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The Improvement of Phenolic Contents, Antioxidant Activity, and the Appearance of Black Tea Extract by Combination Viscozyme and Tyrosinase Addition

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Abstract

The problem with ready-to-drink (RTD) black tea is a long manufacturing process and contains phenolic compounds and low antioxidant activity due to the enzymatic oxidation process. Viscozyme is an enzyme that degrades plant cell walls during extraction so that it releases active components from the cell wall, such as phenolic contents and antioxidant activity. Meanwhile, tyrosinase includes the polyphenol oxidase enzyme, which can take advantage of catechins conversion into theaflavins and thearubigins. This study aimed to determine the addition of viscozyme and tyrosinase combination to increase the content of phenolic compounds, antioxidant activity, and improve the appearance of black tea (color). The study used young leaves that had their enzymes inactivated was added with viscozyme to fresh tea leaves extract. The tea extract that had been given viscozyme was then added with tyrosinase, and the incubation was carried out. The addition of tyrosinase with higher concentrations (3,571 U/mL) and longer incubation times (40 min) decreased total phenolic contents, antioxidant activity using DPPH free radical scavenging method, increased tea cream formation (precipitation that forms as the tea cools), the compound of theaflavins, thearubigins, and TF:TR ratio (comparison of theaflavins and thearubigins contents) in tea extracts that had been treated with viscozyme. The combination of viscozyme and tyrosinase gave higher total phenolic compounds, antioxidant activity, theaflavins, thearubigins content, and TF:TR ratio compared to control and could prevent the formation of tea cream. The use of viscozyme 250 µL and tyrosinase 3,571 U/mL for 20 min on ready-to-drink black tea gave the best effects in this study.

Keywords: Antioxidant activity, Phenolic contents, Ready-to-drink tea, Tyrosinase, viscozyme.

Introduction

Teas are classified into 3 types based on manufacturing stages, mainly in enzymatic oxidation processes. They are green tea (non-enzymatic oxidation), oolong tea (semi-enzymatic oxidation), and black tea (full enzymatic oxidation) [1]. Black tea is one of the tea most popular beverages worldwide,

accounting for up to 80 % [2]. Its unique color and flavor are influenced by the composition of tea catechins and

their derivatives, such as theaflavins and thearubigins, which affect the quality of black tea products [3-5].

Ready-to-drink (RTD) tea is one of the product derivatives people favor [6]. Ready-to-drink (RTD) black tea, prepared from dried black tea by tea plantations, has various variants, but black tea and jasmine tea dominate the market [7,8]. The aroma and taste of black tea are crucial attributes of consumer preference [9], making it the most consumed ready-to-drink tea (RTD) beverage due to its distinctive taste [10]. Ready-to-drink tea (RTD) processing is typically done far from tea farms, but efforts are being made to shorten the process by using fresh leaves at the plantation site [11]. Tyrosinase has been studied for its ability to improve the color of tea extract by oxidizing phenolic compounds into theaflavins and thearubigins. Incubation with tyrosinase increased theaflavin production, though prolonged incubation reduced it [12].

Viscozyme as an exogenous enzyme can degrade cell walls in plant materials and release bioactive compounds such as polyphenols, antioxidant compounds, flavonoids, and catechins. The addition of viscozyme to green tea extract was superior in increasing polyphenols (2.82 mg/mL), antioxidant activity (90 %), and total catechins (4.01 mg/mL) for 120 min of incubation time. Viscozyme can release antioxidant compounds other than phenolic compounds, and this has an effect on antioxidant activity. Green tea extract with viscozyme addition increased the total polyphenols, flavonoids, catechins contents, and radical scavenging activities to a greater extent than the other commercial enzyme treatments. Viscozyme can impove the extraction yields of green tea and give the best treatment. The result showed that total polyphenol contents and antioxidant activity increased compare to the extract without enzyme at the same condition [13,14].

Many efforts have been made to improve the quality of black tea by using exogenous enzymes such as polyphenol oxidases such as, laccase, bilirubin oxidase, crude tea polyphenol oxidase, and tyrosinase [15]. Black tea was treated according to conventional methods, i.e. withered, CTC, sprayed with enzyme and then fermented and dried [16]. The exogenous enzymes are aimed to increase theaflavin. Laccase and bilirubin oxidase only produce theaflavin in a small amount about 5 % and crude tea polyphenol oxidase of 27 %. Tyrosinase produced higher theaflavin content and produced a better yellow color. Tyrosinase is the best enzyme in forming theaflavins more than 80 % among any other enzymes [15]. The black tea prepared from the leaves gave the darkest brown color [12]. Tyrosinase has specificity for ungallated catechin compounds [17,18].

A combination of tyrosinase and β-glucosidase was able to improve the quality of black tea prepared from tea leaf extract. The best treatment was treated with β-glucosidase. There are many volatile compounds detected in tea leaf extract. Volatile compounds can be improved by adding β-glukosidase [11]. Considering that the each advantages of enzyme added are known, a combination experiment has been made, namely tyrosinase and β-glucosidase. However, considering that viscozyme has the advantage of being able to break down leaf cell tissue so that more catechin compounds are obtained. Meanwhile, the research about the phenolic contents and antioxidant activity by tyrosinase addition has not been carried out so far. Thus, it is expected that a combination of viscozyme and tyrosinase to produce black tea from tea leaf extract can increase the total phenolic content and antioxidant activity and improve the appearance of black tea.

Materials and methods

Materials

Main ingredient was green tea leaves of PGL-15 were picked after the ages of 5 years and 25 days after, the shoot tip was grown. The tea shoots used are buds shoot and 2 young leaves underneath (P+2) were used

as raw material which harvested from Pagilaran Tea Plantation-Batang, Central Java, Indonesia. Broken Orange Pekoe (BOP) black tea was produced by Pagilaran Plantation used as a commercial black tea to be compared with tyrosinase treatments. Viscozyme (108 FBGU/mL), tyrosinase (7.164 U/mg solid), Folin-Ciocalteu, sodium carbonate (Na_2CO_3), absolute methanol, 2,2-diphenyl-1-picrylhydrazyl) (DPPH), sodium bicarbonate, ethyl acetate, acid oxalate, and citric acid were purchased from Sigma Aldrich (St. Louis, USA).

Methods

Preparation of fresh tea leaves

Fresh tea leaves of clone of PGL-15 were steamed first for 15 min before freezing with liquid nitrogen and transferred in a frozen state to the laboratory for further analysis.

Drying of fresh tea leaves

The frozen tea leaves were lyophilized overnight in a freeze dryer at a pressure of 0.125 mbar at −50 ℃.

Application of viscozyme in fresh tea leaves extract

Freeze-dried tea leaves (2 g) were crushed and mixed with 40 mL of distilled water in a 50 mL conical tube, and 250 µL of viscozyme was added, then incubated for 30 min at 40 ℃. The reaction mixture heated for 10 min at 90 ℃ to stop the reaction. The supernatant was used for analysis after centrifuged at 3000 xg at 5 ℃ for 15 min [14]. The application was repeated 3 times.

Application of tyrosinase on fresh tea leaves extract

1 mL of the tea leaves extract was mixed with 1 mL of tyrosinase (223, 893, and 3,571 U/mL) of tyrosinase, then the mixture was incubated at 25 ℃ for 20 and 40 min. After incubation, 100 µL of 25 mM citric acid was added to stop the reaction. Furthermore, the sample was centrifuged at a speed of 10.000 xg with a temperature of 20 ℃ for 5 min [12,18]. The application was repeated 3 times.

Application of viscozyme and tyrosinase on fresh tea leaves extract

1 mL of the tea leaves extract treated with viscozyme was mixed with 1 mL of tyrosinase enzyme (223, 893, and 3,571 U/mL), then the mixture was incubated at 25 ℃ for 20 and 40 min. After incubation, 10 mL of 25 mM citric acid was added to stop the reaction. Furthermore, the sample was centrifuged at a speed of 10.000 xg with a temperature of 20 ℃ for 5 min [12,18]. The application was repeated 3 times.

Determination of total phenolic content

0.5 mL of diluted tea extract and 2.5 mL of Folin Ciocalteu (1/10) dilution in a distilled water) reagents were mixed. The solutions were incubated for 5 min, and it was added by 2 mL of sodium carbonate (7.5 % w/v). The mixture was allowed at dark and room temperature for 60 min before measuring the absorbance at 765 nm. The calculation of phenolic content was expressed as the gallic acid equivalent/g of fresh tea leaves (db) [19].

Determination of DPPH radical scavenging activity

Each tea extract of 1 mL was added with 1 mL of DPPH 0.1 M solution mixed with methanol absolute and incubated at dark for 30 min. 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 [20].

Tea cream formation

The tea cream formation test was carried out by centrifugation using a refrigerated centrifuge at a

temperature of 8 ℃, a speed of 5.600 xg for 20 min. The resulting precipitate was then dried using a hot air oven at 100 ℃ until a constant weight was obtained. Tea cream formation was determined as the sediment's weight per 100 mL of tea extract [21].

Estimation of theaflavins and thearubigins contents

The tea extract (20 mL) was mixed with 6 mL 2.5 % (w/v) of disodium hydrogen carbonate solution. The reaction mixture was extracted with 20 mL of ethyl acetate and subjected to vortex for 1 min. The lower layer was separated, and the upper layer, namely the ethyl acetate layer (theaflavins fraction) obtained as a solution extract (E1), was used in the analysis. 10 mL of solution extract (E1) was diluted to 25 mL with methanol absolute before reading the optical density at 380 nm. The preparation of solution extract 2 (E2) was 1 mL of tea extract, 1 mL of aqueous saturated oxalic acid, and 8 mL of water are mixed and made up to 25 mL with methanol absolute and determined the optical density at 380 nm.

The optical density of solution E1 and E2 were used for calculating theaflavins and thearubigins with the following equation:

$$TF\ (\%) = 2.25 \times E1 \quad (1)$$

$$TR\ (\%) = 7.06 \times (4E2 - E1)\ [22] \quad (2)$$

Data analysis

The experimental design used was a Paired T-Test (extract with viscozyme and control), a completely randomized design (CRD) with 2 factors (concentration and incubation time) with 3 replications used in the experiment. The data obtained were then processed using 1-way 2-factor ANOVA with Duncan's continued test. The treatment, which resulted in a significance of $p < 0.05$, indicated that the samples were significantly different. Then the data are presented in the form of SD (standard deviation). Statistical analysis was performed using Microsoft Excel 2013 software and SPSS Statistic 23.0.

Results and discussion

Effect of viscozyme on total phenolic contents and DPPH antioxidant activity in fresh tea leaves extract

Extract with viscozyme treatment showed the highest total phenolic content of 114.71 mg GAE/g was significantly higher compared to extract without viscozyme which has value of 90.15 mg GAE/g as shown in **Table 1** Viscozyme degrades the cell walls of tea leaves, releasing more compounds, including catechins, thus increasing the total phenolic content. The result was in line with a previous study that showed that viscozyme treatment 250 µL (100 FBGU/mL) for 2 h of incubation time could increase total polyphenol content to green tea extract [14]. Viscozyme treatment to old tea leaves extract (2 % v/w) at a concentration of 700 FBGU/mL resulted in maximum total polyphenol content of 82.25 mg GAE/g polyphenol content increased by 1.18 compared to tea leaf extract without viscozyme [13].

Table 1 Total phenolic contents and antioxidant activity DPPH in fresh tea leaves extract.

Samples	TPC (mg GAE/g)	DPPH (mg GAE/g)
Extract without viscozyme	90.15 ± 1.93^{a}	44.75 ± 1.03^{a}
Extract with viscozyme	114.71 ± 9.11^{b}	78.28 ± 2.27^{b}

Values in the same column followed by a different letter represent a significant difference at $p < 0.05$.

The results from the DPPH assay, shown in **Table 1**, indicate that the antioxidant activity was higher in the viscozyme-treated extract. The DPPH value of 78.28 mg GAE/g in the viscozyme-treated extract was significantly higher than the 44.75 mg GAE/g in the untreated extract. This increase in antioxidant activity corresponds with the higher total catechin content in the viscozyme-treated extract. These findings are consistent with the research by Hang et al. [13], which showed that green tea extract with viscozyme (0.06 v/w, 100 FBGU/ml) had higher antioxidant activity (76.29 %) compared to extracts without viscozyme (66.88 %). As the total polyphenol content in green tea extract increased, so did its antioxidant capacity, demonstrating that the increase in polyphenol content due to viscozyme treatment directly contributes to higher antioxidant activity.

The effect of tyrosinase addition on total phenolic contents (TPC) and DPPH antioxidant activity in tea extracts treated with viscozyme

Tyrosinase addition on fresh tea extract with viscozyme treatment was obtained total phenolic contents according in **Table 2**. The concentration of tyrosinase on tea extract with viscozyme treatment decreased total phenolic contents significantly. The addition of the lowest tyrosinase concentration of 223 U/ml resulted in the highest total phenolic content value of 87.95 mg GAE/g, while the highest tyrosinase concentration was 3.571 U/ml resulting in the lowest total phenolic content value of 56.50 mg GAE/g. The result was in line with Faustina et al. that stated total phenolic content was decreased during the increase of the tyrosinase concentration [11]. Tyrosinase includes polyphenol oxidase enzymes, which can utilize the conversion of polyphenol, especially catechins [17]. Tyrosinase roled oxidized individual catechins, considering some of the catechin compounds would be oxidized to become theaflavin therefore the remained compounds to be less [12]. Tea extract has a high catechin content. Catechins comprise approximately 25 % of the dry weight of fresh tea leaves [7]. The higher concentration of tyrosinase, the lower phenolic content produced. The catechin content in black tea decreases as it occurs enzymatic oxidation process which results in degradation. In black tea production, catechins are oxidized and catalyzed by polyphenol oxidase to form theaflavin and thearubigin pigments [23].

The incubation time of tyrosinase also affected the decrease in total phenolic contents in tea extracts treated with viscozyme. The incubation time of 20 min resulted in the highest total phenolic content value of 87.95 mg GAE/g, while the incubation time of 40 min resulted in the lowest total phenolic content value of 56.50 mg GAE/g. These results indicate that tyrosinase acted as polyphenol oxidase which oxidizes the catechins in fresh tea extracts to theaflavins and thearubigins so that the long incubation time affects the reduction of total phenolic contents. Theaflavins formation increased with a long incubation time of 180 min [15]. Based on **Table 2**, the interaction between concentration and incubation time of tyrosinase added to the tea extract with the addition of viscozyme showed a significant decrease in the total value phenolic contents. The highest concentration and the longest incubation time of tyrosinase produced the lowest total value of phenolic compounds in line with each given factor.

Table 2 The effect of tyrosinase addition on total phenolic contents and antioxidant activity of fresh tea extract clone of PGL-15 treated with viscozyme.

Tyrosinase Concentration (U/mL)	Incubation Time (min)	TPC (mg GAE/g)	DPPH (mg GAE/g)
223	20	87.95 ± 5.61^{d}	92.83 ± 3.47^{c}
	40	84.16 ± 4.85^{d}	89.38 ±3.43^{c}
893	20	75.59 ± 4.17^{c}	81.94 ± 4.42^{b}
	40	67.96 ± 5.32^{bc}	75.57 ± 6.35^{b}
3,571	20	61.86 ± 3.11^{ab}	54.99 ± 2.50^{a}
	40	56.50 ± 3.94^{a}	47.85 ± 2.81^{a}

Values in the same column followed by a different letter represent a significant difference at $p < 0.05$.

The effect of tyrosinase concentration in antioxidant activity DPPH on fresh tea leaves extracts with viscozyme treatment could be seen in **Table 2**. The concentration of tyrosinase on tea extract with viscozyme treatment decreased in antioxidant activity significantly. The addition of the lowest tyrosinase concentration of 223 U/mL resulted in the highest antioxidant value of 92.83 mg GAE/g, while the highest tyrosinase concentration was 3,571 U/mL resulting in the lowest antioxidant activity value of 47.85 mg GAE/g. The decrease of antioxidant activity was influenced by the processing of fresh leave to black tea during fermentation. The decrease was strongly suspected to be caused by the change of individual catechins into oxidized products such as theaflavins and thearubigins [24]. Whereas the antioxidants activity of oxidized substances were lower than individual catechins. Therefore, in tea extracts that had experienced a reduction in the number of individual catechins due to the oxidation process, the antioxidant value would tend to be lower (**Table 2**). The antioxidant activity value is lower compared to natural compounds, namely individual catechins. Antioxidants are influenced by the level of hydroxylation of the B ring and/or the hydroxyl group at the position of C-3 of the catechin structure thus affecting the ability to inhibit free radicals. Therefore, the amount of tea extract has been reduced individual catechins due to the oxidation process cause the hydroxyl groups to become more low, the antioxidant value also tends to be lower [25]. This results were in line was Zhang *et al.* [26] research that green tea had DPPH antioxidant activity of 78.41 % and it was higher than black tea's antioxidant activity of 44.12 %.

Based on **Table 2**, the incubation time also affected the change in DPPH antioxidant activity values in tea extracts treated with viscozyme. The longest incubation time of tyrosinase resulted in the lowest DPPH antioxidant activity value in the tea extract treated with viscozyme. The incubation time of 20 min resulted in the highest antioxidant activity value of 92.83 mg GAE/g, while the incubation time of 40 min resulted in the lowest antioxidant activity value of 47.85 mg GAE/g [17]. The interaction between concentration and incubation time of tyrosinase added to tea extracts treated with viscozyme decreased the value of antioxidant activity DPPH significantly. The highest concentration and the longest incubation time of tyrosinase produced the lowest of the value of antioxidant activity DPPH during the transformation process of green tea into black tea along with each given factor. In this study, the lowest tyrosinase concentrations and fastest incubation times gave the highest antioxidant activity results. It showed that green tea with 223 U/mL and 20 min for incubation of tyrosinase has the best treatment in antioxidant activity

and phenolic compound results. The antioxidant activity of tea leaves is reduced due to tea fermentation. The tea fermentation process to produce black tea has catechin oxidation into theaflavins and thearubigin so that reducing antioxidant activity compared to green tea [26].

Effect of tyrosinase on tea creaming and TF:TR ratio in tea extracts treated with viscozyme

Tyrosinase concentration significantly increased tea creaming formation in tea extracts treated with viscozyme as shown in **Table 3**. Increasing incubation time also showed increased the formation of tea creaming. These results were indicated by the incubation time of 40 min resulting in higher tea creaming than the 20 min incubation time. The increase in tea cream after enzymatic oxidation was due to the catechin oxidized product in the form of theaflavins and thearubigins which had a higher ability to bind to existing proteins than the green tea catechin form. Therefore, when the amount of theaflavins and thearubigins increase the amount of tea cream will also increase [27].

Table 3 Effect of tyrosinase on tea creaming and TF:TR ratio in tea extracts treated with viscozyme.

Tyrosinase Concentration (U/mL)	Incubation Time (min)	Tea Cream (mg)	TF (%)	TR (%)	Rasio TF:TR
223	20	4.8 ± 0.4^{a}	1.19 ± 0.04^{a}	2.10 ± 0.64^{a}	0.57
	40	5.3 ± 0.5^{a}	1.24 ± 0.06^{a}	2.21 ± 0.23^{a}	0.56
893	20	6.0 ± 1.1^{a}	2.55 ± 0.10^{b}	2.37 ± 0.28^{a}	1.08
	40	8.1 ± 0.1^{b}	2.77 ± 0.07^{c}	3.04 ± 0.44^{a}	0.91
3,571	20	9.2 ± 0.5^{b}	4.95 ± 0.05^{d}	2.48 ± 0.15^{a}	1.99
	40	11.5 ± 0.9^{c}	5.99 ± 0.10^{e}	4.27 ± 0.81^{b}	1.40

Values in the same column followed by a different letter represent a significant difference at $p < 0.05$.

Table 3 showed that the increase in the concentration of tyrosinase also affects the yield of tea cream. As previously stated, the amount of tea cream corresponds to the presence of theaflavins and thearubigins [28,29]. Thus, both concentration and incubation time greatly affect the formation of theaflavins and thearubigins and tea cream. At tyrosinase concentrations of 3,571 U/mL and an incubation time of 40 min gave the highest theaflavin results of 5.99 % compared to other treatments (**Table 3**). Tea extracts with high theaflavin content would produce a yellowish-brown color and gave an astringent or bitter taste [23], while tea extracts with high thearubigins content would produce a reddish-brown color with ashy and slight astringent or slightly bitter [30]. These results in line with research of Verloop *et al*. [12] which shows that tyrosinase had a role in the formation of theaflavins as compounds product from the condensation of catechins in the processing of green tea into black tea due to oxidation process. In addition, several reported studies also show that tyrosinase as PPO shows an increase in catecholase and cresolase activity along with substrate added resulting in monophenol catalysis becomes o-dhipenol which then forms o-quinone [31]. The highest thearubigin content produced in this study was 4.27 %, but this value was lower than that theaflavin. This in in line with research conducted by Ngure *et al.* [32], which shows that the thearubigin fraction in black tea is around 13 - 22 %. Thearubigin content is low due to influenced by the presence of horseradish peroxidase (POD), which is a crude enzyme from fresh tea leaves and had a role in enzymatic oxidation. When fresh tea leaves are steamed, the crude POD enzyme in the fresh tea leaves will have

inactivation.

The TF/TR ratio has been used to describe the brewing quality of black tea. generally categorized as follows; TF/TR ratio 0.04 indicates good brewing quality, 0.04-0.08 indicates better brewing quality, and > 0.08 indicates best brewing quality [33]. As the value of the TF/TR ratio of all treatments was greater than 0.08, all extracts could be grouped into best quality. This indicates the highest tyrosinase concentration was the best activity to transform viscozyme-treated green tea into black tea. Theaflavins found in black tea have the same antioxidant potential as catechin in green tea. Theaflavins have potential health effects in protecting human LDL from oxidation [34]. *In vitro* or chemical treatment, theaflavins have more potential antioxidant activity when compared to EGCG. This is because there are more hydroxy groups (OH) in theaflavins than catechins. The higher the theaflavin content in the dose, the stronger the free radical scavenging activity, which is reflected in the smaller the inhibition number in the EC50 [35]. Theaflavin-based diet causes a maximum and better reduction in lipid profile compared to thearubigin [36]. Thus, the combination treatment of viscozyme and tyrosinase had a positive effect on the quality of green tea extract. Extract quality is getting better. In this study, the lowest tyrosinase concentrations and fastest incubation times gave the lowest tea cream results. It showed that green tea with 223 U/mL and 20 min for incubation of tyrosinase can inhibit the formation of tea cream.

The combination of viscozyme and tyrosinase in enhancing phenolic contents and the antioxidant activity DPPH of black tea

The effects of treatments used in black tea processing include tyrosinase without viscozyme, a combination of viscozyme and tyrosinase, as well as commercial grade BOP black tea are shown in **Table 4**. The total phenolic content and antioxidant activity of DPPH in black tea from the combination of viscozyme and tyrosinase treatments gave the highest value compared to treatment using tyrosinase alone and BOP commercial black tea control.

Table 4 Total phenolic contents and DPPH antioxidant activity of black tea with various treatments.

Treatments	TPC (mg GAE/g)	DPPH (mg GAE.g)
Tyrosinase	55.96 ± 1.22^{a}	54.89 ± 1.56^{b}
Viscozyme-Tyrosinase	61.86 ± 3.11^{b}	54.99 ± 2.50^{b}
BOP*)	53.31 ± 3.14^{a}	49.94 ± 2.80^{a}

Values in the same column followed by a different letter represent a significant difference at $p < 0.05$.
*a commercial black tea (Broken orange pekoe-BOP) used as reference.

Black tea extract resulted from the combination of viscozyme and tyrosinase treatment showed the highest total phenolic content and antioxidant activity of DPPH. The addition of the viscozyme enzyme was able to release more individual catechins from tea leaf tissue through a more serious mechanism of cell wall damage. As a consequence, the content of phenolic compounds was higher and resulted in higher antioxidant activity of DPPH as well [37].

The combination of viscozyme and tyrosinase on the formation of tea cream and the TF:TR ratio in black tea

The formation of tea cream in black tea with various treatments was significantly different as showed in **Table 5**. The highest formation of tea cream was obtained in black tea extract with a BOP treatment of 0.0163. This is because BOP has a higher thearubigins content compared to other black tea treatments. The

increase in tea cream after enzymatic oxidation showed that the tea extract containing catechins which produced oxidation products in the form of theaflavins and thearubigins had a higher creaming capacity compared to unoxidized catechins because they had more hydroxyl groups on hydrogen bonds which needed for the formation of tea cream [27]. Beside that, Raghuwanshi *et al*. [21] stated that gallated catechins such as EGCG and ECG have the ability of stronger tea cream forming. The treatment of viscozyme and tyrosinase will be hydrolyzed to produce non-gallated catechins which have fewer hydroxyl groups for hydrogen bonding so that the ability to form tea cream will be lower.

Table 5 Tea creaming and TF:TR ratio on black tea with various treatments

Treatments	Tea cream (g)	TF (%)	TR (%)	TF:TR Ratio
Tirosinase Viscozyme-	0.0129 ± 0.0005^{b}	4.47 ± 0.06^{b}	2.64 ± 0.20^{a}	1.69
Tirosinase	0.0092 ± 0.0005^{a}	4.95 ± 0.05^{c}	2.48 ± 0.15^{a}	1.99
BOP	0.0163 ± 0.0003^{c}	1.29 ± 0.01^{a}	21.46 ± 0.22^{c}	0.06

Values in the same column followed by a different letter represent a significant difference at $p < 0.05$.

Black tea with the combination treatment of viscozyme and tyrosinase resulted in lower tea cream formation than other treatments. It is suspected that the combination of viscozyme and tyrosinase in black tea extract produced non-gallic theaflavins. The research of Liang *et al*. [27] stated that the ungallolated catechins and theaflavins were lower to form tea creaming, while the gallated catechins and gallated theaflavins have a stronger creaming ability. The treatment with viscozyme can increase individual catechins, while tyrosinase will oxidize non-gallate catechins so that the ability to form tea creaming will be lower.

The highest theaflavin content was produced in black tea with a combination treatment of viscozyme and tyrosinase of 4.95 % as in **Table 5**. The highest thearubigin content was obtained from BOP based on **Table 5**. Thearubigin fraction in black tea was around 13 - 22 %. While the thearubigin content in this study was lower than the theaflavin. This is because it is influenced by the presence of indigenous polyphenol oxidase [32] and oxidation time for almost 2 h. In research those enzyme has been inactivated and replaced with tyrosinase, and the oxidation time was 20 min. Indegenous polyphenol oxidase in fresh tea leaves has high potential to oxidize theaflavins into a more complex form resulting in higher thearubigin as the main product of black tea [38]. The highest TF:TR ratio was produced in black tea extract with a combination treatment of viscozyme and tyrosinase of 1.99. The results showed that the concentration of the TF:TR category of black tea was the best (best quality). Meanwhile, TF:TR ratio in BOP was included in the black tea category with a better quality ratio of 0.06. The low thearubigins content in tyrosinase-treated tea extract compared to BOP commercial tea was because of the presence of peroxide (POD), meanwhile in this research, the enzyme was inactivated to prevent the process from oxidation occurring. POD in fresh tea leaves has the potential to oxidize theaflavin becomes more complex form producing thearubigin higher as the main product of black tea when pH condition are low (< 5.5) with the addition H_2O_2. Thearubigin increases along with high activity of peroxidase at high temperatures. Initial the brightness level still increases when the temperature increases, but decreases when the enzymatic oxidation time reaches, This shows that the color formed in the tea is darker or brownish [39].

Conclusions

The manufacture of liquid black tea can be carried out directly with the raw material of green tea extract using the help of extracellular enzymes. The use of the viscozyme enzyme was able to increase individual catechin yields through the mechanism of greater tea leaf tissue damage. An addition of viscozyme significantly increased phenolic contents and antioxidant activity. The combination of extracellular viscozyme and tyrosinase enzymes were able to produce liquid black tea with the best quality properties accompanied by other properties such as total phenolic compounds and high antioxidant activity. these properties are better than those brewed from BOP commercial black tea.

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