Effect of Ferrous Ion on Heat-Induced Aroma Deterioration of Green Tea Infusion

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Abstract: Aroma deterioration is one of the biggest problems in processing tea beverages. The aroma of tea infusion deteriorates fast during heat sterilization and the presence of ferrous ion (Fe\(^{2+}\)) aggravates it. The underlying mechanism remains unveiled. In this study, Fe\(^{3+}\) was verified to deteriorate the aroma quality of green tea infusion with heat treatment. Catechins were necessary for Fe\(^{3+}\)-mediated aroma deterioration. By enhancing the degradation of catechins, Fe\(^{2+}\) dramatically increased the production of hydrogen peroxide (H\(_2\)O\(_2\)). Fe\(^{2+}\) and H\(_2\)O\(_2\) together exacerbated the aroma of green tea infusion with heat treatment. GC-MS analysis revealed that the presence of Fe\(^{2+}\) enhanced the loss of green/grassy volatiles and promoted the formation of new volatiles with diversified aroma characteristics, resulting in a dull scent of green tea infusion. Our results revealed how Fe\(^{2+}\) induced aroma deterioration of green tea infusion with heat treatment and could help guide tea producers in attenuating the aroma deterioration of tea infusion during processing.

Keywords: green tea infusion; aroma deterioration; ferrous ion; catechins; hydrogen peroxide

1. Introduction

Green tea has been traditionally and widely used as a beverage in China. Ready-to-drink tea is preferable in modern society because of its convenience. Compared with freshly-brewed tea, the aroma of ready-to-drink tea is less fresh, sometimes even dull, impairing its sensory quality. The aroma of tea infusion deteriorates fast during heat sterilization [1,2]. Green tea infusion, which contains a higher level of unoxidized catechins (Figure 1), tends to experience more severe aroma deterioration than black tea and dark tea infusions during heat treatment [3]. Aroma deterioration is positively correlated to the degradation of unoxidized catechins and the accumulation of hydrogen peroxide (H\(_2\)O\(_2\)) [4], suggesting catechins might be vital in the aroma deterioration. Nevertheless, there are other factors.

Many metal ions affect the aroma of tea infusion. Among common ions in tea infusion, calcium ion (Ca\(^{2+}\)), ferrous ion (Fe\(^{2+}\)), and zinc ion (Zn\(^{2+}\)) have adverse effects on aroma [5,6]. In particular, Fe\(^{2+}\) deteriorates the aroma of tea infusion at concentrations as low as 0.1 ppm [6], implying aroma are very sensitive to Fe\(^{2+}\). However, the mechanism of Fe\(^{2+}\)-mediated aroma deterioration remains unclear. To figure it out, the effects of Fe\(^{2+}\) on aroma quality, degradation of catechins, and generation of H\(_2\)O\(_2\) in green tea infusion with heat treatment were assessed. The roles of catechins and H\(_2\)O\(_2\) on Fe\(^{2+}\)-mediated aroma deterioration were explored, respectively. The impacts of Fe\(^{2+}\) on volatile profiles were also evaluated. The results would deepen the knowledge of how Fe\(^{2+}\) deteriorated the aroma of tea infusion, and provide a new aspect for improving the aroma quality of ready-to-drink tea.
Figure 1. Structural formula of catechins. C, EC, GC, GCG, ECG, and EGCG are short for (+)-catechin, (−)-epicatechin, (−)-gallocatechin, (−)-epigallocatechin, (−)-catechin gallate, (−)-gallocatechin gallate, (−)-epicatechin gallate, and (−)-epigallocatechin gallate.

2. Results and Discussion

2.1. Fe$^{2+}$ Deteriorates the Aroma Quality and Promotes the Degradation of Catechins in Green Tea Infusion with Heat Treatment

The untreated infusion had fresh green tea aroma (defined as “fresh”) (Table 1). Heat treatment of green tea infusion reduced the freshness of the aroma (defined as “slightly dull”). The presence of Fe$^{2+}$ dose-dependently aggravated heat-induced aroma deterioration. It not only enhanced the reduction of the freshness, but also produced an off-aroma of the infusion (defined as “dull”).

Table 1. Effect of Fe$^{2+}$ on aroma quality of green tea infusion with heat treatment.

| Treatment                        | Description    | Score        |
|----------------------------------|----------------|--------------|
| Untreated                        | Fresh          | 8.7 ± 0.2 $^a$ |
| Heat treatment                   | Slightly dull  | 7.0 ± 0.4 $^b$ |
| 2 µM FeCl$_2$ + Heat treatment   | Slightly dull  | 6.5 ± 0.5 $^b$ |
| 4 µM FeCl$_2$ + Heat treatment   | Dull           | 5.5 ± 0.4 $^c$ |

The description and score of infusions were given by a team of qualified panelists. A higher score indicates a better aroma acceptability. The same letter within each column indicates no significant difference ($p > 0.05$).

Aroma deterioration is associated with degradation of catechins [4]. Catechins are heat-sensitive. When incubated at 100 °C, epimerization was the predominant reaction of epi-form catechins whereas ester hydrolysis was the predominant reaction of GCG [7,8]. In this study, the concentration of total catechins was not significantly changed but the composition of catechins was changed after heat treatment (Table 2).

The concentrations of two epi-form catechins, i.e., EGCG and EC, were decreased after heat treatment. Meanwhile, the concentrations of four non-epi-form catechins (GC, C, GCG, CG) and GA—a degradation product of catechins—were increased. The decrease of EGCG along with the increase of GCG indicated that the epimerization of EGCG took place under this condition. Notably, the loss of EGCG was higher than the increase of GCG. GC was increased though EGC was hardly changed. It suggested that part of GCG further degraded to GC. The change trends of EC and C were similar to that of EGCG and GCG, demonstrating the epimerization of EC also occurred. Taken together, heat treatment promoted the epimerization of EGCG and EC to their corresponding non-epi-forms, and triggered the production of non-gallated catechins. Compared with heated green tea infusion, levels of total catechins, EGCG, GCG, GC, and CG were decreased in heated green tea infusion with Fe$^{2+}$ addition. It implied that Fe$^{2+}$ predominantly promoted the degradation of catechins in green tea infusion with heat treatment.
Table 2. Effect of Fe$^{2+}$ on the transformation of catechins in green tea infusion with heat treatment.

| Concentrations (µg/mL) | Untreated | Heat Treatment | 2 µM FeCl$_2$ + Heat Treatment | 4 µM FeCl$_2$ + Heat Treatment |
|------------------------|-----------|----------------|-------------------------------|-------------------------------|
| GA                     | 4.41 ± 0.19$^a$ | 12.6 ± 0.8$^b$ | 13.0 ± 0.8$^b$ | 13.4 ± 1.1$^b$ |
| GC                     | 18.6 ± 0.1$^a$ | 41.6 ± 1.3$^b$ | 38.3 ± 0.6$^c$ | 37.3 ± 0.1$^d$ |
| EGC                    | 53.9 ± 3.6$^a$ | 52.6 ± 2.7$^a$ | 50.6 ± 3.3$^a$ | 49.5 ± 2.0$^a$ |
| C                      | 5.14 ± 0.18$^a$ | 12.0 ± 0.8$^b$ | 11.2 ± 0.1$^b$ | 11.5 ± 0.2$^b$ |
| EGCG                   | 818 ± 9$^a$   | 582 ± 5$^b$   | 568 ± 22$^bc$ | 561 ± 5$^c$   |
| EC                     | 45.5 ± 1.8$^a$ | 33.2 ± 0.5$^b$ | 33.0 ± 1.5$^b$ | 32.9 ± 0.6$^c$ |
| GCG                    | 5.07 ± 0.25$^a$ | 219 ± 4$^b$   | 200 ± 10$^c$  | 197 ± 10$^c$  |
| ECG                    | 93.3 ± 0.2$^a$ | 95.7 ± 2.1$^a$ | 93.7 ± 1.0$^a$ | 92.9 ± 3.0$^a$ |
| CG                     | 6.10 ± 0.11$^a$ | 16.9 ± 0.2$^b$ | 15.6 ± 1.0$^bc$ | 15.5 ± 0.9$^c$ |

Non-gallated catechins referred to GC, EGC, C, and EC, while gallated catechins referred to GCG, EGCG, CG, and ECG. Non-epi-catechins referred to GC, C, GCG, and CG, while epi-catechins referred to EGC, EC, EGCG, and ECG. The experiments were carried out in triplicate.

The degradation of catechins is coupled to the formation of H$_2$O$_2$ [9]. In a previous study, the concentrations of catechins in tea infusion were decreased and the concentrations of H$_2$O$_2$ were increased during storage [4]. A similar phenomenon was detected during heat treatment of tea infusion in this study (Figure 2). The addition of Fe$^{2+}$ dose-dependently increased the levels of H$_2$O$_2$ in green tea infusion. In contrast, the levels of H$_2$O$_2$ in catechins-removed green tea infusion were not significantly elevated by the addition of Fe$^{2+}$. It proved that H$_2$O$_2$ in heated green tea infusion was partially derived from catechins and Fe$^{2+}$ promoted the generation of H$_2$O$_2$ dependently on catechins.

Non-gallated catechins referred to GC, EGC, C, and EC, while gallated catechins referred to GCG, EGCG, CG, and ECG. Non-epi-catechins referred to GC, C, GCG, and CG, while epi-catechins referred to EGC, EC, EGCG, and ECG. The experiments were carried out in triplicate.

The same letter within each group indicates no significant difference ($p > 0.05$).

![Figure 2](image-url). Effect of Fe$^{2+}$ on the accumulation of H$_2$O$_2$ in green tea infusion with heat treatment. The same letter within each column indicates no significant difference ($p > 0.05$).

2.2. Effect of Catechins and H$_2$O$_2$ on Fe$^{2+}$-Mediated Aroma Deterioration in Green Tea Infusion with Heat Treatment

To investigate the roles of catechins on Fe$^{2+}$-mediated aroma deterioration in green tea infusion with heat treatment, the effect of Fe$^{2+}$ on aroma quality of catechins-removed green tea infusion was assessed. According to the results (Table 3), the effect of Fe$^{2+}$ on aroma deterioration was diminished in catechins-removed green tea infusion, implying that catechins were necessary for Fe$^{2+}$-mediated aroma deterioration.
Table 3. Effect of Fe\(^{2+}\) on aroma quality of catechins-removed green tea infusion.

| Treatment                          | Score       |
|-----------------------------------|-------------|
| Untreated                         | 8.2 ± 0.3 \(a\) |
| Heat treatment                    | 7.6 ± 0.4 \(ab\) |
| 2 \(\mu\)M FeCl\(_2\) + Heat treatment | 7.6 ± 0.4 \(ab\) |
| 4 \(\mu\)M FeCl\(_2\) + Heat treatment | 7.3 ± 0.5 \(b\) |

The same letter within each column indicates no significant difference \((p > 0.05)\).

To further test whether catechins participated in Fe\(^{2+}\)-mediated aroma deterioration via generating H\(_2\)O\(_2\), the effect of Fe\(^{2+}\) on aroma quality of catechins-removed green tea infusion containing exogenous H\(_2\)O\(_2\) was evaluated. The results (Table 4) revealed that exogenous H\(_2\)O\(_2\) or Fe\(^{2+}\) alone hardly deteriorated the aroma of catechins-removed green tea infusion. However, the combination of exogenous H\(_2\)O\(_2\) and Fe\(^{2+}\) effectively impaired the aroma. It suggested that H\(_2\)O\(_2\) played an essential role in Fe\(^{2+}\)-mediated aroma deterioration.

Table 4. Effect of H\(_2\)O\(_2\) on Fe\(^{2+}\)-mediated aroma deterioration in catechins-removed green tea infusion.

| Treatment                                       | Score       |
|------------------------------------------------|-------------|
| Untreated                                      | 8.2 ± 0.3 \(a\) |
| Heat treatment                                 | 7.5 ± 0.6 \(a\) |
| 100 \(\mu\)M H\(_2\)O\(_2\) + Heat treatment    | 7.5 ± 0.5 \(a\) |
| 4 \(\mu\)M FeCl\(_2\) + Heat treatment         | 7.3 ± 0.6 \(a\) |
| 4 \(\mu\)M FeCl\(_2\) + 100 \(\mu\)M H\(_2\)O\(_2\) + Heat treatment | 6.0 ± 0.4 \(b\) |

The same letter within each column indicates no significant difference \((p > 0.05)\).

Previous studies have mentioned that Fe\(^{2+}\) can react with H\(_2\)O\(_2\) to generate highly reactive radicals (e.g., hydroxyl radicals) [10]. Fe\(^{2+}\) can catalyze the reaction of H\(_2\)O\(_2\) and superoxide ion to generate hydroxyl radicals and hydroxide ions [10]. Due to the massive production of reactive radicals, the two reactions play important roles in the degradation of various organic compounds, including hydrocarbons, alcohols, and organic acids [11]. Both H\(_2\)O\(_2\) and superoxide ion are generated during the degradation of catechins [9]. Therefore, it is possible that Fe\(^{2+}\) also relies on these two reactions to induce aroma deterioration of green tea infusion. EGCG, the most abundant catechin in green tea infusion, was capable of enhancing the efficiency of Fe\(^{2+}\)-mediated oxidative systems on the degradation of organic compounds, probably by accelerating the transformation of Fe\(^{3+}\) to Fe\(^{2+}\) [12]. This implied that catechins might not only participate in Fe\(^{2+}\)-mediated aroma deterioration by generating H\(_2\)O\(_2\), but also by promoting the regeneration of Fe\(^{2+}\). However, further experiments are needed to verify this hypothesis.

2.3. Effect of Fe\(^{2+}\) on the Volatile Profile of Green Tea Infusion with Heat Treatment

GC-MS analysis (Figure 3 and Table 5) revealed that 49, 54, and 61 volatiles were detected in unheated tea infusion, heated tea infusion, and heated tea infusion with Fe\(^{2+}\) addition, respectively. Among them, 42 volatiles were observed in all infusions. It indicated that the majority of volatile components in the three infusions were similar and the presence of Fe\(^{2+}\) promoted the formation of new volatiles.

The volatile profiles of the three infusions were quite different. It was supported by the results of PCA. Points representing samples with different treatments in the PCA score plot were clearly separated (Figure 4). The types and relative abundance of alkenes, alcohols, and heterocycles were gradually increased in unheated tea infusion, heated tea infusion, and heated tea infusion with Fe\(^{2+}\) addition, respectively. Meanwhile the relative abundance of aldehydes, ketones, and esters were gradually decreased in the three infusions, respectively. When categorized by aroma characteristics, green/grassy
volatiles were gradually decreased and woody volatiles were gradually increased in the three infusions, respectively.

Figure 3. GC-MS total ion count chromatograms of green tea infusion with different treatments.

Figure 4. PCA score plot based on volatiles in tea infusions with different treatments.
| RT     | CAS No.   | Molecular Formula | Name                                   | Relative Abundance (%) | 4 μM FeCl₂ + Heat Treatment | Aroma Properties                     |
|--------|-----------|-------------------|----------------------------------------|------------------------|-----------------------------|--------------------------------------|
|        |           |                   | Unheated                               | Heat Treatment         |                             |                                      |
| 5.310  | 141-79-7  | C₅H₁₀O            | Mesityl oxide                           | 1.27 ± 0.05 a          | 0.68 ± 0.05 b              | <LOQ c                               |
| 5.437  | 66-25-1   | C₆H₁₂O            | Hexanal                                | 11.70 ± 0.92 a         | 3.86 ± 0.53 b              | 3.39 ± 0.13 b                        |
| 5.764  | 617-92-5  | C₇H₁₄N            | 1-Ethylpyrroline                       | 0.42 ± 0.05 a          | 0.32 ± 0.01 b              | 0.19 ± 0.05 c                        |
| 5.944  | 123-86-4  | C₈H₁₈O₂           | Butyl acetate                          | 0.49 ± 0.06 a          | <LOQ b                      | <LOQ b                               |
| 7.557  | 544-12-7  | C₈H₁₆O₂           | Trans-3-hexen-1-ol                    | 1.06 ± 0.03 a          | 0.71 ± 0.07 b              | 0.58 ± 0.03 c                        |
| 9.654  | 6728-31-0 | C₉H₁₄O₂           | (Z)-4-Heptenal                          | 0.48 ± 0.03 a          | 0.29 ± 0.05 b              | 0.19 ± 0.05 c                        |
| 9.918  | 111-71-7  | C₁₀H₁₈O₂           | Heptanal                               | 3.67 ± 0.28 a          | 1.98 ± 0.41 b              | 2.00 ± 0.24 b                        |
| 9.943  | 222866    | C₁₀H₁₄NO₂         | Oxime-, methoxy-phenyl-               | 1.56 ± 0.07 a          | 0.99 ± 0.19 b              | 1.31 ± 0.18 ab                       |
| 12.882 | 57266-86-1| C₁₁H₂₂O           | (Z)-2-Heptenal                         | 2.17 ± 0.13 a          | 1.41 ± 0.05 b              | 1.49 ± 0.10 b                        |
| 12.998 | 100-52-7  | C₁₂H₁₈O           | Benzaldehyde                           | 1.44 ± 0.08 a          | 1.36 ± 0.02 a              | 2.34 ± 0.22 b                        |
| 13.988 | 26456-76-8| C₁₃H₁₆            | 3,5,5-Trimethyl-2-hexene              | 1.10 ± 0.10 a          | 0.81 ± 0.02 b              | 0.83 ± 0.03 b                        |
| 14.183 | 2918-13-0 | C₁₄H₂₂O           | 1-Hepten-3-one                         | 0.44 ± 0.06 a          | 0.28 ± 0.04 b              | 0.33 ± 0.00 c                        |
| 14.442 | 3391-86-4 | C₁₅H₂₆O           | 1-Octen-3-ol                           | 4.55 ± 0.05 a          | 2.86 ± 0.23 a              | 2.72 ± 0.16 b                        |
| 14.693 | 110-93-0  | C₁₆H₂₄O           | 5-Hepten-2-one, 6-methyl-              | 2.19 ± 0.14 a          | 1.38 ± 0.08 b              | 1.13 ± 0.08 e                        |
| 14.822 | 3050-69-9 | C₁₇H₂₄O₂          | Vinyl hexanoate                        | 4.14 ± 0.65 a          | 5.65 ± 0.47 b              | 4.79 ± 0.27 a                        |
| 15.366 | 13643-08-8| C₁₈H₃₄             | 2,4-Octadiene                          | 1.05 ± 0.30 ab         | 0.72 ± 0.07 a              | 0.91 ± 0.11 b                        |
| 15.903 | 124-13-0  | C₁₉H₃₈O           | Octanal                                | 2.15 ± 0.01 a          | 1.45 ± 0.08 b              | 1.36 ± 0.00 c                        |
| 16.082 | 3681-71-8 | C₂₀H₄₂O₂          | cis-3-Hexenyl acetate                  | 3.58 ± 0.15 a          | 1.10 ± 0.12 b              | 1.02 ± 0.06 b                        |
| 16.297 | 4313-03-5 | C₂₁H₄₀O           | (E,E)-2,4-Heptadienal                  | 1.81 ± 0.02 a          | 1.37 ± 0.05 b              | 1.41 ± 0.11 b                        |
| 17.473 | 5989-27-5 | C₂₂H₄₄O₃           | D-Limonene                             | <LOQ a                 | <LOQ a                      | 0.43 ± 0.09 b                        |
| 17.663 | 2114-48   | C₂³H₄₆O₂           | 2-Ethyl-1-hexanol                     | 0.51 ± 0.15 a          | <LOQ a                      | 0.22 ± 0.07 b                        |
| 17.764 | 100-51-6  | C₂₄H₄₈O           | Benzyl alcohol                         | <LOQ a                 | <LOQ a                      | 0.58 ± 0.22 b                        |
| 17.768 | 2408-37-9 | C₂₅H₅₆O           | 2,6,6-Trimethylocyclohexane            | 0.92 ± 0.01 a          | <LOQ b                      | <LOQ b                               |
| 18.322 | 122-78-1  | C₂₆H₅₂O           | Benzene acetaldehyde                   | 0.43 ± 0.02 a          | 0.97 ± 0.06 b              | 1.15 ± 0.13 b                        |
| 18.551 | 2167-14-8 | C₂₇H₅₄NO          | 1H-Pyrole-2-carboxylic acid, 1-ethyl-  | 0.44 ± 0.04 a          | 0.33 ± 0.06 b              | 0.37 ± 0.05 ab                       |
| 19.223 | 65405-80-3| C₂₈H₄₄O₂           | (E,Z)-2-Butenoic acid, 3-hexenyl ester| <LOQ a                 | <LOQ a                      | 0.13 ± 0.01 b                        |
| 19.357 | 78-59-1   | C₂₉H₅₄O           | Isophorone                             | 0.27 ± 0.01 a          | <LOQ b                      | 0.11 ± 0.00 c                        |
| 19.515 | 2548-87-0 | C₃₀H₇₂O           | (E)-2-Octenal                          | 0.79 ± 0.06 a          | 0.68 ± 0.05 b              | 0.74 ± 0.07 a                        |
| 19.731 | 96-86-2   | C₃₁H₇₄O           | Acetophenone                           | 0.35 ± 0.02 a          | 0.44 ± 0.06 b              | 0.46 ± 0.01 b                        |
| 20.275 | 38284-27-4| C₃₂H₇₆O           | 3,5-Octadien-2-one                    | 2.08 ± 0.34 a          | 1.91 ± 0.64 ab             | 1.59 ± 0.01 b                        |
| 20.556 | 111-87-5  | C₃₃H₇₈O           | 1-Octanol                              | 1.72 ± 0.02 a          | 1.22 ± 0.03 b              | 1.38 ± 0.22 b                        |
| 21.889 | 30086-02-3| C₃₄H₇₄O           | (E,E)-3,5-Octadien-2-one              | 0.25 ± 0.06 a          | 0.23 ± 0.03 a              | 0.25 ± 0.03 a                        |
| 22.477 | 78-70-6   | C₃₅H₈₂O           | Linalool                               | 3.45 ± 0.16 a          | 19.79 ± 0.68 b             | 19.99 ± 0.89 b                       |
| 22.673 | 29957-43-5| C₃₆H₈₄O           | 3,7-Dimethylocta-1,5,7-trien-3-ol      | 0.68 ± 0.11 a          | 0.64 ± 0.04 a              | 0.63 ± 0.05 a                        |
| 22.836 | 124-19-6  | C₃₇H₉₂O           | Nonanal                                | 6.57 ± 0.93 ab         | 6.84 ± 0.18 a              | 5.62 ± 0.24 b                        |

**Table 5.** Effect of Fe²⁺ on the volatile profile of green tea infusion with heat treatment.
| RT     | CAS No. | Molecular Formula | Name                                      | Relative Abundance (%) | Aroma Properties                  |
|--------|---------|-------------------|-------------------------------------------|------------------------|-----------------------------------|
|        |         |                   | Unheated                                  | Heat Treatment         | 4 µM FeCl₂ + Heat Treatment       |
|        |         |                   |                                           |                        |                                   |
|        |         |                   | 0.35 ± 0.00 a                             | 0.52 ± 0.04 b          | 0.48 ± 0.01 b Floral, rose        |
| 29.922 | 60-12-8 | C₆H₁₂O            | Phenylethyl Alcohol                       |                        |                                   |
| 28.960 | 119-36-8| C₆H₁₂O₃           | Methyl salicylate                         | <LOQ a                 | <LOQ a                            | Wintergreen mint                  |
| 29.321 | 53398-84-8| C₆H₁₂O₂          | Butanoic acid, 3-hexenyl ester, (E)-      | 4.33 ± 0.06 b          | 1.22 ± 0.10 b 0.76 ± 0.10 c pine, lilac |
| 29.49  | 98-55-5 | C₁₀H₁₈O            | α-Terpine                                 | <LOQ a                 | <LOQ a                            | Pine, lilac                       |
| 30.423 | 116-26-7| C₁₀H₁₈O            | Safanal                                   | <LOQ a                 | <LOQ a                            | Pine, lilac                       |
| 30.903 | 112-31-2| C₁₀H₂₅O            | Decanal                                   | 1.47 ± 0.59 a          | 2.95 ± 0.25 b 1.28 ± 0.06 b Fresh, herbal |
| 31.059 | 432-25-7| C₁₀H₁₄O            | β-Cyclocitrinal                           | 1.91 ± 0.01 a          | 1.19 ± 0.08 b 0.23 ± 0.06 b Fresh, herbal |
| 31.253 | 496-16-2| C₆H₈O            | 2,3-Dihydrobenzofuran                     | <LOQ a                 | 0.24 ± 0.01 b 0.34 ± 0.03 c Sweet, nut |
| 32.183 | 2177-77-7| C₆H₁₄O₂          | Methyl 2-, Methylpentanoate               | 4.15 ± 0.66 a          | 0.70 ± 0.14 b 1.37 ± 0.02 c Fruity |
| 33.450 | 35852-46-1| C₁₁H₂₂O₂       | n-Valeric acid cis-3-hexenyl ester        | 1.06 ± 0.05 a          | 0.20 ± 0.03 b <LOQ c Green fruity, unripe banana |
| 33.575 | 624-15-7| C₁₀H₁₈O            | 3,7-Dimethyl-2,6-octadienol               | <LOQ a                 | 0.80 ± 0.11 b 1.09 ± 0.06 b Woody |
| 36.176 | 120-72-9| C₆H₁₈N            | Indole                                    | 0.28 ± 0.01 a          | 0.34 ± 0.10 ab 0.38 ± 0.06 b Floral, waxy |
| 36.176 | 36431-72-8| C₁₅H₂₃O        | Theaspirane                               | <LOQ a                 | 0.84 ± 0.06 b 0.58 ± 0.00 c Woody, spicy |
| 37.193 |         |                   | 2,6,10,10-Tetramethyl-1-oxaspiro[4.5]dec-7-ene | <LOQ a                 | 1.27 ± 0.02 b 0.96 ± 0.05 c Woody |
| 41.043 | 31501-11-8| C₁₂H₂₅O₂       | Hexanoic acid, 3-hexenyl ester, (Z)-      | 5.08 ± 0.19 a          | 1.27 ± 0.14 b 0.79 ± 0.04 c Fruity green, pear |
| 41.606 | 61444-38-0| C₁₂H₂₀O₂       | cis-3-Hexenyl cis-3-hexenoate            | 0.87 ± 0.05 a          | 0.27 ± 0.00 b 0.19 ± 0.02 c Green |
| 41.721 | 488-10-8| C₁₁H₁₈O            | Jasmonate                                 | 2.09 ± 0.00 a          | 2.47 ± 0.19 b 2.64 ± 0.01 c Floral, Jasmin |
| 44.928 | 3879-26-3| C₁₁H₂₈O            | Nerylacetone                              | 0.58 ± 0.05 a          | 0.94 ± 0.12 b 0.47 ± 0.08 b Woody |
| 46.344 | 79-77-6 | C₁₅H₂₆O            | β-Ionone                                  | 2.24 ± 0.02 a          | 1.97 ± 0.16 b 2.14 ± 0.14 ab Floral, woody |
| 47.931 | 166273-38-7| C₁₅H₃₃O₃        | 5-Hydroxypentanoate                       | <LOQ a                 | 0.38 ± 0.07 a 0.41 ± 0.05 a Woody |
| 48.219 | 500-66-3| C₁₂H₁₈O            | 1,3-Benzenediol, 5-pentyl-               | 9.56 ± 0.04 a          | 13.13 ± 1.13 b 13.70 ± 0.21 c Olive |
| 48.354 | 483-76-1| C₁₃H₃₄            | (+)-3-Cadinene                           | 0.79 ± 0.07 a          | 3.00 ± 0.44 b 4.01 ± 0.29 c Herbal, woody |
| 48.469 | 483-77-2| C₁₃H₃₂            | (−)-Calamenene                            | <LOQ a                 | <LOQ a                            | Herb spice                        |
| 48.545 | 41702-63-0| C₁₃H₃₄          | Epizonare                                 | <LOQ a                 | <LOQ a                            | Herb spice                        |
| 49.244 | 21391-90-7| C₁₃H₃₄          | α-Calone                                  | <LOQ a                 | <LOQ a                            | Herb spice                        |
| 50.619 | 142-50-7| C₁₃H₂₆O            | Nerolidol                                 | <LOQ a                 | 0.92 ± 0.28 b 0.51 ± 0.01 b Floral |
| 52.427 | 77-53-2 | C₁₃H₂₆O            | Cedrol                                    | 0.52 ± 0.05 a          | 0.81 ± 0.02 b 0.88 ± 0.05 b Cedarwood, woody, soft sweet |
| 53.501 | 16728-99-7| C₁₅H₃₂        | Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-   | <LOQ a                 | 0.89 ± 0.20 b 0.92 ± 0.06 b Cedarwood, woody, soft sweet |
| 54.188 | 19912-67-5| C₁₅H₂₆O        | Epicubol                                  | <LOQ a                 | 1.23 ± 0.08 b 1.34 ± 0.16 b Faint odor |
| 62.562 | 66408-55-7| C₁₇H₂₆O        | 5,9,13-Trimethyl-tetradeca-4,8,12-trienal | <LOQ a                 | 0.33 ± 0.11 b 0.36 ± 0.05 b Waxy, fatty |
| 63.552 | 84-74-2 | C₁₃H₂₆O            | Dodecyl palhalate                         | <LOQ a                 | <LOQ a                            | Wintergreen mint                  |
| 67.845 | 57-10-3 | C₁₃H₂₆O            | n-Hexadecanoic acid                      | 0.53 ± 0.13 a          | <LOQ a                            | Wintergreen mint                  |
| 71.894 | 83834-59-7| C₁₃H₂₆O        | 4-Methoxycinnamic acid 2-ethylhexyl ester | <LOQ a                 | 0.71 ± 0.11 b 0.71 ± 0.11 b Waxy, fatty |

LOQ is short for the limit of quantification. The experiments were carried out in triplicate. The same letter within each row indicates no significant difference (p > 0.05).
A total of 28 volatiles were decreased after heat treatment. Eleven of them were further decreased in heated tea infusion with Fe$^{2+}$ addition, most of which had green/grassy scents. Twenty-one volatiles were increased after heat treatment. Five of them were further increased in heated tea infusion with Fe$^{2+}$ addition. They smelt sweet, fruity, or woody. Notably, there were nine volatiles only detected in the heated infusion with Fe$^{2+}$ addition and their aroma characteristics were so different from each other, ranging from fruity, floral, minty, fresh, herbal, to woody. The loss of green/grassy volatiles might be associated with the less fresh aroma of green tea infusion. The gain of volatiles with too many different aroma characteristics might lead to an unpleasant complex scent. They together presented a dull scent in heated tea infusion with Fe$^{2+}$ addition.

3. Materials and Methods

3.1. Reagents

Green tea was purchased from Jiangsu Xinpin Tea Co., Ltd (Changzhou, China). (−)-Epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-epicatechin (EC), (+)-catechin (C), (−)-epicatechin gallate (ECG), (−)-catechin gallate (CG), (−)-epigallocatechin (EGC), (−)-gallocatechin (GC), and gallic acid (GA) were obtained from Sigma-Aldrich Co. LLC (Saint Louis, MO, USA). Polyvinylpolypyrrolidone (PVPP) and ferrous chloride (FeCl$_2$) were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China). The 30% H$_2$O$_2$ was purchased from Sinopharm group Co., Ltd (Beijing, China).

3.2. Preparation of Green Tea Infusion

Green tea was brewed with deionized water (1:100, w/v) at 60 °C for 20 min and filtered through a 0.45 µm membrane to obtain tea infusion.

To assess the effects of Fe$^{2+}$ on the aroma quality, degradation of catechins, and generation of H$_2$O$_2$ of green tea infusion with heat treatment, FeCl$_2$ solution was added to green tea infusion to reach the final concentrations of 0, 2, and 4 µmol/L (µM). According to the requirement of the standards for drinking water quality (GB 5749-2006), the ferrous ion concentration in drinking water shall not exceed 5.36 µM. Therefore, the Fe$^{2+}$ concentrations used for experiments in the study were defined as 2 and 4 µM.

To investigate the effect of catechins on Fe$^{2+}$-mediated aroma deterioration, green tea infusion was mixed with PVPP (5%, w/w) to remove catechins. After PVPP treatment, catechins in green tea infusion were no longer detectable using the HPLC method [13] (Figure 5). FeCl$_2$ solution was added to the catechins-removed green tea infusion to reach the final concentrations of 0, 2, and 4 µM.

To investigate the effect of H$_2$O$_2$ on Fe$^{2+}$-mediated aroma deterioration, H$_2$O$_2$ was added to the catechins-removed green tea infusion to reach the final concentration of 100 µM. Infusions with different additions were incubated at 100 °C for 40 min and cooled to room temperature for further tests.

3.3. Sensory Evaluation of Green Tea Infusion

The aroma quality of tea infusion was assessed according to the national standard GB/T 21733-2008, and scored by a team of seven qualified panelists (three men and four women, 25–50 years old), all of whom achieved certificates for tea-quality evaluation from the Tea Scientific Society of China. A ten-point scale was used for scoring. The score 0 is for least acceptable and 10 is for most acceptable [14].

3.4. Determination of Catechins and GA in Green Tea Infusion

The concentrations of catechins and GA were measured by an established HPLC method [13].

The separation was performed on a Symmetry C$_{18}$ column (5 μm, 4.6 mm × 250 mm, Waters, Milford, MA, USA) using a Shimadzu LC-20A system (Shimadzu, Tokyo, Japan) equipped with a UV detector set at 280 nm. The gradient separation was carried out using 2% acetic acid in water and acetonitrile as mobile phases A and B, with the flow rate at
1 mL/min for 30 min and the column temperature at 35 °C. Separation was conducted under the following conditions: 0–16 min, 6.5–15% B; 16–25 min, 15–25% B; and 25–30 min, and 25–6.5% B.

GA (3.54–56.7 mg/L), EGCG (3.23–51.7 mg/L), GCG (5.00–80.0 mg/L), EC (5.31–85.0 mg/L), C (5.31–85.0 mg/L), ECG (4.11–65.8 mg/L), CG (1.56–25.0 mg/L), EGC (5.21–83.3 mg/L), and GC (2.55–40.8 mg/L) were used as standards to prepare calibration curves.

3.5. Determination of H$_2$O$_2$ in Green Tea Infusion

The levels of H$_2$O$_2$ were determined using a Hydrogen Peroxide Assay Kit (Beyotime Biotechnology, Nantong, China) according to the manufacturer’s instructions. Briefly, 50 µL of the infusion was added to a 96-well plate, mixed with 100 µL of the hydrogen peroxide reaction reagent, stayed at room temperature for 30 min, and then the absorbance was read at 560 nm using the BioTek Synergy 2 multi-mode microplate reader (Winooski, VT, USA).

3.6. Analysis of Volatiles in Green Tea Infusion

Volatiles in tea infusion were determined by gas chromatography–mass spectrometry (GC-MS) as previously reported [15].

Thirty milliliters of the infusion containing ethyl caprate as the internal standard was added into a 50 mL glass vial. The glass vial was sealed and incubated at 60 °C. A SPME needle (Supelco, Bellefonte, PA, USA) was used to absorb volatiles for 60 min. Then the SPME needle was inserted into the injection port of GC and volatiles were desorbed at 250 °C for 5 min.

Volatiles were analyzed using an Agilent6890 gas chromatograph coupled with an Agilent HP 5973 mass selective detector (Agilent, Wilmington, DE, USA). The separation was performed on a DB-5MS capillary column (30 m × 250 µm × 0.25 µm). The GC condition was as follows: the GC inlet temperature of 250 °C, and the split ratio of 15:1, the carrier gas (high purity helium) flow of 1 mL/min. The separation was conducted under the following conditions: 0–2 min, 40 °C; 2–24.5 min, 40–85 °C; 24.5–26.5 min,
85 °C; 26.5–64.5 min, 85–180 °C; 64.5–66.5 min, 180–230 °C; and 71.5–73.5 min, 230 °C.

For MS analysis, the temperature of the ion source was 230 °C, the voltage was 70 eV and the scan range was 40 to 400 m/z.

Tentative identification of volatiles was made by comparing the MS fragmentation patterns with data from the National Institute for Standards and Technology database (NIST 08, match percentage >80%). The relative abundance of each compound was calculated by comparing the peak area of each compound to the total peak area.

3.7. Statistical Analysis

Data are presented as the mean ± standard error of the mean (SEM). All experiments were carried out in triplicate. The principal component analysis (PCA) was performed using SIMCA-P 13.0 software (Umetrics, Umea, Sweden). The results were analyzed with SPSS Version 18.0 for Windows using the one-way analysis of variance with 2-sided Dunnett’s post hoc test to determine overall differences between groups. p-values < 0.05 were considered to be statistically significant.

4. Conclusions

Fe$^{2+}$ significantly exacerbated the aroma quality of green tea infusion with heat treatment. It promoted the degradation of catechins and the generation of H$_2$O$_2$. Catechins were prerequisites for Fe$^{2+}$-mediated aroma deterioration. They were important sources of H$_2$O$_2$. H$_2$O$_2$ alone showed limited effects on aroma. Fe$^{2+}$ and H$_2$O$_2$ worked together to induce aroma deterioration. Fe$^{2+}$ significantly modified the volatile profiles of green tea infusion. Green/grassy volatiles were reduced and several volatiles with different aroma characteristics were increased or newly generated, leading to a dull scent. Based on the above results and previous references [12,16,17], a reaction scheme was proposed (Figure 6). It was deduced that under heat treatment, catechins could deprotonate and lose electrons to form semiquinone and quinone, which was reversible. The electrons reacted with dissolved oxygen and hydrogen ions, leading to the generation of H$_2$O$_2$. The presence of Fe$^{2+}$ triggered the Fenton reaction and produced multiple free radicals, including hydroxyl radicals, which were regarded as the most active radicals and were capable of oxidizing vulnerable volatiles. Fe$^{3+}$ produced during the process could be rapidly reduced to Fe$^{2+}$ by EGCG, which promoted the transformation of semiquinone to quinone. Taken together, the presence of Fe$^{2+}$ might promote the degradation of catechins to generate H$_2$O$_2$ and reacted with H$_2$O$_2$ to produce highly active radicals to deteriorate the aroma of green tea infusion. Our results partially revealed how Fe$^{2+}$ induced aroma deterioration of green tea infusion with heat treatment and implied that elimination of Fe$^{2+}$ in green tea infusion during processing might help to improve the sensory quality of ready-to-drink tea.

![Figure 6. Proposed reaction scheme for the effect of ferrous ion on heat-induced aroma deterioration of green tea infusion.](image-url)
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