Improvement of Antioxidant Activity and Sensory Quality of Pagilaran's Tea Clones Treated by Tannase

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ABSTRAK

PT. Pagilaran sedang mengembangkan lima klon baru yang diberi nama PGL 9, PGL 10, PGL 11, PGL 12, dan PGL 15 sebagai klon unggulan untuk teh hitam. Klon tersebut sangat potensial untuk dijadikan minuman teh instan siap minum (RTD). *Ready to Drink* (RTD) dari teh hitam memiliki kelemahan, yaitu aktivitas antioksidan yang rendah. Tannase adalah enzim industri yang memiliki potensi besar untuk aplikasi dalam industri makanan dan minuman. Tannase telah digunakan untuk menghidrolisis ester katekin teh hijau. Belum ada penelitian tentang perubahan komposisi kimia dengan perlakuan enzimatis tannase pada klon teh PT Pagilaran. Tujuan dari penelitian ini adalah untuk mengamati efek dari perlakuan tannase pada lima ekstrak teh hitam klon pagilaran terhadap aktivitas antioksidan, profil senyawa fenolik, warna minuman teh, sifat sensoris, dan senyawa volatil. Daun teh pemetikan sedang (P+2) secara tradisional diolah menjadi teh hitam. Minuman teh ditambahkan dengan tannase pada konsentrasi 100 mg/100 mL, diinkubasi selama 1 jam pada suhu 37 °C. Penambahan tannase dapat meningkatkan aktivitas antioksidan, kandungan asam galat, EC, dan konsentrasi EGC karena hidrolisis ECG, EGCG, atau turunannya. Warna minuman teh semakin cerah dan menguning daripada yang tidak diberi perlakuan enzim tannase. Meski begitu, tingkat penerimaan konsumen masih bagus. Profil aroma dan senyawa volatil minuman teh menunjukkan bahwa tidak ada perubahan signifikan setelah penambahan enzim tannase.

Kata kunci: Antioksidan; klon Pagilaran; tannase; teh

ABSTRACT

PT. Pagilaran developed five new clones named PGL 9, PGL 10, PGL 11, PGL 12, and PGL 15 for black tea. These clones can be potentially made into ready-to-drink (RTD) instant tea beverages with low antioxidant activity. Tannase is an industrial enzyme with great potential for application in the food industry used to hydrolyze gallated ester catechin in green tea. There were no findings on the effects of this enzyme treatment on the chemical composition of PT Pagilaran's tea clones. Therefore, this study is aimed to observe the effects of tannase treatment on five clones of black tea extracts to the antioxidant activity, profile of phenolic compounds, the tea

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brew color, consumer acceptance, and volatile compounds. The leaves of the plucking medium were traditionally processed into black tea. Furthermore, the tea brew was added at a 100 mg/100 mL concentration and then incubated for 1 hour at 37 °C. Additional tannase increased their antioxidant activities, the gallic acid, EC, and EGC concentrations due to hydrolysis of ECG, EGCG, or their derivatives. The color of the tea brew was brighter and yellower than the untreated, but the level of consumer acceptance was still good. The profile aroma and volatile compounds showed no significant changes after enzymatic treatment.

Keywords: Antioxidant; Pagilaran clones; tannase; tea

INTRODUCTION

Pagilaran tea processing and research institute in Universitas Gadjah Mada have developed new clones to improve the quality and production levels. Five new tea clones named PGL 9, PGL 10, PGL 11, PGL 12, and PGL 15 have high productivity and disease resistance (Krisyando et al., 2012). The clones were first developed for black tea material since the parents were known to be good materials for black tea. Ichsan (2019) reported that total phenolic content was high, reaching about 17,97 mg GAE/g dry weight.

Recently, Ready to drink tea has become a trend and lifestyle of most consumers who want practicality and ease. Most consumers are looking for healthier alternatives to food and drinks by considering the nutritional content and functional properties (Kearney, 2010; Küster and Vidal, 2017) This has become one of the opportunities to provide black tea drinks with high antioxidant content. However, this tea is affected by formation of white deposit due to reaction between protein and high molecular weight of gallate polyphenol derivatives during storage. Tannase or tannin acyl hydrolase is an enzyme that can hydrolyze tannin/ catechin, producing gallic acid (Baik et al., 2014; Beniwal et al., 2014). Tannase enzymes may change gallated polyphenols to ungallated polyphenols, increasing the solubility index and increasing the amount of gallic acid in tea extracts. Increased content of gallic acids by tannase is associated with increased antioxidant activity, and are components of medicinal plants or herbs with anti-mutagenic, anti-tumor, antioxidant, anti-inflammatory, and anti-bacterial properties (Liu et al., 2012). Since the amount of gallated polyphenols is reduced in the tea brew, the problem of creaming formation in RTD black tea can be minimized (Raghuwanshi et al., 2013). Furthermore, the new tea clones of Pagilaran are intended for making black tea, and it also has the potential to be made into ready-to-drink black tea. Since the clones are still new, information about the tea infusion properties and the possibility of creaming formation occurring during storage is unknown.

Therefore, the use of the tannase enzyme to determine black tea infusion properties such as antioxidant activity and polyphenol content as well as changes in the individual catechin, creaming formation, and sensory analysis is important.

MATERIAL AND METHOD

Material

Fresh tea leaves of five clones (PGL 9, PGL 11, PGL 12, and PGL15) were harvested on August 19th, 2019 in PT. Pagilaran's afdeling Sanderan and Kayulandak field and immediately processed manually. The shoots resulted from the plucking medium, and the tannase enzyme (300 U/g) was purchased from Sangherb Bio-Tech (Xi'an, Shaanxi, China). Individual standards of EC, EGC, C, ECGG, and ECG were obtained from Sigma Aldrich. Other chemicals used were HPLC- and or analytical-grade quality.

Black tea preparation

Black tea preparation was according to the procedure of Pou (2016) with modification.

Withering process

Withering was carried out in a pagilaran tea processing trough with parameters set at a temperature of 28 °C. A 5 kg of fresh leaves of each clone was provided for processing and were evenly separated and spread on a screen with a thickness of 10 cm and then placed in a withering trough for withering. Meanwhile, the tea leaves was reversed every every 5 hours intervals to even the process of withering.

Rolling and fermentation process

The rolling process was conducted manually by adjusting the working principle in using a simple tool made of 2 stainless steel plates. The Tol was used for crushing, tearing, and curling tea leaves, and the process was continued with the enzymatic fermentation/ oxidation process, which involves cell fluid and oxygen meet. Therefore, oxidation occurs using a tray with a

layer thickness of about 2 cm, and the process was carried out for 2 hours.

Drying

Tea leaves obtained from the fermentation process were then performed in a drying process using an aluminum skillet by first heating to a temperature of 100 °C on the stove. Subsequently, they were roasted for 30 minutes with a continuous reversal process, hence tea leaves were dry evenly to all parts and did not burn.

Cooling

The cooling process was by spreading the sample on a flat surface/tray after the roasting was finished for 10 minutes with a fan until room temperature was reached. The dried tea samples of each clone were immediately weighed, packaged and stored at 25 °C until further analysis was performed.

Preparation of tea infusion

The extraction process involved black tea leaves (5 g) with 100 mL boiling water, which was then mixed in 250 mL Erlenmeyer flasks and kept for 20 minutes. The steeping tea obtained was filtered using Whatman No. paper. 1, and ready for analysis.

Tannase treatment

The filtrate obtained from tea infusion of black tea, i.e., PGL Clones tea extracts, was used as substrates. The biotransformation process was carried out with the tannase enzyme according to Raghuwanshi et al. (2013) method. Furthermore, 100 mL of tea extract was added with 100 mg tannase. Mixed and incubated at a temperature of 37 °C for 1 hour, and the reaction mixture was stopped by heating at 90 °C for 5 minutes.

Antioxidant Activity Using DPPH Method

Antioxidant activity was carried out according to Sudaryat et al. (2015). About 1 ml of the diluted (1000 x with methanol) tea extract was piped into a solution of 0.1 mM DPPH and incubated in a dark room for 30 minutes. The absorbance solution was measured at a wavelength of 517 nm, and the antioxidant activity was calculated using Equation 1.

$$\text{AA } (\%) = \frac{(\text{Abs. control} - \text{Abs. sample})}{\text{Abs. control}} \text{x } 100\% \qquad (1)$$

IC_{50} Evaluation

IC50 of each tea extract sample was calculated according to the procedure of Shimamura et al. (2014)1-diphenyl-2-picrylhydrazyl (DPPH as follows: (1) The ratio of inhibition of DPPH (y) was plotted to the concentration of the sample (x) at all five points. A regression line was drawn (y = ax + b), (2) x (sample concentration) and calculated when y in the regression equation was replaced by 50. The value (2) on each measurement was calculated and set as each tea extract sample's IC50 value (ppm). Meanwhile, ascorbic acid was used as a comparison.

Antioxidant Activity Using FRAP Method

Ferric Reducing Antioxidant Power (FRAP) reagent consist of 300 mM acetate buffer pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM FeCl3.6H2O. The ratio of acetate buffer, TPTZ, and FeCl3.6H2O was 10:1:1 (v/v/v), respectively. The reagent of 3.0 mL was added to 150 µL of tea extract and incubated for 30 minutes in a dark room at room temperature. The absorbance of the solution containing extract and FRAP was measured by UV spectrophotometer -Vis at 598 nm. The results are converted to transmittance using the equation Abs = -log T, hence T = e^{-abs}. Inhibition results obtained from the formula: percentage (%) antioxidant capacity = (1-T) x 100% (Ichsan, 2019).

Total Phenolic Content (TPC) identification Using Folin's Ciocalteu Method

A total of 1 mL of tea extracts was added with a 5 mL Folin Ciocalteu reagent of 5 mL and a 7.5% Na_2CO_3 solution of 4 mL to obtain a volume of 10 mL. The solution was vortexed and stored in a dark room for 2 hours, and then its absorbance was measured at a wavelength of 765 nm. Total phenol content (mg GAE/g) was calculated based on the standard gallic acid curve. The standard solution in the gallic acid solution was concentrated at 0 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, 80 ppm and 100 mg/L (Kaur et al., 2019).

Estimation of catechin monomers and gallic acid with HPLC-PDA

A total of 20 µL of 5 PGL clones tea extract samples were first filtered with a 0.45 µm nylon filter membrane and then automatically injected into the Shimadzu Japan DAD HPLC series injectors equipped with PDA detectors. The columns used are the 5 µm C18 Shimadzu column (SHIMPACK GIST 4.6 x 150 mm), oven temperature 40 °C, wavelength 210 nm, and retention time 35 minutes. The isocratic mobile phase was used, a mixture of 0.1% ortho-phosphoric acid, water, acetonitrile, and methanol (14:7:3:1; v/v/v/v). Quantifying of catechin and gallic acid compounds with standard catechin and gallic acid compounds were performed using external standard catechin compounds (EGC, C, EGCG, EC, ECG) and

gallic acid. The mobile phase flow rate was maintained at 1 mL /min (Martono and Martono, 2013).

Evaluation of Tea Cream Formation

Each black tea infusion, i.e., PGL Clones tea extracts, was treated with 100 mg of tannase as against control. The treated and untreated tea infusion samples obtained from both the teas were centrifuged at 8 °C, 5600g for 20 min. The residue was dried in a hot-air oven at 100 °C until constant weight. The tea cream (g/100 mL) was expressed as the weight of precipitate obtained per 100 mL of tea infusion (Raghuwanshi et al., 2013).

Estimation of Total Theaflavin (TF) and Thearubigin (TR) by Spectrometric Methods

Theaflavin (TF) and thearubigins (TR) in black tea extracts were measured according to Ullah's (1986) spectrophotometric method. Tea extract (20 mL) was mixed with 6 mL 2.5% (w/v) aqueous sodium hydrogen carbonate solution, and the mixture obtained was extracted with 20 mL ethyl acetate and vortexed for 1 minute. The bottom layer was separated, and the upper layer, the ethyl acetate layer (TF fraction) obtained, was used in the analysis. Extract Solution 1 (E1): 10 mL TF extract was diluted to 25 ml with methanol (MeOH). Solution 2 (E2): For 1 mL of tea extract, 1 mL of 10% (w/v) aqueous saturated oxalic acid and 8 mL of water were added and made up to 25 mL with methanol. Furthermore, optical Density E1 and E2 at 380 nm were obtained in the two extracts after applying the appropriate correction for the extract strength and the actual volume used. TF and TR were then calculated with Equations 2 and 3.

$$\text{TF}\ (\%) = 2.25 \text{ x } \text{E1} \quad (2)$$

$$\text{TR}\ (\%) = 7.06 \text{ x } (4\text{E2} - \text{E1}) \quad (3)$$

Volatile Analysis with GC-MS

Volatile analysis refers to the method of Sereshti et al. (2013). Chloroform was used as a non-polar solvent to extract the volatile components in the PGL clone tea extract. Meanwhile, 10 mL of tea extract was added with 30 mL of organic solvent chloroform, mixed using vortex for 5 minutes, then separated using a separating funnel. Non-polar fractions were separated by polar fractions based on specific gravity. The chloroform fraction was dried using Na_2SO_4 anhydrous before being concentrated by flowing nitrogen gas until a volume of 2 mL was obtained. Subsequently, a 1 µL chloroform fraction was injected into the GC-MS, equipped with an HP-5MS UI column with a length of 30m diameter (ID) 0.25mm. The injector and detector temperatures were 260ºC and 250ºC, respectively. The carrier gas used was Helium (He), with a flow rate of 50 ml/ min, and the identification of volatile compounds carried out screening by LPPT-UGM.

Sensory Analysis

Tea samples of about 5.68 g were poured into a test cup measuring 280 mL, boiling water, covered, and left for 6 minutes. Tea brewing was poured into a bowl to keep the steeping pulp from participating. The observations of the brewing water's color, taste, and odor were carried out with the appearance of black tea steeping pulp. Furthermore, sensory analysis was performed to determine the quality of black tea extracts of clones PGL 9, PGL 10, PGL 11, PGL 12, and PGL 15 with or without enzyme treatment. This was performed by tea testers from PT Pagilaran, who had been trained and testing procedures based on SNI 01-1902-1995 on Black Tea.

Statistical Analysis

SPSS version 23.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses, and the data were expressed as mean ± standard deviation (SD). Mean differences were tested using a one-way analysis of variance (ANOVA). A further Tukey's test was applied, and P values <0.05 were considered significant.

RESULTS AND DISCUSSION

Antioxidant Activity Using DPPH Method

The results of antioxidant activity in black tea extract 5 PGL clones according to Figure 1. The value without and with enzyme treatment showed that PGL 15 clone had the highest initial antioxidant activity, followed by PGL 9, PGL 11, PGL 10, and PGL 12.

The antioxidant activity of black tea with the treatment of the enzyme tannase (TR) shows an increase from its initial activity. Figure 1 shows the highest activity was PGL 15, which increased by 10.60% from the initial 52.31%. The second, third and fourth positions were PGL 9, PGL 11, PGL 10, and PGL 12, which increased by 11.16%, 8.08%, 7.15% and 7.99% from the initial 46.05%, 46.24%, 37.78%, and 32.74%.

An increase in antioxidant activity by enzyme treatment was also found in research (Raghuwanshi et al., 2012). The study stated that the tannase enzyme (CTC and Kangra orthodox) samples had 61.6% antioxidant activity and the orthodox Kangra sample had 73.9%. The antioxidant activity in the tea sample

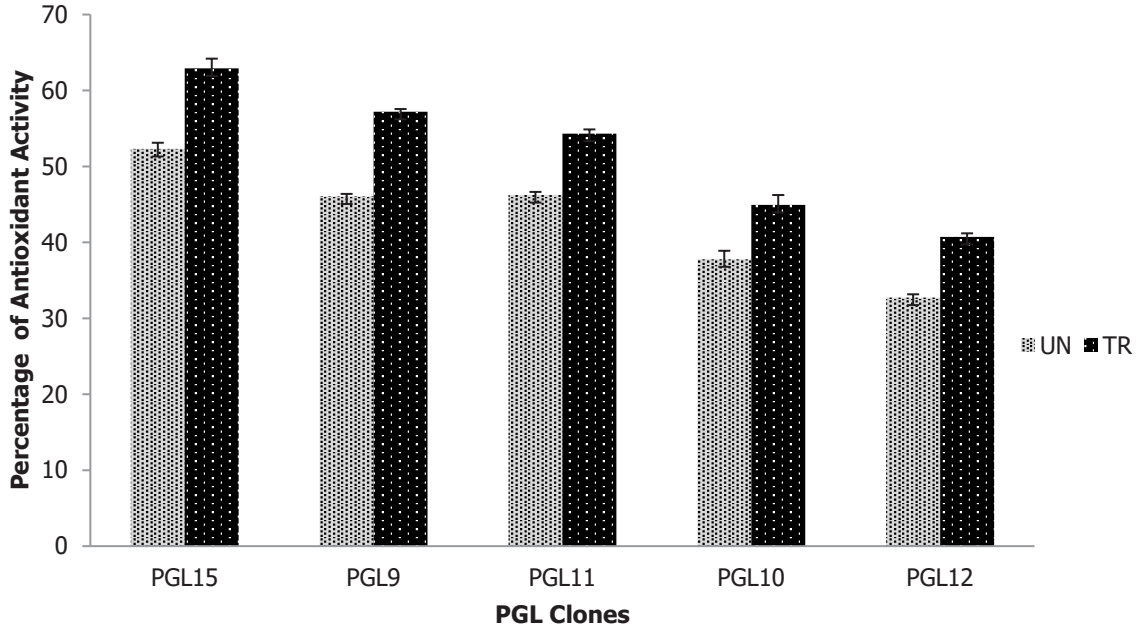

Figure 1. Percentage of antioxidant activity of 5 PGL clones using the DPPH Method (UN: Untreated; TR: Treated)

without tannase enzyme treatment for the CTC tea sample was 35% and Kangra orthodox samples 45.7% each.

The addition of the tannase enzyme in the five PGL clones significantly increased antioxidant activity compared to without the enzyme treatment. Based on (Han et al., 2017; Lu & Chen, 2008)and the effect of tannase-aided treatment on their antioxidative activity was then examined. Their antioxidant capacity was evaluated using assays for DPPH, superoxide anion, trolox-equivalent antioxidant capacity (TEAC, tannase enzymes catalyze the hydrolysis of gallated catechin (EGCG and ECG) to increase the radical scavenging activity against superoxide anions, hydrogen peroxide, and DPPH radicals.

The difference in the increase in antioxidant activity between tea extracts and tannase enzyme treatment can be influenced partly because of the different types of tea clones and altitude. Clones of PGL 15, PGL 9, PGL 10 were taken from the Afdeling Kayulandak, and PGL 11 and PGL 12 were taken from the Afdeling Sanderan of PT. Pagilaran has a different height, while Kayulandak and Sanderan have a height of around 1200 m and 860 m above sea level. Han et al. (2017)its impact on tea quality is less acknowledged. To understand the divergence in tea quality, we collected green tea samples from five sites (with varying altitude from 212 to 1020 m stated the quality of green tea was better in higher places with relatively cooler temperatures. However, Martono et al. (2016) research stated that GMB 7 which was planted at an altitude of 690 masl, was higher than total phenolic and antioxidant activity compared to GMB 7, which was planted at an altitude of 1280 masl and 1890 masl. In this study, PGL 15, PGL 9 and PGL 11 tend to have higher antioxidant activity than PGL 10 and PGL 12 due to differences in the genetic origin of each clone. This affects the number of catechin compounds associated with antioxidant activity in tea brewing. Research Mitrowihardjo et al. (2012) stated that the total high catechins for PGL 10 are at an altitude of 1200 - 1300 meters above sea level. Meanwhile, in this study, PGL 10 was taken at an altitude of 860 m above sea level. Hence the total catechins were lower than those of PGL 15, PGL 9 and PGL 11. Differences in height and type of clones will affect the flavor and appearance of tea grounds after brewing.

IC50 Scores

The results of the calculation of the IC50 value of each 5 PGL clones without and with enzyme tannase (Tr) treatment are in Table 1. The IC50 values of the five black tea clone samples were at a strong antioxidant level (50-100). As for the PGL clone tea extract sample with tannase enzyme treatment in the range of 0 - 50, which can be categorized as a very strong antioxidant. Ascorbic acid was used to positively control the antioxidant content expressed in IC50, which shows the large sample concentration that can inhibit 50% absorbance of DPPH. The smaller the value of IC50 means, the stronger antioxidants.

Table1. IC50 Scores of 5 PGL black tea clones

Clones	IC_{50} (ppm)	
	Untreated (Un)	Treated (Tr)
PGL 9	50.39	36.09
PGL 10	62.33	51.19
PGL 11	53.81	40.06
PGL 12	72.89	46.39
PGL 15	54.75	42.27
Positive control		
Ascorbic acid	7.89	

IC50 values can be increased by using enzymes under changes in each clone that received 0.1% tannase enzyme treatment. Meanwhile, as a positive control, IC50 used ascorbic acid with a value of 7.89, which was at a very strong antioxidant level (less than 50) based on Jun et al. (2003). The most powerful antioxidant activity of PGL clones was PGL 9 (Tr), followed by PGL 11 (Tr), PGL 15 (Tr), PGL 10 (Tr), PGL 12 (Tr), PGL 9 (Un), PGL 11 (Un), PGL 15 (Un), PGL 10 (Un) and PGL 12 (Tr).

Antioxidant Activity Using FRAP Method

The FRAP method produces a percentage of antioxidant activity as in Figure 2.

From Figure 2, the highest oxidant activity was PGL 15 with an enzyme treatment which increased by 8.94% from the initial 53.06%. The second, third, fourth and fifth positions were PGL 11, PGL 9, PGL 10 and PGL 12 with enzyme treatment which increased by 7.57%, 8.42%, 8.58%, and 5.65% from the initial 51.70%, 49.22%, 41.51% and 39.17%.

This study confirmed an increase in antioxidant activity by treating tannase enzymes in 5 PGL clones of black tea extracts. The results of the antioxidant analysis using the DPPH and FRAP methods showed that the black tea extract added with a concentration of 0.1% experienced a significant increase in antioxidant activity.

Total Phenolic Content (TPC)

The total phenolic content (TPC) was estimated by the Folin Ciocalteu method, and the results are shown in Table 2.

Table 2. Total phenolic content (TPC) of PGL black tea clones

Klon	TPC (mg GAE/g)	
	Untreated (Un)	Treated (Tr)
PGL 9	10.58 ± 0.0155^{e}	12.48 ± 0.1969^{g}
PGL 10	8.09 ± 0.0397^{a}	9.73 ± 0.1275^{d}
PGL 11	9.40 ± 0.0124^{c}	11.89 ± 0.0329^{f}
PGL 12	8.08 ± 0.0153^{a}	8.62 ± 0.1955^{b}
PGL 15	9.49 ± 0.0502^{c}	12.05 ± 0.1918^{f}

Notes: different letters in the same row indicate a significant difference (p>0.05). Results of the parameter determined were expressed as a mean of the triplicate determination.

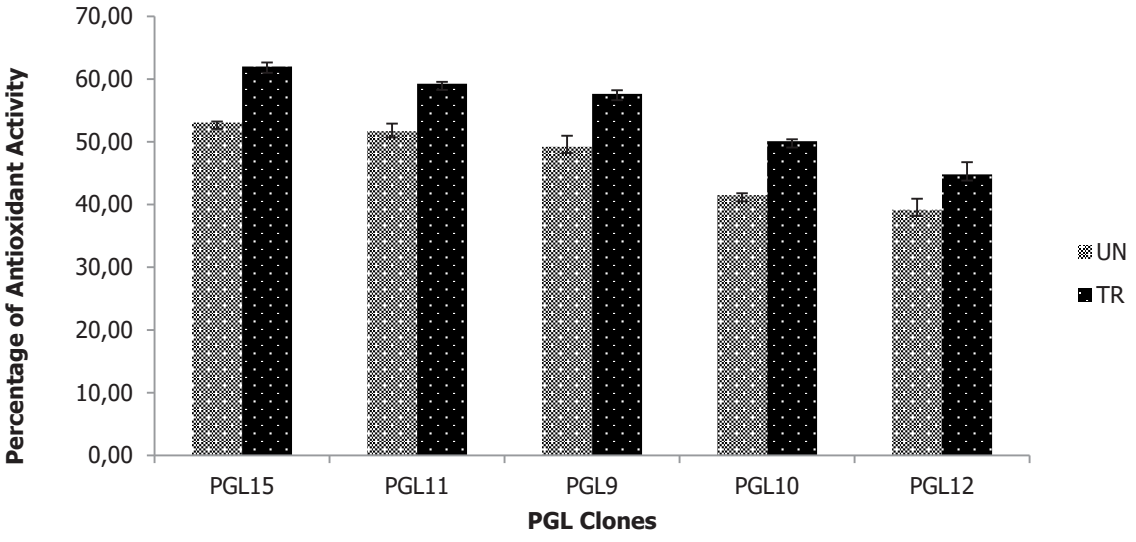

Figure 2. Percentage of antioxidant activity of 5 PGL clones using FRAP method (UN: Untreated; TR: Treated)

From Table 2, there were significant differences between clones and between treatments. The highest total phenolic content included PGL 9 (Tr) enzyme, which was 12.48 mg GAE/g (dry weight), then PGL 15 (Tr) was 12.05 mg GAE/g, PGL 11 (Tr) was 11.89 mg GAE/g, as shown in Table 2.

The results of statistical data analysis show significant differences in the total phenolic content between tea extracts without and with tannase enzymes, indicated by the different letters of each sample.

The increase in the total phenolic value was caused by tannase activity associated with complex polyphenol compounds. Tannases hydrolyze the ester (the galloyl ester of an alcohol moiety) and depside bonds (the galloyl ester of gallic acid) from tannic acid substrates, EGCG, ECG, and chlorogenic acid. It was also reported to break ester bonds between galloyl groups and compounds such as EGCG and ECG in green tea leaves (Hong et al., 2013). Furthermore, the enzyme can increase the extraction of polyphenols in black tea extract to increase the total polyphenols.

Individual Catechin and Gallic Acid Using HPLC (High-Performance Liquid Chromatography)

Estimation of catechins and gallic acid was carried out using Shimadzu HPLC. The standard compounds used were gallic acid, catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG).

Table 3 shows the percentage of 6 compounds in 5 PGL clones samples. The percentage of compound values without treatment (Un) from the largest to the smallest were catechin, ECG, EC, EGCG, gallic acid, and EGC. After enzymatic treatment, the percentage of EGCG and ECG decreased while gallic acid, EGC, and EC content increased in each PGL clone.

Murugesh and Subramanian's (2014) research stated that EGCG and ECG were hydrolyzed by tannase to produce EGC, EC, and general products such as gallic acid. The study showed that the EGC, EC, and GA contents increased by 2.5-, 5.8- and 4.1- fold, accompanied by an almost complete reduction in EGCG and ECG (enzyme hydrolysis content).

Increasing content of gallic acid, EGC, and EC in 5 PGL clones of black tea extract in the sample were products of hydrolysis of gallated polyphenols (EGCG and ECG), as indicated by an increase in the percentage. Gallic acid, the main product of the hydrolysis of polyphenol compounds, gives a fairly high percentage of treated tea extracts, which increases 5 - 12 times. For EC and EGC, it had increased but not significantly as gallic acid.

Theaflavin and Thearubigin

Theaflavin and thearubigin tests were carried out based on the method (Ullah, 1986), producing data as in Table 4.

He (2017) stated that the amount of theaflavin in black tea is around 2-6%, but it significantly affects the quality of tea. Karori et al. (2014) reported the composition found in tea cultivars from Kenya at a maximum of 2.08%. The study was almost identical to the theaflavin fraction in PGL15 clone samples of 2.03% and PGL 15 (Tr) of 2.14%.

Another important component of black tea was thearubigins, resulting from further oxidation of quinone, theaflavins, benztropolones, and theasinensins (Haslam, 2003). Karori et al. (2014) reported that the composition in tea clones from

Table 3. Percentage of 6 compounds in a PGL clone sample (%w/w) dry weight

Compounds	PGL 9		PGL 10		PGL 11		PGL 12		PGL 15	
	Un	Tr	Un	Tr	Un	Tr	Un	Tr	Un	Tr
Gallic acid	1.02	12.14	0.97	9.28	0.97	11.76	1.44	8.03	2.91	15.06
EGC	0.46	0.65	0.19	0.33	0.03	0.23	0.06	0.12	0.14	0.18
C	7.95	7.38	6.92	6.41	6.03	5.69	7.22	6.97	6.41	6.38
ECG	5.06	4.95	4.16	4.48	5.06	4.20	4.14	4.33	5.06	4.45
EC	3.64	5.25	2.52	4,.1	2.67	3.77	1.42	1.88	2.15	2.82
EGCG	2.72	0.22	0.68	0.20	4.27	0.23	0.41	0.22	0.93	0.25
Total Catechins	19.83	18.44	14.47	15.42	18.06	14.12	13.25	13.53	14.67	14.08
Total	20.86	30.58	15.44	24.70	19.03	25.88	14.68	21.55	17.58	29.13

Notes: Un: Untreated; Tr: Treated

Table 4. Estimation of theaflavin and thearubigin

Parameters		PGL 9	PGL 10	PGL 11	PGL 12	PGL 15
TF (%)	Untreated (Un)	1.60±0.02^{c}	0.38±0.01^{i}	1.47±0.01^{d}	0.86±0.01^{g}	2.03±0.03^{b}
	Treated (Tr)	1.61±0.01^{c}	0.75±0.02^{h}	1.45±0.01^{e}	1.14±0.01^{f}	2.14±0.02^{a}
TR (%)	Untreated (Un)	14.48±0.05^{e}	14.95±0.03^{e}	13.80±0.02^{f}	15.62±0.08^{d}	19.53±0.11^{b}
	Treated (Tr)	14.52±0.01^{e}	21.79±0.81^{a}	14.50±0.03^{e}	17.59±0.02^{c}	19.42±0.19^{b}
TF/TR	Untreated (un)	0.11±0.00^{a}	0.02±0.00^{h}	0.11±0.00^{c}	0.05±0.00^{f}	0.10±0.00^{c}
	Treated (Tr)	0.11±0.00^{a}	0.03±0.00^{g}	0.10±0.00^{c}	0.06±0.00^{e}	0.11±0.00^{a}

Notes: different letters in the same row indicate a significant difference (p>0.05). Results of the parameter determined were expressed as a mean of the triplicate determination.

Kenya varied between 12% and 17%. The PGL clones' thearubigins fraction composition ranged from 13-21%. The variation of theaflavins and thearubigins fractions was influenced by the location, climate, and type of each clone, and their composition positively correlates with TPC. The percentage value tends to be higher in clones PGL 15, PGL 9, and PGL 11, affecting the degradation results of catechin oxidation compounds, namely theaflavins and thearubigins. The greater the proportion of polyphenol compounds, particularly catechins, in black tea, the greater the likelihood of oxidation compounds in the form of theaflavin and thearubigin.

Borse (2012) stated that black tea's TF/TR ratio was divided into 3 categories. TF/TR ratio values up to 0.04 are included in the category of good black tea, TF/TR ratio of 0.04 to 0.08 including the category of better quality tea and TF/TR ratio values above 0.08 were the best tea categories. Based on Table 5, the best quality tea range values are black tea clones PGL 9, PGL 11, and PGL 15 clones, while the better and lowest black tea categories were PGL 12 and PGL 10 clones.

Tea Infusion Color

The Color analysis was carried out on 10 tea samples without and with enzyme treatment. Tea samples without and with treatment were compared in color using a chromameter. It shows that the treated sample's L, a, and b value tends to increase, as shown in Table 5.

L value indicated the degree of embezzlement (Zamora et al., 2016)like tea, are widely used for preparing herbal infusions. These plants have an interesting antioxidant capacity that may change after harvesting depending on the technological processing and the storage conditions. We determined the antioxidant capacity (ABTS, DPPH and FRAP methods. PGL 15 with enzyme treatment was the highest L value, meaning that the brewing of PGL 15 tea clones tends to be brighter than other clones. The lowest L value was found in the PGL 10 sample without enzyme treatment, with an L value of 36.37. The redness value (a) illustrates the color of the sample on the red-green axis, and the positive values indicate that the PGL clone samples tend to be reddish. The highest value was in

Table 5. Color analysis of five PGL black tea clone extract

Parameters		PGL 9	PGL 10	PGL 11	PGL 12	PGL 15
L	Untreated	36,57 ± 0,84^{f}	36,37 ± 0,52^{f}	36,90± 0,47^{d}	36,94± 0,47^{ef}	38,06± 0,47^{e}
	Treated	40,12 ± 0,87^{d}	41,38 ± 0,16^{bc}	42,41± 0,29^{ab}	41,04± 0,83^{cd}	42,93± 1,39^{a}
a	Untreated	9,44 ± 0,43^{f}	10,93 ± 0,45^{e}	12,36± 0,11^{d}	11,19± 0,49^{e}	11,98± 0,16^{e}
	Treated	13,25 ± 0,12^{c}	14,35 ± 0,35^{a}	14,43± 0,53^{a}	14,59± 0,93^{a}	13,65± 1,00^{b}
b	Untreated	3,34 ± 0,51^{f}	4,94 ± 0,74^{ef}	10,80± 0,56^{c}	5,60 ± 1,00^{e}	7,95± 0,87^{d}
	Treated	11,44 ± 1,56^{c}	12,74 ± 1,06b^{c}	15,14± 0,44^{a}	13,01± 1,63^{b}	15,93± 1,74^{a}

Notes: different letters in the same row indicate a significant difference (p>0.05). Results of the parameter determined were expressed as a mean of the triplicate determination.

the PGL 12 clone sample with an enzyme treatment and (+) value of 14.59, while the smallest value was PGL 9 with no treatment (+) 9.44. Another hunter system parameter was the yellowness value (b) which shows the yellow-blue color change. The highest yellowness value in the PGL clone sample was PGL 15, with a b value of 16.64. The smallest value of b was in the PGL 9 sample with no enzyme treatment of 3.34.

The dominant compounds in black tea extract were theaflavins and thearubigins, contributing to orange-reddish and red-brown colors. The value in steeping black tea extract PGL clones was positive in a and b. A positive value indicates the dominant color was red, and a positive value b indicates the dominant color was yellow. Furthermore, L, a, and b values in PGL clone black tea extracts that received tannase enzyme treatment increased. This shows that other compounds contribute to color other than theaflavins and thearubigins. The addition of the tannase enzyme causes an increase in the extraction of red and yellow pigments.

Tea Creaming

This study decreased tea cream formation in tea extracts treated with enzymes, as in Table 6. The difference in the decrease in each clone can be caused by gallated catechin content. PGL 11, PGL 9, and PGL 15 have gallated catechin (EGCG and ECG) content higher than PGL 10 and PGL 12, according to Table 6, which then undergoes hydrolysis by tannase enzymes to become ungallated catechin (EC and EGC). This resulted in a reduced ability to form creams higher than PGL 10 and PGL 12.

The ability of tannase enzymes to reduce tea cream was in line with the study of Chandini et al. (2011). Tea creaming in black tea extract decreased 65-72% with tannase enzyme treatment. Chandini et al. (2011) decreased tea creaming due to the degallation process of gallated polyphenols to decrease the ability of these polyphenol compounds to bind to proteins. In line with research, Chandini et al. (2011); Raghuwanshi et al. (2013) stated that gallated catechins such as EGCG and ECG have stronger tea creaming formation capabilities. Treatment with the enzyme will be hydrolyzed to produce ungallated catechins with fewer hydroxyl groups for hydrogen bonding to form lower tea cream. The tannase enzyme can also prevent bonding between theaflavin and thearubigins with caffeine which causes a foggy appearance that can reduce the quality of tea drinks (Viswanath et al., 2015), especially in ready-to-drink tea.

Volatile Compounds

Volatile compounds from black tea extract PGL 15 with and without tannase enzyme treatments were extracted using the method of separating the compound from a water solvent (PGL 15 clone tea extract) using organic chloroform solvent. Based on research by Sereshti et al. (2013), the highest extraction efficiency was obtained using organic chloroform solvents. Chromatogram results from PGL 15 clone black tea extract without and with enzyme treatment are shown in Figure3 (a) and (b).

Figure 3 shows that there are 12 volatile compounds that GC-MS can identify by the extraction method used. The twelve compounds were α-Pinene, ß-Pinene, trans-Linalool oxide (furanoid), 2-Propenoic acid, 1-methylundecylester,Caffeine,n-Octylidencyclohexane, Cyclooctene, 1,4-Dimethyl-4,5,7,8-tetrahydroimidazo - [4,5-E] -1,4-diazepin-5,8 (6H) -ione, 1,4-Dimethyl-4,5,7,8-tetrahydroimidazo - [4,5-E] -1, 4- diazepin-5,8 (6H) -dione, Cyclopentene, 1-octyl, Hexadecanoic acid, methyl ester, 19.12-Octadecadienoic acid, methyl ester, (E, E) -, 11-Octadecenoic acid, and methyl ester.

Table 6. Tea creaming formation of PGL clones

Clones	Untreated (g)	Treated (g)	Tea cream reduction (%)
PGL 9	0.1095±0.0205	0.0445±0.0106	60.66±0.36^{b}
PGL 10	0.0925±0.0120	0.0550±0.0042	49.34±0.42^{d}
PGL 11	0.1265±0.0530	0.0460±0.0085	73.74±0.24^{a}
PGL 12	0.1340±0.0764	0.0850±0.0608	31.61±0.53^{e}
PGL 15	0.0955±0.0064	0.0440±0.0042	58.93±0.58^{c}

Notes: different letters in the column indicate a significant difference (p>0.05). Results of the parameter determined were expressed as a mean of the triplicate determination.

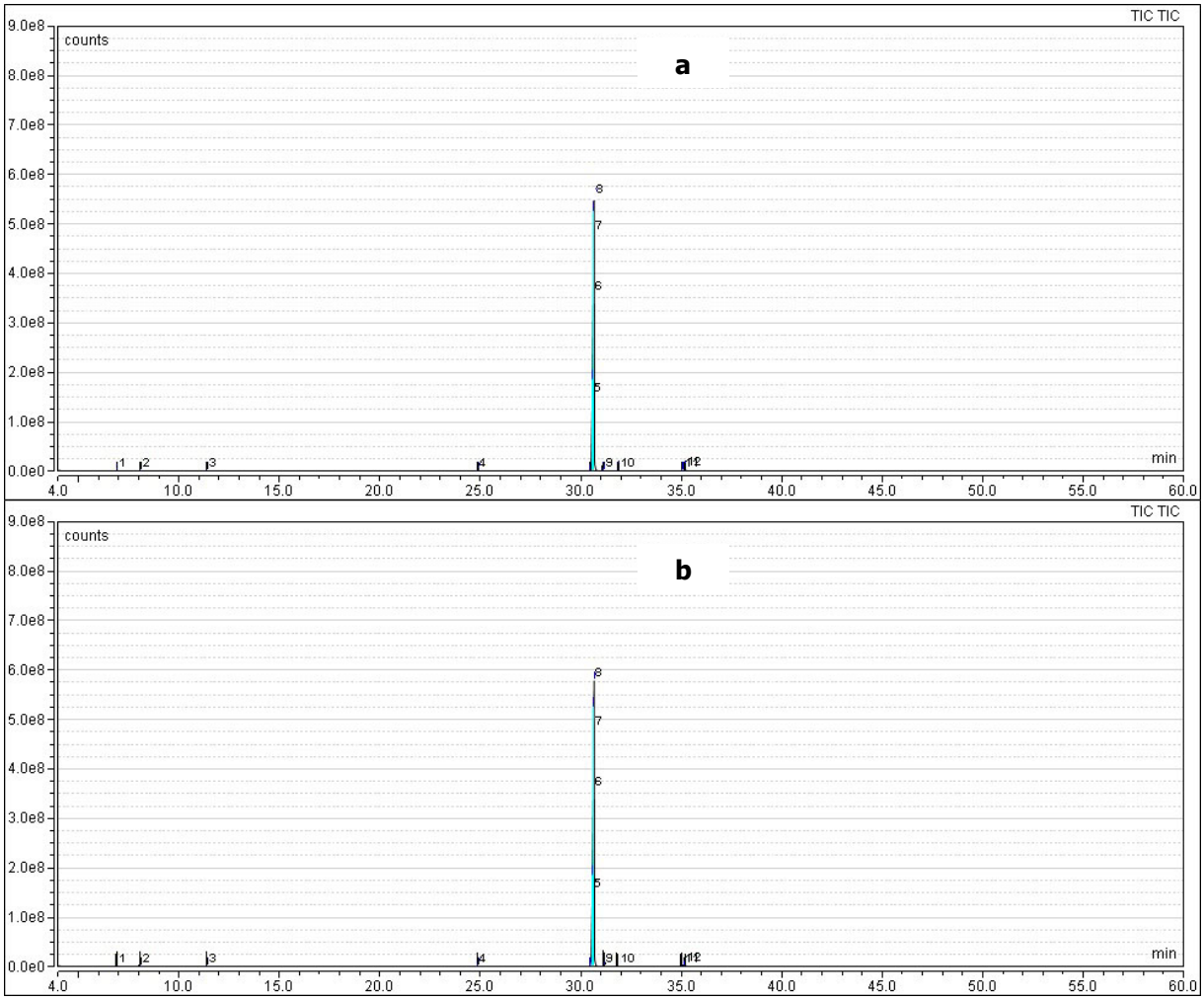

Figure 3. (a) Chromatogram of black tea extract PGL 15 clone without treatment (Un), (b) Chromatogram of black tea extract PGL 15 clone with enzyme treatment (Tr). Peak: 1= α-Pinene, 2= ß-Pinene, 3= trans-Linalool oxide (furanoid), 4= 2-Propenoic acid, 1-methylundecyl ester, 5= Caffeine, 6= n-Octylidencyclohexane, 7= Cyclooctene, 8= 1,4-Dimethyl-4,5,7,8 -tetrahydroimidazo- [4,5-E] -1,4-diazepin-5,8 (6H) -dione, 9= Cyclopentene, 1-octyl, 10= Hexadecanoic acid, methyl ester, 11 = 9,12-Octadecadienoic acid, methyl ester, (E, E) -, 12= 11-Octadecenoic acid, methyl ester

The compound profile was obtained based on peaks formed such as terpenoids consisting of α- and ß-Pinene, trans-Linalool oxide (furanoid), furan, terpene alkaloids (caffeine) and carboxylic acid derivative in the form of acid esters (hexadecanoic acid, octadecenoic acid, methyl esters).

Figures 3 (a) and (b) show a slight difference in the peak chromatogram results. GC-MS analysis in the first PGL black tea clones research was carried out on the black tea extraction of the untreated PGL 15 clone (Un) and the PGL 15 clone with enzyme treatment (Tr), which was the best clone based on statistical analysis resulting in 12 identified volatile compounds. PGL 15 clone chromatograms that received enzyme treatment had higher elevations but were insignificant. This study showed that the tannase enzyme did not significantly affect odor because there were no changes in volatiles compounds.

Qiu et al. (2017) identified 53 volatile compounds in black tea with the HS-SPME (Head Space - Solid Phase Micro Extraction) extraction method. The most identified volatile compounds from alcohol groups were more than 70%, then aldehydes, hydrocarbons, ketones, and ester. The dominant compounds in black tea include linalool, geraniol, benzyl alcohol, α-damascenone, methyl salicylate, α-ionone, ß-ionone, and others can be detected using HS-SPME extraction. In contrast, research Sereshti et al. (2013) identified

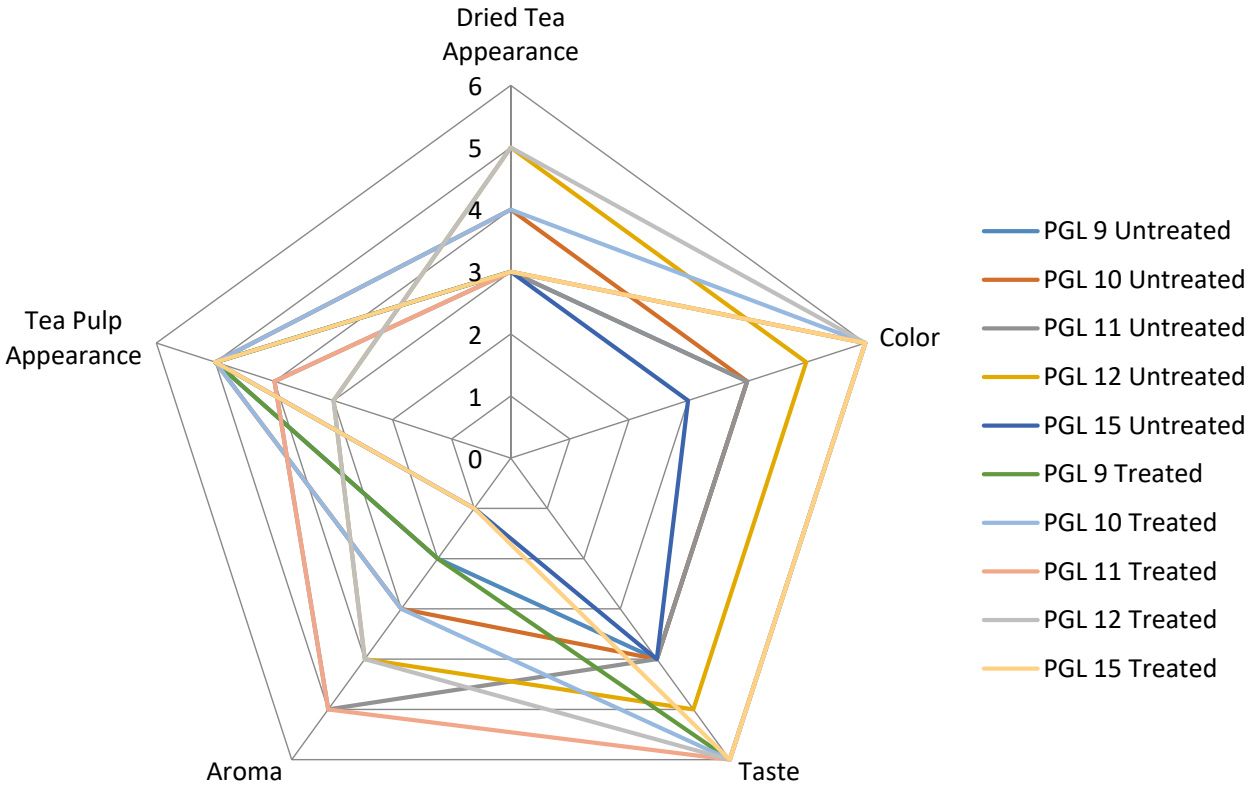

Figure 4. Spider web chart of 5 (five) PGL Clones steeping tea

42 volatile compounds by the UAE-DLLME (Ultrasound Assisted Extraction-Dispersive Liquid-Liquid Micro Extraction) extraction method from six tea samples. The total volatile compounds identified were 12 untreated and treated tea extracts. In black tea, these compounds include linalool, geraniol, benzyl alcohol, α-damascenone, methyl salicylate, α-ionone, and ß-ionone. Maillard reactions such as dimethyl disulfide and 2-Acetyl-3-methyl pyrazine in black tea PGL clones cannot be detected. Therefore, developing an extraction method for GC-MS analysis is necessary to detect more volatile compounds.

Sensory Attributes

A tea tester conducted sensory analysis from to determine the appearance, color, taste/odor of tannase and non-tannase enzyme treatment. The sensory analysis of black tea extract PGL clones showed changes in the color of steeping and taste before and after tannase enzyme treatment.

Four tea extracts of the clones without tannase enzyme treatment showed strong taste characteristics, namely PGL 10, PGL 9, PGL 11, and PGL 15. The color of steeping was between bright to slightly dark red, and dried tea appeared brown-gray. Meanwhile, the tea extract PGL 12 clone showed a very bright red appearance and a little astringent and felt fresh with a sweet/ caramel aroma with a brown-black appearance.

The Strong astringent taste might be caused by the content of polyphenol compounds, especially gallated catechin contained in each clone. Epicatechin (EC) and epigallocatechin (EGC) can also contribute to the aroma and flavor, giving rise to a slightly bitter (sweet) taste after being drunk. The gallated form (EGC and EGCG) gives rise to a strong sense of astringent (Mitrowihardjo et al., 2012). Apart from catechins, in black tea, the character of astringency was largely determined by the content of theaflavins. Furthermore, PGL 9, PGL 10, PGL 11, and PGL 15 clones have higher total catechin and theaflavins content than PGL 12. Therefore, it was suspected that a strong astringent was caused by the influence of these components of each clone.

The aroma character after getting tannase enzyme treatment did not change. This was consistent with the analysis of volatile compounds, having no significant changes. The taste of steeping of PGL black tea clones with tannase enzyme treatment also changed to fruity, and astringency was significantly reduced. The tannase enzyme in steeping tea will hydrolyze gallated polyphenols to ungallated polyphenols and gallic acid. Hence, the taste of astringent becomes significantly reduced and turns fruity. The Spider web chart of 5

PGL clones of treated and untreated tannase enzyme is shown in Figure 4.

The pH value of black tea extract without and with enzyme treatment ranged between 4.6 – 4.8 and 4.0 – 4.3. These results showed a decrease in the pH of the tea extract-treated by the enzyme. Due to the hydrolysis process by tannases that release gallic acid from their ester bonds, the decreased pH was due to increased gallic acid content. Zeng et al. (2017) stated that the total stability of polyphenols in tea depended on Ph. The higher the pH, the more unstable the total polyphenols, and pH 3-6 is stable at 4 and 25 °C. The addition of the enzyme tannase to tea extracts stabilized total polyphenols from degradation and oxidation in the storage process to maintain the health benefits of tea.

CONCLUSION

Tannase 0.1% treated tea infusion of five PGL black tea clones increased antioxidant activity by 7,15 – 11,16 % in the DPPH Method and 5,65 – 8,94 % in FRAP Method phenolic content (TPC), increasing gallic acid content, and reduction in IC50 value. It showed a significant change in the theaflavins (TF)/ thearubigins (TR) ratio. Furthermore, the treatment resulted in no significant change in the quality of volatile compounds and infusion aroma. The sensory analysis resulted in significant changes in the color and taste of black tea infusion with the enzymatic treatment.

The volatile compounds extraction method needs to be improved to obtain more information on extracted black tea PGL clones. The utilization can be developed in black tea ready-to-drink (RTD) for a more attractive steeping color character.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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