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Contribution of Additional Glutamic Acid and Fructose in The Formation of Flavor Compounds in Green Tea

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ABSTRACT:
Green tea in Indonesia has an astringent and bitter taste due to its high catechin content. Adding amino acids and reducing sugars in green tea processing will increase the desirable flavor and cover the astringent and bitter flavors by increasing the Maillard reaction. Research on the manufacture of green tea with the addition of glutamate and fructose in fresh tea leaves has never been explored. This study aims to find a combination of glutamic acid (0%, 0.35%, 0.7%, 1.05%, 1.4%) and fructose (0%, 0.13%, 0.26%, 0.39%, 0.52%) to improve the flavor profile in green tea. The samples were evaluated for their antioxidant activity, amino acid, volatile, sensory evaluation, and color analysis. The results showed that adding glutamate and fructose increased the antioxidant activity of amino acid compounds and decreased the catechin content in green tea. Thus, the best treatment obtained was glutamate (1.05%) and fructose (0.26%), which increased the Maillard reaction in the formation of flavor compounds in green tea.

Keywords: Green Tea; Glutamic Acid; Fructose; Maillard Reaction

INTRODUCTION

Tea is one of the most popular and highly consumed beverages in Indonesia. For tea consumers, this commodity is considered to have a comparative advantage due to its attractive characteristics, including its distinctive taste and aroma. It does not cause adverse effects when consumed and provides freshness after consumption (Shukla, 2007). Besides that, infusing tea leaves (*Camellia sinensis*) can be considered a functional drink because it has an antioxidant effect (Eveline, 1997). Catechins, the compounds responsible for the antioxidant effect, also provide a unique flavor of tea. According to (Sundari, 2009), the chemical composition of fresh tea leaves (in % dry weight) consists of crude fiber, cellulose, lignin (22%); protein and amino acids (23%); fat (8%); polyphenols (30%); caffeine (4%); pectin (4%). Tea leaves contain three essential components that affect beverage quality, namely caffeine, tannins, and polyphenols.

Besides functional properties, taste, aroma, and color are essential parameters to determine the quality of green tea. Precursor compounds significantly affect the aroma of tea products. This is because volatile compounds greatly influence the sensory attributes of tea. The formation of taste compounds can occur through the Maillard reaction. The Maillard reaction involving certain amino acids and reducing sugars can produce caramel, fruity, and floral impression (Wong *et al*., 2008). During the rolling process in green tea production, suitable conditions for the Maillard reaction can be intervened due to the cell fluid from the damaged leaf surface (Ye *et al*., 2018; Zhang *et al*., 2019). Damage to the leaves can allow the Maillard reaction involving the amino acids theanine and reducing sugars to occur so that it can increase the chestnut-like flavor during the drying process (Zhang *et al*., 2020). Amino acids and reducing sugars are essential in enhancing the Maillard reaction as a precursor. Glutamate is an amino acid derivative of theanine. It can be added to green tea to enhance the Maillard reaction (Sugiyama *et al*., 2004). In addition, the addition of fructose as a reducing sugar also plays a role in the formation of aroma and taste through a series of Maillard reactions (Bhumiratana *et al*., 2011; Sari *et al*., 2012; Cecilia *et al*., 2012).

Apart from volatile compounds, tea also has non-volatile compounds formed from three essential components, namely caffeine, tannins, and polyphenols. Caffeine gives a bitter taste, while tannins, which comprise about 7–15% of tea's non-volatile components (caffeine, tannins, and polyphenols), provide an astringency or distinctive taste. Tannins can precipitate proteins on cell surfaces and polyphenols, which have many health benefits because they have antioxidant activity with strength 100 times more effective than vitamin C and 25 times higher than vitamin E (Prastiwati *et al*., 2010). Additionally, EGCG is a polyphenol in green tea that possesses anti-inflammatory, anti-microbial, and anti-allergic properties (Hosnuter *et al*., 2015).

However, the local green tea variety in Indonesia is classified as having an astringent and bitter taste because

Table 1. Experimental design

	F1 Fructose (0%)	F2 Fructose (0.13%)	F3 Fructose (0.26%)	F4 Fructose (0.39%)	F5 Fructose (0.52%)
G1 Glutamic acid (0%)	G1F1	G1F2	G1F3	G1F4	G1F5
G2 Glutamic acid (0.35%)	G2F1	G2F2	G2F3	G2F4	G2F5
G3 Glutamic acid (0.70%)	G3F1	G3F2	G3F3	G3F4	G3F5
G4 Glutamic acid (1.05%)	G4F1	G4F2	G4F3	G4F4	G4F5
G5 Glutamic acid (1.40%)	G5F1	G5F2	G5F3	G5F4	G5F5

Note: glutamic acid (G), fructose (F)

it has a high catechin content (10.04%) (Karori *et al*., 2007). Therefore, it is necessary to modify the green tea processing to improve the flavor profile and cover the astringent and bitter flavors by increasing the Maillard reaction by adding glutamic amino acid and fructose-reducing sugar. This study aims to enhance the profile of compounds, which are glutamic amino acid and fructose-reducing sugar in green tea.

MATERIALS AND METHOD

Materials

The fresh tea leaves of the TRI 25 clone type were harvested from PT. Pagilaran in Samigaluh, Central Java, Indonesia. Glutamate and D-fructose were obtained from Nitra Chemical (Merck, Germany). Several reagents for analysis were purchased from Sigma-Aldrich (St. Louis, USA).

Sample preparation

Fresh TRI 25 clone tea leaves (*Camellia sinensis* L.) were picked in mid-February 2021 from PT. Pagilaran, Samigaluh, Kulon Progo, Yogyakarta. The fresh tea leaves were subjected to a series of manufacturing processes. First, they were spread out on a baking sheet to wither. Then, they were steamed and cooled before finally being rolled. Before the drying process, glutamate and fructose were added. Then, the tea was dried at 95 °C for 45 minutes.

Application of glutamic acid and fructose in green tea extract

To prepare the tea, 100 grams of tea leaf shoots were steamed for 5 minutes to wither them. They were then rolled to damage the surface of the leaves and remove the cell fluid. It was then followed by spraying with a solution of glutamic acid with various concentrations (0%, 0.35%, 0.7%, 1.05%, 1.4%) and fructose solution with concentrations (0%, 0.13%, 0.26%, 0.39%, 0.52%). After being sprayed, the tea leaves were dried at 95 °C for 45 minutes. The experimental design used is shown in Table 1.

Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity of the tea extracts was measured by the method described by Zaiter *et al*. (2016) with modifications. Each tea extract of 1 ml was added with 1 ml of DPPH 0.1 M solution mixed with methanol absolute, and the mixture was incubated in the dark for 30 minutes. As a control, 1 ml of DPPH 0.1 M solution was mixed with 1 ml of methanol as a blank. The absorbance was measured at 517 nm. Radical scavenging activity was calculated as a percentage (%) of radical scavenging of extracts.

Catechin analysis

Some tea samples were homogenized by grinding with the addition of liquid nitrogen. A total of 0.5 grams of tea samples from each clone were brewed with 25 mL of cellular segments. The cellular segments used were isocratic cellular segments, particularly a combination of 0.1% ortho-phosphoric acid:water:acetonitrile:methanol (14:7:3:1 v/v/v/v). Catechin extraction was performed by using a sonicator for 10 minutes. After the extraction, the analyte underwent filtration using a 0.45 µm membrane filter. Finally, it was diluted ten times with the mobile phase. A total of 20 µL was injected into the HPLC injector equipped with a PDA detector. To calculate the levels of the analyzed substance, each peak area was compared with the standard compound peak area, consisting of six individual catechins (Wu *et al.*, 2010).

Table 2. Effect of Addition of Glutamate and Fructose on Antioxidant Activity Inhibiting DPPH Green Tea Leaf Extract

		Fructose				
	%	0	0.13	0.26	0.39	0.52
Glutamate	0	56.8±6.3^{Bc}	69.0±1.4^{Bc}	56.8±1.6^{Bb}	66.8±0.5^{Ba}	70.1±2.7^{Bc}
	0.35	59.3±0.2^{Ac}	55.4±0.3^{Ac}	56.0±0.2^{Ab}	39.8±0.9^{Aa}	57.2±0.7^{Ac}
	0.7	64.2±7.9^{Bc}	68.0±5.9^{Bc}	59.4±7.5^{Bb}	63.7±3.6^{Ba}	62.1±2.0^{Bc}
	1.05	59.3±0.2^{Ac}	55.4±0.3^{Ac}	56.0±0.2^{Ab}	39.8±0.9^{Aa}	57.2±0.7^{Ac}
	1.4	59.3±0.2^{Ac}	55.4±0.3^{Ac}	56.0±0.2^{Ab}	39.8±0.9^{Aa}	57.2±0.7^{Ac}

Note: The values presented are the mean ± standard deviation. Different notations in uppercase letters in the same column indicate significant difference (p<0.05) in the same fructose concentration, while different lowercase letters in the same row indicate significant difference (p<0.05) in the same glucose concentration based on the One-Way ANOVA.

Amino acid content

Free amino acid analysis was performed using HPLC instruments with a DAD detector. The 0.5 g of sample was extracted using 25 mL at 80 ℃ for 30 minutes, then centrifuged to obtain the supernatant. The supernatant was added to distilled water and filtered through a 0.45 µm filter. The filtrate was passed through Sep-PAK $C_{18,}$ and 5 ml of ethanol was added. The sample was then filtered through to the 0.45 µm micro filter and given 20 µL of OPA (*o*-phthaldialdehyde) reagent before being injected into the HPLC for derivatization. The column used was Zorbax Eclipse XDB-C_{18} (150×4.6 mm, 5 µm, Agilent). The oven temperature was conditioned at 40 °C. The mobile phases were made of methanol/acetonitrile/water (45/45/10, A) and phosphate buffer (pH 7.5, B) with a flow rate of 1.0 ml/min. The volume of the sample injected was 20 µl. DAD detectors used a wavelength of 338 nm (Wu *et al.*, 2010).

Sensory evaluation

Determination of the sensory properties of green tea extract was carried out by descriptive analysis test. This test used a score/scale approach associated with specific descriptions of product quality attributes. About 5 g of tea sample was prepared and poured with boiling water, covered, and left for 6 minutes. The brewing tea was then poured into a test bowl. Then, observations were made on the color, taste, smell, appearance and the dregs of the green tea. Sensory evaluation was carried out to determine the quality of the green tea extract of TRI 25 clones which were treated with the addition of glutamate and fructose. Sensory evaluation was carried out by a tea tester from PT. Pagilaran, who had been trained and the testing procedure was based on SNI 01-1902-1995 regarding tea that was applicable in Indonesia.

Volatile compounds analysis

The method used for extracting volatile compounds was based on Apriyantono and Bakti's method (2004) with some modifications. The tea leaf powder was then given 400 mL of pentane using a 1:8 (w/v) ratio. After that, the mixture was stirred with a magnetic stirrer for 15 minutes and let it sit for 24 hours. After that, the extract was filtered using Whatmann No 41 filter paper, then Na_2SO_4 was added to remove water. The extract obtained was evaporated by a rotary evaporator. After obtaining the concentrated extract, the volatiles were analyzed using GS-MS to identify the volatile compounds.

Then, there is another extraction method using HS-SPME-GC-MS. First, crushed green tea at a size of 0.1–0.5 mm, then put into a 15 ml vial as much as one-third of the volume of the vial. After that, the extraction was carried out using the solid phase microextraction (SPME) method and then analyzed using GS-MS to identify the volatile compounds.

Color analysis

Color analysis was performed using a chromameter. Each green tea sample that had been extracted was put in a glass container and then attached to the chromameter sensor. Once detected, the tool will display the value of Lightness (L), appearance (a), and blueness (b).

Statistical Analysis

Data analysis used one-way ANOVA statistics with a significance level of 95% with SPSS for Windows software (SPPS Inc. Chicago, USA). The experimental design was carried out using a non-factorial, completely randomized design (CRD).

Table 3. Effect of Addition of Glutamate and Fructose on Catechin Content in Green Tea Extract

No	Sample code	Result (µg/mL)
1	G1F1	2.65 ± 0.16^{Be}
2	G2F2	2.25 ± 0.014^{Dc}
3	G3F1	2.72 ± 0.00^{De}
4	G4F3	1.33 ± 0.001^{Ca}
5	G4F4	$1.89\pm0.01_{Cb}$
6	G5F1	1.22 ± 0.00^{Ae}
7	G2F5	1.37 ± 0.00^{Dd}
8	G3F5	1.05 ± 0.00^{Dd}

Note: Glutamic acid 0% + fructose 0% (G1F1), glutamic acid 0.35% + fructose 0.13% (G2F2), glutamic acid 0.70% + fructose 0% (G3F1), glutamic acid 1.03% + fructose 0.26% (G4F3), glutamic acid 1.05% + fructose 0.39% (G4F4), glutamic acid 1.40% + fructose 0% (G5F1), glutamic acid 0.35% + fructose 0.52% (G2F5), glutamic acid 0.70% + fructose 0.52% (G3F5). The values presented are the mean ± standard deviation. Different notations in uppercase letters indicate significant difference ($p<0.05$) in the same fructose concentration, while different lowercase letters indicate significant difference ($p<0.05$) in the same glutamate concentration based on the One-Way ANOVA.

RESULT AND DISCUSSION

Antioxidant Activity DPPH (radical scavenging activity)

The value of DPPH radical scavenging activity in fresh tea leaf extract is presented in Table 2. The result showed that there was a significant difference in antioxidant activity between samples. Fresh tea leaf extract, which was given glutamate and fructose, generally increased with a certain concentration limit. The highest DPPH content was 70.1% in the G1F5 treatment while the lowest was in G5F4 which was 39.8%. Heating treatment can accelerate the oxidation of antioxidants contained in the material and result in a decrease in antioxidant activity with different levels according to the type of component that plays a role in antioxidation (Muawanah *et al.*, 2012).

Individual Catechins

The content of catechins in the treated green tea leaf extract can be seen in Table 3. Based on Table 3. Fresh tea leaf extract added with glutamate and fructose produced lower individual catechins than fresh tea leaf extract without treatment because adding sugar would reduce the catechin content in green tea. Catechin levels of the tea leaf extracts from almost all treatments decreased compared to that of control (Table 3). Green tea treated with G3F1 had the highest catechin content, which was 2.72 g/ml. However, most green teas with added glutamate and fructose have low catechin content. This shows that the process of adding glutamate and fructose can reduce the catechin content in green tea compared to control (untreated) samples. It might be due to the influence of the dissolved polyphenol components presence. Catechins/tannins are flavonoid compounds in green tea. Increasing sugar concentration might be reducing the solubility of polyphenol components.

Amino acid content

The total content of free amino acids in tea is also categorized into several flavor-forming groups, including MSG-like AA (amino acid), sweet AA (amino acid), and other AA (amino acid). MSG-like AA consists of aspartate, glutamate, and theanine (Kaneko et al., 2006). Sweet AA consists of alanine, glycine, serine, threonine; Bitter AA consists of phenylalanine, tryptophan, tyrosine, and other amino acids are arginine, histidine, isoleucine, leucine, methionine, valine, lysine, tyrosine. MSG-like compound will contribute to the umami taste of the later produced tea.

In Table 4, shows the amino acids identified in several tea leaves. The tea sample coded G4F3 (glutamate 1.05%, fructose 0.26%) had the highest value of glutamic acid at 4.66 mg/100g. According to the research of Yilmaz et al., (2020), this glutamate content can give a pleasant/sweet and fruity aroma to tea leaves. Likewise, the highest alanine amino acid was 0.95 mg/100g. Alanine gives a fruity, pleasant/sweet, caramel-like aroma. It can also be seen that the lysine in G4F3 is the highest compared to other tea samples, namely 1.31 mg/100g. This value is also more significant when compared to tea leaves in the research of Jiang et al. (2019) of 0.36 mg/100g. Lysine gives a caramel-like, biscuit-like, malty, chocolate, bitter taste aroma. The content of amino acid compounds produced is very diverse. In addition, based on research results, the G4F3 sample contained almost all amino acids. Thus, it was expected that this sample would have a more diverse and complex aroma if there were a Maillard reaction between the amino acids and the sugar contained in this tea.

Sensory evaluation

Four samples of the eight green tea extracts showed a dark tea color character while the rest showed a less dark color. The samples with dark steeping colors were G1F1, G2F2, G3F1, and G5F1. While green tea, which has a less dark color, was found in samples G2F5, G3F5, G4F3, and G4F4. The result of sensory evaluation can be seen in Table 5.

Table 4. Effect of addition of glutamate and fructose on total amino acid compounds of green tea extract

No	Amino Acid	Concentration (g/100g Protein)							
		G1F1	G2F2	G2F5	G3F1	G3F5	G4F3	G4F4	G5F1
MSG like AA (umami)									
1	Aspartate	1.47	1.48	1.34	1.31	1.23	1.76	1.26	1.42
2	Glutamate	3.29	4.25	3.07	2.6	2.4	4.66	2.98	4.25
Sweet AA (sweetness)									
3	Alanine	0.77	0.79	0.71	0.73	0.69	0.95	0.69	0.75
4	Serine	0.68	0.68	0.62	0.63	0.58	0.81	0.6	0.65
5	Glycine	0.74	0.75	0.68	0.69	0.66	0.91	0.65	0.72
6	Threonine	0.65	0.65	0.6	0.61	0.57	0.77	0.58	0.62
Bitter AA (bitterness)									
7	Phenylalanine	0.91	0.87	0.81	0.84	0.79	1.05	0.87	0.88
8	Tyrosine	0.47	0.49	0.43	0.44	0.41	0.61	0.4	0.45
Other AA									
9	Arginine	1.11	1.02	0.96	0.98	0.88	1.11	1.03	1.04
10	Histidine	0.79	0.62	0.65	0.62	0.59	0.05	0.8	0.64
11	Methionine	1.54	1.43	1.54	1.02	1.15	1.72	1.53	1.1
12	Valine	0.97	0.96	0.89	0.91	0.85	1.16	0.88	0.93
13	Ileucine	0.7	0.72	0.65	0.66	0.62	0.86	0.62	0.69
14	Leucin	1.28	1.31	1.19	1.23	1.16	1.58	1.13	1.25
15	Lycine	1.15	1.12	0.99	1.02	0.85	1.31	0.94	1.06
	MSG like	4.76	5.73	4.41	3.91	3.63	6.42	4.24	5.67
	Sweet AA	2.84	2.87	2.61	2.66	2.5	3.44	2.52	2.74
	Bitter AA	1.38	1.36	1.24	1.28	1.2	1.66	1.27	1.33
	Other AA	7.54	7.18	6.87	6.44	6.1	7.79	6.93	6.71
	Total AA	16.52	17.14	15.13	14.29	13.43	19.31	14.96	16.45

Note: amino acid (AA), glutamic acid 0% + fructose 0% (G1F1), glutamic acid 0.35% + fructose 0.13% (G2F2), glutamic acid 0.35% + fructose 0.52% (G2F5), glutamic acid 0.70% + fructose 0% (G3F1), glutamic acid 0.70% + fructose 0.52% (G3F5), glutamic acid 1.03% + fructose 0.26% (G4F3), glutamic acid 1.05% + fructose 0.39% (G4F4), glutamic acid 1.40% + fructose 0% (G5F1),

The aroma and taste of green tea extract without G1F1 treatment showed the same characteristic fruity aroma and strong taste as samples G2F2, G3F5, G4F3, G4F4, and G5F1 but samples G2F5 and G3F1 had a foreign aroma with a mild taste.

The strong astringent taste is caused by the content of polyphenol compounds, especially catechins contained in each. Epicatechin (EC) and epigallocatechin (EGC) can also contribute to aroma and flavor, eliciting a slightly bitter (bitter) taste with a slight sweetness after drinking. While the form of gallate (EGC and EGCG) gives rise to

Table 5. The effect of addition of glutamate and fructose on green tea leaves sensory characteristics

Sample	Parameter									
	Tea brew color		Tea brew aroma		Tea brew taste		Tea leaves appearance		Tea leaves dregs	
	Score	Description	Score	Description	Score	Description	Score	Description	Score	Description
G1F1	B	Dark	B	Fruity	B	Strong	B	Good	B	Bright
G2F2	B	Dark	B	Fruity	B	Strong	B	Good	B	Bright
G2F5	C	Less Dark	C	Foreign aroma	C	Weak	C	Leafy	C	Dark
G3F1	B	Dark	C	Foreign aroma	C	Weak	C	Leafy	B	Bright
G3F5	C	Less Dark	B	Fruity	B	Strong	B	Good	C	Dark
G4F3	C	Less Dark	B	Fruity	B	Strong	C	Leafy	B	Bright
G4F4	C	Less Dark	B	Fruity	B	Strong	C	Leafy	B	Bright
G5F1	B	Dark	B	Fruity	B	Strong	B	Good	B	Bright

Note: glutamic acid 0% + fructose 0% (G1F1), glutamic acid 0.35% + fructose 0.13% (G2F2), glutamic acid 0.35% + fructose 0.52% (G2F5), glutamic acid 0.70% + fructose 0% (G3F1), glutamic acid 0.70% + fructose 0.52% (G3F5), glutamic acid 1.03% + fructose 0.26% (G4F3), glutamic acid 1.05% + fructose 0.39% (G4F4), glutamic acid 1.40% + fructose 0% (G5F1), B: score, C: score

a strong astringent taste (Mitrowihardjo *et al*., 2012). Apart from catechins, in black tea, astringent is determined mainly by theaflavin content. Samples G2F2, G3F5, G4F3, G4F4, and G5F1 were thought to have high theaflavins content compared to samples G2F5 and G3F1. Therefore, the strong astringent taste is caused by the influence of the components of each sample.

Green tea samples (G1F1, G2F2, G3F5, and G5F1) gave a good leaves appearance but were different from samples G2F5, G3F1, G4F3, and G4F4 due to the differences in the interaction of water content and rolling process. However, the quality of black tea cannot be judged on a limited number of characteristics. In other words, when one characteristic is at the highest level, another characteristic may be at a lower level. Thus, to produce good quality black tea, all characteristics must be at optimal levels. Based on the green tea pulp parameters in the table, only samples G2F5 and G3F5 had dark pulp characteristics, while the other six samples had bright pulp.

Color

Table 6. showed that the values of L (Lightness), a (redness), and b (yellowness) in the green tea leaf extract treated were higher than the green tea leaf extract without treatment. The lowest L (Lightness) value was 52.9±0.1 in the untreated sample (G1F1), indicating that the extract had a darker color than the treated sample. Likewise, the highest b value was 16.7±0.6, indicating that the sample was the yellowest. The brightness value of green tea increased after being given glutamate and fructose due to the Maillard reaction

Table 6. The effect of addition of glutamate and fructose on green tea leaf extract color

Sample	Hunter score		
	L	a	b
Control	52.9 ± 0.1^{Aa}	-2.27 ± 0.2^{ABa}	16.7 ± 0.6^{Ed}
G2F2	55.2 ± 1.7^{Aa}	-1.83 ± 0.1^{Bc}	6.2 ± 0.1^{Bb}
G2F5	53.6 ± 0.1^{Aa}	-2.48 ± 0.06^{Ba}	12.6 ± 0.2^{Bc}
G3F1	54.7 ± 0.1^{Aa}	-2.18 ± 0.01^{Aa}	8.5 ± 0.03^{Dd}
G3F5	54 ± 1.5^{Aa}	-2.25 ± 0.04^{Aa}	9.4 ± 0.2^{Dc}
G4F3	54.7 ± 0.1^{Ab}	-1.71 ± 0.2^{Cd}	7.1 ± 0.1^{Ca}
G4F4	54.7 ± 0.4^{Aa}	-2.28 ± 0.03^{Cb}	9.6 ± 0.2^{Cc}
G5F1	54.5 ± 0.2^{Aa}	-1.98 ± 0.03^{Da}	9 ± 0.2^{Ad}

Note: The values presented are the mean ± standard deviation. Different notations in uppercase letters indicate significant difference ($p<0.05$) in the same fructose concentration, while different lowercase letters indicate significant difference ($p<0.05$) in the same glutamate concentration based on the One-Way ANOVA.

Table 7. The effect of addition of glutamate and fructose on aromatic volatile compounds of green tea leaf extract

Compounds	CAS	LRI	Concentration (ppb)	
			Tea leaves extract without treatment	Tea leaves extract with treatment
Dimethyl sulfide	75-18-3	**0**	11.164	16.2217
Cyclobutanol	2919-23-5	**0**	2.4308	8.7304
1-Pentanol	71-41-0	**774**	0.9433	1.657
2-Pyrazoline. 1.5-dimethyl-	5775-96-2	**810**	0.2111	1.6469
m-Xylene	108-38-3	**872**	0.9674	1.1979
p-Xylene	106-42-3	**885**	0.091	0.4199
o-Xylene	95-47-6	**898**	0.1937	0.2753
Heptanal	111-71-7	**905**	0.1662	0.8869
Benzaldehyde	100-52-7	**966**	1.5411	1.5411
2-Methyl-2-butenol	4675-87-0	**913**	-	0.1275
2.6-dimethylpyrazine	108-50-9	**919**	-	0.7957
2-formyl-1-methylpyrrole	1192-58-1	**932**	-	0.7593
1-Octen-3-ol	3391-86-4	**965**	0.3284	1.4045
β-Myrcene	123-35-3	**995**	2.0758	6.6044
1.3-dichloro-benzene	541-73-1	**1015**	5.6431	7.5439
2.3.4-trimethyl-hexane	921-47-1	**1017**	1.6048	6.833
D-limonene	5989-27-5	**1031**	1.8869	8.7612
α-ocimene	502-99-8	**1041**	0.8964	3.002
β-ocimene	13877-91-3	**1053**	2.1306	10.3037
α-terpinolene	586-62-9	**1091**	1.5662	4.8319
Linalool	78-70-6	**1105**	9.2221	12.9446
Nonanal	124-19-6	**1110**	3.922	16.6457
2-Methoxy-4-methylaniline	39538-68-6	**1117**	2.6775	3.8445
Allo-Ocimene	673-84-7	**1133**	0.5054	1.777
D-Camphor	464-49-3	**1147**	0.5918	0.5918
Neo-allo-ocimene	7216-56-0	**1145**	-	1.2025
Naphthalene	91-20-3	**1188**	6.8415	65.1105
α-Terpineol	98-55-5	**1195**	0.9469	3.0595
Methyl salicylate	119-36-8	**1198**	0.8521	1.7762

Compounds	CAS	LRI	Concentration (ppb)	
			Tea leaves extract without treatment	Tea leaves extract with treatment
Safranal	116-26-7	**1203**	0.5564	1.949
Tetradecane	629-59-4	**1204**	0.3576	1.3325
5-Ethyl-1-nonene	19780-74-6	**1233**	1.1656	2.6238
1-methyl-napthalene	90-12-0	**1297**	0.5632	1.0259
Indole	120-72-9	**1302**	1.797	2.2385
2.6.10.10-tetramethyl-1-Oxaspiro[4.5]dec-6-ene	36431-72-8	**1319**	0.7701	2.0389
Dehydro-ar-ionene	30364-38-6	**1358**	-	0.9777
Ionene	475-03-6	**1361**	0.4907	1.0136
10-Methylnonadecane	56862-62-5	**1364**	0.5495	0.5495
β-Damascenone	23726-93-4	**1388**	0.1834	0.4975
1-Pentadecene	13360-61-7	**1391**	0.3326	0.2101
2.7-dimethyl-naphthalene	582-16-1	**1407**	-	0.1502
Undecane	1120-21-4	**1411**	-	0.2582
1.2-dihydro-1.5.8-trimethyl-naphthalene	4506-36-9	**1413**	-	0.3173
2-Methylhexadecane	1560-92-5	**1420**	0.2497	0.3315
Sulfurous acid. butyl tridecyl ester	1000309-18-0	**1481**	-	0.858
Dehydro-β-ionone	1203-08-3	**1487**	-	0.6086
3.9-Dimethylundecane	17301-31-4	**1481**	0.6653	0.6653
Octadecane	593-45-3	**1505**	0.4	0.6392
Butylhydroxytoluene	128-37-0	**1517**	-	0.4951
δ-Cadinene	483-76-1	**1528**	-	0.2515
1-Ethyl-2-propylcyclohexane	62238-33-9	**1534**	1.0675	1.2042
Pentacosane	629-99-2	**1542**	-	0.5668
2.5-Diisobutylthiophene	54845-33-9	**1560**	-	0.3026
Phytane	638-36-8	**1563**	0.7935	0.605
4.7-Dimethylundecane	17301-32-5	**1577**	0.0658	0.1023
2-Methylhexacosane	1561-02-0	**1583**	-	0.1864

Compounds	CAS	LRI	Concentration (ppb)	
			Tea leaves extract without treatment	Tea leaves extract with treatment
Ethyl-α-naphthylamine	118-44-5	**1595**	-	0.1082
6-Methyl-2.3-dihydrofuro[2.3-b]quinoline	64124-83-0	**1628**	-	0.221
γ-Cadinene	39029-41-9	**1650**	0.237	0.4055
α-Cadinol	481-34-5	**1663**	0.1123	0.2447
8-Hexylpentadecane	13475-75-7	**1706**	0.111	0.1529
di-tert-dodecyldisulfide	27458-90-8	**1763**	0.1173	0.5449
1-Ethyl-2.4-dimethylcyclohexane	61142-69-6	**1768**	0.3046	0.6892
4-Ethyltetradecane	55045-14-2	**1777**	0.2234	0.3876
Hexahydrofarnesyl acetone	502-69-2	**1828**	0.0815	0.38
Caffeine	58-08-2	**1855**	2.672	3.3067

which took place at high temperature, thus increasing the lightness (L) value. Meanwhile, in this study, the G4F3 treatment, namely the addition of Glutamate (1.05%) and Fructose (0.26%) showed a significant difference to other treatments due to the Maillard reaction after treatment. The value of a redness is the chromatic color of a mixture of red and green. The red color is in the range of values 0 to +100 while the green color is in the range of values 0 to -80. The greater the positive value of a means the redder the color, while the higher the negative value, the greener the color (Anjani *et al*., 2015). Based on research, the color of brewed green tea leaves had a negative value. This means that the color of the steeped tea tended to be green. Based on the analysis of statistical results, the addition of glutamate and fructose gave a significant effect on the value of steeping green tea (p<0.05). The green color of fresh tea leaf extract given glutamate and fructose is determined by the presence of chlorophyll and as the main color in fresh tea leaves. In addition, this is in accordance with the research of Wang *et al*., (2004) that a negative a (redness) value indicates that there are bioactive compounds that contribute to the greenish color of fresh tea leaf extract. However, the results showed that the "a" value in the control was higher than all treatments. This was because the sample added with glutamate and fructose increased the occurrence of the Maillard reaction, and it was estimated that the product of the Maillard reaction had an adverse effect on tea color (Wang *et al*., 2019). The value of "b" (yellowness) is a mixed chromatic color of blue-yellow (Ardiyansyah and Apriliyanti, 2016). The +b (positive) value is from 0 to +70 for yellow and -b (negative) is from 0 to -70 for blue (Manera *et al*., 2012). Based on the research results, the b value obtained was positive. This indicates that the color of the green tea was yellowish. Based on the results of statistical analysis, the addition of glutamate and fructose had a significant effect (p < 0.05) on the b value in green tea. This indicates that the extract had a bright yellowish green color (Liang *et al*., 2008).

Volatile compounds

In view of the positive effect and enhancement of the chestnut-like aroma, the aroma components of the tea samples under different additional treatments were determined. As can be seen from Table 7, a total of 171 compounds were detected, including 50 hydrocarbons, 20 alcohols, 4 esters, 14 ketones, 19 aldehydes, 3 phenolic and 1 pyrrole, 6 pyrazine, and 54 other compounds. The total contents of the types of compounds in the different additive treatments are shown in the table. It was found that there was an increase in the concentration of volatile compounds when tea was treated with the addition of glutamate and fructose. The total volatile in the control treatment of green tea extract was 289.8278 ppb while the total volatile compound in the

addition of glutamate and fructose was 369.6741 ppb. The role of adding glutamate and fructose in this research was to increase the volatile compounds that will give a certain aroma to the green tea extract. In this study, the addition of glutamate and fructose increased the occurrence of the Maillard reaction, which changed and increased the volatile compounds in green tea.

CONCLUSION

The samples with the highest amino acid content were approved as the best treatment in this study, adding 1.05% glutamate and 0.26% fructose. Adding glutamate and fructose could reduce catechins' content but increased the content of amino acids and antioxidant capacity in green tea. The addition of glutamate and fructose also increased the flavor compounds through the Maillard reaction when compared to commercial green tea.

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REFERENCES

Anjani, Puncak, P., Andrianti, S., Widyaningsih, T.D. (2015). Pengaruh Penambahan Pandan Wangi dan Kayu Manis Pada Teh Herbal Kulit Salak Bagi Penderita Diabetes. *Jurnal Pangan dan Agroindustri*, *3* (1).

Ardiyansyah, & Apriliyanti, M. (2016). Karakteristik kimia teh kulit melinjo. *Jurnal Ilmiah Inovasi*, *16*(2). https://doi.org/10.25047/jii.v16i2.289

Bhumiratana, N., Adhikari, K. and Chambers, E. (2011) Evolution of sensory aroma attributes from coffee beans to brewed coffee. *LWT - Food Science and Technology*, *44*(10), 2185–2192. https://doi.org/10.1016/j.lwt.2011.07.001.

Cecilia, K., K. Glanton, M. Simon, B. Renaud, and N. Fredrick. (2012). Volatile organic compounds in brewed Kenyan arabica coffee genotypes by solid phase extraction gas chromatography mass spectrometry. *Food Science and Quality Management*, *8*, 18 –22

Eveline, R. (1997). *Kajian Produk Olahan Teh di Indonesia.* Jurusan Sosial Ekonomi Pertanian, Bogor.

Hosnuter M, Melikoglu C, Aslan C, Saglam G,Sutcu, C. (2015). The potective effects of epigallocatechin gallate against distantorgan damage after severe skin burns-experimental study using a rat model of thermal trauma. *Adv Clin Exp Med 24*(3), 409–417. https://doi.org/10.17219/acem/43761

Jiang, H., Yu, F., Qin, L., Zhang, N., Cao, Q., Schwab, W., Li, D., & Song, C. (2019). Dynamic change in amino acids, catechins, alkaloids, and gallic acid in six types of tea processed from the same batch of fresh tea (*Camellia sinensis* L.) leaves. *Journal of Food Composition and Analysis*, *77*, 28–38. https://doi.org/10.1016/j. jfca.2019.01.005

Kaneko, M. K., Kato, Y., Kitano, T., & Osawa, M. (2006). Conservation of a platelet activating domain of Aggrus/podoplanin as a platelet aggregation-inducing factor. *Gene*, *378*, 52–57. https://doi.org/10.1016/j.gene.2006.04.023

Karori, S. M., Wachira, F. N., Wanyoko, J. K., & Ngure, R. M. (2007). Antioxidant capacity of different types of tea products. *African Journal of Biotechnology*, *6*(19), 2287-2296, https://doi.org/10.5897/AJB2007.000-2358

Liang, Y. R., Ye, Q., Jin, J., Liang, H. Lu, J. L. Du, Y. Y., and Dong, J. J. (2008). Chemical and instrumental assessment of green tea sensory preference, *International Journal of Food Properties, 11*, 258–272, https://doi.org/10.1080/10942910701299430.

Mitrowihardjo, Suyadi, Woerjono Mangoendidjojo, Hari Hartiko, dan Prapto Yudono. 2012. Kandungan Katekin dan Kualitas (Warna Air Seduhan, Flavor, Kenampakan) Enam Klon Teh (Camellia sinensis (L.) O. Kuntze) Di Ketinggian yang Berbeda. *Agritech*, *32*(2), 199–206

Muawanah, A., Djajanegara, I., Sa'duddin, A., Sukandar, D., Radiastuti, N. (2012). Penggunaan bunga kecombrang (*Etlingera elatior*) dalam proses formulasi permen jelly. *Jurnal Kimia VALENSI*, *2*(4), https://doi.org/10.15408/jkv.v2i4.270.

Prastiwati, R., Rahayu, W. S., Hartanti, D. (2010). Perbandingan daya antioksidan ekstrak metanol daun tembakau (*Nicotiana tabacum l.*) dengan rutin terhadap radikal bebas 1,1-diphenil-2-pikrilhidrazil (DPPH). *Jurnal Farmasi Indonesia, 7*(1), 109–118 https://doi.org/10.30595/pji.v7i1.549.

Sari, A. B. T., C. Ismayadi, T. Wahyudi, and A. Sulihkanti. (2012). Anaysis of luwak coffee volatile by using solid phase microextraction and gas chromatography. *Pelita Perkebunan*, 28 (2), 111-118.

Shukla, Y. (2007). Tea and cancer chemoprevention: A comprehensive review. *Asian Pacific Journal of Cancer Prevention, 8*(2), 155–166.

Sugiyama, T., & Sadzuka, Y. (2004). Theanine, a specific glutamate derivative in green tea, reduces the adverse reactions of doxorubicin by changing the glutathione level. *Cancer letters*, *212*(2), 177–184,

https://doi.org/10.1016/j.canlet.2004.03.040

Sundari, Dian, *et al*. (2009). Toksisitas Akut (LD50) dan Uji Gelagat Ekstrak Daun Teh Hijau (*Camellia sinensis*) Pada Mencit. *Jurnal Media Penelitian dan Pengembangan Kesehatan*. Vol XIX, No 4, 198-203.

Wang, L. F., Park, S. C., Chung, J. O., Baik, J. H., and Park, S. K. (2004). To the greenness of green tea. S*ensory and Nutritive Qualities of Food*, *69*(8), 301–305.

Wang, L. *et al*. (2019). Protective effect of green tea catechin against urban fine dust particle-induced skin aging by regulation of NF-KB, AP-1, and MAPKs signaling pathways. *Environmental Pollution*, *252*, 1318–1324, https://doi.org/10.1016/j.envpol.2019.06.029.

Wong, K. H., Abdul Aziz, S. and Mohamed, S. (2008). Sensory aroma from maillard reaction of individual and combinations of amino acids with glucose in acidic conditions. *International Journal of Food Science and Technology*, *43*(9), 1512–1519, https://doi.org/10.1111/j.1365-2621.2006.01445.x.

Wu, L. Y., Gao H. Z., Wang, X. L., Ye, J. H., Lu, J. L., Liang, Y. R. (2010). Analysis of chemical composition of chrysanthemum indicum flowers by GC/MS and HPLC. *Journal of Medicinal Plants Research*, *4*(5), 421–426.

Ye, Y.L., Yan, J.G., Cui, J.L., Mao, S.H., Li, M.F., Liao, X.L., Tong, H.R., 2018. Dynamic changes in amino acids, catechins, caffeine and gallic acid in green tea during withering. J. Food Anal. 66, 98–108.

Yılmaz, C. and Gökmen, V. (2020). Neuroactive compounds in foods: occurrence, mechanism and potential health effects. *Food Research International*, *128*, 108–744, https://doi.org/10.1016/j.foodres.2019.108744.

Zaiter, A., Becker, L., Karam, M., and Dicko, A. (2016). Effect of particle size on antioxidant activity and catechin content of green tea powders. *Journal of Food Science and Technology*, *53*(4): 2025–2032, https://doi.org//10.1007/s13197-016-2201-4.

Zhang, L., Ho, C. T., Zhou, J., Santos, J. S., Armstrong, L., Granato, D. (2019). Chemistry and biological activities of processed camellia sinensis teas: A Comprehensive Review. *Comprehensive Reviews in Food Science and Food Safety*, *18*(5), 1474–1495, https://doi.org/10.1111/1541-4337.12479.

Zhang, M, Yang, Y., Yuan H., Hua J., Deng, Y., Jiang, Y., Wang, J. (2020). Contribution of addition theanine/sucrose on the formation of chestnut-like aroma of green tea. *LWT*, *129*(18), 109-512, https://doi.org/10.1016/j.lwt.2020.109512.