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Study on the Effects and Mechanisms of Action of Biological Enzymes on the Quality of Summer Rock Tea Extract

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Abstract: The gallated catechin content in Wuyi Rock Tea is higher in the summer, leading to a more bitter and less desirable taste. Here, tannase, tyrosinase, and laccase were used to treat summer Rougui Wuyi Rock Tea extracts. The effects of single and combined enzymes on the taste of the tea extracts and their mechanisms of action were analyzed. Compared with the no-enzyme-treated sample, the sensory score results showed that tannase was the most effective, increasing the bitterness and astringency scores by 113.9% and 255.3%, respectively. Among the combined enzyme treatments, the samples treated with tannase and tyrosinase yielded the best sensory scores, with bitterness and astringency scores increasing by 141.2% and 289.0%, respectively. Data obtained using an electronic tongue confirmed the role of these enzymes, showing that, in addition to bitterness and astringency, enzyme treatment also influenced the bitterness aftertaste and astringency aftertaste, as well as its sourness and sweetness. Further product analysis revealed that tannase hydrolyzes the ester bonds on the gallacyl groups in gallated catechins, converting them to non-gallated catechins, while tyrosinase and laccase oxidize the phenolic hydroxyl groups on catechins to form o-quinone, leading to the production of theaflavins and improved tea quality. This study presents an effective approach to improving the quality of summer tea using biological enzymes.

Keywords: Rougui Wuyi Rock tea; tannase; catechins; theaflavins; tea cream; electronic tongue

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1. Introduction

Wuyi Rock Tea is a high-quality oolong tea produced in Wuyi Mountain, Fujian Province, China. In recent years, it has been chosen by consumers because of its unique "rock flavor" [1]. Spring Rock tea is of the best quality, rich in nutrients and with a moderate content of tea polyphenols and amino acids, and a high content of aromatic linalool and linalool oxides. However, the content of tea polyphenols, such as catechins, as well as the bitterness of tea has increased significantly during the summer [2,3]. Therefore, reducing the bitterness of summer Rock Tea is of great significance for improving its taste quality.

Summer oolong tea contains higher levels of catechins, including (−)-epigallocatechingallate (EGCG), (−)-epicatechingallate (ECG), (−)-epigallocatechin (EGC), and (+)-catechin (C), among others. The bitterness of summer Wuyi Rock Tea is primarily attributed to gallated catechins, specifically EGCG and ECG [4]. In contrast, non-gallated catechins, such as EGC and C, are key contributors to the tea aftertaste. The color and brightness of tea are also key factors for evaluating its quality, and these attributes depend on the contents of theaflavins

(TFs) and thearubigins (TRs) [5]. Catechins such as EGCG, ECG, and EGC in fresh leaves can be oxidized to polyphenols, such as theaflavins and thearubigins, under the action of endogenous polyphenol oxidase [6]. Theaflavins and thearubigins improve human health by reducing the blood sugar levels, having antioxidant [7,8] and antibacterial properties [9], and regulating the blood lipid levels [10].

Biological enzymes have good functional characteristics, such as high specificity and high catalytic efficiency, and have attracted great attention due to their environment-friendly properties [11]. Tannase (EC 3.1.1.20, TAN), whose full name is tannin acyl hydrolase, can hydrolyze the ester bonds of tea compounds, such as gallic acid esters and hydrolysable tannins, leading to the production of gallic acid and a small amount of glucose [12]. Tannase has a crucial role in juice and beverage processing. Cavia-Saiz, M. et al. [13] found that treating grapefruit juice with tannase can effectively reduce its bitterness and improve its antioxidant properties. Chavez-Gonzalez, M. et al. [14] reported that tannase can reduce the amount of precipitate produced with calcium, magnesium, and other metal ions, as well as protein substances, due to the high tannin concentration in beer and beverages. Polyphenol oxidase also plays an important role in the degree of fermentation and oxidation in tea processing, significantly affecting the color of tea beverages [15,16]. Tyrosinase (EC 1.14.18.1, TYR) can oxidize the o-hydroxyl group of phenolic substances to o-diphenol, and then oxidize it to o-quinone. Laccase (EC 1.10.3.2, LAC), which also has polyphenol oxidase activity, leads to the formation of free radicals after oxidation, and quinone is formed after two such oxidation reactions occur. Laccase has broader substrate specificity than tyrosinase [17]. Yabuki, C. et al. found that tyrosinase from mushrooms can be effectively used for the synthesis of theaflavins [6]. Moreover, Li, W. et al. showed that a co-immobilization system incorporating laccase can enhance the development and industrial application of theaflavins [18].

The use of biological enzymes to improve tea quality has become a tea research hot spot. The content of gallated catechins in green tea infusions treated with tannase is reduced, demonstrating the effective ability of tannase to degrade precipitates [19]. Tyrosinase and laccase can promote the rapid consumption of catechins, thereby enabling the continuous production of theaflavins [20]. High-quality instant black tea with desired properties can also be produced using bioenzyme-assisted extraction technology [21]. However, systematic research on the synergistic effect of biological enzymes in tea is lacking. Therefore, here, tannase, tyrosinase, and laccase were selected as the research objects. Sensory evaluation and electronic tongue technology were employed to investigate the effects of single and complex enzyme treatments on the taste improvement of Wuyi Rock Tea extract. Moreover, the mechanisms of action of the three enzymes in the tea extract were thoroughly analyzed.

2. Materials and Methods

2.1. Materials and Chemicals

Wuyi Rock Tea was provided by H.K.I. (Fujian, China) Tea Company Limited, China. The fresh leaves were processed through the stages of withering, fixation, rolling, and baking. The tea samples used in this study were prepared in June 2023. Tannase (500 U/g) was purchased from Shanghai Maclin Biochemical Technology Co., Ltd. (Shanghai, China) Tyrosinase (1120 U/mg) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China) Laccase (120 U/g) was obtained from Merck Sigma Chemical Reagents Co., Ltd. (Darmstadt, Germany)

The catechin standard products (EGCG, ECG, C, and EGC), the Total Antioxidant Capacity (T-AOC) Assay Kit (ABTS), and the Total Antioxidant Capacity (T-AOC) Assay Kit (DPPH) were purchased from Shanghai Maclin Biochemical Technology Co., Ltd.

(Shanghai, China) The salicylic acid, the hydrogen peroxide, and the ferrous sulfate (AR) were purchased from Merck Sigma Chemical Reagents Co., Ltd. (Darmstadt, Germany) The ethyl acetate, the oxalic acid, and the sodium bicarbonate (AR) were purchased from Shanghai Titan Technology Co., Ltd. (Shanghai, China).

2.2. Preparation of the Tea Extracts and Incubation with the Biological Enzymes

The tea sample weighed 10 g and was extracted with 100 mL of pure water at 100 °C. Five minutes after the extraction, the mixture of tea leaves and water was filtered using medium-speed qualitative filter paper, and the obtained tea extract was kept warm in the water bath for use. The incubation process for the enzyme treatment of the tea extracts involved adding a specified amount of enzyme preparations to 100 mL of the extract, maintaining the mixture in a water bath at a specific temperature for a designated time, and then raising the temperature to 95 °C for 10 min to inactivate the enzymes. The process conditions were set as follows: temperature gradient: 30 °C, 40 °C, and 50 °C [fixed enzyme treatment time of 2 h, concentration of the enzymes added: tannase (4 U/mL) + tyrosinase (4 U/mL) + laccase (4 U/mL)]; time gradient: 1, 2, and 3 h [fixed enzyme treatment temperature of 40 °C, concentration of the enzymes added: tannase (4 U/mL) + tyrosinase (4 U/mL) + laccase (4 U/mL); enzyme addition gradient: tannase (TAN) (1.2, 2.4, and 3.6 U/mL); tyrosinase (TYR) (3, 4, and 5 U/mL); and laccase (LAC) (3, 4, and 5 U/mL)]. The combinations of enzymes tested were as follows: tannase 2.4 U/mL + tyrosinase 4 U/mL (TAN-TYR); tannase 2.4 U/mL + laccase 4 U/mL (TAN-LAC); tyrosinase 4 U/mL + laccase 4 U/mL (TYR-LAC); and tannase 2.4 U/mL + tyrosinase 4 U/mL + laccase 4 U/mL (TAN-TYR-LAC) (fixed enzyme treatment temperature of 40 °C and enzyme treatment time of 2 h). The action temperature, time, and concentration ranges for the three enzymes were established based on the preliminary experimental findings of the authors. The enzyme-treated tea extracts in this step were stored for subsequent use.

2.3. Sensory Evaluation

According to the GB/T 23776-2018 [22] tea sensory evaluation method, 10 professional reviewers, who possess more than 10 years of experience in sensory evaluation in the tea field, were selected for evaluation. The tea bitterness, astringency, aftertaste, and overall flavor were evaluated on a scale of 0–10. The sensory ratings were characterized as follows: bitterness [extremely bitter (1–2), very bitter (3–4), bitter (5–6), somewhat bitter (7–8), and not bitter (9–10)]; astringency [extremely astringent (1–2), very astringent (3–4), astringent (5–6), somewhat astringent (7–8), and not astringent (9–10)]; aftertaste [almost no aftertaste (1–2), slight aftertaste (3–4), average aftertaste (5–6), obvious aftertaste (7–8), and extremely strong aftertaste (9–10)]; overall flavor [very poor (1–2), poor (3–4), average (5–6), good (7–8), and excellent (9–10)] [23].

2.4. Electronic Tongue Analysis

The SA402B electronic tongue sensory intelligent analysis system (INSENT, Fukuoka, Japan) containing bitterness, astringency, sourness, saltness, umami, and sweetness sensors, was used, and the data were collected at 25 °C. Each sample was analyzed three times, and the results of the three measurements were averaged [24]. The measured value of the electronic tongue was positively correlated with the perceived intensity of the taste, and the higher the value was, the stronger the taste appeared to be.

2.5. Evaluation of Tea Cream Formation

The tea extracts of the control and enzyme treatment groups (10 mL) were placed in a centrifugal tube, kept at 4 °C for 24 h, and centrifuged at 4 °C (200× g) for 20 min. The supernatant was discarded and the precipitate was placed in an air drying oven for drying

at a constant temperature (100 °C) until a constant weight was achieved, after which the tea cream was collected and weighed [25].

2.6. Antioxidant Analysis

Salicylic acid reacts with hydroxyl radicals (·OH) in the reaction system to produce 2,3-dihydroxybenzoic acid, which has maximum absorption peak at 510 nm. The ·OH scavenging rate is calculated based on this absorbance [26]. The free radical scavenging rates of DPPH and ABTS were measured using a test kit. After being oxidized, ABTS was able to generate the blue-green cationic radical ABTS+, which exhibited a maximum absorption at 734 nm. The DPPH radical, which possessed a single electron, exhibited a purple color in its alcoholic solution and demonstrated strong absorption at 515 nm. In the presence of antioxidants, the DPPH radical was scavenged, resulting in a lighter colored solution. The change in absorbance at 515 nm was directly proportional to the degree of radical scavenging that had occurred. The antioxidant property was measured using the spectrophotometer (MAPADA, Shanghai, China).

2.7. Determination of the Gallic Acid and Catechin Levels Using High Performance Liquid Chromatography (HPLC)

The catechin level was determined using an HPLC system (Agilent 1260, Agilent Corporation, Santa Clara, CA, USA) equipped with an Agilent C18 (4.6 mm × 150 mm × 5 μm) column. The sample volume was 10 μL, the flow rate used was 1 mL/min, the detector used was a UV detector, the wavelength was set to 270 nm, the mobile phase A was a 1% acetic acid aqueous solution, and the mobile phase B was methanol. The initial composition of the mobile phase was 5% phase B. From 0 to 28 min, it linearly increased to 17% phase B. The mobile phase remained at 17% phase B until 38 min. Between 38 and 40 min, it linearly decreased back to 5% phase B. Finally, the composition maintained at 5% phase B until the end of the experiment at 45 min. The gallic acid and catechin contents were calculated according to the peak area [27,28].

2.8. The Contents of TFs and TRs Were Determined Using Spectrophotometry

Samples to be tested (3 mL) were soaked in 125 mL of boiling water for 10 min. Then, 25 mL of the test solution was pipetted and placed in a separation funnel, to which 25 mL of ethyl acetate was added. The two phases were separated after being oscillated for 5 min. The ethyl acetate extract (4 mL) was then diluted with 95% ethanol to a volume of 50 mL to obtain liquid A. Additionally, 25 mL of the test solution and 25 mL of n-butanol were collected and placed in the liquid separation funnel. After shaking for 3 min, 4 mL of the water layer was collected, and 4 mL of saturated oxalic acid solution (oxalic acid had a solubility of 9.5 g at 20 °C; based on this, the oxalic acid was dissolved in pure water) and 12 mL of water were added. This mixture was then diluted with 95% ethanol to a volume of 50 mL to obtain liquid B. Next, 15 mL of ethyl acetate extract and 15 mL of a 2.5% sodium bicarbonate solution were added to the liquid separation funnel. After shaking for 30 s, 8 mL of the upper liquid was collected and diluted with 95% ethanol to a volume of 50 mL to obtain liquid C. Lastly, 4 mL of the standby liquid from the first water layer was mixed with 4 mL of saturated oxalic acid and 12 mL of water, and then diluted with 95% ethanol to a volume of 50 mL to obtain liquid D. The absorbance of liquids A, B, C, and D were all measured at 380 nm. The TF content was calculated using the following formula [5]:

$$\mathrm{TFs}(\%) = A_c \times 2.25 \div (m \times \omega) \times 100\% \quad (1)$$

$$\mathrm{TRs}(\%) = (2A_a + 2A_d - A_c - 2A_d) \times 7.76 \div (m \times \omega) \times 100\% \quad (2)$$

where m and ω are the mass of sample and dry matter content of sample (%), respectively.

2.9. Statistical Analysis

Excel 2010 (Microsoft, Albuquerque, NM, USA) was used to calculate the mean and standard deviation values, SPSS Statistics 25.0 (IBM, San Francisco, CA, USA) was used to conduct a one-way analysis of variance, multiple comparisons (Duncan test, $p < 0.05$), and correlation analysis of the data, and Origin 2021 (OriginLab, Northampton, MA, USA) was used to draw radar and column charts. The orthogonal projections to latent structures-discriminant analysis (OPLS-DA) was performed using Simca 14.1 (Umetrics, Malmo, Sweden), and the molecular structure diagram was drawn using ChemDraw 22.0.0 (PerkinElmer, Waltham, MA, USA).

3. Results and Discussion

3.1. Effect of the Enzyme Treatment on the Sensory Quality of the Rock Tea Extract

3.1.1. Effects of the Enzyme Action Temperature and Time on the Sensory Properties of the Tea Extract

To explore the effects of enzyme reaction temperature and time on the sensory quality of the tea extracts, gradient experiments were conducted, and a review team was assembled to assign sensory scores to the tea extracts. The four indexes were scored on a scale from zero to ten, from worst to best, with higher bitterness and astringency scores indicating a better reduction in bitterness and astringency in the sample.

Combined enzymes (tannase 4 U/mL + tyrosinase 4 U/mL + laccase 4 U/mL) at 30 °C and 40 °C increased the bitterness score of the extract by 86.6% and 141.2%, and the astringency score by 144.2% and 288.4%, respectively, compared with those of the control group. The aftertaste increased by 10.5% and 34.8%, respectively (Figure 1a). Therefore, 40 °C was determined as the optimal temperature for enzyme action. Cao, Q.Q. et al. proposed that after the tannase treatment, autumn green tea has a sweeter aftertaste, with the bitterness and astringency intensities being reduced from 3.3 and 2.4 to 2.5 and 2.0, respectively, while the overall acceptance is improved [4]. In addition, when the enzyme reaction time was 2 h, the scores for bitterness, astringency, aftertaste, and overall flavor were optimal (Figure 1b), increasing by 143.9%, 288.4%, 37.4%, and 41.2%, respectively. Therefore, in the subsequent experiments, the conditions were set as follows: an enzyme reaction temperature of 40 °C and an enzyme reaction time of 2 h.

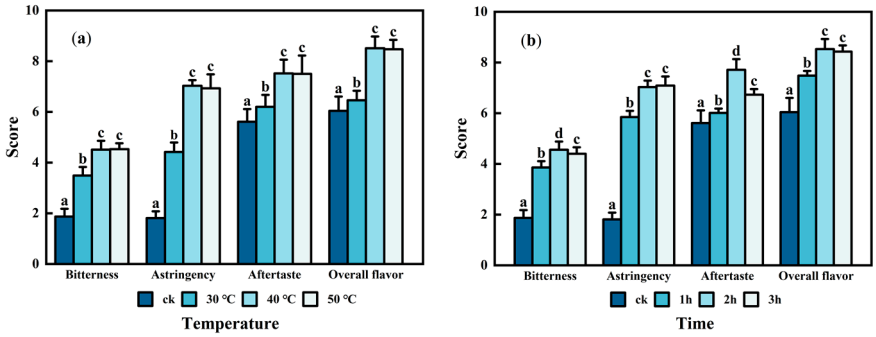

Figure 1. Sensory scores of the tea extracts for the different enzyme reaction temperatures (**a**) and times (**b**). ck represents the sample that has not been treated with the enzyme. The error bar represents the standard error of the mean, and by the LSD test, the average of different lowercase letters indicates a significant difference ($p < 0.05$).

3.1.2. Sensory Effects of the Enzyme Concentration on the Tea Extracts

Different concentration gradients were established for tannase, tyrosinase, and laccase to investigate the sensory effects of enzyme concentration on the tea extracts using sensory scores. For tannase, the best improvement effect in the sensory indexes of tea extract was

obtained at a concentration of 2.4 U/mL. At this concentration, the bitterness, astringency, and aftertaste increased by 20.5%, 19.7%, and 26.5%, respectively, compared to when the concentration was 1.2 U/mL (Figure 2a). Some researchers have noted that the content of gallated catechins is closely related to the bitterness and astringency of tea, while non-gallated catechins and gallic acid are the main components responsible for the aftertaste of tea. Under the action of tannase, the composition of catechins and the content of gallic acid change significantly under the action of tannase [12]. These findings might be related to changes in the taste of the tea extracts. When the concentration was 4 U/mL, tyrosinase and laccase showed similar trends in reducing the bitterness and astringency; however, tyrosinase had a slightly better effect on the overall flavor of tea extract, with the bitterness and astringency scores being 8.6% and 10.9% higher, respectively. The effects of the two polyphenol oxidases on the aftertaste differed; however, 4 U/mL was still determined to be the appropriate concentration for both enzymes (Figure 2b,c). Flavonol glycosides in tea are closely related to its bitterness and astringency, and polyphenol oxidase can enhance the oxidation of flavonol glycosides, thereby improving these qualities [16]. The 2.4, 4, and 4 U/mL concentrations of tannase, tyrosinase, and laccase, respectively, were used in subsequent experiments to facilitate in-depth analysis. From the perspective of the influence of a single enzyme in the tea extracts, tannase had a better effect on the overall sensory enhancement than tyrosinase and laccase ($p < 0.05$), with the overall flavor scores for the three enzymes being 7.02, 6.43, and 6.33, respectively (Figure 2).

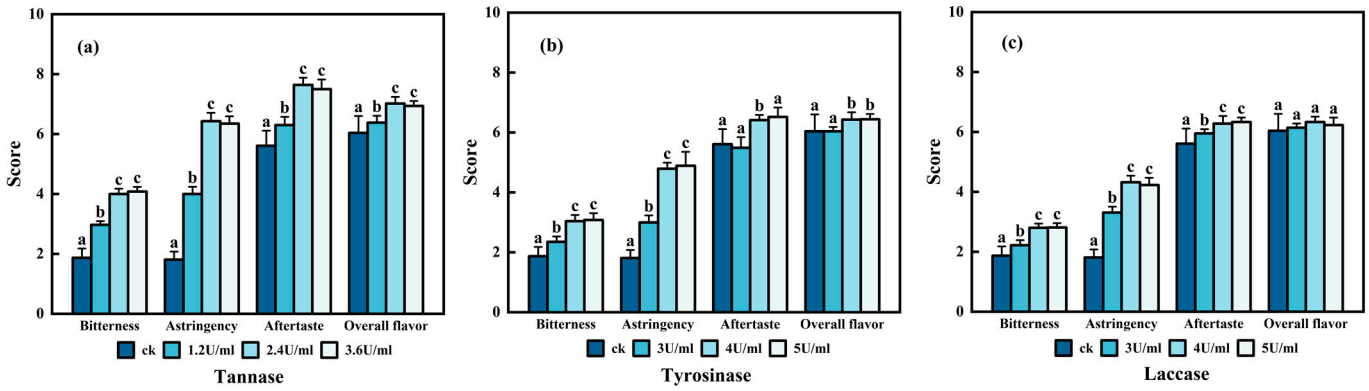

Figure 2. Sensory scores of tea extract for different enzyme treatment concentrations. a, b, and c represent tannase (**a**), tyrosinase (**b**), and laccase (**c**), respectively. ck represents the sample that has not been treated with the enzyme. The error bar represents the standard error of the mean, and by the LSD test, the average of different lowercase letters indicates a significant difference ($p < 0.05$).

3.1.3. Sensory Effects of the Synergistic Action of Enzymes on Tea Extract

Tannase, tyrosinase, and laccase have a notable effect on improving the taste of tea, prompting further exploration of the combined action of these three enzymes. From the perspective of sensory scores for complex enzymes, the TAN-TYR and TAN-TYR-LAC combinations exhibited the best taste improvement, although no significant difference between the two in terms of improvement level ($p > 0.05$) was observed. The overall flavor score increased by 42.7% and 40.7% compared with ck, respectively, and the aftertaste score increased from 5.61 to 7.52 and 7.6. The overall flavor score of TAN-TYR was also 22.8% higher than that of the most effective single enzyme (TAN). The improvement effect of TAN-LAC on the tea extract was slightly inferior to that of TAN-TYR. Compared with the best enzyme combination, TYR-LAC showed no significant improvement in the taste of the tea extract ($p > 0.05$) (Figure 3). Combining the effects of single and combined enzymes on the extract revealed that the enzyme treatment significantly contributes to taste improvement, not only in bitterness and astringency but also in aftertaste. This phenomenon may be

closely related to changes in the amount of phenolic substances, such as gallated catechins, non-gallated catechins, theaflavins, and thearubigins [29], in tea extracts [4]. Therefore, it is speculated that the increase in aftertaste may result from changes in the gallic acid content. The addition of tannase and polyphenol oxidase increases the content of gallic acid and non-gallated catechins, which positively affect the soft taste and produce a pleasant aftertaste [11].

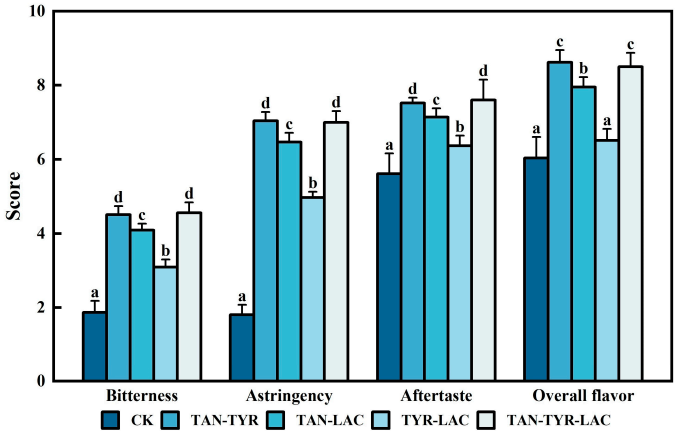

Figure 3. Sensory scores of the tea extracts for the different enzyme compound methods used. ck indicates the sample that has not been treated with the enzyme; TAN-TYR indicates tannase 2.4 U/mL + tyrosinase 4 U/mL; TAN-LAC indicates tannase 2.4 U/mL + laccase 4 U/mL; TYR-LAC indicates tyrosinase 4 U/mL + laccase 4 U/mL. TAN-TYR-LAC indicates tannase 2.4 U/mL + tyrosinase 4 U/mL + laccase 4 U/mL. The error bar represents the standard error of the mean, and by the LSD test, the average of different lowercase letters indicates a significant difference ($p < 0.05$).

3.2. Electronic Tongue Determination and Analysis of the Rock Tea Extract After the Enzyme Treatment

The electronic tongue is a new analytical method based on a multi-sensor array, characterized by its high objectivity and interactive sensitivity. [30]. By simulating the human taste mechanism, it can identify samples that are difficult for the human senses to distinguish, thereby obtaining more objective and comprehensive sample information and having improved efficiency and accuracy. It is widely used in various fields of the food industry, such as tea, wine, and milk production [31]. Here, bitterness, astringency, sourness, saltness, umami, and sweetness sensors were used to collect data.

Regarding the results obtained from the electronic tongue detection of the tea extracts, the properties of the enzyme-treated samples were significantly different ($p < 0.05$). When the three enzymes were each individually applied to the tea extract, tannase had the greatest effect on the taste of the tea extract, with the sourness and sweetness increasing by 31.39% and 10.02%, respectively, while the bitterness, astringency, bitterness aftertaste, and astringency aftertaste decreased by 29.25%, 37.63%, 37.31%, and 62.53%, respectively. The umami and richness were also reduced by 23.56% and 25.71%, respectively. Tyrosinase and laccase had similar effects on the taste of the tea extract, but their impact was much lower than that of tannase (Figure 4a). Among the samples treated with complex enzymes, the improvement effects of TAN-TYR, TAN-LAC, and TAN-TYR-LAC on the tea extract taste tended to be consistent, with the best effect being observed for TAN-TYR. This enzyme combination increased the sourness and sweetness by 34.70% and 7.72%, respectively. The bitterness, astringency, bitterness aftertaste, and astringency aftertaste decreased by 35.05%, 48.21%, 51.54%, and 69.92%, respectively. Umami and richness decreased by 21.94% and 26.67%, respectively. The effect of TAN-LAC and TAN-TYR-LAC on the reduction in bitterness and astringency was slightly lower than that of TAN-TYR (Figure 4b), likely due

to the action of laccase. The precursor oxidation products of thearubigins are theaflavins, theacitrins, and theanaphtoquinones, among others. When laccase is added to the tea extracts, due to its broader substrate specificity, the content of thearubigins is likely to increase; moreover, thearubigins are also one of the main contributors to the bitterness of tea [17]. The increase in sourness and sweetness is probably related to a change in the contents of gallic acid and glucose. When tannase acts on the ester bonds to break them, gallic acid and a small amount of glucose are produced [32]. The results of the electronic tongue test were generally consistent with the sensory score ones.

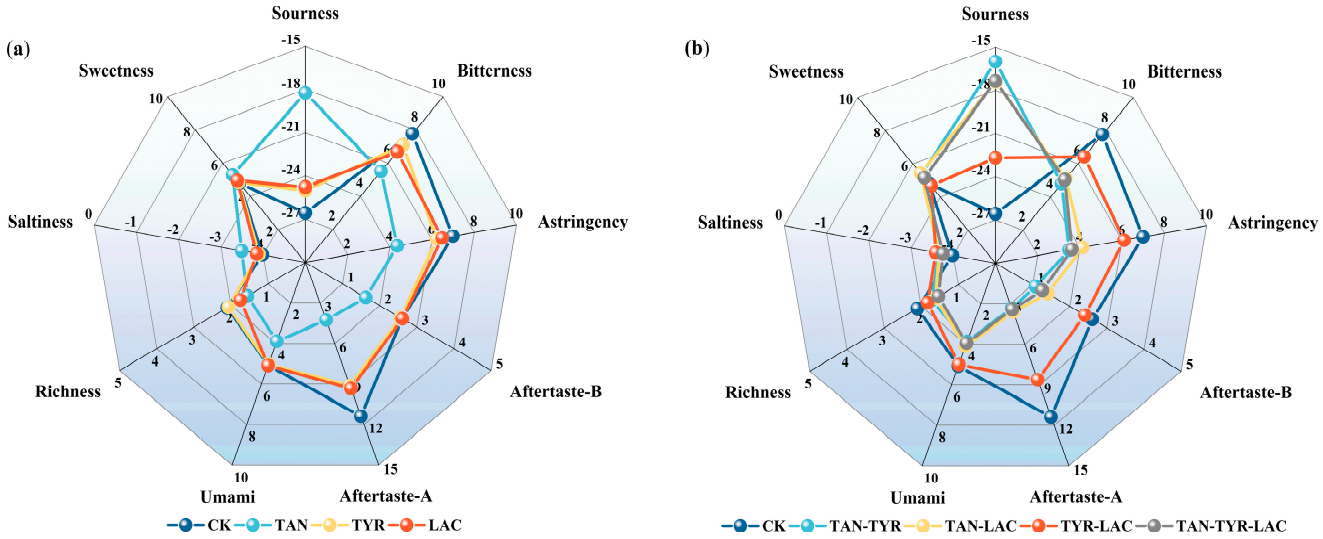

Figure 4. Radar map of the electronic tongue results obtained for the action of single enzymes (**a**) and combined enzymes (**b**) on the tea extracts. Aftertaste-B represented the bitterness aftertaste; Aftertaste-A represented the astringency aftertaste.

3.3. Effects of Enzyme Treatment on the Formation of Tea Cream in Rock Tea Extract

"Cold and muddy" refers to the phenomenon where turbidity is created in the tea extract and precipitates are formed upon cooling. It is an important quality issue related to liquid tea extracts [33]. This turbidity and precipitation, known as tea cream, is primarily formed by the combination of catechins and tea polyphenols with proteins [34,35]. For liquid tea extracts, eliminating the "cold and muddy" phenomenon is crucial to improving the taste of tea extracts.

The status of tea extracts stored at room temperature and at 4 °C for 24 h are shown in Figure 5. The weight of tea cream in all enzyme-treated samples was significantly lower than that in the control group ($p < 0.001$). When comparing the weight of tea cream obtained after the treatment with the three single enzymes (TAN, TYR, and LAC), tannase had the best effect on reducing precipitation, with a tea cream weight of 2.21 mg/L, which was 91.46% lower than that obtained in the control group (Figure 6). Jie QiongWang et al. also found that tannase can reduce the precipitation amount in green tea concentrate by 74.63% and improve the storage taste of green tea concentrate [19]. According to the results obtained for the combined enzymes, TAN-TYR-LAC showed the greatest decrease in tea cream weight (0.85 mg/L), corresponding to a 96.71% reduction compared with that of the control group. This was followed by TAN-TYR and TAN-LAC, which reduced the tea cream weight by 93.39% and 84.81%, respectively, compared with that of the control group. Among the combination enzymes, TYR-LAC led to the smallest tea cream weight reduction, with a decrease of only 60.11% compared with that of the control group (Figure 6). Gallated catechins, such as EGCG and ECG, contain more galloyl and B-cyclohydroxyphenyl groups, which easily bind to caffeine and other compounds, making them more likely to form

precipitates than non-gallated catechins, such as EGC and C [19]. By reacting with tannase, tyrosinase, and laccase in tea extracts, gallated catechins are likely transformed into non-gallated catechins, thus weakening the binding ability of proteins and caffeine in the tea extracts containing gallated catechins [25]. Consequently, the tea cream weight is reduced. This is likely the reason why enzyme treatment positively affects the clarity of tea extract and the formation of tea cream.

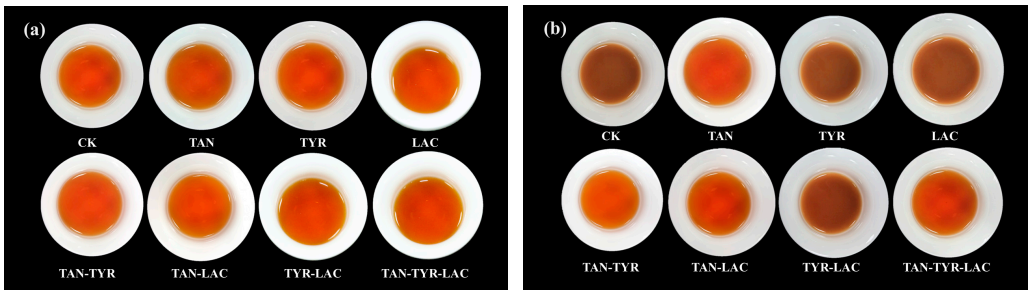

Figure 5. Pictures showing the tea extracts stored at room temperature (**a**) and 4 °C for 24 h (**b**).

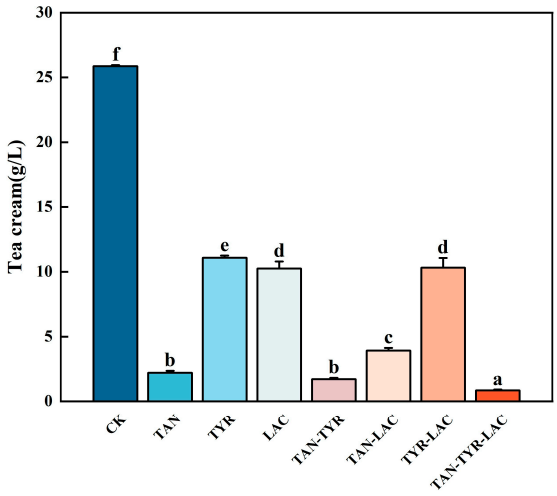

Figure 6. Weight of the tea cream in the enzyme-treated tea extracts. The error bar represents the standard error of the mean, and by the LSD test, the average of different lowercase letters indicates a significant difference ($p < 0.05$).

3.4. Effects of the Enzyme Treatments on the Antioxidant Properties of Rock Tea Extract

Tea polyphenols are natural substances with antioxidant properties. The presence of tea polyphenols gives tea excellent physiological properties, such as antibacterial, anti-aging, and anti-cancer properties [36]. Changes in the contents of phenolic substances in tea change its antioxidant properties.

DPPH is commonly used as a substrate to evaluate the free radical scavenging ability of antioxidants. Except for TAN-TYR-LAC, the free radical scavenging ability of DPPH was significantly higher in all samples than that of the control group ($p < 0.05$). The highest free radical scavenging rates of DPPH were observed for TAN and TAN-TYR, which were higher than that of the control group by 23.34% and 21.87%, respectively (Figure 7a). Regarding the free radical scavenging rates of ABTS, as shown in Figure 7b, those obtained for TAN, TAN-TYR, TAN-LAC, and TAN-TYR-LAC were significantly higher than those of the control group ($p < 0.05$), with TAN showing the highest value. Although ·OH has a short survival time in the system, its activity is extremely high. When substances with the ability to remove ·OH are added to the system to compete with salicylic acid, the clearance rate of ·OH can be determined by a reduction in the content of colored substances [37]. The

clearance rate of ·OH in all enzyme-treated samples was significantly higher than that in the control group, with the highest values being obtained for TAN-TYR-LAC, followed by TAN-LAC, TAN, TAN-TYR, and TYR-LAC (Figure 7c). In summary, TAN, TAN-TYR, and TAN-LAC led to higher antioxidant levels. Relevant studies suggest a good correlation between the content of gallated catechins and non-gallated catechins and the antioxidant activity [38]. The antioxidant activity of catechins in tea is ranked in the following order: EGC > EGCG > EC > ECG [39]. Other studies also indicate that tannase is an important enzyme for enhancing the antioxidant properties of tea [40]. Therefore, it is speculated that the improvement in antioxidant activity is due to the effects of tannase and polyphenol oxidase on gallated catechins, leading to an increase in the EGC content of non-gallated catechins and the concentration of gallic acid, thereby improving the antioxidant activity of tea extract.

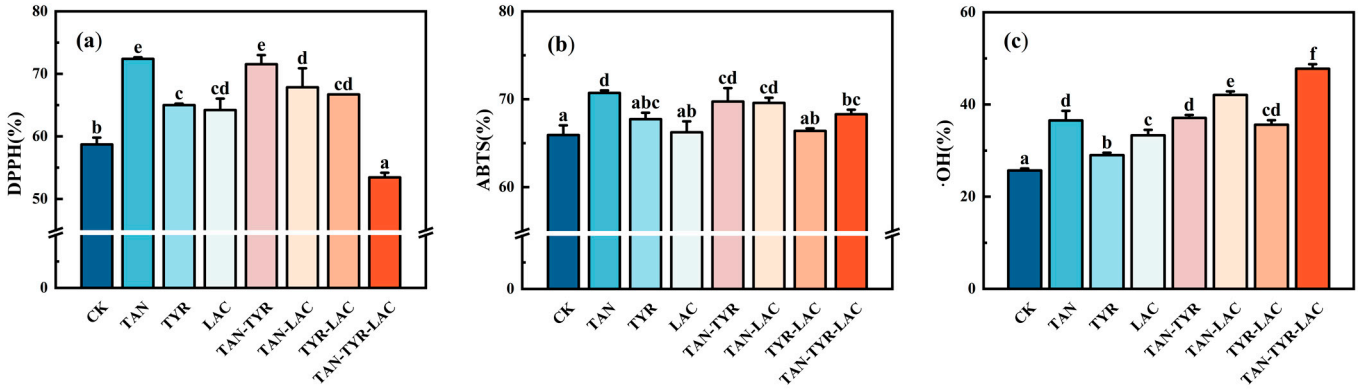

Figure 7. Free radical scavenging rate of DPPH (**a**), ABTS (**b**), and (**c**) ·OH. The error bar represents the standard error of the mean, and by the LSD test, the average of different lowercase letters indicates a significant difference ($p < 0.05$).

3.5. Effects of Enzyme Treatments on the Levels of Gallic Acid and Catechins in Tea Extract

Catechins, including gallated and non-gallated catechins, are the most abundant polyphenols in tea, which play a vital role in the taste and health benefits of tea. EGCG and ECG are the main contributors to bitterness, while non-gallated catechins and gallic acid provide a refreshing aftertaste [41]. Therefore, the catechin content of the tea extract after enzyme treatment was further analyzed.

Based on the literature, the general pathways of action of tannase, tyrosinase, and laccase on four catechins (EGCG, ECG, EGC, and C) were plotted (Figure 8). Tannase can act on the galloyl groups of EGCG and ECG, breaking the ester bonds and producing EGC, C, and gallic acid [5] (Figure 8a). According to the catechin level results, the contents of the gallated catechins EGCG and ECG in TAN, TAN-TYR, TAN-LAC, and TAN-TYR-LAC treated tea extracts all decreased significantly, with the largest decreases being observed for EGCG and ECG, which dropped by 81.93% and 94.62%, respectively (Figure 9b). For these four samples, the content of the non-gallated catechin EGC significantly increased, with TAN showing the highest increase, which was 3.15 times that of the untreated group (Figure 9c). Additionally, the content of gallic acid in TAN was significantly increased, being 9.31 times that of the untreated group (Figure 9a). Catechins contain multiple phenolic hydroxyl groups in their molecular structure; moreover, polyphenol oxidase is believed to contribute to the decrease in the content of gallated catechins during oxidation [16]. Both tyrosinase and laccase can convert catechins into dimers (such as theaflavins), trimers, and other substances, which enhance the antioxidant properties and health benefits of tea extracts [18]. Tyrosinase acts only on the dihydroxyl B-ring on ECG and C, while laccase has broader substrate specificity. In addition to the dihydroxyl B-ring on ECG and C, laccase

can also act on the trihydroxy B-ring and the gallic acid groups on the trihydroxy C-ring of EGC and EGCG [17] (Figure 8b). As shown in Figure 9b, the TYR, LAC, and TYR-LAC also reduced the EGCG and ECG contents to varying degrees, while increasing the concentration of gallic acid. Therefore, tyrosinase and laccase can also reduce the content of gallated catechins; however, their ability to convert gallated catechins is relatively lower than that of tannase.

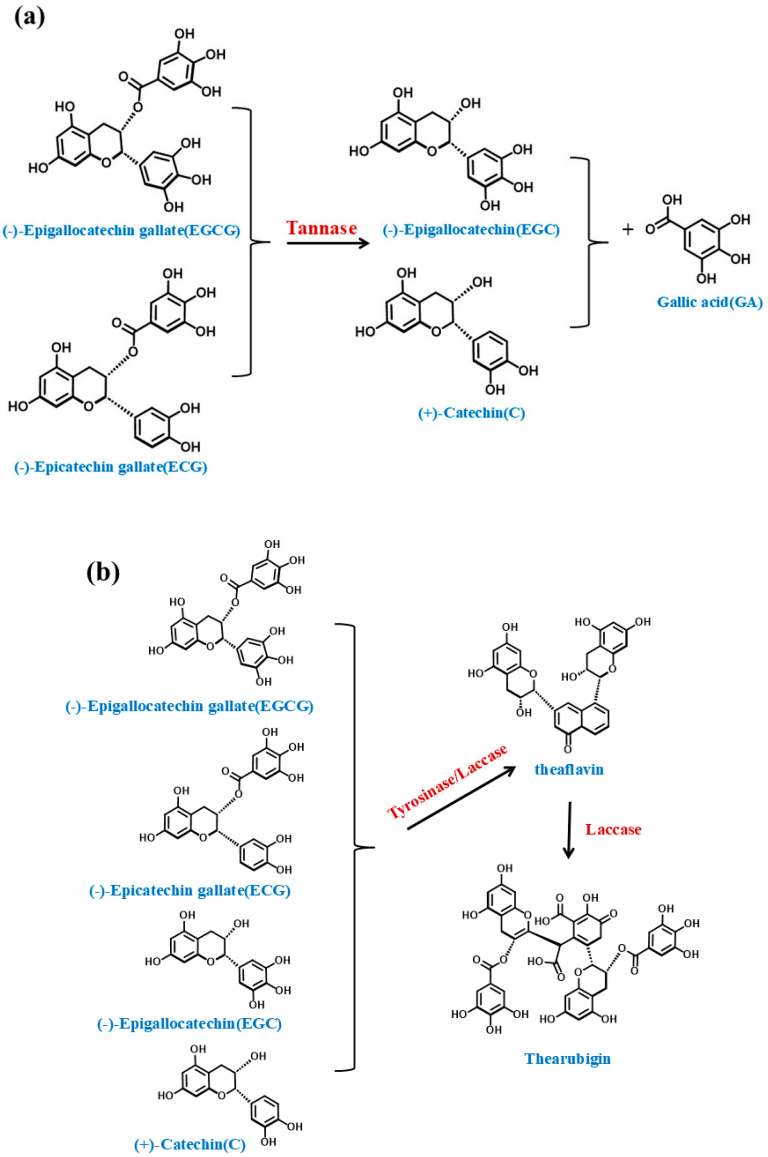

Figure 8. Pathway of action of tannase (**a**) and tyrosinase and laccase (**b**) on catechins [5,17,21].

In summary, EGCG was significantly positively correlated with ECG (r = 0.983, $p < 0.001$) and was significantly negatively correlated with gallic acid (GA) (r = −0.974, $p < 0.001$) and EGC (r = −0.928, $p < 0.001$). This result further verifies the gradual transformation of gallated catechins to non-gallated catechins during the reaction with the enzyme, accompanied by an increase in the gallic acid content. This fully explains the series of changes in the taste of the tea extracts after the enzyme treatments. The sensory score indicated that TAN-TYR had the best taste improvement effect, with the overall flavor score increasing by about 40%. The contents of EGCG and ECG, which reflect the bitterness of tea, were the lowest in the TAN-TYR-treated extracts, being only 157.03 and 6.5 mg/kg, respectively (Figure 9b). The electronic tongue results showed that the bitterness, astringency,

bitterness aftertaste, astringency aftertaste, and sourness of all samples were significantly affected, with TAN-TYR leading to the most significant bitterness reduction. Additionally, the sourness increased by 34.70%, which can be explained by the increase in the gallic acid content, which was 6.42 times higher than that of the control group (Figure 9a). Regarding the quality of tea cream, a significant positive correlation was found with the EGCG (r = 0.891, $p < 0.001$) and ECG (r = 0.954, $p < 0.001$) contents. This indicates that as gallated catechins are converted to non-gallated catechins, their ability to bind to other substances such as protein and caffeine weakens, resulting in reduced tea cream quality. TAN, TAN-TYR, TAN-LAC, and TAN-TYR-LAC showed stronger antioxidant properties, likely due to the significant increase in EGC content (Figure 9c). EGC is the catechin with the strongest antioxidant capacity in tea. Therefore, it was the effect of enzyme treatment on the contents of gallated and non-gallated catechin that changed the taste and quality of tea extracts.

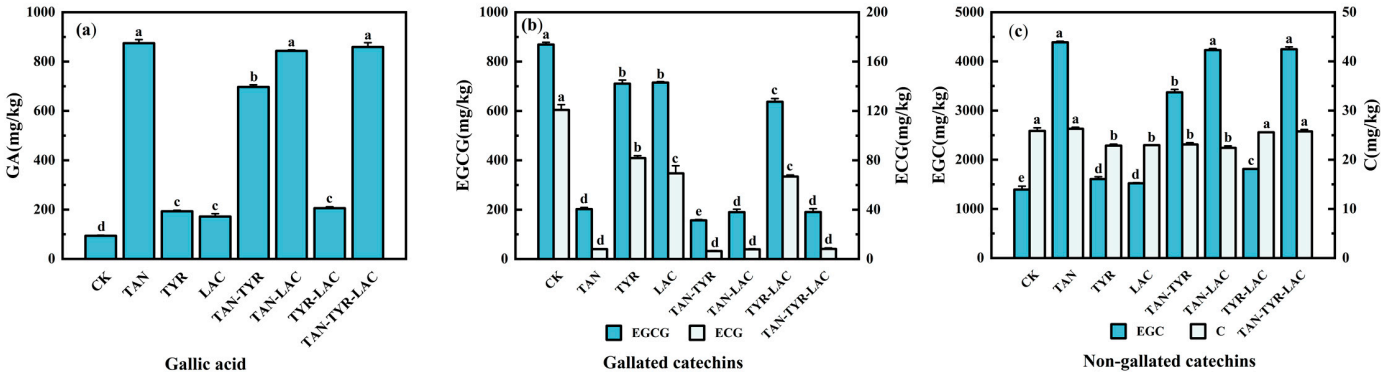

Figure 9. Contents of gallic acid (**a**), gallated catechins (**b**), and non-gallated catechins (**c**) in tea extract. GA: gallic acid; EGCG: (−)-epigallocatechin gallate; ECG: (−)-epicatechin gallate; EGC: (−)-epigallocatechin; and C: (+)-catechin. The error bar represents the standard error of the mean, and by the LSD test, the average of different lowercase letters indicates a significant difference ($p < 0.05$).

3.6. Effect of the Enzyme Treatments on TFs and TRs in Tea Extracts

Tyrosinase and laccase have enzymatic oxidation functions and can catalyze catechins to form theaflavins, thearubigins, theabrownines (TBs), and other compounds [6], which positively impact the aroma and functional quality of tea. As shown in Figure 10, the theaflavin content of TYR, TYR-LAC, and TAN-TYR-LAC increased by 55.56%, 66.67%, and 51.11%, respectively, compared to the control group, while the samples with the highest theaflavin content were those treated with LAC and TYR-LAC, having a theaflavin content that was 3.29 and 3.57 times higher than that of the control group. EGCG, ECG, EGC, and C can be oxidized to theaflavins by tyrosinase and laccase. Tyrosinase produced theaflavins at higher levels than laccase (Figure 10). Pyrogallol- and catechol-type catechins are conjugated by ECG and EGC to produce theaflavin 3′-O-gallate (TF2B), and by EGCG and ECG to produce theaflavin 3,3′-di-O-gallate (TF3) [21]. While theaflavins, a good substrate for laccase, continue to be oxidized into thearubigins and other catechin polymers, tyrosinase itself cannot oxidize theaflavins. Laccase continues to participate in the subsequent reactions, presumably because theaflavins no longer have an O-dihydroxyl B-ring [17] (Figure 8b). Thearubigins are considered ill-defined polyphenol components that contain heterogeneous polymers; therefore, their structure remains largely unclear [6]. The theaflavin content resulting from the TAN-TYR and TAN-TYR-LAC treatments was lower than that resulting from the TYR treatment, and the thearubigin content resulting from the TAN-LAC and TAN-TYR-LAC treatments was lower than that resulting from the LAC treatment (Figure 10). Therefore, it is speculated that tannase may have an inhibitory effect on laccase. This inhibition may be reflected in the inhibition of the activity of laccase

by tannase, as well as in the competitive inhibition of the substrate, because tannase has an excellent ability to convert gallated catechins. At the same time, phenolic substances such as EGC and EGCG may also inhibit the activity of tyrosinase, while EC, C, and their oxidation products may also affect the activity of laccase [17]. Therefore, the single addition of tyrosinase or laccase, or a combination of both enzymes, seem to have a better ability to increase the theaflavin and thearubigin contents than adding a combination of tannase and another enzyme.

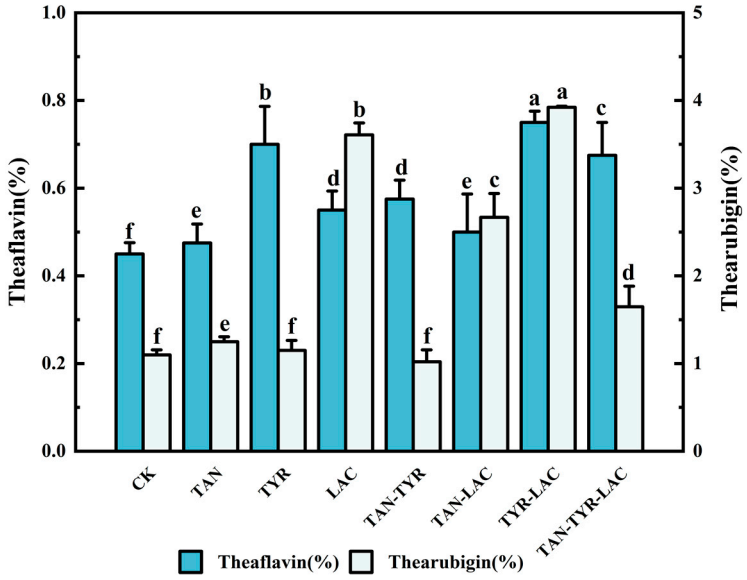

Figure 10. Content of theaflavins and thearubigins in the tea extract. The error bar represents the standard error of the mean, and by the LSD test, the average of different lowercase letters indicates a significant difference ($p < 0.05$).

3.7. OPLS-DA Analysis

To better analyze the differences in the effects of tannase, tyrosinase, and laccase on Rock Tea extract, the OPLS-DA method was utilized in this study. Based on the contents of GA, EGCG, ECG, EGC, C, theaflavins, and thearubigins in the extract, the difference in the contribution rate of the three enzyme treatments to the extract components was analyzed. To verify the accuracy of the established OPLS-DA model, 200 permutation tests were performed for internal validation (Figure 11a). The permutation test results showed that $R^2 = (0.0, 0.173)$ and $Q^2 = (0.0, -1)$, where $R^2 < 0.4$ and $Q^2 < 0.05$. The slope was positive, and the regression line of Q^2 intersected the vertical axis below the origin, indicating no overfitting of the model, making the results reliable [42]. The model scores chart, shown in Figure 11b, had values of $R^2x = 0.999$, $R^2y = 0.989$, and $Q^2 = 0.977$, indicating a good fit to the model [1]. In the evaluation group, significant separation was observed between the different groups, indicating significant changes between the enzyme-treated samples and the control group. The changes caused by tyrosinase and laccase were similar; however, a considerable difference between tannase and the other two enzymes was observed. In the OPLS-DA loading diagram, tannase was the furthest away from and in the negative direction of EGCG and ECG, indicating that tannase effectively reduced the levels of these two gallated catechins. Moreover, the theaflavin and thearubigin contents were more closely related to those of tyrosinase and laccase (Figure 11c), likely because these two polyphenol oxidases significantly influence the formation of theaflavins and thearubigins.

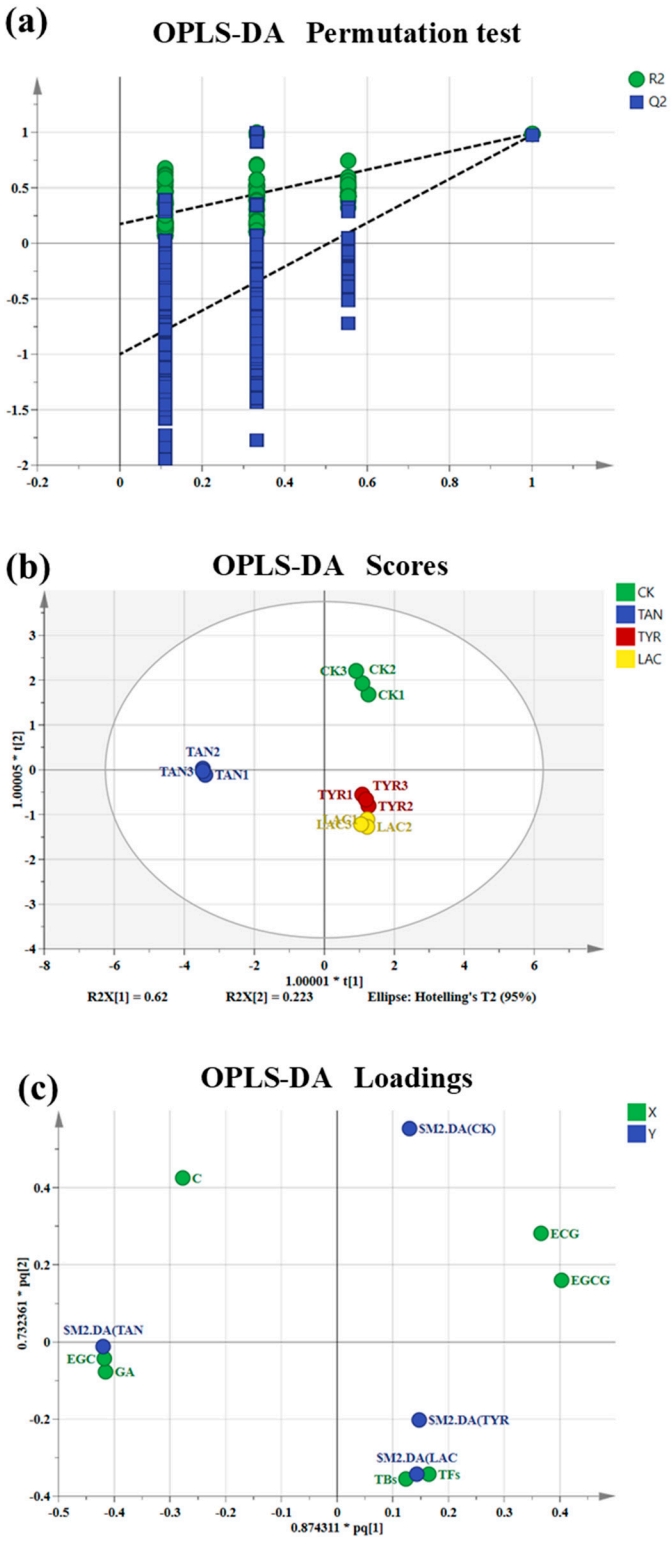

Figure 11. Results of the multivariate statistical analysis of the enzyme treatments. OPLS-DA permutation test diagram ($R^2 = 0.173$, $Q^2 = -1$) (**a**); the orthogonal projections to latent structures-discriminant analysis results ($R^2x = 0.999$, $R^2y = 0.989$, $Q^2 = 0.977$) (**b**); and OPLS-DA loading diagram (**c**).

4. Conclusions

The tannase, tyrosinase, and laccase treatments can significantly improve the appearance, sensory, and functional quality of tea extracts. The sensory evaluation results showed

that the taste of the tea extracts was significantly improved after the enzyme treatment for 2 h at 40 °C. Among the single enzymes, tannase had the best effect on the sensory indexes. Among the combined enzymes, TAN-TYR led to the best sensory score. Product analysis showed that tannase could reduce the content of gallated catechins, thereby reducing the bitterness of the tea extracts by hydrolyzing the ester bonds. Moreover, tyrosinase and laccase could promote the oxidation of catechins to theaflavins, improving the tea quality. Theaflavins, as a substrate, are continuously oxidized by laccase to thearubigins. In addition, enzyme treatment can also reduce the weight of tea cream formed in the tea extract, and improve its antioxidant activity.

This study provided a theoretical basis and practical guidance for improving the quality of summer tea by using biological enzymes, and offered a new idea for the full utilization of summer tea resources.

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Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Consent to Participate Informed consent was obtained from all individual participants included in this study.

Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest: We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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