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Research Article

Effects of Variety, Season, and Region on Theaflavins Content of Fermented Chinese Congou Black Tea

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The effects of variety, region, and season on the theaflavins content of fermented Congou black tea were determined. Fresh tea leaves from 5 varieties, 3 tea-growing regions, and 3 harvesting seasons were treated by withering, rolling, fermenting, and drying. Contents of theaflavins, polyphenol oxidase (PPO), peroxidase (POD), and individual catechins in each treatment process were determined. The results showed that the varieties of Yinghong 9 and Hongyan 12 produced high concentrations of theaflavin, as much as 0.5% and 0.6%, respectively. The production capacity of theaflavin in different regions had the trend of Yingde > Changsha > Hangzhou. Fresh leaves plucked in spring season produced the strongest theaflavins. In tea leaves, high ratio of PPO to POD activity and high contents of certain individual catechins lead to high yield of theaflavin production during the whole process.

1. Introduction

Tea is the second most consumed beverage around the world after water. The annual consumption of tea may reach about 2.5 million metric tons (processed leaves), 75% of which is black tea [1]. Congou black tea is a unique variety of Chinese black tea, which is made with careful skill to produce thin, tight strips without breaking the leaves [2]. The tea processing includes withering, rolling, fermenting, and drying. Withering enables the moderate physical and chemical changes of internal substances and emits part of the water content. Rolling appropriately damages the leaf tissue and promotes various material changes. Fermentation as a key step that can enhance the activation level of enzymes, promote the oxidative condensation of polyphenols, and produce a strong fragrance, thus forming the special desirable color and flavor of black tea ready for drying and packaging. The final drying will assure that the leaves will produce the expected dark color and strong fragrance when "brewed" with boiling water [2, 3].

The content of theaflavins is the most important evaluation index of the quality of Congou black tea. The theaflavins are compounds with a benzophenone structure. They are generated through oxidative condensation of polyphenols (catechins) affected by enzyme sources and damp-heat environment in the fermentation process of the leaves. The theaflavins are important components affecting the freshness degree and taste intensity of the black tea beverage. Additionally, the theaflavins have health-protecting properties such as cancer prevention, antisepsis, virus prevention, and oxidation resistance [4, 5]. Therefore, the content of theaflavins greatly affects the quality of the black tea.

The critical factors affecting the generation of theaflavins are the contents of reaction substrates (catechins) and enzymes (polyphenol oxidase and peroxidase) of fresh tea leaves. These factors also affect the sensory quality of the final tea beverage [6]. Research studies have found that black tea processed with fresh tea leaves of different tea leaf sources significantly influences the tea quality [7, 8]. This may be due to the diverse contents of catechins and enzymes in different tea leaves. China is a traditional big black tea-producing and -exporting country with rich varieties of tea plants and large tea-growing regions. Many traditional tea-making workshops make tea by experience; therefore, the qualities of Congou black tea are significantly different.

The objectives of this study are (1) to investigate the effects of variety, region, and season on theaflavins content of Congou black tea and (2) to determine the influences of the enzyme activity and catechins content on theaflavins content of black tea during tea processing.

2. Materials and Methods

2.1. Plucking of Leaves. Fresh leaves from 5 plant varieties: Yinghong 9 and Hongyan 12 (Tea Research Institute, Guangdong Academy of Agricultural Science), Chuyeqi (Hunan Tea Research Institute), Fuding Dabai, and Longjing 43 (Hangzhou Academy of Agricultural Science) with the tenderness of initial spread of one bud and two leaves were plucked on April 25, 27, and 29, 2015.

Fresh leaves from 3 regions: a variety of Fuding Dabai, with the tenderness of initial spread of one bud and two leaves were, respectively, plucked from Tea Research Institute, Guangdong Academy of Agricultural Science (Yingde, southern China), Hunan Academy of Agricultural Science (Changsha, southwest China), and Hangzhou Academy of Agricultural Science (Hangzhou, eastern China) on September 24, 26, and 28, 2015.

Fresh leaves from different seasons: a variety of Yinghong 9, with the tenderness of initial spread of one bud and two leaves, were plucked from Tea Research Institute, Guangdong Academy of Agricultural Science on April 25, July 11, and September 24, 2015, respectively.

2.2. Tea Processing

2.2.1. Withering. Withering was carried out in an artificial climate chest (PRX-450D, Saifu Experimental Apparatus Technology Ltd., Ningbo, China). The withering parameters were set as temperature of 28°C, illumination intensity of 6000 lx, and relative humidity of 70%. A 5 kg of fresh leaves of each variety was provided for processing; they were evenly mixed and spread on a screen with the thickness of 5 cm and then placed in the artificial climate chest for withering.

2.2.2. Rolling. Rolling would be started when the moisture content of fresh leaves reaches 62%–64% measured by infrared moisture meter (MA-150C, Sartorius Stedim Biotech GmbH, Germany). A 2.5 kg of fresh leaves was rolled by the 6CR-25 Rolling Machine (Sunyoung Machinery Ltd., Zhejiang, China) in the following order: nonload rolling (20 min) ⟶ light rolling (10 min) ⟶ heavy rolling (10 min) ⟶ light rolling (10 min) ⟶ heavy rolling (10 min) ⟶ light rolling (10 min) ⟶ deblocking, which would last for 70 min in total.

2.2.3. Fermenting. Fermenting was carried out in an artificial climate chest at the temperature of 28°C and relative humidity of 95% (covered with a damp cloth for regulation); the fermenting period was set as 4 h.

2.2.4. Drying. After initial roasting of 15 min at 120°C in the aroma-enhancing machine (JY-6CHZ-7B, Jiayou Ltd., Fujian, China), the leaves were properly spread out for cooling and then roasted to full dryness at 85°C for about 20 min.

2.2.5. Sampling. During withering in every 3 h and fermenting in every 1 h, 10 g of leaves was collected into a liquid nitrogen container to be used to detect the polyphenol oxidase and peroxidase activity; 60 g of leaves was cryodryed to be used to detect the conventional biochemical components.

2.3. Chemical Analysis

2.3.1. PPO and POD Activities. The activities of PPO and POD in the fermenting process were measured as described by Hua et al. [9].

2.3.2. Analysis of Catechins by HPLC. The HPLC analyses were carried out using the method described by Hua et al. [10]. A 3 g of tea sample was placed in an evaluation cup and added 150 mL of boiling water; after digestion for 5 min, the cooled aqueous extract was filtered with the 0.22-μm aqueous microfiltration membrane to the liquid vial for determination of individual catechins. HPLC (A1100, Agilent Technologies Co. Ltd., USA) with VWD detector; chromatographic column: WAT054275-C18 column of 5 μm and 4.6 mm × 250 mm; mobile phase A was 2% acetic acid, and mobile phase B was acetonitrile, with the flow velocity of 1.0 mL/min; test wavelength: 280 nm, column temperature: 40°C, and sample size: 10 μl; gradient elution: mobile phase B subjected to the linear variation 15% to 25% between 16 min and 25 min, maintained at 25% between 25 min and 25.5 min, subjected to the linear variation from 25% to 6.5% between 25.5 min and 30 min, and maintained at 6.5% between 30 min and 35 min.

2.3.3. Theaflavin, Thearubigin, and Theabrownin Determination. Tea-pigment separation and extraction: A 3 g of tea sample was placed in an evaluation cup, and 150 mL of boiling water was added. After digestion for 5 min, the aqueous extract was filtered. 30 mL of tea-cooled beverage was put into a separating funnel of 60 mL, and then 30 mL of ethyl acetate was added. It was vibrated for 5 min and placed still for layering; later, the water layer (the lower layer) was discharged, and the ethyl acetate layer (the upper layer) was poured out. 2 mL of the upper layer was absorbed, and 25 mL of 95% ethyl alcohol was added to get solution A of 25 mL; 2 mL of the lower layer was absorbed, and 2 mL of saturated oxalic acid solution and 6 mL of distilled water were added, and then 95% ethyl alcohol was added to get solution B of 25 mL. 15 mL of the upper layer was absorbed and put into a separating funnel of 30 mL, and 15 mL of 2.5% sodium bicarbonate solution was added; it was vibrated for 30 s and placed still for layering; later, the upper layer was discarded, 4 mL of the lower layer was absorbed, and 25 mL of 95%

ethyl alcohol was added to get solution C of 25 mL; 15 mL of tea beverage was put into the separating funnel of 30 mL, 15 mL of n-butyl alcohol was added, and then it was vibrated for 3 min and placed still for layering; later, 2 mL of the water layer was absorbed, 2 mL of saturated oxalate solution and 6 mL of distilled water were added, and finally 95% ethyl alcohol was added to get solution D of 25 mL. OD values of the four solutions were measured at the place of 380 nm and were recorded as EA, EB, EC, and ED, which are calculated according to the following formulas:

$$\begin{aligned} \text{theaflavin}\,(\%) &= 2.25 \times \text{EC}, \\ \text{thearubigin}\,(\%) &= 7.06 \times (2\text{EA} + 2\text{EB} - \text{EC} - 2\text{ED}), \\ \text{theabrownin}\,(\%) &= 2 \times \text{ED} \times 7.06. \end{aligned} \quad (1)$$

2.4. Statistical Analysis. Experimental data were analyzed using the statistical package SAS 9.1 (Cary, NC, USA). A one-way ANOVA procedure followed by the least significant difference (LSD) test was used to determine the significant difference ($P < 0.05$) between treatment means. Each mean was calculated from triplicate values.

3. Results and Discussion

3.1. PPO and POD Activities. The changes of PPO and POD activities of fresh leaves plucked from different plant varieties in different regions and seasons in the entire tea production process are shown in Figure 1. The activities of the enzymes increased and decreased. During withering, the activity was slightly increased; after rolling, the enzyme activity was significantly lost due to the full contact of enzymes and substrates, and the activity was decreased [9]. The varieties influenced the enzymatic activity significantly. In Figures 1(a) and 1(b), the PPO and POD activities of Yinghong 9 and Hongyan 12 were greatly increased during withering, which was significantly higher than that of the other three varieties. It meant that different varieties would determine the difference of gene intensity between the two kinds of enzymes, thus resulting in different activation ability during tea processing [11]. Therefore, the fermentation performance of different varieties could not be determined on the basis of enzymatic activity of fresh leaves.

In different regions (Figures 1(c) and 1(d)), PPO activity was the highest in Yingde, and POD activity was the highest in Changsha; PPO and POD activities in Hangzhou were both the lowest. Although the fresh leaves of the same variety were plucked in the same season, significant difference of PPO and POD activities would be caused by the different soil, water, and climatic conditions.

Comparing fresh leaves of the same variety plucked in different seasons (Figures 1(e) and 1(f)), although the PPO and POD activities of fresh leaves plucked in spring was low, they were greatly increased during withering. The activities of the two enzymes were the lowest in fresh leaves plucked in summer. The low enzymatic activity would prevent the polyphenols from full oxidation into theaflavins and thearubigins, which explained the low organoleptic quality and low market value of the Congou black tea made of fresh leaves plucked in summer. For the fresh leaves of the same variety in the same region, if they were plucked in different seasons, their PPO and POD activities would be significantly different due to the influence of temperature, humidity, light, and other climatic conditions.

In conclusion, variety, region, and season would significantly affect the PPO and POD activities. The PPO and POD activities of fresh leaves cannot reflect the fermenting performance. The degree of PPO and POD activities depended not only on the genes in leaves but also on the external soil and climatic conditions. The suitable soil and climatic conditions would greatly increase the enzymatic activity during tea processing, which would be conducive to the full conversion of polyphenols and promote the formation and accumulation of theaflavins and thearubigin, thus producing the high-quality Congou black tea.

3.2. Catechin. Figure 2 shows the changes of contents of 8 catechins in fresh tea leaves from different sources during tea processing. The contents and composition of catechins in fresh leaves plucked from different varieties were significantly different from each other. On average, in the 8 catechins, epigallocatechin gallate (EGCG) contents were significantly the highest, and catechine (C) content was significantly the lowest. It needs to be noticed that, Hongyan 12 has the highest total catechins content; EGC and EGCG components were significantly higher than other varieties. Yinghong 9 has the highest while Chuyeqi has the lowest total simple catechins. Yinghong 9 has the highest EC and CG components. Therefore, the varieties of tea plants had a significant influence on the contents of catechins in fresh leaves. The total ester-type catechins (TETC) is the total amount of ester-type catechins, including EGCG, epicatechin gallate (ECG), catechin gallate (CG), and gallocatechin gallate (GCG). The total simple catechin (TSC) is the total amount of simple catechins, including C, epicatechin (EC), epigallocatechin (EGC), and gallocatechin (GC). The ratios of TETC to TSC were different in each sample. The ratios directly affect the proportion of oxidation reaction substrate in fresh tea leaves, thus affecting the formation and transformation of theaflavins and other components.

As for the total amount of catechins (TAC, TAC = TETC + TSC) in fresh leaves plucked in different regions, Hangzhou > Changsha > Yingde. The TETC in Hangzhou was the highest (8.281%). The content of TSC in Changsha was the highest (3.924%). The ratio of TETC to TSC in Hangzhou was relatively the highest (higher than 3.5), and that in Changsha was relatively the lowest, which meant that the different growth and environmental conditions could result in the significant changes in the composition and contents of catechins in tea leaves. Fresh leaves in Changsha were collected from mountain tea gardens at an altitude of 500 meter. The climate was humid, and the direct sunlight was relatively low. Therefore, the content of TSC was higher, and the content of TETC was lower. However, fresh leaves from plain tea plantations in Hangzhou have relatively low

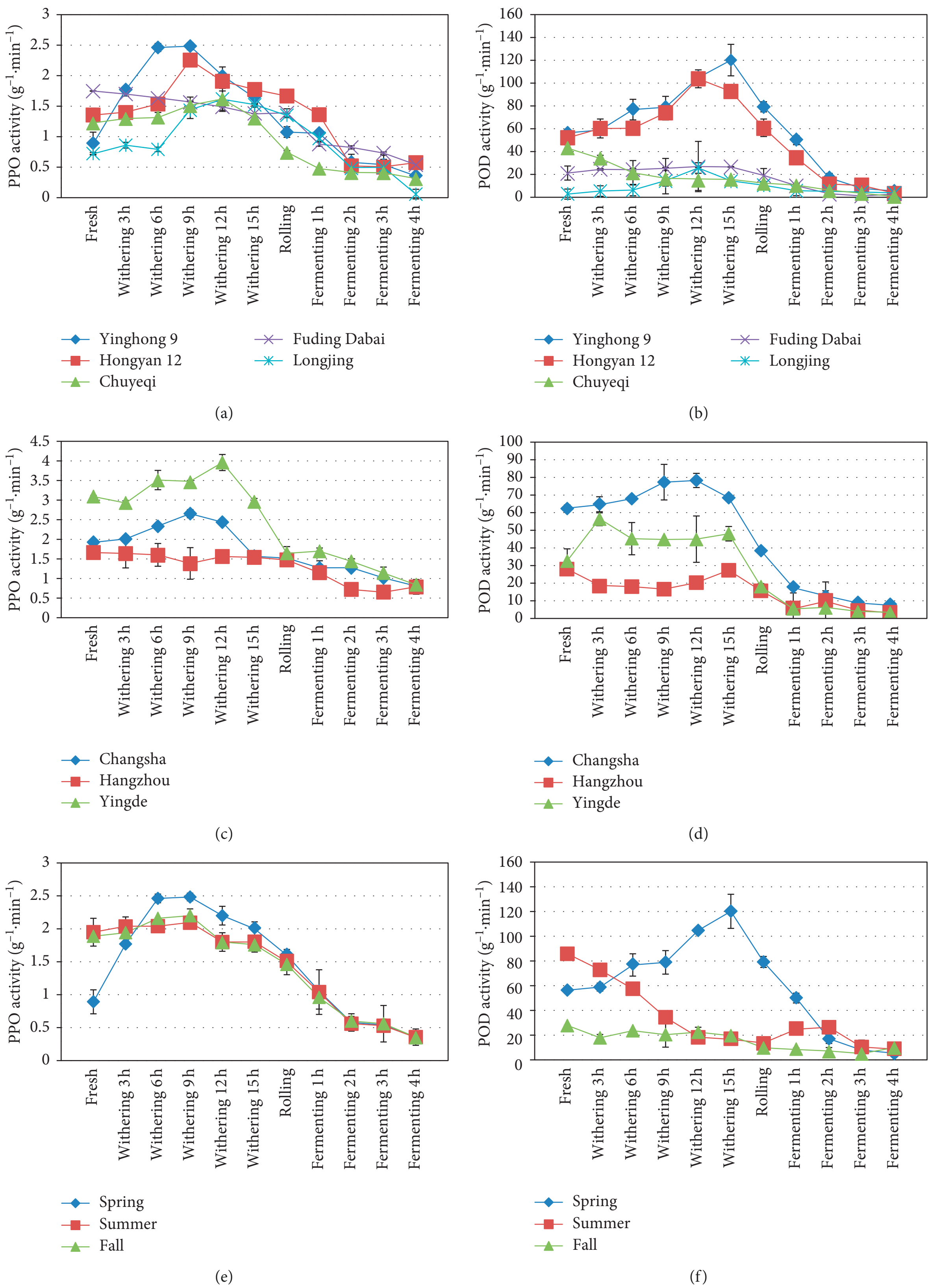
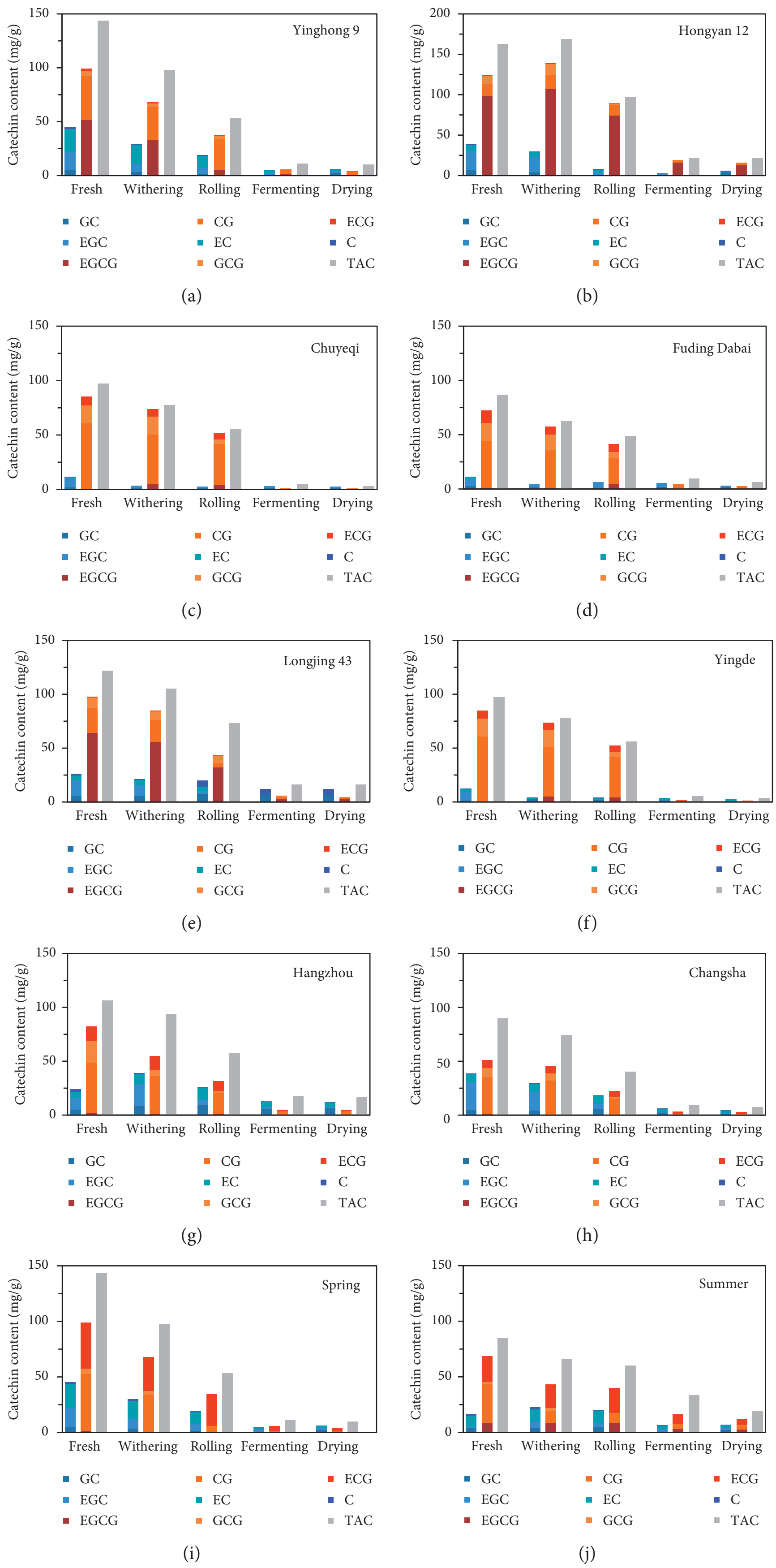


Figure 1: PPO and POD activities in fresh leaf cultivars during tea processing. (a, b) Different varieties; (c, d) different regions; (e, f) different seasons.



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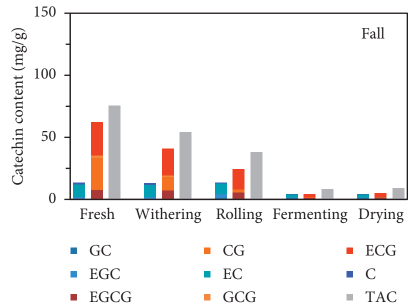

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Figure 2: Changes of catechin components content of fresh leaf cultivars from different varieties, region and seasons during tea processing (mg/g). C: catechin; GC: gallocatechin; EGC: epigallocatechin; EC: epicatechin; ECG: epicatechin gallate; GCG: gallocatechin gallate; CG: catechin gallate; EGCG: epigallocatechin gallate; TSC: total simple catechins; TETC: total ester-type catechins; TAC: total amount of catechins.

climatic humidity and more direct sunlight, resulting in higher TETC content.

The contents of the 8 catechins were significantly the highest in fresh tea leaves plucked in spring, which meant that fresh tea leaves plucked in spring had the most catechins due to the accumulation in the growing process.

Rolling and fermenting are important processes for oxidation conversion of catechins. In these two processes, the crushed leaf cells could promote enzymes to fully contact the substrates and accelerate the oxidation reaction, thus leading to the large consumption of catechins. Various types of catechins were reduced to different degrees. At 1 h prior to fermenting, the oxidation consumption of catechins was relatively the highest. The consumption of TETC was significantly higher than the consumption of TSC, which was corresponding to the reaction principle of oxidation of ester-type catechins to generate theaflavins and simple catechins, and the consumption of EGCG was significantly the highest.

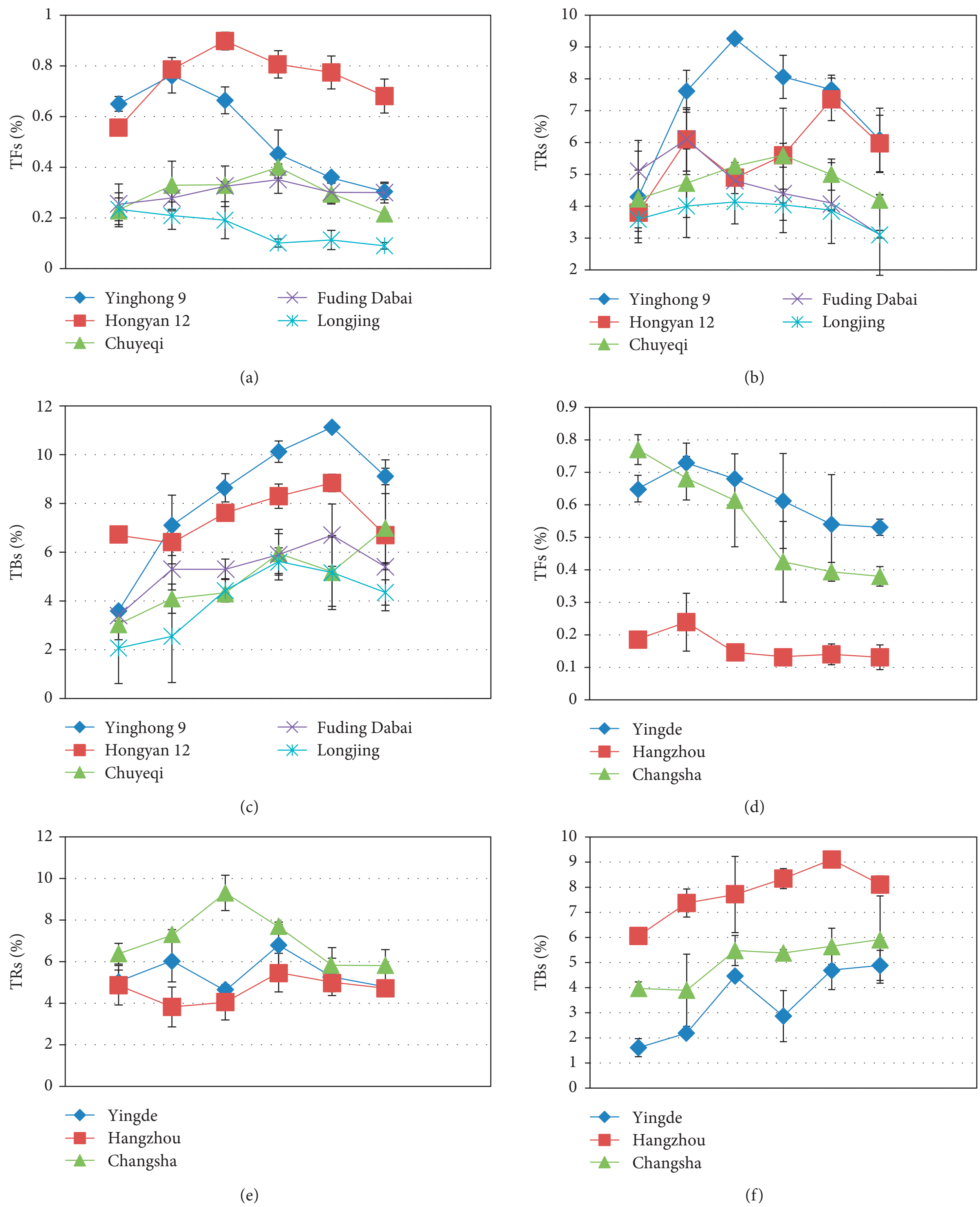
To sum up, the contents and proportion of catechins and the consumption of oxidative catechins were significantly different due to the varieties, regions, and seasons; the absolute amount and oxidation consumption of EGCG in fresh leaves were significantly the highest, and the consumption of TETC was significantly greater than that of TSC. At the earlier stages of rolling and fermenting, the oxidation consumption of catechins was the highest. The oxidation consumption of catechins in fresh leaves of Yinghong 9 plucked in Yingde in spring was significantly the highest. From Figure 1, it can be seen that highly active PPO and POD were in favor of oxidation consumption of catechins.

3.3. Theaflavin, Thearubigin, and Theabrownin Production. The contents of theaflavin and thearubigin would positively affect the quality and market price of the Congou black tea [12, 13]. The theabrownin created the dark color and nonconvergent taste, thus affecting the quality of the black tea [14].

Figure 3 shows the theaflavins (TFs), thearubigins (TRs), and theabrownins (TBs) in tea leaves plucked from different varieties in different regions and seasons during fermenting. These three components were all generated during fermenting and showed a trend of rising and falling, but their peak values appeared at different time. In general, TFs were firstly generated, then TRs and TBs were generated the latest, which meant that the long-term fermentation would be adverse to the accumulation of TFs and TRs but would continuously produce TBs.

Comparing the production capacity of TFs, TRs, and TBs in different varieties of tea leaves (Figures 3(a)–3(c)), Yinghong 9 and Hongyan 12 had a higher capacity of producing TFs than other varieties, which was closely related to the highly active PPO and POD (Figures 1(a) and 1(b)) and higher content of catechins (Figure 2); Yinghong 9 had a higher enzymatic activity than other varieties, and in addition to the higher content of catechins, it could produce the most TRs; although Longjing 43 had a higher content of catechins, its enzymatic activity was low, so the production of TRs was significantly the lowest; the production of TRs was significantly associated with the content of catechin substrate and PPO and POD activity [15].

Considering the changes of the production of TFs, TRs, and TBs in fresh leaves plucked in different regions in Figures 3(d)–3(f), the samples in Yingde had the highest production capacity of TFs, and the samples in Hangzhou had the lowest production capacity. As compared with other two regions, the production capacity of TRs in fresh leaves plucked in Changsha was significantly higher. The samples in Changsha with high POD activity could rapidly produce TRs under the combined action of catechins and TFs, but the total amount of catechins was sharply reduced at the interim of fermenting, and there were no sufficient reaction substrates, so the production of TRs started declining at this moment. The insufficient PPO and POD activities in Hangzhou could not fully convert catechins to TFs and TRs, so the production of TRs was quite slow, and the content was low. The amount of TBs was significantly the highest in Hangzhou, as predicted in combination with Figure 2 and Figures 3(d) and 3(e); if catechins could not be fully converted to produce TFs and TRs during fermenting, TBs would be produced through other oxidative polymerization means, which would be adverse to the quality.



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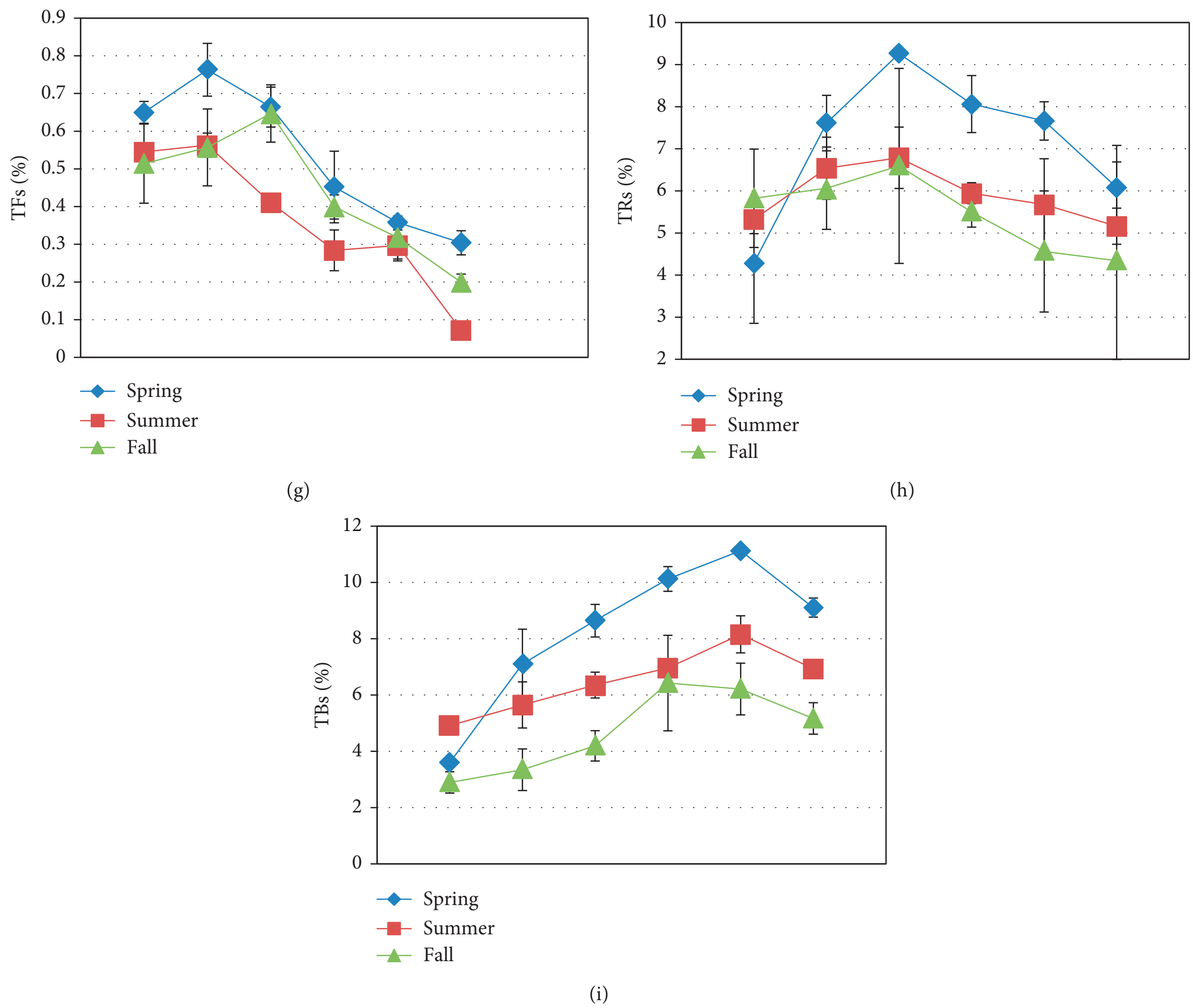

Figure 3: Theaflavins (TFs), thearubigins (TRs), and theabrownins (TBs) in fresh leaf cultivars during fermentation (a–c) different varieties; (d–f) different regions; (g–i) different seasons.

The changes of the production of TFs, TRs, and TBs during fermenting of Yinghong 9 plucked in different seasons can be seen in Figures 3(g)–3(i). The production of TFs was the highest in spring, and then in fall, and the lowest in summer, which was consistent to the study results of Sud and Baru [16]. PPO activity in spring was significantly the highest (Figure 1(e)), and the high POD activity (Figure 1(f)), in addition to the significant maximal consumption of catechins (Figure 2), would continuously reduce the total amount of TFs at 1h after fermenting and would also continuously produce more TRs and TBs than in summer and fall, and the peak values were reached at 2 h and 4 h after fermenting, respectively.

To sum up, the efficient and sustained production of TFs required the high PPO activity and high concentration of catechin substrates; high POD activity would rapidly transfer TFs into TRs and TBs, which was adverse to the accumulation of TFs. The production of TRs was also positively related to the content of catechin substrate and PPO and POD activity. However, the contents of TRs would be reduced along with time, resulting in gradual increase of TBs, namely, the long-term fermentation would not be conducive to the accumulation of TFs and TRs, but would cause the continuous production of TBs, which was adverse to the improvement of quality.

4. Conclusions

This study tested the influence of the fresh leaves plucked from different varieties of tea plants, different regions, and different seasons on the conversion of TFs. By comparing different varieties of tea plants, the results showed that Yinghong 9 could produce the highest content of TFs in a very short time, Hongyan 12 could produce high TFs continuously, and Longjing 43 could produce lowest TFs. By comparing the fresh leaves of the same variety plucked in different regions, the production ability of TFs showed the following trend: Yingde > Changsha > Hangzhou; as for the comparison of the fresh leaves of the same variety plucked in different seasons, the fresh leaves plucked in spring had significantly the strongest TFs production capacity.

Abbreviations

TSC: total simple catechins
TETC: total ester-type catechins
TAC: total amount of catechins
C: catechin
EC: epicatechin
EGC: epigallocatechin
EGCG: epigallocatechin gallate
ECG: epicatechin gallate
CG: catechin gallate
GC: gallocatechin
GCG: gallocatechin gallate
PPO: polyphenol oxidase
POD: peroxidase
TF: theaflavin
TFs: theaflavins
TRs: thearubigins
TBs: theabrownins.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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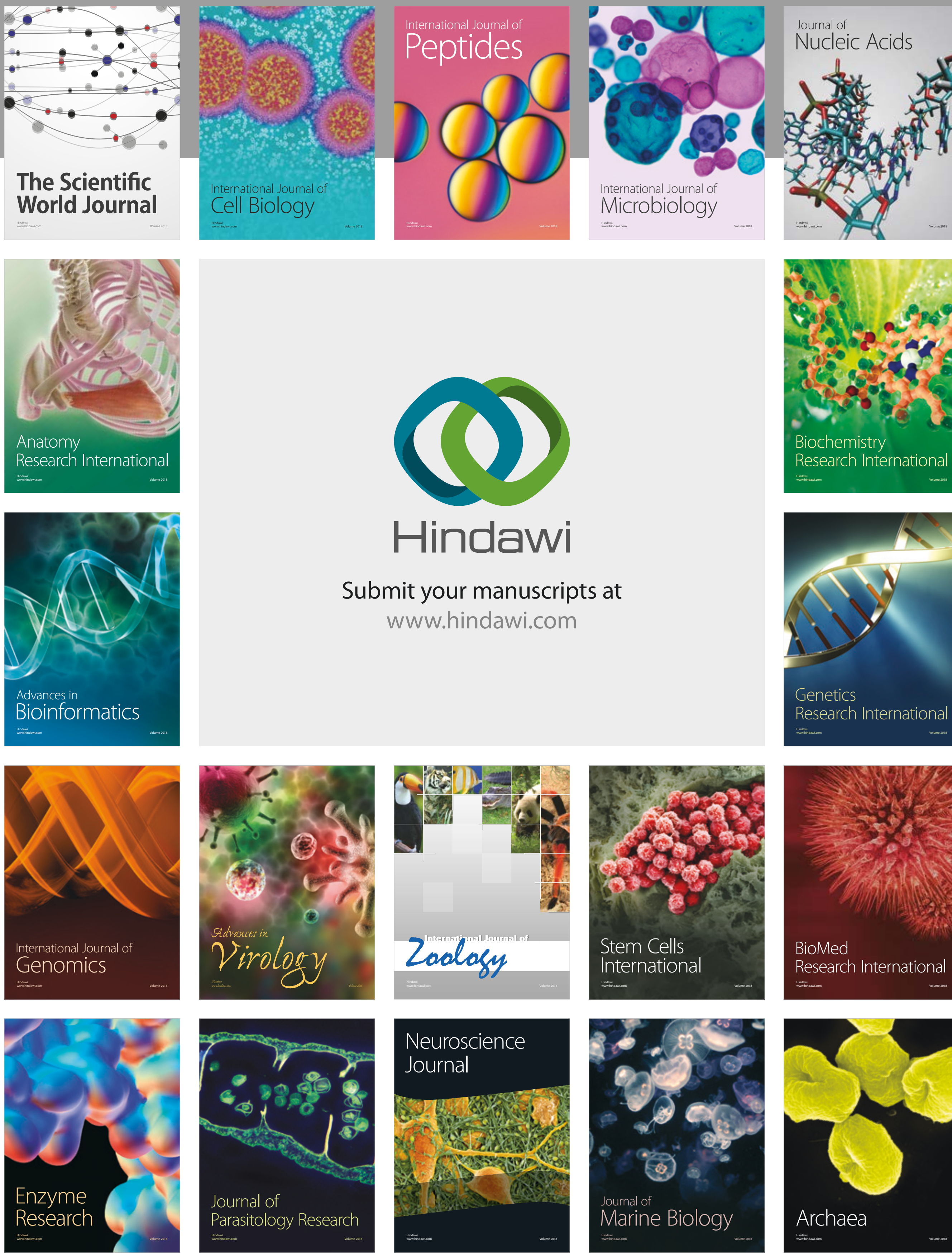

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