



Article

Determining the Relationship between Aroma and Quality of Bao-Chung Tea by Solid-Phase Microextraction (SPME) and Electronic Nose Analyses

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Abstract: Despite extensive studies, the relationship between the quality/quantity of tea odorants and oolong tea quality remains unclear. To investigate the key components affecting Bao-chung tea quality, we collected samples of different grades from a tea-tasting competition and determined the content and composition of volatile components and individual catechins using gas chromatography–mass spectrometry and high-performance liquid chromatography. We used an electronic nose (E-nose) to collect odor component signals and established a quality recognition model. The different tea grades did not significantly differ in catechin content, but their specific odor intensity and proportion of odor components varied significantly. Linear discriminant analysis showed that the intensity and proportion of volatile organic compounds could be used for distinguishing the different grades of Bao-chung tea. By combining different quantities of indole, linalool, and butanoic acid and proportions of *p*-cymene, *cis*- β -ocimene, nonanal, allo-ocimene, *cis*-jasnone, and α -farnesene, the ability to distinguish among Bao-chung tea grades was significantly improved. Our results revealed that the quality of Bao-chung tea should be evaluated based on the combined perception of odor component intensity and proportion rather than solely relying on the concentration or composition of specific compounds. Therefore, individuals can judge a Bao-chung tea grade based on the combined perception of odor component intensity and proportion. The E-nose can be used to identify Bao-chung tea grades based on its ability to determine the odorant composition.

Keywords: Bao-chung tea; electronic nose; oolong tea; quality; volatile organic compounds



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1. Introduction

Oolong tea generally refers to a wide range of partially oxidized (partially fermented) varieties of tea, which are categorized based on their aroma characteristics and the production process used to prepare them [1]. In Taiwan, partially oxidized teas include Bao-chung, ball-type oolong, oriental beauty, and Tieh-Kuan-Yin teas. Bao-chung tea consists of strip-shaped, partially oxidized tea leaves with pleasant floral notes [2]. By contrast, ball-shaped oolong tea consists of spherical, partially oxidized tea leaves, which usually exude floral, fruity, and roasted aromas [3].

Understanding oolong tea quality and characteristics is important for deconstructing the combination of compounds that afford its unique olfactory signature and for developing new tea characterization technologies. In recent years, the effects of various tea quality measures, such as the type of tea production technology used, tea variety, cultivation environment, fertilizer management, and severity of insect damage, have been previously investigated [4,5]. Several previous studies have focused on specific tea aroma compounds (such as indole, linalool, nonanal, and *cis*-jasnone) attributable to different production processes [2,6] and have mainly explored variations in tea composition originating from the cultivation and manufacturing perspectives. To improve tea quality, the key components

that affect tea quality must first be identified before the component proportions that give rise to a given set of tea characteristics can be determined [7–9].

The relationship between the quality of green and black tea varieties and their volatile and non-volatile components has been intensively investigated. High-quality black tea generally contains high contents of theaflavin [10,11] and some particular aroma compounds [12,13]. However, higher-grade green teas taste bitter and astringent despite having high amounts of sweet and umami components [14,15]. Specific non-volatile compounds such as catechins, theaflavins, and thearubigins may be used to distinguish between quality grades of green or black tea. However, few studies have explored the relationship between the quality and chemical composition of partially oxidized teas [5].

In this study, we investigated the relationship between aroma components and the quality of a partially oxidized tea. Our study focused on Bao-chung tea (an elegant tea with a flower-like fragrance and refreshing taste [3]) produced from the Chin-Shin-Oolong variety, which is considered the most popular variety of Bao-chung tea among tea drinkers. Deconstructing some of the fermented components that affect tea quality is challenging. To reduce the number of factors that influence the quality-related components of Bao-chung tea, we collected stable-grade tea samples of the same variety, which were prepared using the same production process as those used by participants from different competitions. These participants may have used other field management and manufacturing processes for the same variety of Bao-chung tea.

Gas chromatography–mass spectrometry (GC–MS) has long been used to analyze the quality and quantity of specific tea aroma components [16–18] because of its ability to separate, identify, and quantify the volatile components present in tea. We used GC–MS to examine the relationship between specific aroma compounds and Bao-chung tea grades to prove that this aroma information could serve as a determinant index for grading Bao-chung tea quality and be the foundation of the electronic nose (E-nose) technology. An E-nose is capable of aroma sensing and identification and can be used as a rapid technique for grading Bao-chung tea quality.

The metal oxide semiconductor-based E-nose is a type of E-nose technology that facilitates a rapid differentiation of aromas [19]. Although this E-nose cannot distinguish individual chemical components, it can identify complex odors using sensor arrays and has been extensively proven to be effective in discriminating tea aromas [19,20]. Recently, some studies have used E-noses with electronic tongues or spectral analyses to distinguish the quality, variety, and origins of various teas [19,20]. Useful results have been obtained previously, but the precise origin of the odor differential effects remains challenging because of the difficulty in analyzing individual odor components using the E-nose. Although the E-nose can be used for various odor discrimination applications, it cannot be used to identify which odor molecules are responsible for the discrimination. By contrast, GC–MS analysis can provide additional experimental data to help elucidate the E-nose discrimination results.

Human olfaction can distinguish a wide range of odors; however, understanding how these compounds can be utilized to assess tea quality remains challenging. Comprehending the olfactory compositions that influence tea quality is an essential pathway to improving overall characteristics of tea. Therefore, this study employs the widely used linear discriminant analysis (LDA) to link GC–MS, the E-nose technology, and human sensory evaluations. By doing so, we aim to gain a deeper understanding of how the concentration and proportional composition of different odor compounds affect tea quality, thereby providing valuable insights for enhancing tea quality in the future.

In addition, tea aroma may be affected by the properties of tea extraction solvents [21]. Therefore, to prevent solvents from affecting the properties of the teas, most previous studies used deionized water or NaCl additives in the extraction step [21,22]. Although earlier studies have confirmed that different extracts affect the composition of tea aroma [17,21], data on whether different solvents affect the performance of volatile organic compounds (VOCs) in tea quality grading are scarce. To confirm and collect the maximum information on the quality

and quantity of aroma components that are similar to those assessed by judges evaluating brewed tea, we studied two extracts with different mineral compositions to understand how different extracts affect the aroma of tea dregs after extraction. In addition, we analyzed the tea aroma components to establish the correlation between aroma components and tea quality.

2. Materials and Methods

2.1. Tea Source

We used 24 tea samples collected from the 1500 tested and graded by a panel of judges at the 2021 Pinglin Bao-chung Tea Spring Tea Competition held by the New Taipei City Government. The panel of judges of the tea competition consisted of trained and selected evaluators appointed by the government [3]. The judges conducted the reviews in a double-blind manner and assessed each tea sample individually. The brewing process followed the specifications outlined in the ISO 31031 standard, which involved steeping 5 g of tea leaves in 150 mL of water. The samples were classified into five grades: first, second, third, formal, and disuse. The tea we purchased had the following grades: grade 1 (combination of 1st and 2nd grades), grade 2 (3rd grade), and grade 3 (formal grade). For each grade, 300 g of eight different tea samples were randomly purchased and stored under the same vacuum conditions before evaluation. An appropriate amount of tea was retrieved from the vacuum packaging for each GC–MS or E-nose analysis. The remaining tea was again vacuum packed to maintain its aroma.

2.2. Analysis of the VOCs in Tea

To perform tea extraction, we added 5 g of each tea sample and 150 mL of water filtered by reverse osmosis (RO; 475 M Ω cm resistivity at 25 °C) or deionized water (18.2 M Ω cm resistivity at 25 °C) at 100 °C into a 150 mL serum bottle. The bottle was then covered with a polyethylene (PE) material. We performed the tea extraction step for chemical analysis using the same amount of the tea sample and the same brewing liquid as in the competition sensory evaluation. After 5 min of extraction, the water was decanted, and the serum bottle with the tea leaves was immediately covered with a Teflon cap. Then, the bottle was placed at the center of a heating tray at 40 °C (the human evaluation was conducted at the same temperature). A solid-phase microextraction (SPME) needle was inserted into the serum bottle through the Teflon cap to collect the VOCs released from the brewed tea leaves placed in a dry bath maintained at 40 °C. The SPME needle was removed after 20 min of VOC collection, and the collected sample was analyzed by GC–MS.

GC–MS analysis was performed as follows: the SPME fiber [50/30 μ m divinylbenzene/carboxen/polydimethylsiloxane, Supelco, St. Louis, MO, USA] with adsorbed VOCs was inserted into the inlet of the GC–MS apparatus (Agilent 6890 GC coupled with an Agilent-5973 mass spectrometer, Agilent Technologies Inc., Santa Clara, CA, USA) for desorbing the VOCs. The inlet temperature was 250 °C. The mass spectrometer was equipped with a capillary column (30 m $\times$ 0.25 mm and 0.25 μ m thickness; HP-5MS UI), which was used for the chromatographic separation. The temperature of the oven was initially maintained at 40 °C for 2 min, increased to 90 °C at a rate of 15 °C min^{−1}, and further increased to 125 °C at a slower rate of 3 °C min^{−1}. Finally, the temperature was increased to 220 °C at a rate of 10 °C min^{−1} and held for 2 min; a method used by Lin et al. [2] and Lin, Ya-Hsin, and Chen [6] was modified and used herein. Helium was used as the carrier gas at a constant pressure of 6 psi (>99.999%) during chromatography. Mass spectrometry was conducted at an ionization voltage of 70 eV.

The mass spectra were obtained through automatic scanning, and the fragmentation pattern of each compound was compared with that obtained from the National Institute of Standards and Technology (NIST 11) database. The matching factor was ≥ 80 , indicating the presence of the following putatively identified compounds: benzaldehyde (grass and floral aroma [23]), *p*-cymene (rosemary aroma [24]), D-limonene (citrus-like and herbal aroma [25]), *cis*- β -ocimene (citrus or spicy aroma [26]), *trans*- and *cis*-linalool oxide (earthy, floral, and creamy aroma [27]), linalool (floral aroma [28]), hotrienol (floral aroma [17]),

nonanal (sweet, floral and fruity aroma [23,29]), allo-ocimene (floral aroma [30]), butanoic acid, 3-hexenyl ester (floral and fruity aroma [31]), (E)-hexanoic acid, 3-hexenyl ester (rancid aroma [29]), (Z)-geraniol (rose-like aroma [28]), indole (animal-like aroma [28]), hexanoic acid (sweaty, green aroma [32]), *cis*-jasmone (floral, jasmine-like aroma [28]), caryophyllene (woody-spicy, dry, and clove-like aroma [33]), *trans*- β -Ionone (woody and violet aroma [28]), α -farnesene (fruity, herbaceous aroma [33]), and nerolidol (floral aroma [34]).

The retention index (RI) of the VOCs was calculated using the retention time of the compounds and a standard saturated *n*-alkanes mixture (C8–C20) (Sigma-Aldrich, St. Louis, MO, USA) obtained under the same GC–MS conditions, and the values were published by the NIST library [6].

The extraction and analysis of each tea sample were conducted in two batches, and the external standard curves (ethyl decanoate ($\geq 98\%$), Sigma-Aldrich Co., St. Louis, MO, USA) were used to quantify the concentrations of each volatile compound. The external standard curves ranged from $0 \mu\text{g mL}^{-1}$ to $220 \mu\text{g mL}^{-1}$, with an R^2 value of 0.9985. The average of the two batches of each sample was referred to as the intensity index of that compound in each case. The concentrations of all the volatile aroma compounds mentioned above were integrated, and the proportion of each compound was calculated as the relative content of that chemical component.

2.3. Analysis of Catechins in Tea

The tea sample was ground, and 0.5 g of the powder was added to 50 mL of boiling deionized water and allowed to stand for 20 min for extraction. The entire extraction process was conducted in a water bath at 90°C , and the tea extract was cooled and filtered using a $0.45 \mu\text{m}$ syringe filter. The filtered liquid was subsequently analyzed by high-performance liquid chromatography (HPLC, Agilent 1290 infinity II, 1290 DAD FS detector, Agilent Technologies Inc., Santa Clara, CA, USA) using a C18 column ($5 \mu\text{m} \times 4.6 \text{ mm} \times 250 \text{ mm}$; Waters, Milford, MA, USA). The column was eluted using 0.1% formic acid (*v/v*) in water (solvent A) and acetonitrile (solvent B) as the mobile phases, using the following gradient: a linear increase of B from 1% to 10% over 15 min and then from 10% to 20% over 14 min, followed by an increase from 20% to 22% within 6 min. Finally, the mobile phase was maintained at 22% of B for 5 min at a flow rate of 1.0 mL min^{-1} . Absorbance at 280 nm was used for the real-time monitoring of peak intensities [2,6]. The concentrations of epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), gallic catechin (GC), gallic catechin gallate (GCG), catechin (C), and catechin gallate (CG) were determined by comparing their peak areas with those of their respective standards (Sigma-Aldrich, St. Louis, MO, USA).

2.4. E-Nose Analysis of Tea Leaves

Tea powder (5 g) and filtered RO water (150 mL) at 100°C were added to a 150 mL serum bottle, and the bottle was covered with a closed PE material for tea extraction. After 5 min of extraction, the water was decanted. The bottle with the tea leaves was immediately covered with a Teflon cap that allowed the insertion of the 3 mm long adsorption pipeline of the E-nose device. Then, the bottle was placed in a heating tray at 40°C during each E-nose analysis.

The E-nose used in this study was a device with 14 metal oxidation sensors (ENOSIM BIO-TECH CO, LTD), which were used to collect the aroma compounds of tea leaves after brewing. Aroma analysis using the E-nose was performed on every tea sample. Prior to collecting the data from each sample, an environmental odor balance operation was performed for 10 min using the E-nose. After the equilibration process, the background air in the environment was drawn from the serum bottle into the sensor analysis chamber at an airflow rate of 300 mL min^{-1} for 1 min of data collection. After the odor data of the brewed tea leaves were collected, the E-nose adsorption pipeline was drawn away from the tea sample. The aroma compounds were desorbed until the sensor returned to the level before sensing. Then, sufficient time was allowed before the next sample was analyzed.

The E-nose signal was acquired using the automatic acquisition system of the equipment. The formula for calculating the change in resistance is as follows:

$$\text{Resistance difference ratio} = (R_{\max} - R_{\text{base}}) / R_{\text{base}}$$

$$\text{Maximal slope of resistance} = \text{Max}\{R_{\text{slope}}\}$$

$$\text{Maximal slope of voltage} = \text{Max}\{V_{\text{slope}}\}$$

2.5. Statistical Analysis

The experimental data were analyzed using analysis of variance, using CoStat 6.451 (Cohort Software, Monterey, CA, USA), and the difference in significance was determined using the least significant difference (LSD) method. We performed Pearson's correlation analysis using CoStat 6.451 and regression analysis of the scatter plots using SigmaPlot. We used SYSTA version 13 to perform LDA. All data were plotted using SigmaPlot version 14.0.

3. Results and Discussion

3.1. Effect of the Extraction Solvent on the Aroma of Tea

We used water purified by RO and deionized water to investigate the effect of different extraction solvents on the aroma volatilization of tea residues after extraction.

The results of using two types of water to brew tea revealed the emission of 19 main aroma compounds from the tea residue of the Bao-chung tea extracted with RO water: benzaldehyde, *p*-cymene, D-limonene, *cis*- β -ocimene, *trans*- and *cis*-linalool oxide, linalool, hotrienol, nonanal, allo-ocimene, butanoic acid, 3-hexenyl ester, (E)-hexanoic acid, 3-hexenyl ester, (Z)-geraniol, indole, hexanoic acid, *cis*-jasmane, caryophyllene, *trans*- β -ionone, α -farnesene, and nerolidol. By contrast, only eight aroma components in the tea residues were extracted with deionized water: benzaldehyde, *cis*- β -ocimene, linalool, allo-ocimene, butanoic acid, 3-hexenyl ester, (E)-hexanoic acid, 3-hexenyl ester, (Z)-indole, hexanoic acid, and α -farnesene (Table 1).

Table 1. Odor descriptions and odor threshold values of the identified volatiles in Bao-chung tea from collected samples.

No.	Volatile Compounds	RI ^z	Odor Description ^y	Threshold ^x
1	Benzaldehyde	880	Grass, floral	350
2	<i>p</i> -Cymene	1010	Rosemary	11.4
3	D-Limonene	1013	Citrus-like, herbal	200
4	<i>cis</i> - β -ocimene	1027	Citrus, spicy	1.2036
5	<i>trans</i> -Linalool oxide	1049	Earthy, floral, creamy	3000
6	<i>cis</i> -Linalool oxide	1049	Earthy, floral, creamy	3000
7	Linalool	1072	Floral	0.22
8	Hotrienol	1076	Floral	110
9	Nonanal	1076	Sweet, floral	1.1
10	Allo-ocimene	1111	Floral	N.A. ^w
11	Butanoic acid, 3-hexenyl ester, (E)-	1149	Floral, fruity	781
12	Geraniol	1213	Rose-like	7.5
13	Indole	1251	Animal-like	40
14	Hexanoic acid, 3-hexenyl ester, (Z)-	1342	Rancid	40
15	<i>cis</i> -Jasmone	1375	Floral, jasmine-like	7
16	Caryophyllene	1388	Woody-spicy, dry, clove-like	14
17	<i>trans</i> - β -ionone	1470	Woody, violet	0.007
18	α -Farnesene	1495	Fruity, herbaceous	87
19	Nerolidol	1574	Floral	250

^z RI, retention index, which is calculated, refers to the retention time of C5–C28 *n*-alkanes under the same conditions. ^y Odor descriptions were found in the literature [16,24–34]. ^x All odor threshold information was found in the literature [25,35]. ^w 'N.A.', no odor threshold information was found in the literature.

The indole content of the volatiles from the tea dregs after the deionized water extraction had the highest correlation with the indole content of the volatiles from the tea dregs obtained after the RO water extraction ($R^2 = 0.71$). β -Ocimene and allo-ocimene had moderate correlations ($R^2 = 0.58$ – 0.68), whereas almost no correlation was found for the other components (Figure 1).

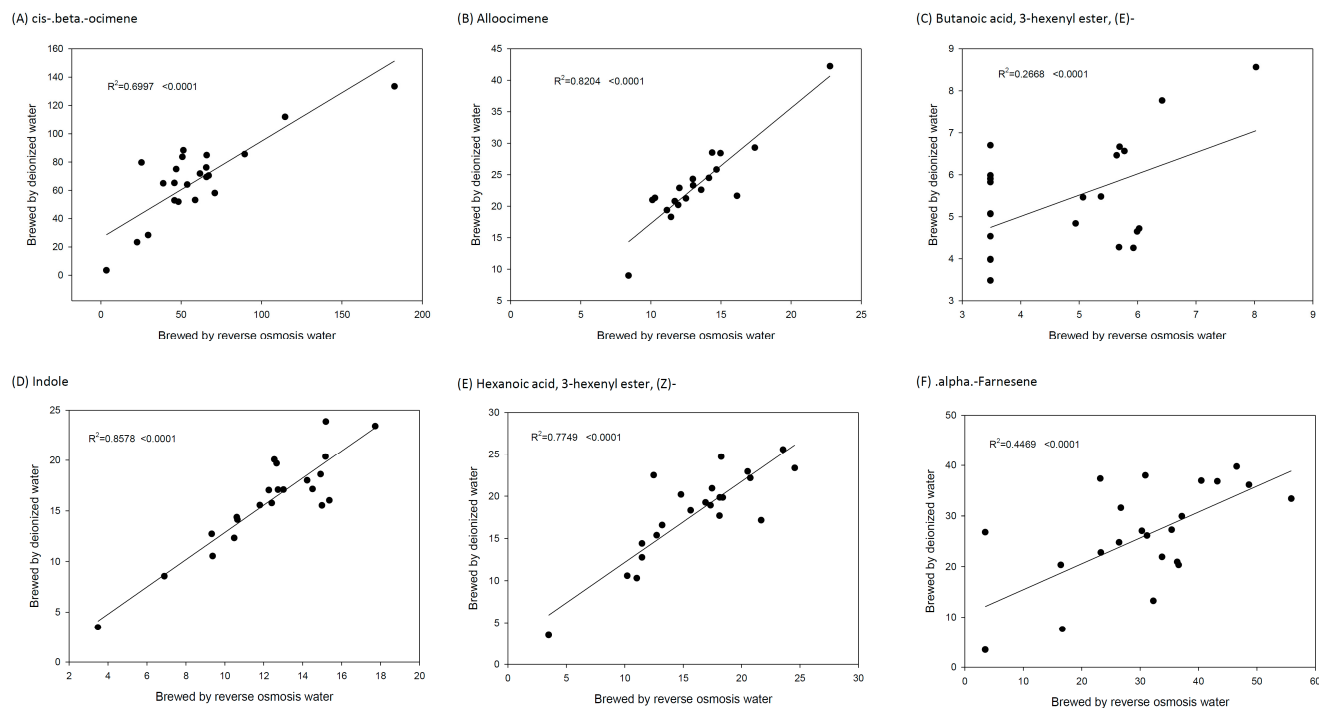


Figure 1. Relationship between each volatile organic compound (VOC) of Bao-chung tea leaves brewed using reverse osmosis (RO) water and deionized water. Each point indicates the average of two batches of analysis for each tea sample by RO or deionized water separately. The concentration ($\mu\text{g mL}^{-1}$) represents the quantitative result of each compound relative to the external standard (Ethyl decanoate).

These results reveal that different extraction liquids affect the volatilization state of the components of brewed tea leaves, affecting the results of GC–MS analysis and sensory evaluation. To compare the results of the instrumental analysis with those of the sensory evaluation performed by the panel of judges and to determine the key aroma components used for grading Bao-chung tea, we used RO water in this study. RO water is similar to that used by tea tasters for tea brewing and aroma extraction. We performed GC–MS and E-nose odor analysis to obtain the aroma composition state closest to the sensory evaluation conducted by the tea experts. We used the results of the GC–MS and E-nose measurements to identify the key aroma components affecting the quality of Bao-chung tea.

3.2. Volatile Components of Different Bao-Chung Tea Grades

Among the 19 main volatile compounds extracted from the Bao-chung tea dregs, the intensity of the floral aroma compounds, which are considered key quality-related compounds [16,22–25] such as allo-ocimene, *cis*-jasnone, and α -farnesene (contributing to floral, jasmine-like, and fruity aroma, respectively [28,30,33]), was significantly higher in higher-quality grade tea samples. The intensity of the fresh aroma compound, *cis*- β -ocimene [26], was also higher in superior-grade tea samples. These results demonstrate that Bao-chung tea possesses unique floral and fruity aromas while exhibiting a refreshing fragrance.

Further, as shown in Figure 2, the average values of VOCs in each tea grade were different, as determined by ANOVA analysis. However, a significant overlap in the aroma intensity was observed between tea samples of different grades. This result clearly suggests

that a specific aroma compound intensity alone cannot distinguish the tea grade. These results also explain why identifying a particular compound as a quality indicator has been difficult in previous oolong tea studies and why the key component indicators (chemical markers) determined by extensive studies are difficult to apply in the tea industry [3,5,9,36].

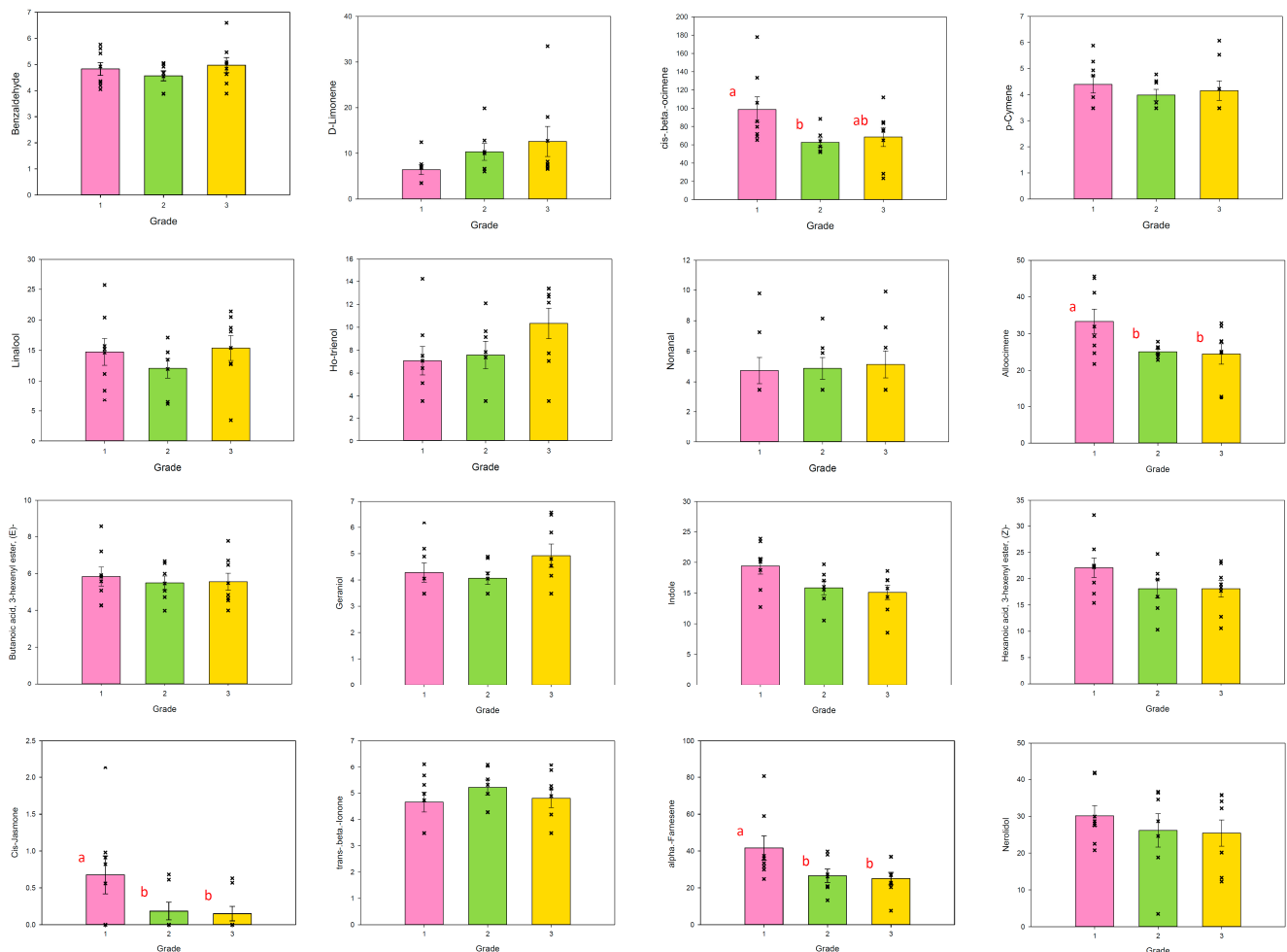


Figure 2. Intensity of VOCs released from brewed tea leaves of different Bao-chung tea grades. Each dot indicates the average of two batches of analysis for each tea sample brewed with RO water. Each bar represents the average of each tea grade set, and the error bar indicates the standard error of each grade set. The concentration ($\mu\text{g mL}^{-1}$) represents the quantitative result of each compound relative to the external standard (ethyl decanoate). Different lower case letters suggest significant differences among different quality levels indicated by the LSD test at $p \leq 0.05$.

The ratios of D-limonene and α -farnesene were different in tea samples of different grades. Similar to aroma intensity, the ratio of these components alone cannot be directly used as an absolute indicator for judging the tea grade (Figure 3).

Based on the results presented earlier, we conclude that no individual VOC intensity or proportion can fully differentiate different quality grade teas. However, when considering the human perception of aromas, the combined influence of both the intensity and proportion of aroma molecules is a crucial consideration. The aroma compound α -farnesene displayed significant differences in content and proportion in the tea sample grades that we analyzed; therefore, it may be considered an important aroma indicator as it satisfies both the “quality” and “quantity” aspects necessary in human aroma perception. Our results indicate that no individual VOC intensity or proportion can fully discriminate among different tea quality grades. However, when considering the human perception of an aroma, the combined contribution of various elements that influence the intensity

and proportion of the aroma molecules must be considered. Among the different grades of the tea samples analyzed, α -farnesene displayed significant differences in both content and proportion; therefore, it may be considered an important indicator as it satisfies both the “quality” and “quantity” aspects necessary for human aroma perception. Thus, we hypothesize that α -farnesene could be a key aroma molecule for indicating the quality of Bao-chung tea.

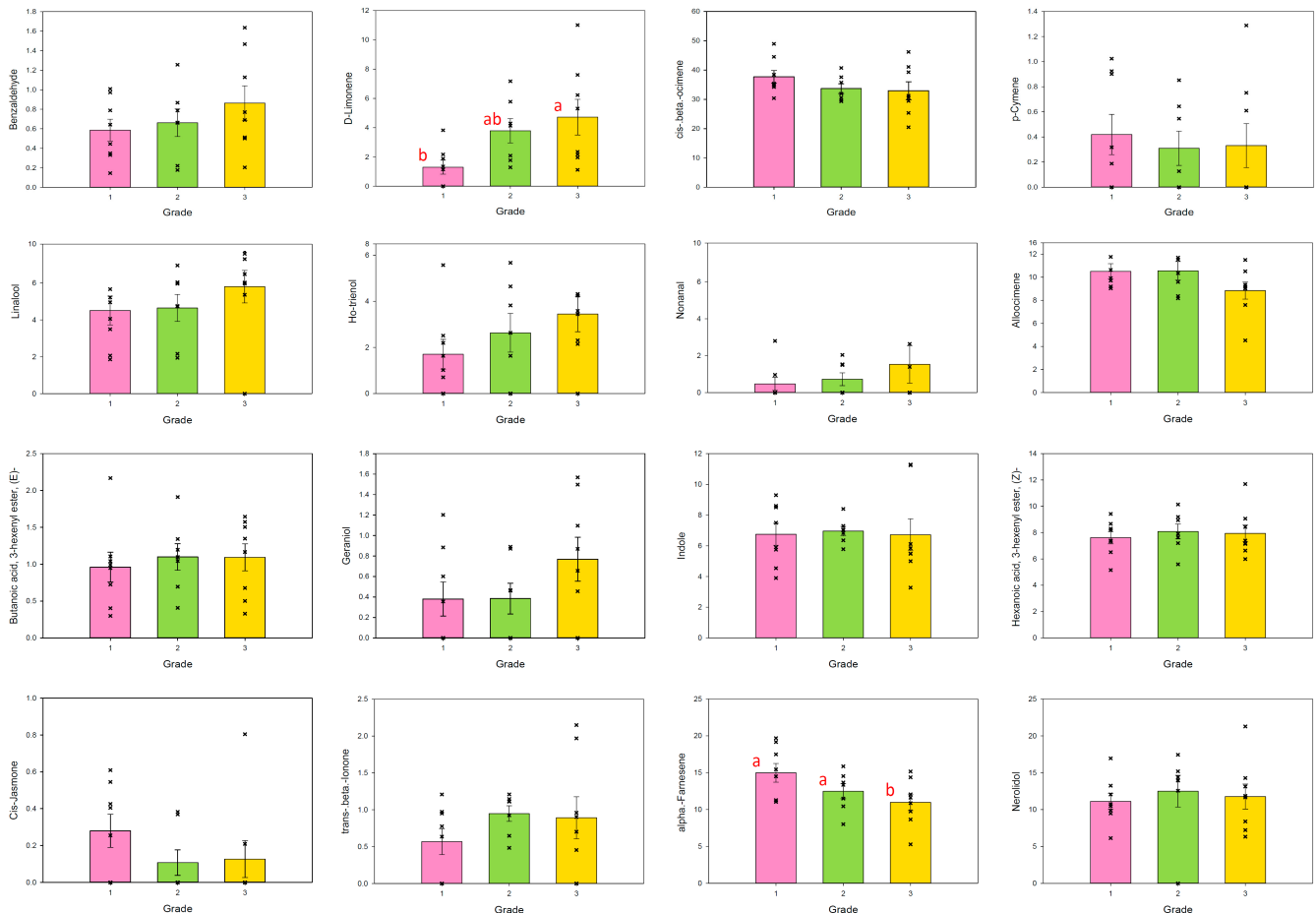


Figure 3. Ratio of VOCs released from brewed tea leaves of different Bao-chung tea grades. Each dot indicates the average of two batches of analysis for each tea sample brewed with RO water. Each bar represents the average of each tea grade set, and the error bar indicates the standard error of each grade set. The ratio (%) indicates that the peak area of a specific compound is proportional to the total peak area of all compounds. The concentration represents the quantitative result of each compound relative to the external standard (ethyl decanoate). Different lower-case letters suggest significant differences among different quality levels indicated by the LSD test at $p \leq 0.05$.

Given the difficulty of performing grade discrimination for a single specific component, we applied forward stepwise LDA to distinguish different grades of tea. The results indicate that different grades of Bao-chung tea cannot be well distinguished based on the VOC intensity or proportion. However, three grades could be well distinguished based on the combination of the VOC intensity and proportion (Figure 4). The LDA results obtained using both the intensity and proportion parameters indicate that higher proportions of *p*-cymene, *cis*- β -ocimene, nonanal, allo-ocimene, *cis*-jasmone, and α -farnesene, a higher quantity (intensity index) of indole, and lower amounts of linalool and butanoic acid would improve tea quality.

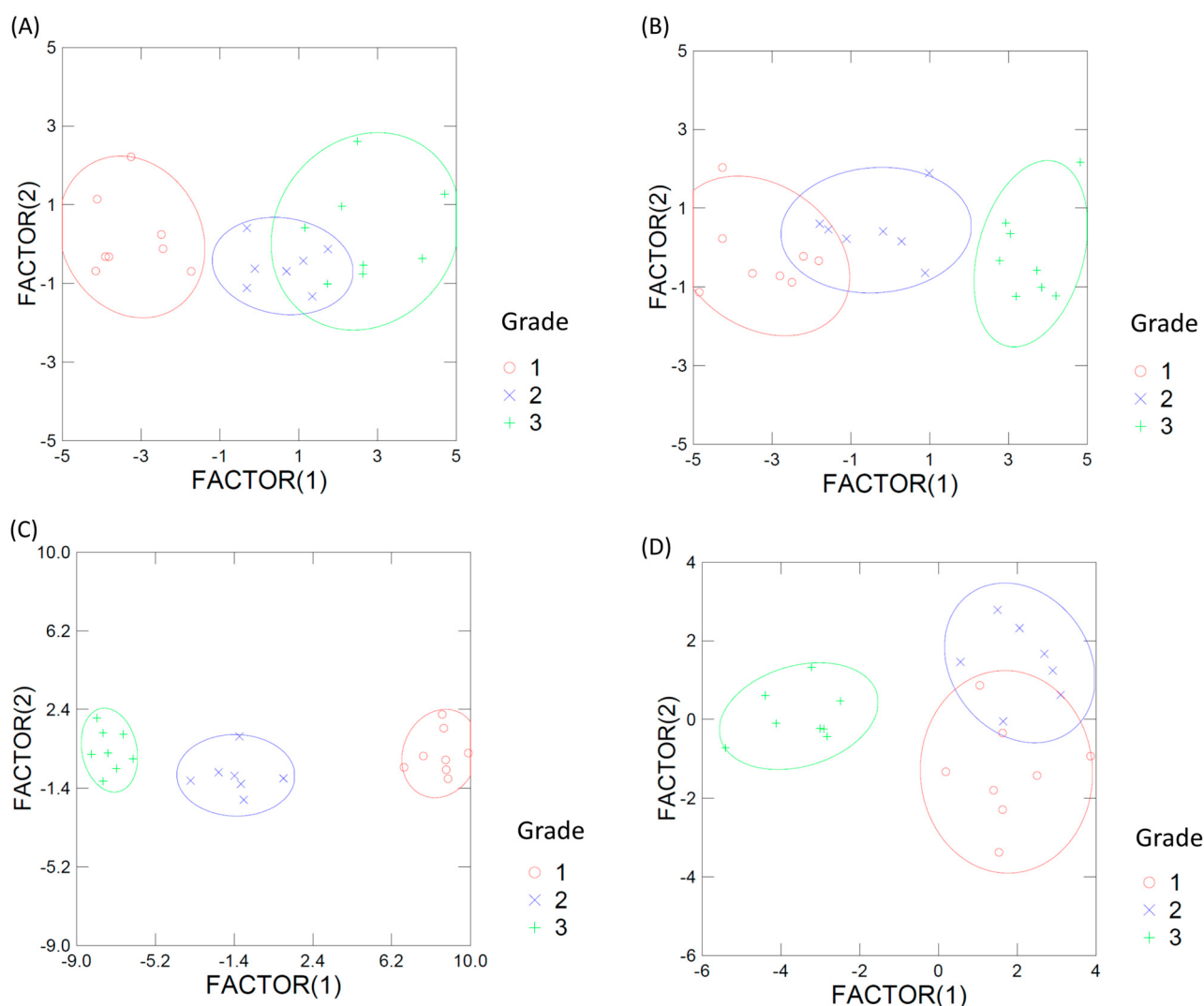


Figure 4. Distinguishing results of different Bao-chung tea grades based on LDA: (A) intensity of VOCs, (B) ratio of VOCs, (C) intensity and ratio of VOCs, and (D) contents of eight individual catechins of brewed tea leaves.

The GC–MS results indicate that although a single specific VOC cannot be used to judge the quality of Bao-chung tea, the tea grade can be distinguished from its aroma compound composition. The results also suggest that the individual aroma intensity or proportion may not be the key quality indicator; instead, the aroma compound composition should be considered in quality evaluation. This may be because humans focus more on comprehensive sensation, which includes a collective influence of the intensity and ratio of aroma molecules released from tea rather than only the aroma strength. Based on these results, the aroma characteristics can be used as an index to judge the quality of Bao-chung tea, providing an essential theoretical basis for using an E-nose to distinguish Bao-chung tea quality.

3.3. Catechin Contents of Different Bao-Chung Tea Grades

Catechin content is a key factor affecting the bitterness and astringency of a tea [37,38]. Therefore, catechin content is often used as an indicator to judge tea grades. However, in the superior-grade Bao-chung tea samples (i.e., the tea samples qualified in the competition) collected in this study, we found no significant differences in the content of individual catechins in the tea leaves of different grades, and the distribution of individual catechins in differently graded Bao-chung tea was highly similar (Figure 5).

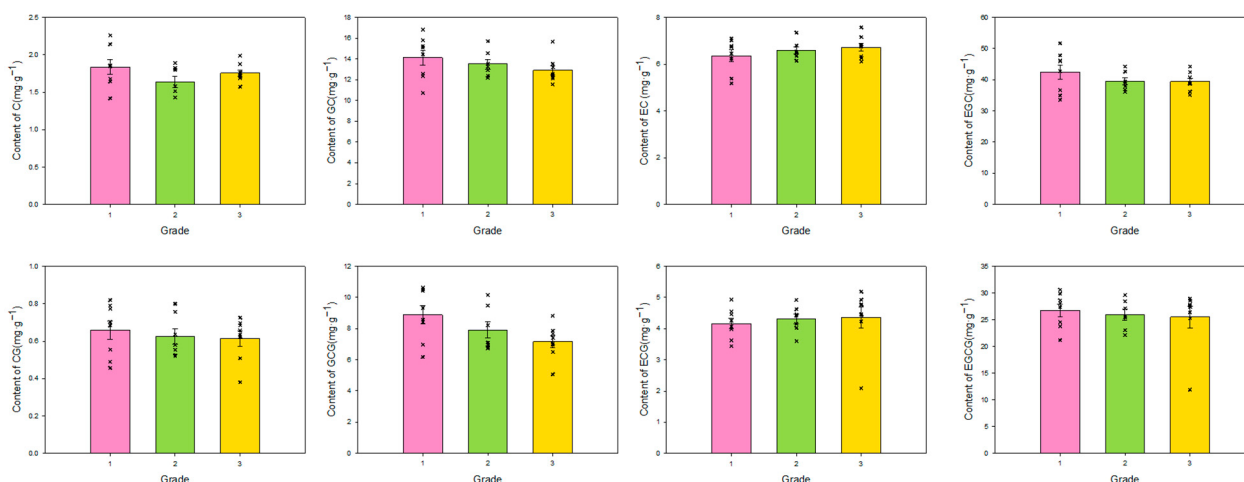


Figure 5. Catechin content in the brewed tea samples of different Bao-chung tea grades. Each dot indicates the average of two batches of analysis for each tea sample brewed with RO water. Each bar represents the average of each tea grade set, and the error bar means the standard error of each grade set.

Many studies have demonstrated that different catechins can be used for grading green and black teas [39–41]. In addition, other studies [42,43] have suggested that catechins are grading indicators for specific partially oxidized tea types. However, the catechin content may not necessarily serve as a quality indicator in oolong tea or partially fermented teas. Some studies have indicated that catechins cannot serve as quality indicators for partially oxidized teas [44,45]. Our study revealed similar results; when LDA was used for grouping, only grade 3 tea could be discriminated from grade 1 and 2 tea samples, and the samples belonging to grades 1 and 2 could not be discriminated (Figure 4). This result may have occurred because previous studies primarily analyzed tea samples with significant differences in grades, where the degree of bitterness may have been the key factor affecting tea quality. However, catechin content is difficult to use as an indicator for discriminating tea of grades 1 and 2 because samples of higher quality have a similar range of bitterness.

LDA results indicate that although we cannot use individual catechins as key indicators of Bao-chung tea grades, they have a specific impact on quality. In higher quality Bao-chung tea, the smell components may be more important than the taste components; this also indirectly suggests that compared to other types of partially oxidized teas, Bao-chung tea was developed to emphasize aroma expression.

3.4. Relationship between the VOC of Bao-Chung Tea and the Contents of Individual Catechins

To further elucidate the relationship between the quality of Bao-chung tea and its composition and to identify the scientific basis for the grading of Bao-chung tea by experts, a correlation study and a forward stepwise regression analysis of the VOC data of tea samples used in the test and the catechin data were conducted. The results revealed that the correlation between individual VOCs and catechins was only as high as $R = 0.58$. However, VOC data can be used to predict the contents of individual catechins, particularly in the prediction of free form catechins ($R^2 > 0.819$) (Figure 6).

Therefore, we can infer the individual catechin content of tea leaves from the state of the odor composition. When the ratio of D-limonene to nonanal was higher, the contents of the respective catechins were lower. Similarly, when the ratio of *cis*- β -ocimene to hotrienol was higher, the contents of the respective catechins were lower. Therefore, when the aroma composition of Bao-chung tea is more fragrant and floral, the catechin content will be lower, probably because the oxidation of catechins can form specific aroma compounds [46,47]. However, when the catechin content is too low, the degree of oxidation may be too high [2]. As the oxidation level increases, the tea may transform into other types of oolong tea, which may have different aromas, and deviate from the unique characteristics of Bao-chung tea [3]. In this case, the taste becomes too weak [21], and a smell that does not belong to

Bao-chung tea is produced [3]. Therefore, based on the combined results of the aroma data mentioned above, we infer that the smell of Bao-chung tea has a greater impact on its quality than its taste. Because the catechin content is the key source of bitterness and flavor in sensory evaluation, we deduce the possible reasons why tea taste experts use the smell of Bao-chung tea to estimate its taste and quality.

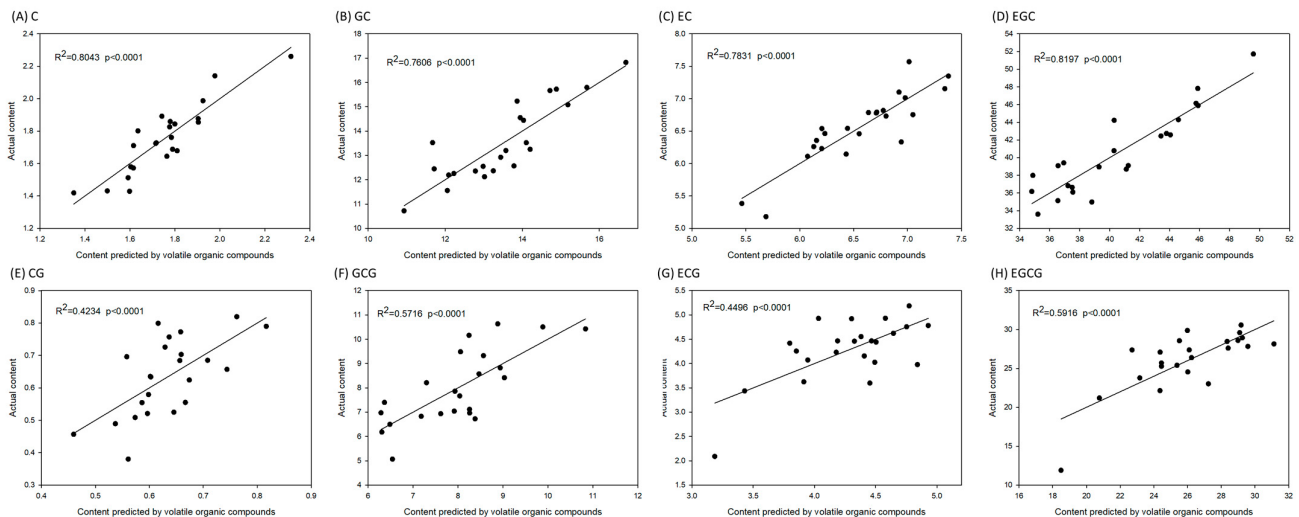


Figure 6. Relationship between the relevant catechin content predicted by the regression equation of volatile organic compounds and the actual catechin content of Bao-chung tea. Each point indicates the average of two batches analysis for each tea sample.

3.5. Quality Grading of Bao-Chung Tea Using E-Nose

According to previously discussed results, the quality of Bao-chung tea can be judged based on its aroma, which lays the foundation for classifying Bao-chung. Using the nine characteristic data obtained by four E-nose sensors, the 24 tea samples evaluated in this study could be efficiently graded (Figure 7). This observation indicates that the E-nose is not only suitable for grading green [48] and black [49] tea but also for classifying Bao-chung tea.

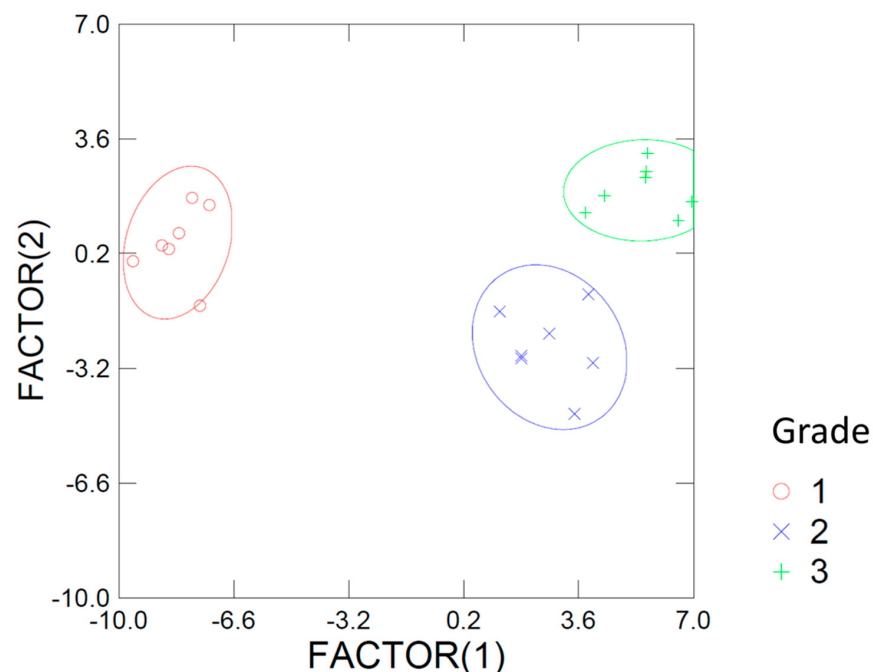


Figure 7. LDA distinguishing results of different Bao-chung tea grades from the E-nose data.

4. Conclusions

This study is the first comprehensive work to investigate the relationship between the VOCs, catechins, and Bao-chung tea quality grades. The results indicated that the key aroma components in Bao-chung tea include indole, linalool, butanoic acid, *p*-cymene, *cis*- β -ocimene, nonanal, allo-ocimene, *cis*-jasmane, and α -farnesene; the concentration ranges of these aroma components in the highest-grade Bao-chung tea samples were 12.73–23.87, 6.8–25.71, 4.26–8.56, 3.48–5.88, 65.22–177.92, 3.48–9.79, 21.76–45.73, 3.48–5.62, and 24.78–80.64 $\mu\text{g mL}^{-1}$, respectively. However, the concentration or ratio of individual compounds cannot serve as the basis for quality differentiation.

The results were obtained by combining the quality and quantity of specific Bao-chung tea aromas (indole, linalool, butanoic acid, *p*-cymene, *cis*- β -ocimene, nonanal, allo-ocimene, *cis*-jasmane, and α -farnesene) for a comprehensive tea aroma evaluation. Our results also demonstrate that this method can be used to efficiently discern different tea grades. This combined-parameter method performed better than a grading method based on individual catechins, indicating that Bao-chung tea is a particular type of oolong tea that emphasizes floral and fresh aromas. These specific aromas are important characteristics of Bao-chung tea and can be used as a reference for further research on improved tea cultivation and processing techniques. Using an E-nose for quickly collecting odor data and subsequent LDA can facilitate a quicker classification of Bao-chung tea grades.

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