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Research article

Tannase application in secondary enzymatic processing of inferior Tieguanyin oolong tea

Jiajia Li [a,1], Qiong Xiao [a,b,1], Yufeng Huang [a,1], Hui Ni [a,c], Changzheng Wu [a,c], Anfeng Xiao [a,b,c,*]

[a] College of Food and Biological Engineering, Jimei University, Xiamen 361021, China
[b] Fujian Provincial Engineering Technology Research Center of Marine Functional Food, Xiamen, Fujian Province 361021, China
[c] Fujian Provincial Key Laboratory of Food Microbiology and Enzyme Engineering, Xiamen, Fujian Province 361021, China

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ABSTRACT

Background: Inferior Tieguanyin oolong tea leaves were treated with tannase. The content and bioactivity of catechins in extracts from the treated tea leaves were investigated to assess the improvement in the quality of inferior Tieguanyin oolong tea.
Results: Analysis showed that after treatment, the esterified catechin content decreased by 23.5%, whereas non-galloylated catechin and gallic acid contents increased by 15.3% and 182%, respectively. The extracts from tannase-treated tea leaves showed reduced ability to bind to BSA and decreased tea cream levels. The extracts also exhibited increased antioxidant ability to scavenge OH and DPPH radicals, increased ferric reducing power, and decreased inhibitory effects on pancreatic α-amylase and lipase activities.
Conclusions: These results suggested that tannase treatment could improve the quality of inferior Tieguanyin oolong tea leaves.

1. Introduction

Tea is one of the most regularly consumed beverages worldwide. Tea has beneficial properties such as anti-oxidative and anti-tumor effects and improved cardiovascular function [1]. Fresh tea leaves are processed in a series of steps to develop different tea types, depending on the extent of fermentation. Different types of tea include green (unfermented), oolong (semi-fermented), and black teas (fully fermented). "Fermentation" refers to the natural enzymatic browning reaction induced by oxidative enzymes in the cells of tea leaves [2]. Green tea is consumed in many Asian countries, especially in China and Japan. Oolong tea is popularly consumed in Taiwan, whereas black tea is popularly consumed in America and Europe [3]. Tea contains as much as 30% soluble ingredients, which differ with cultivar, climate, production region, and processing and handling process. The active constituents of tea are polyphenols, commonly known as tea catechins. Catechins account for 75%–80% of soluble ingredients in tea. The four types of tea cathechins include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). EGCG is the most important of them because of its high content in tea leaf and excellent bioactivity [4].

Tea catechins have received increased research interest because of their positive physiological and pharmacological effects combined with antimutagenic, anticarcinogenic, and antitumorigenic activities [5]. Catechins also exhibit strong antioxidant and free radical scavenging activities and have been tested as antimicrobial and antiviral agents [6]. Despite their health advantages, catechins are major contributors to the astringency and bitterness of green tea infusions. High catechin concentration enhances taste intensity but also results in decreased taste palatability [7]. Catechin bioavailability is very poor because of its large molecular size and the number of hydrogen bonds. Fan et al. [8] reported that the molecular structure and steric configuration of catechins are associated with their bioactivities and intermolecular interactions, thereby possibly inducing differential behavior of individual catechins. Hot and clear tea infusions produce tea cream and visible and turbid precipitates upon cooling during the manufacturing of ready-to-drink teas. Tea cream influences storage quality and reduces product appeal for consumers. Polyphenol complexation is a major contributor to tea cream formation, which is influenced by the number of galloyl and hydroxyl groups in polyphenols [9]. Moreover, tea polyphenols possess anti-nutritional properties as they bind and precipitate some

* Corresponding author
E-mail address: xxaaffeng@jmu.edu.cn (A. Xiao).
[1] These authors contributed equally to this work.
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digestive enzymes, such as pepsin, α-amylase, and lipase. Tannase treatment is one of the best solutions to the drawbacks mentioned above.

Tannase, or tannin acyl hydrolase (EC, 3.1.1.20), is an inducible extracellular enzyme produced by a variety of microorganisms, such as fungi, bacteria, and yeast. Tannase catalyzes ester and disulfide bond hydrolysis in hydrolysable tannins or gallic acid esters, such as EGCG or ECG, to release glucose or gallic acid [10]. Many studies have reported the application of tannase in instant tea to reduce tea cream formation and improve color appearance. The hydrolytic action of tannase decreases EGCG binding with protein due to ester bond cleavage, thus preventing macromolecule aggregation and precipitate formation during tea beverage storage [11]. Moreover, tannase applications in food and beverage industry products may eliminate tannins' undesirable effects. Lata and Rani [12] reported that enzymatic treatment of fruit juices with tannase reduced bitterness and resulted in higher juice quality by lowering haze and non-deterioration. Some studies have also previously reported that tannase treatment during processing improved the taste quality and mouthfeel of green tea beverages, green tea concentrates, and instant green tea powders [13]. Lu and Chen [14] reported that tannase-treated green tea had stronger inhibitory ability against dimethyline nitrosation. In addition, Hagerman et al. [15] reported that condensed and hydrolysable tannins have higher antioxidant activity than simple phenolics as the former quench peroxyl radicals.

The tea industry in Fujian province is not only a traditional and prominent industry, but it is also an important industry for rural economics. However, a considerable portion of low-quality tea has a low extraction rate of active components and results in astringent tea infusions, thus decreasing the freshness and briskness of taste. Recently, many reports have focused on the application of tannase in instant tea manufacturing to reduce tea cream formation [16,17]. However, limited information is available on changes in tea leaves after tannase treatment. The present study conducted the secondary processing of tea leaves using tannase obtained from solid-state fermentation. Analyses of constituents and biological activities of catechins in tea extracts were conducted to assess quality improvement of inferior tea.

2. Materials and methods

2.1. Materials

Inferior Tieguanyin oolong tea was obtained from an ecological tea plantation located at Anxi County of Fujian Province, China. Pancreatic α-amylase (2800 U/mg) was obtained from Sinopharm Shanghai Medical Instrument Co., Ltd. Pancreatic lipase (100–400 U/mg) was obtained from Sigma Corporation of America (USA). Propyl gallate and rhodanine were procured from Tokyo Chemical Industry Co., Ltd. (Japan). All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Ltd., Corp. (China).

2.2. Production of tannase under solid-state fermentation

Tannase was produced by *Aspergillus tubingensis* CICC 2651 under solid-state fermentation as our previous article mentioned [11]. *A. tubingensis* CICC 2651 was obtained from the China Center of Industrial Culture Collection. Fermentation conditions and culture medium formula were as follows: dried tea stalk powder (size: 20-mesh sieve) 5 g, NH_4Cl 5% (*w*/*w*), α-lactose 5% (*w*/*w*), initial pH of 6.0, solid–liquid ratio of 1:2, and autoclaving at 121°C for 20 min. After cooling, the fermentation medium were inoculated with spore inoculum (4.35 mL, 1×10^8 spores/mL) and incubated at 28°C for 118 h. Then, the fermented mass was mixed with citrate buffer (0.05 M, pH 5.0) to obtain a volume of 100 mL in each flask and then agitated at 180 rpm for 1 h. The slurry was squeezed through cheesecloth, followed by centrifugation at 10,000 × g for 10 min at 4°C. The supernatant was subsequently decolorized by adding activated charcoal and purified by ammonium sulfate precipitation. Finally, tannase activity (1.5 U/mL) was determined by the colorimetric method described by Sharma et al. [18]. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the release of 1 μmol gallic acid per minute under specific conditions.

2.3. Treatment of tea leaves with tannase

Several tannase concentrations were prepared with a specific solid–liquid ratio and then sprayed evenly on dried tea leaves with appropriate rolling. The enzymatic reaction was allowed to proceed at 50°C for 1 h. The tea leaves were immediately placed in a drying oven to inactivate the enzyme and dry the leaves. The drying process was divided into two steps: the first step involving drying at 120°C and the second step involved drying at 100°C. After the drying was completed, the tea leaves were ground into powder for further analysis.

2.4. Preparation of tea extracts

One gram of tea powder was extracted with 80 mL of distilled water at 90°C for 30 min. The extract was filtered under reduced pressure centrifugation. The supernatant was transferred to a 100-mL volumetric flask and brought to volume with distilled water.

2.5. Identification of tea catechins

Catechins were analyzed by high-performance liquid chromatography (HPLC). The tea infusion was centrifuged at 10000 × g for 10 min and filtered through a 0.22-μm millipore filter before injection. HPLC separation was performed on a symmetry C_{18} column (3.0 × 250 mm, 5 μm) at 30°C. The mobile phase consisted of (A) water containing 0.5% acetic acid and (B) methanol. The elution was run in a linear gradient as follows: 88% A (0 min), 88% A (5 min), 81% A (16 min), 76% A (28 min), 70% A (32 min), 88% A (40 min), and 88% A (45 min). The flow rate of the mobile phase was 0.5 mL/min. The UV absorbance detection wavelength was set at 278 nm.

2.6. Evaluation of protein-tannin aggregation

Protein-tannin aggregation was measured according to a previously described method with minor modifications [3]. Briefly, bovine serum albumin (BSA) was dissolved in 40 ml of 1 g/100 ml $NaHCO_3$ (pH 8.2). After that, Remazol Brilliant Blue R was added and incubated overnight to dye BSA. Tannase-treated or untreated tea infusion (1 mL) and dyed BSA (4 mL) were mixed to obtain a reaction. The precipitate was collected and dissolved in 3.5 mL sodium dodecyl sulfate:triethanolamine:isopropanol (1:5:20/100). Absorbance of the solution was then measured at 590 nm.

2.7. Evaluation of tea cream formed

Tea cream content was determined by a previously described method [3]. Tieguanyin oolong tea extract was centrifuged at 11,000 × g and 8°C for 25 min. The precipitate was collected and weighed after oven-drying at 100°C. Tea cream (g/100 mL) was expressed as the weight of precipitate obtained per 100 mL of tea infusion.

2.8. Determination of ferric reducing antioxidant potential

The reducing power of the tea extract was determined by a previously described method, with slight modifications [19]. This method is based on the reduction of Fe^{3+} in stoichiometric excess relative to antioxidants. Briefly, different concentrations of tea extracts in 0.5 mL volumes were mixed with 1.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and

1.5 mL (1%, m/v) potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 20 min. After 20 min of incubation, the reaction mixture was acidified with 1.5 mL trichloroacetic acid (10%) with shaking and then centrifuged at 2625 × g for 15 min. Finally, 2.5 mL of the supernatant was mixed with 0.5 mL $FeCl_3$ (0.1%) and 2.5 mL distilled water and allowed to settled for 10 min. Distilled water was used as a blank and the control. Absorbance of the tea mixture was measured at 700 nm using a UV spectrophotometer. A higher absorbance indicated higher ferric reducing power.

2.9. Determination of hydroxyl radical scavenger activity

Hydroxyl radical scavenging activity was determined by the Fenton method as described previously [20]. Briefly, 1 mL sample, 1 mL $FeSO_4$ (2.25 mM), 1 ml H_2O_2 (8.8 mM), and 1 mL salicylic acid-ethanol solution (9 mM) were mixed and incubated at 37°C for 30 min. The reaction mixture's absorbance was determined at 510 nm. Hydroxyl radical scavenging activity was calculated as follows:

$$\text{Scavenging activity } (\%) = (1 - \text{As}/\text{Ac}) \times 100$$

where As and Ac are the absorbance of the sample group and blank control group, respectively.

2.10. Determination of DPPH radical scavenger activity

DPPH radical scavenger activity was evaluated according to a previous method with minor modifications [21]. Two milliliters of diluted tea infusion was transferred into 2 mL of DPPH working solution, followed by incubation for 30 min at room temperature in the dark. Absorbance was measured at 517 nm against a mixture of 2 mL methanol, with 2 mL DPPH working solution as blank. Half maximal inhibitory concentration (IC_{50}), or the concentration of a tea infusion that decreased the absorbance by 50%, was calculated to evaluate antioxidant capacity.

2.11. Determination of α-amylase inhibition by tea extracts

α-Amylase activity was measured following a previously described method with minor modifications [22]. A total of 0.25 mL of α-amylase (0.05 mg/mL) was mixed with 0.25 mL of different concentrations of tea extract at 37°C for 10 min. Then 0.5 mL of 0.5% (*w*/*w*) starch solution was added, and the mixture was incubated at 37°C for 30 min. After reaction, 1 mL of 3, 5-dinitrosalicylic acid reagent was added. The mixture was boiled for 8 min, then cooled, and, finally, diluted with water to a volume of 10 mL. Absorbance was measured at 540 nm. Percentage of pancreatic α-amylase inhibition was calculated according to the equation below:

$$E(\%) = (T_1 - T_2)/T_1 \times 100$$

where T_1 and T_2 are the α-amylase activities of the test and control groups, respectively, and E(%) is the inhibition rate of α-amylase in tea extracts.

2.12. Determination of pancreatic lipase inhibition by tea extracts

Pancreatic lipase activity was determined following the modified method of Wilcox et al. [23]. One milliliter of tea infusion was mixed with 1 mL of pancreatic lipase solution (2 mg/mL) at 37°C for 5 min. Substrates containing 4 mL of phosphate buffer (0.05 mol/L, pH 7.7) and 2 mL of olive oil were then added into the mixture and stirred at 37°C for 30 min. The reaction was stopped by adding 10 mL xylene and was subsequently centrifuged at 4000 × g for 10 min. Finally, 4 mL of the supernatant organic phase was withdrawn and mixed with 1 mL chromogenic agent [0.5% (*w*/w) copper acetate, pH 6.1] with stirring for 5 min and then centrifuged at 4000 × g for 10 min. The absorbance of 1 mL of the supernatant was measured at 710 nm using a microplate reader. The percentage of pancreatic lipase inhibition was calculated according to the equation below:

$$E(\%) = (T_1 - T_2)/T_1 \times 100.$$

where T_1 and T_2 are the lipase activities of the test and control groups, respectively, and E(%) is the inhibition rate of lipase in tea extracts.

2.13. Statistical analysis

All results were recorded as mean ± standard deviation of three replicates. Significance of the differences between each sample was analyzed by ANOVA and Duncan's multiple range test by SPSS software, with the significance set at $P < 0.05$ (version 17, SPSS Inc., Chicago, USA).

3. Results and discussion

3.1. Effects of tannase:tea ratio on catechins in inferior oolong tea leaves

Table 1 presents the changes in catechin concentrations after enzymatic hydrolysis of tea leaves at various ratios of tannase to tea (1:1 to 1:9). Enzymatic treatment significantly decreased EGCG, ECG, and GCG and increased GA, EGC, and EC contents of tea infusions. According to previous findings, EGCG was transformed to EGC and GA, GCG was hydrolyzed to GC and GA, and ECG was degraded to EC and GA after tannase cleaved the ester bonds in EGCG and ECG [24]. When the tannase to tea ratio was 1:1 (*v*/*w*), EGCG decreased from 246.5 μg/mL to 153.9 μg/mL. ECG also decreased from 23.1 μg/mL to 9.9 μg/mL, but gallic acid increased from 21.9 μg/mL to 83.9 μg/mL. After treating inferior Tieguanyin oolong tea leaves with tannase, EGCG and ECG contents were reduced by 37.6% and 58.4%, respectively. However, a higher enzyme dosage could be an expensive proposition for the process, suggesting that it is desirable to limit enzyme dosage relative to tea leaf content. Moreover, Corey et al. [25] showed that catechins are more susceptible to degradation at higher moisture conditions because of the greater reactant mobility, dissolution, or deliquescence of organic acids. Therefore, a 1:3 ratio of tannase to tea ratio may be ideal, considering the process economics.

3.2. Effects of tannase concentration on catechins in inferior oolong tea leaves

Changes in catechin concentrations after enzymatic hydrolysis of tea leaves by tannase at different concentrations are presented in Table 2. A direct correlation between catechin hydrolysis and tannase concentration was discovered. EGCG and ECG concentrations from tannase-treated tea leaves decreased with tannase concentration, and this was accompanied by increased EGC, EC, and GA. Approximately 43% of esterified catechins were hydrolyzed with a 12% increase in non-galloylated catechins after 60 min of tannase (1.5 U/mL) treatment. In addition, gallic acid content increased by 77%, whereas gallocatechin amount changed without any obvious trends.

3.3. Effects of temperature on the enzymatic hydrolysis of catechins in inferior oolong tea leaves

Changes in tea catechin concentrations after the enzymatic hydrolysis of tea leaves at various enzymolysis temperatures are presented in Table 3. During tannase treatment, ECG is converted into EC and gallic acid, whereas EGCG is hydrolyzed into EGC and gallic acid because of ester bond cleavage. Therefore, EC and EGC are the simplest forms of catechins without undergoing further hydrolysis. Hydrolytic activity increased with enzymolysis temperature. The amount of hydrolytic products clearly depicted this trend (Table 3). For example,

Table 1
Effects of the ratio of tannase to tea on the catechins in oolong tea leaves.

The ratio of tannase to tea	GA (μg/mL)	Contents of catechins (μg/mL)								
		GC	EGC	EGCG	EC	GCG	ECG	Nonestern*	Estern*	Total
control	21.9^{f}	9.8^{a}	275.6^{b}	246.5^{a}	49.9^{b}	32.7^{b}	23.1^{a}	335.4^{a}	302.4^{a}	637.7^{a}
1:9	36.1^{e}	9.3^{b}	294.0^{ab}	230.6^{a}	45.5^{b}	37.3^{a}	21.8^{ab}	348.8^{a}	289.7^{a}	638.5^{a}
1:7	47.3^{d}	8.9^{b}	307.0^{ab}	213.4^{ab}	47.0^{b}	32.6^{b}	19.1^{b}	362.9^{a}	265.1^{a}	628.0^{a}
1:5	65.9^{c}	8.2^{c}	309.7^{ab}	183.5b^{c}	47.7^{b}	26.1^{c}	15.5^{c}	365.6^{a}	225.2^{b}	590.7^{a}
1:3	72.7^{b}	7.5^{d}	319.9^{a}	173.9^{c}	49.6^{b}	19.0^{d}	11.8^{d}	377.0^{a}	204.6^{bc}	581.6^{a}
1:1	83.9^{a}	6.6^{e}	318.0^{a}	153.9^{c}	57.5^{a}	12.3^{e}	9.9^{d}	382.1^{a}	176.2^{c}	558.3^{a}

* Tannase concentration: 1.5 U/mL, temperature: 50°C, time: 60 min; different letters in the same column indicate significant difference ($P < 0.05$); EGCG: epigallocatechin gallate; EGC: epigallocatechin; ECG: epicatechin gallate; EC: epicatechin; GCG: gallocatechin gallate; GC: gallocatechin; GA: gallic acid; Content of nonestern catechins = GC + EGC + EC; Content of estern catechins = EGCG + GCG + ECG.

Table 2
Effects of tannase concentrations on the catechins in oolong tea leaves.

Tannase (U/mL)	GA (μg/mL)	Contents of catechins (μg/mL)								
		GC	EGC	EGCG	EC	GCG	ECG	Nonestern	Estern	Total
0	18.4^{e}	7.1^{b}	326.1^{a}	255.7^{a}	50.4^{b}	22.0^{a}	29.7^{a}	383.54^{c}	307.44^{a}	691.0^{ab}
0.3	37.1^{d}	6.7^{ab}	367.9^{a}	234.7^{a}	53.1^{b}	18.0^{b}	27.0^{b}	427.74^{b}	279.72^{b}	707.5^{a}
0.6	49.6^{c}	8.0^{a}	346.0^{a}	196.6^{b}	52.4^{b}	16.7^{bc}	23.0^{c}	406.33^{a}	236.28^{c}	642.6^{abc}
0.9	57.8^{b}	6.5^{ab}	347.9^{a}	180.8^{bc}	52.9^{b}	15.5^{c}	20.5^{d}	407.32^{a}	216.79^{d}	624.1^{bc}
1.2	60.5^{b}	6.1^{c}	354.7^{a}	162.4^{cd}	54.3^{ab}	13.0^{d}	18.8^{d}	414.99^{a}	194.18^{d}	609.2^{c}
1.5	80.6^{a}	6.8^{ab}	366.0^{a}	150.6^{d}	57.2^{a}	9.1^{e}	15.2^{e}	430.01^{a}	174.98^{e}	605.0^{c}

The ratio of tannase to tea: 1:3, temperature: 50°C, time: 60 min; different letters in the same column indicate significant difference ($P < 0.05$); EGCG: epigallocatechin gallate; EGC: epigallocatechin; ECG: epicatechin gallate; EC: epicatechin; GCG: gallocatechin gallate; GC: gallocatechin; GA: gallic acid; Content of nonestern catechins = GC + EGC + EC; Content of estern catechins = EGCG + GCG + ECG.

under an enzymolysis temperature of 60°C, approximately 37% of EGCG/ECG was hydrolyzed to EGC or EC and total catechin content decreased by 5.3% from 602.7 to 570.7 μg/mL. Ananingsih et al. reported that heating conditions influenced tea catechin epimerization. EGCG, EGC, EC, and ECG underwent epimerization during heating [26]. The decrease in total catechin concentrations after processing might also be due to oxidation during heating. Therefore, because increasing temperature decreased total catechin content, subsequent experiments were conducted at the enzymolysis temperature of 60°C.

3.4. Effects of enzymatic hydrolysis time on the catechin content in inferior oolong tea leaves

Data analysis revealed a direct correlation between catechin hydrolysis and treatment time. Table 4 shows that as tea leaves were hydrolyzed for a longer time, gallated catechin (EGCG, ECG, and GCG) content decreased and ungallated catechin (EC and EGC) and GA content increased. EGCG obviously decreased with tannase treatment until 1.5 h and then the decrease slightly lessened thereafter, most likely due to the gradual decline of tannase activity as increasing enzymolysis time increased tannase denaturation rate and secondary and tertiary structure loss. Adamczyk et al. [27] also reported that pyrogallols, gallic acid, and gallaldehyde competitively inhibit tannase activity. After hydrolysis for 2 h, approximately 26% and 32% of EGCG and ECG were hydrolyzed to EGC and EC, respectively, indicating effective transformation. Thus, subsequent experiments were performed for an enzymolysis time of 2 h.

3.5. Effects of tannase treatment on catechin content in inferior oolong tea leaves

Catechins are not only the dominant contributors to the astringency and bitterness of green tea infusions but are also associated with a sweet aftertaste in oolong tea infusions [7]. Some previous studies reported that the bitterness and astringency of green tea infusions decreased, while the sweet aftertaste was enhanced after EGCG and ECG degalloylation [13,28]. After a preliminary investigation of single factors, four factors affecting tannase hydrolysis of inferior Tieguanyin oolong tea were identified: tannase to tea ratio of 1:3, tannase concentration of 1.5 U/mL, temperature of 60°C, and time of 2 h. The changes in catechin concentrations of inferior Tieguanyin oolong tea leaves with or without tannase treatment were then measured under these conditions (Table 5). After treating tea leaves with tannase, tea extracts displayed decreased gallated catechin levels, including EGCG, ECG, and GCG, and concomitantly increased degallated catechin levels, including EGC, EC, and GC. However, total catechin levels did not change significantly before (644.6 μg/mL) and after (634.5 μg/mL) tannase treatment. EGC, EC, and GA content increased from 308.7,

Table 3
Effects of enzymatic hydrolysis temperature on the catechins in oolong tea leaves.

Temperature (°C)	GA (μg/mL)	Contents of catechins (μg/mL)								
		GC	EGC	EGCG	EC	GCG	ECG	Nonestern	Estern	Total
30	45.9^{e}	8.8^{a}	285.3^{b}	215.8^{a}	50.8^{a}	17.6^{a}	24.5^{a}	344.8^{bc}	257.9^{a}	602.7^{a}
40	54.3^{d}	7.7^{b}	284.5^{b}	202.2^{ab}	48.3^{a}	14.9^{b}	19.0^{b}	340.5^{c}	236.1^{ab}	576.7^{a}
45	60.1^{c}	8.0^{ab}	324.9^{a}	195.7^{ab}	53.1^{a}	15.6^{b}	20.3^{b}	385.9^{ab}	231.6^{ab}	617.4^{a}
50	66.2^{b}	6.7^{c}	312.7^{ab}	176.4^{b}	50.0^{a}	12.2^{c}	18.3^{b}	369.4^{abc}	206.9^{b}	576.3^{a}
60	74.9^{a}	6.4^{c}	349.9^{a}	135.4^{c}	51.9^{a}	11.8^{c}	15.5^{c}	408.1^{a}	163.6^{c}	570.7^{a}

The ratio of tannase to tea: 1:3, tannase concentration: 1.5 U/mL, time: 60 min; different letters in the same column indicate significant difference ($P < 0.05$); EGCG: epigallocatechin gallate; EGC: epigallocatechin; ECG: epicatechin gallate; EC: epicatechin; GCG: gallocatechin gallate; GC: gallocatechin; GA: gallic acid; Content of nonestern catechins = GC + EGC + EC; Content of estern catechins = EGCG + GCG + ECG.

Table 4
Effects of enzymatic hydrolysis time on the catechins in oolong tea leaves.

Time (h)	GA (μg/mL)	Contents of catechins (μg/mL)								
		GC	EGC	EGCG	EC	GCG	ECG	Nonestern	Estern	Total
0	30.8^{d}	9.2^{a}	308.7^{a}	241.2^{a}	46.3^{a}	26.3^{a}	24.7^{a}	364.2^{a}	292.31^{a}	656.5^{a}
0.5	48.6^{c}	8.5^{a}	310.1^{a}	204.9^{b}	45.8^{a}	20.6^{b}	20.5^{b}	364.4^{a}	245.9^{b}	610.4^{b}
1	64.4^{b}	8.6^{a}	325.4^{a}	184.6^{c}	50.1^{a}	19.4^{bc}	19.6^{bc}	384.2^{a}	223.5^{bc}	607.7^{b}
1.5	72.5^{b}	7.1^{b}	326.4^{a}	172.6^{c}	47.7^{a}	16.8^{c}	18.0^{bc}	381.1^{a}	207.5^{c}	588.6^{b}
2	82.8^{a}	6.9^{b}	338.4^{a}	178.2^{c}	50.1^{a}	16.9^{c}	16.8^{c}	395.4^{a}	211.8^{c}	607.2^{b}

The ratio of tannase to tea: 1:3, tannase concentration: 1.5 U/mL, temperature: 60°C; different letters in the same column indicate significant difference ($P < 0.05$); EGCG: epigallocatechin gallate; EGC: epigallocatechin; ECG: epicatechin gallate; EC: epicatechin; GCG: gallocatechin gallate; GC: gallocatechin; GA: gallic acid; Content of nonestern catechins = GC + EGC + EC; Content of estern catechins = EGCG + GCG + ECG.

Table 5
Effect of tannase treatment on contents of catechins in oolong tea leaves.

Treatment	GA (μg/mL)	Contents of catechins (μg/mL)								
		GC	EGC	EGCG	EC	GCG	ECG	Nonestern	Estern	Total
Without tannase	28.3^{b}	8.5^{a}	308.7^{b}	226.3^{a}	47.6^{b}	25.6^{a}	27.9^{a}	364.8^{b}	279.8^{a}	644.6^{a}
Tannase treated*	79.8^{a}	6.7^{b}	359.6^{a}	184.2^{b}	54.3^{a}	15.1^{b}	14.7^{b}	420.5^{a}	214.0^{b}	634.5^{a}

* Conditions of tannase treatment: the ratio of tannase to tea 1:3, tannase concentration 1.5 U/mL, temperature 60°C, time 2 h; different letters in the same column indicate significant difference ($P < 0.05$); EGCG: epigallocatechin gallate; EGC: epigallocatechin; ECG: epicatechin gallate; EC: epicatechin; GCG: gallocatechin gallate; GC: gallocatechin; GA: gallic acid; Content of nonestern catechins = GC + EGC + EC; Content of estern catechins = EGCG + GCG + ECG.

47.6, and 28.3 μg/mL to 359.6, 54.3, and 79.8 μg/mL, respectively. This result demonstrated that EGCG, ECG, and GCG were successfully converted to EGC, EC, and GC, respectively. Oolong tea infusions are less bitter and astringent with a sweeter aftertaste than green tea infusions because oolong tea has more EGC and EC and less EGCG and ECG than green tea [7]. Therefore, EGCG and ECG degalloylation by tannase may improve the taste quality of inferior Tieguanyin oolong tea.

3.6. Effects of enzymatic treatment of inferior oolong tea leaves on precipitation of BSA with tannin

Fig. 1a shows the reactions between BSA and tea extracts from inferior treated or untreated Tieguanyin oolong tea leaves. The binding rate increased as BSA concentration increased. However, BSA binding ability of extracts from treated tea leaves was much lower,

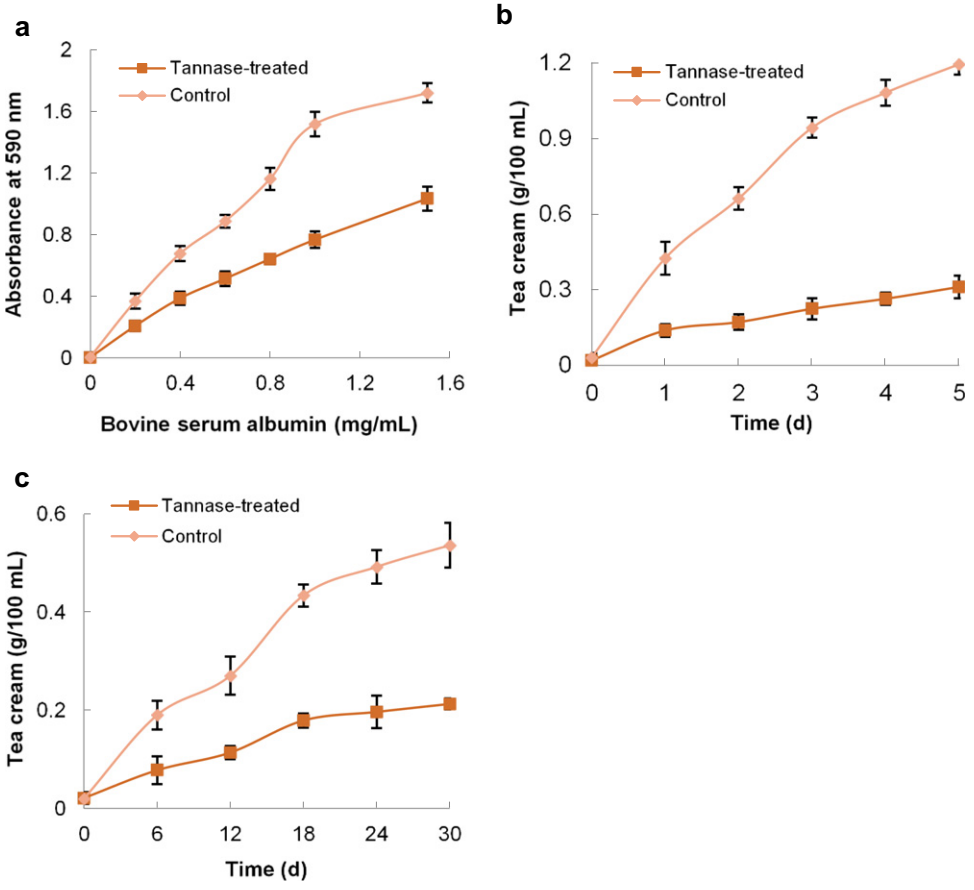

Fig. 1. Effect of enzymatic treatment of oolong tea leaves on the precipitation of BSA with tannin (a) and formation of tea cream during storage at 25°C (b) and 4°C (c).

which may be attributed to the elimination of ester catechins and liberation of ungallated catechins and gallic acid. Tea polyphenols bind with protein by ionic and hydrogen bond formation with polar protein groups (amide, guanidine, pep-1 peptide, amino, and carboxyl groups) [29]. Kawamoto et al. [30] found that a number of galloyl groups in tannin are positively correlated with the degree of protein aggregation in the co-precipitation of hydrolysable tannins with BSA.

3.7. Tea cream formation of tea extracts

All gallated catechins have stronger creaming ability by offering more hydroxyl groups for hydrogen bonding, which is the most important factor for compound aggregation in tea cream [3]. EGCG and ECG are the critical catechins involved in tea cream formation [31]. Tannase action was expected to hydrolyze gallated ester linkages of EGCG and ECG, releasing gallic acid in tea extracts. Fig. 1c shows the effect of tannase treatment on tea cream formation during storage of Tieguanyin oolong tea extracts at 4°C for 30 days. Tea cream formed in tea extracts of tannase-treated tea leaves was approximately 0.213 g/100 g, compared with approximately 0.536 g/100 g in the control. In addition, when the tea extracts of treated or untreated tea leaves were stored at 25°C for 5 days (Fig. 1b), the tea cream content of extract from tannase-treated tea leaves was approximately 0.311 g/100 g, compared with approximately 1.194 g/100 g for the control. The difference observed between enzyme-treated tea leaves and untreated control samples could be due to the degallation of gallated catechins and other gallated polyphenols by tannase, preventing these enzymatically converted tea catechins from interacting with protein. Lu et al. [3] also revealed that the presence of galloyl groups in catechins could facilitate interaction between catechins and proteins in the tea infusion and result in a decrease in the content of EGCG and ECG after storage. In addition, the experimental results indicated that gallated catechins have stronger ability of cream formation when tea infusion stored at room temperature than those stored at low temperature. Therefore, storage in low temperature can probably prolong the tea's shelf-life.

3.8. Effects of tannase treatment on catechin contents and bioactivities

3.8.1. Effects of enzymatic treatment on the reducing capacity of tea

Fe^{3+} reduction is an index of electron-donating activity and is an important mechanism of phenolic antioxidant action [32]. Extracts from both treated and untreated tea leaves exhibited an apparent effect on ferric reducing power (Fig. 2a). Results revealed a dose-effect

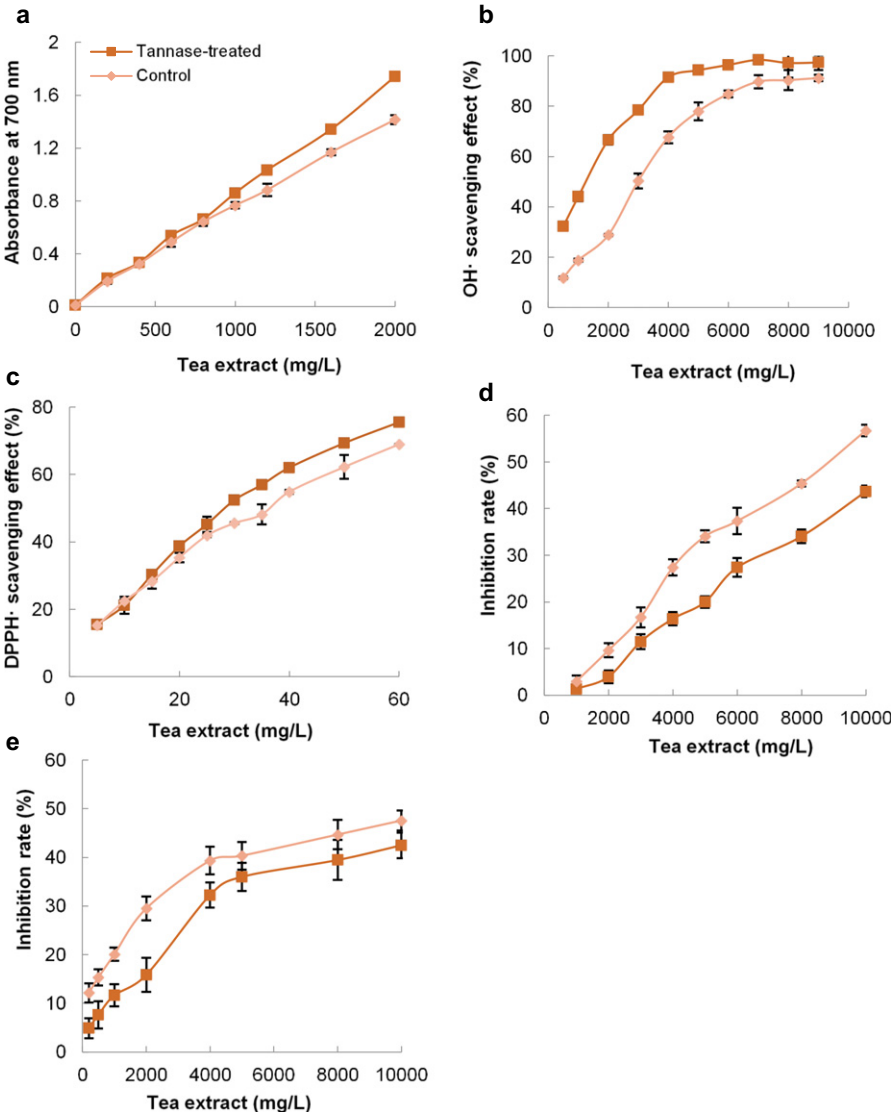

Fig. 2. Effect of enzymatic treatment of oolong tea leaves on the reducing capacity (a), Hydroxyl radical scavenging effect (b), DPPH· scavenging effect (c), inhibition rate of pancreatic α-amylase activity (d), inhibition rate of pancreatic lipase activity (e).

relationship between tea concentrations and reducing effects. When sample concentrations increased from 200 to 2000 mg/L, the reducing effects of tea extract from tannase-treated tea leaves increased from 0.215 to 1.742 at 700 nm. Reducing effects of extracts from untreated tea leaves increased from 0.19 to 1.41 at 700 nm. At a concentration of 2000 g/mL, the scavenging activity of extracts from tannase-treated tea leaves was higher than that of extracts from untreated tea leaves. Ercan and Ekrem [33] demonstrated that the total phenolic content and ferric reducing power are related. However, because total catechin content did not significantly change, tannase treatment enhanced ferric reducing power of the tea extract, which may be attributed to EGC and gallic acid levels. Thus, our data indicated that tannase-derived bioconversion could improve the biological functions of inferior Tieguanyin oolong tea leaves.

3.8.2. Hydroxyl radical scavenging effect of tea extract

Hydroxyl radicals are some of the most harmful and reactive ROS. Hydroxyl radicals are produced in vivo from water by high-energy irradiation or from H_2O_2 in a metal-catalyzed process. Hydroxyl radicals can easily cross cell membranes to damage lipids, proteins, and DNA [34]. Hence, hydroxyl radical removal is important to protect the living system. As shown in Fig. 2b, at 4000 mg/L, both tannase-treated tea leaves and control scavenged hydroxyl radicals in a concentration-dependent manner, exhibiting hydroxyl radical scavenging activities of 91.5% and 67.7%, respectively. Additionally, the IC_{50} of tea extract with or without tannase treatment were 1025 and 2366 mg/L, respectively. Assay results clearly showed that the scavenging activity of extracts from inferior Tieguanyin oolong tea leaves was promoted after tannase treatment. The related structural transformation of catechins might improve and increase the biological activity of tea infusions.

3.8.3. DPPH scavenging effect of tea extract

The DPPH radical is used as a substrate to evaluate the free radical scavenging ability of an antioxidant. The DPPH radical accepts an electron or a hydrogen radical to become a stable diamagnetic molecule [14]. Fig. 2c illustrates the decrease in DPPH concentration in response to the scavenging ability of tea extracts from inferior treated or untreated Tieguanyin oolong tea leaves. Although DPPH radical scavenging increased as tea extract concentration increased, the scavenging ability of tannase-treated leaves was greater than that of untreated leaves. The IC_{50} of tea extract with or without tannase treatment was 31.9 and 36.9 mg/L, respectively, indicating that enzymatic treatment enhanced the radical-scavenging activity. According to a previous study by Lu and Chen [14], tannase-catalyzed hydrolysis of gallated catechins (such as EGCG and ECG) increased the radical-scavenging activity of tea extracts against superoxide anions, hydrogen peroxides, and DPPH. In addition, Battestin et al. [35] also reported a positive association between radical-scavenging capacity and increased EGC and gallic acid content in green tea extracts.

3.8.4. Effects of tea extract inhibition rate of pancreatic α-amylase

Fig. 2d shows the inhibitory activity of tea extracts against pancreatic α-amylase. The inhibitory effects of α-amylase were significantly reinforced with increased tea extract concentration. When the tea extract concentration was 10,000 mg/L, α-amylase inhibition rates in tea extract concentration with or without tannase treatment were 43.7% and 56.8%, respectively. The inhibitory activity of tea extracts against α-amylase is related to hydrogen bond formation between the hydroxyl groups of the ligands and the catalytic residues of the binding site and to the formation of a conjugated π-system that stabilizes the interaction with the active site [36]. Galloylated catechins (EGCG and ECG) had higher binding affinities with α-amylase and appeared to be mainly responsible for α-amylase activity inhibition by binding to active site residues [37], accounting for the higher inhibition ratios of untreated tea extracts.

3.8.5. Effect of tea extract inhibition rate on pancreatic lipase activity

Tea polyphenols may decrease postprandial hyperglycemia by binding and precipitating some digestive enzymes, including pepsin, α-amylase, α-glucosidase, and lipase, through ionic and hydrogen bond formation with amino, hydroxyl, and carboxyl groups of proteins [38]. Lipase inhibition results are shown in Fig. 2e. In vitro, both tea extracts were effective inhibitors of pancreatic lipase. Their inhibitory effect was associated with their concentration and was different before and after tannase treatment. Extracts from treated tea leaves had an inhibition rate of 42.8% when tea extract concentration was 10,000 mg/L, whereas extracts from untreated tea leaves showed a similar trend but with a higher inhibition rate of 47.6%. Gallated catechins have stronger binding ability than ungallated catechins [39]. McDougall et al. [40] also reported that the number of galloyl groups also affect tannins' protein binding ability. EGCG's protein binding ability decreased because of the ester bond cleavage induced by the hydrolytic action of tannase. Thus, tea extracts from treated tea leaves will exhibit a lower lipase inhibition effect.

4. Conclusion

The results suggested that tannase-catalyzed hydrolysis of catechin gallates (EGCG and ECG) in inferior Tieguanyin oolong tea leaves increased the antioxidant activities of the resultant hydrolysates. These activities include scavenging of reducing capacity, DPPH radicals, and hydroxyl radicals. Enzymatic treatment of inferior Tieguanyin tea leaves also reduced tea cream formation and improved storage stability at lower storage temperatures. Untreated tea leaves with EGCG and ECG had higher inhibitory capacities against pancreatic α-amylase and lipase than leaves treated with tannase. Our findings may be useful in the application of tannase to enhance the quality and functionality of inferior Tieguanyin oolong tea leaves.

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