, sour, astringent, chelate), aftertaste (bitter, sour, astringent, chelate), viscosity, and color by using 5 scale point. If the attributes didn't detect by panelists, the scale should be 0 point.

Data Analysis

Chemical and antioxidant data were given as mean value from three replicates for each treatment. Differences among the samples were determined by ANOVA followed by Duncan's Multiple Range tests at *p<0.05* significance level. For the sensory profile analysis, the data was visualized by using principle component analysis (PCA). The PCA biplot graph represented the attribute relationships between the attributes and the samples. All of statistical analysis were conducted using SPSS version 22 (SPSS Inc., USA)

RESULT AND DISCUSSIONS

Crystal Guava Leaf Tea Chemical Profile

The chemical profile of crystal guava leaf (CGL) tea was shown in Table 1. CGL tea was produced by fermented the leaves for 0-20 hours Crystal guava leaves are rich in various micro- and macro-nutrients and health-promoting bioactive compounds (Kumar et al., 2021). It showed that CGL tea had a water content ranging from 5.75 – 6.49%, ash content ranging

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from 6.40 – 7.04%, protein content ranging from 2.16 – 2.59%, fat content 6.87 – 7.29% and carbohydrate content 77.58 – 77.86% (Table 1). Based on these data, the fermentation process for 20 hours generally had no significant effect on the chemical profile levels of CGL tea. The chemical profile of CGL tea also generally meets the quality parameters of SNI 1902-2016, such as water content below 7% and total ash ranging from 4 – 8%.

Table 1. Chemical profile of crystal guava leaf (CGL) tea

Fermentation time (hours)	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
0	6,49	6,40	2,50	6,87	77,74
5	5,75	6,91	2,59	7,13	77,62
10	5,89	6,67	2,57	7,29	77,58
15	5,59	7,04	2,37	7,14	77,86
20	5,96	6,83	2,16	7,22	77,83

Antioxidant Activity of Crystal Guava Leaf Tea

Oxygen is essential in metabolism because it is a terminal electron acceptor during respiration. However, oxygen can also become a free radical agent that can trigger disease in the human body (Mattson, 2000). Therefore, exogenous antioxidants derived from CGL tea can act as agents to neutralize this process (Oktavia et al., 2021). Analysis of antioxidant activity using the DPPH and FRAP methods showed the same trend (Figure 1). In the DPPH test, the antioxidant activity of CGL tea ranged from 90.75 – 94.65%, where the 0th-hour fermentation had the lowest antioxidant activity (90.75%), the 15th-hour fermentation had the highest antioxidant activity (94.65%), the further (> 15 hours) decreased antioxidant activity (92.9%). The same trend of antioxidant activity was also shown by the FRAP method, in which the antioxidant activity of CGL tea ranged from 2,151 – 2,419 µmol ascorbic acid/g. The 0-hour fermentation had the lowest antioxidant activity (2.1510 µmol ascorbic acid/g), the 15-hour fermentation had the highest antioxidant activity (2.4199 µmol ascorbic acid/g), and the further fermentation (> 15 hours) decreased antioxidant activity (2,386 µmol ascorbic acid/g).

The DPPH and FRAP methods are two methods of analyzing antioxidant activity with different approaches. The DPPH method analyzes antioxidant activity based on the number of electrons donated to free radicals (Widyasanti et al., 2016). The FRAP method uses antioxidant compounds as reducing agents in oxidation-reduction reactions (Choirunnisa et al., 2016). Based on the analysis results, the increase in antioxidant activity is thought to be due to the formation of free phenolic components during the fermentation process (Suhardini & Zubaidah, 2016). Meanwhile, an enzymatic oxidation process affected the decrease in antioxidant activity, which caused polyphenols to oxidize and decrease. The decrease in bioactive components such as tannins in tea during fermentation also causes lower antioxidant activity (Kusmiyati et al., 2015).

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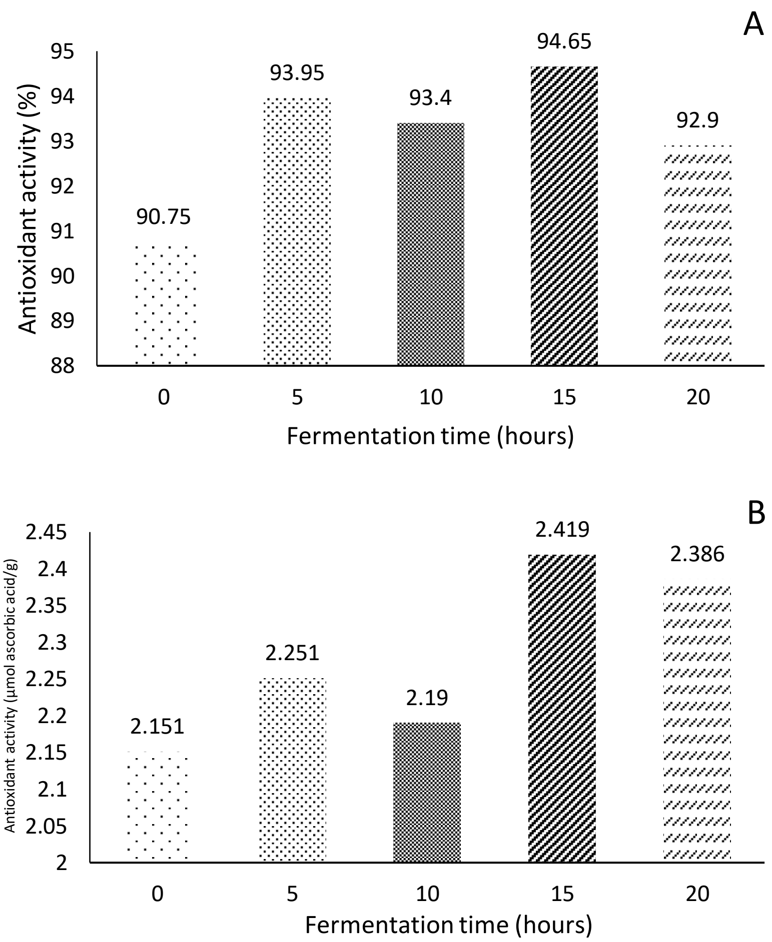

Figure 1. Antioxidant activity of crystal guava leaf tea using DPPH (A) dan FRAP (B) assay

Sensory Profile of Crystal Guava Leaf Tea

Besides having good health benefits, the public widely consumes tea because it has a unique sensory profile (taste, aroma, and color). The sensory profile is the basis for tea product development carried out by the industry (Ananingsih et al., 2013). The preference test using the Friedman Rank for CGL tea with different fermentation durations showed that CGL tea had a taste preference score ranging from 2.13 to 2.64, in which the highest preference score was obtained at 0-hour fermented CGL tea (non-fermented tea) (Table 2).

Table 2. The mean score of preference for guava leaf tea flavor with long fermentation treatment

Fermentation time (hours)	Ranking Total	Preference score
0	239,5	2.64^{b}
5	182,0	2.13^{a}
10	223,5	2.49^{b}
15	232,0	2.60^{b}
20	173,0	2.09^{a}

*numbers followed by different notations show significant differences ($p<0.05$)

The panelists prefer non-fermented CGL tea because it has unique characteristics such as an aroma tends to be floral (aroma associated with flowers), a green (sharp, slightly spicy) and floral (aroma associated with flowers) flavor, bitter (basic taste of caffeine) and astringent

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aftertaste (dry sensation on the tongue and surface of the mouth) (Lee & Chambers, 2010). The preference value of unfermented CGL tea was similar to guava leaf tea fermented at 15 hours (2.60) (Table 2). The panelists preferred the 15-hour fermented CGL tea or semi-fermented/oolong tea because it has a more fragrant aroma than green tea (Han et al., 2016). The study of Anggraini et al., (2018) also reported that oolong tea has a taste that panelists tend to like compared to other types of tea. This is partly caused by the fermentation/oxidation process in tea reducing the catechin content so that oolong tea has a milder bitter/astringent taste than green tea.

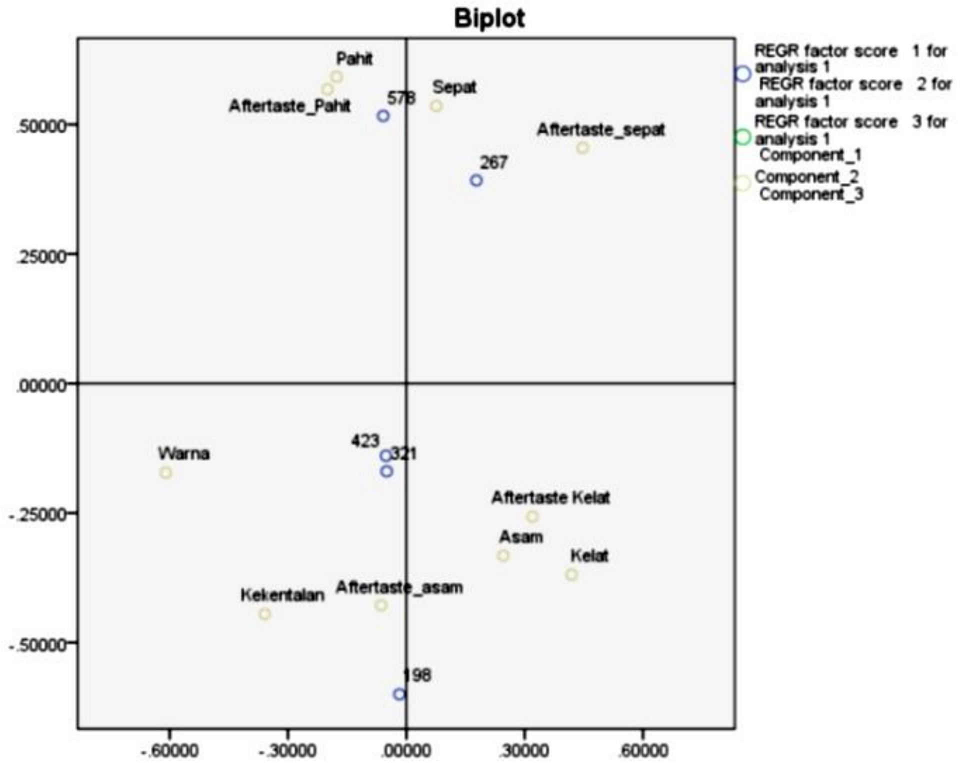

Figure 2. Biplot PCA chart according to all panelist (N=70)

The sensory profile of CGL tea was further analyzed using the RATA (Rate-All-That-Apply) method which aims to determine the description of the product's sensory attributes based on panelist perceptions. The results of the sensory profile test using the RATA method are displayed in PCA biplot graph (Figure 2). The PCA biplot graph showed the relationship between tea samples and sensory attributes of taste (bitter, sour, astringent, chelate), aftertaste (bitter, sour, astringent, chelate), color, and thickness. The treatment of CGL tea with a fermentation time of 5 hours (code 267) was in the first quadrant and had sensory characteristics of astringent taste and astringent aftertaste. The astringent taste and aftertaste are caused by the content of catechins and tannins in tea, in which the 5-hour fermentation time is not enough to reduce or eliminate these two compounds. Study Ares et al., (2009) reported that the astringent taste is influenced by catechins and tannins, which the astringent taste will decrease with increasing fermentation time which causes the levels of catechins and tannins to decrease.

CGL tea with a fermentation time of 20 hours (code 578) was in the second quadrant and is characterized by taste attributes. Tea's bitter taste and aftertaste were due to the presence of catechins. This was in accordance with Han et al., (2016) and Eviza et al., (2021) in which the bitterness of tea brewing is closely related to several groups of non-volatile compounds,

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including polyphenolic catechins and purine alkaloid caffeine. Catechins are responsible for astringency and bitter taste due to their tendency to bind and precipitate salivary proteins and activation of bitter taste receptors. The attributes of chelating, sour, and chelating aftertaste characterized tea treatment in the third quadrant. The taste and aftertaste of this chelate come from tannins and catechins. Paramita et al., (2019) study reported that catechin compounds and their derivatives in the leaves give a chelate taste. Another study by Sariyanto, (2019) also reported that tannins are polyphenolic compounds that carry a bitter and chelating taste. In addition, the taste attribute is also characterized by a sour taste. The sour taste is due to the organic matter content in tea, such as citrate, fumarate, glucuronide, malate, and oxalate (Alasalvar et al., 2012).

CGL tea with fermentation times of 0, 10, and 15 hours (198, 321, and 423) in the fourth quadrant was characterized by its sour aftertaste. The sour aftertaste that arises is due to the presence of citric acid. This is in accordance with the statement Alasalvar et al., (2012) which states that the sour taste in tea is due to citric acid which is an organic acid. According to Zhang et al., (2022) this sour taste is the basic taste that is formed from the fermentation process. The succinic acid in tea also gives off a sour taste.

CONCLUSION

The results of this study indicated that the duration of fermentation affected the level of antioxidant activity, preference score, and sensory profile of CGL tea. The tea from the 15-hour fermentation treatment had the best antioxidant levels and a relatively high preference score. The results showed that the astringent taste of tea could be reduced by increasing the fermentation time because the tannin content was decreasing. In addition, the chemical composition of powdered guava leaf tea produced, such as water content and total ash met the tea quality requirements.

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