Food Chemistry: X 23 (2024) 101534

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Exploring the effect of different tea varieties on the quality of Lu'an Guapian tea based on metabolomics and molecular sensory science

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ARTICLE INFO

Keywords:
Tea varieties
Lu'an Guapian tea
Flavor
Gas chromatography-mass spectrometry
Nonvolatile compounds

ABSTRACT

Lu'an Guapian (LAGP) tea is one of the most famous teas in China. However, research on its suitable processing varieties is still lacking. This study analyzed the quality of LAGP tea made from three different tea varieties, namely, '*Anhui1*' (AH1), '*Quntizhong*' (QTZ), and '*Shuchazao*' (SCZ), using molecular sensory science and metabolomics techniques. The results showed that AH1 had a strong floral aroma and the strongest umami flavor, while QTZ had a distinct roasted aroma and a mellow taste. SCZ had a cooked corn-like aroma and the highest bitterness and astringency owing to the high tea polyphenol contents and low free amino acid contents. The study also identified 12 key aroma-active compounds, with *trans-beta*-ionone and 2-ethyl-3,5-dimethyl-pyrazine contributing the most to floral and roasted aromas, respectively. The results of this study provide a theoretical and practical basis for selecting and breeding high-quality varieties of LAGP tea and stabilizing its quality.

1. Introduction

Green tea is the most produced, varied, and famous tea in China. It has numerous health benefits and a high economic value (Zhang et al., 2024). Each tea cultivar produces green tea with unique characteristics and quality. Therefore, the production of many famous green teas has certain requirements for varieties. For example, Anji white tea, a green tea produced from the '*Baiye1*' tea variety, has high umami and low bitterness and astringency because of its high free amino acid content and low polyphenolic content (Wang, Zhang, Chen, & Jiang, 2020; Yan et al., 2017). Taiping Houkui tea, produced from '*Shidacha*', has a strong orchid aroma and a mellow flavor (Zhou et al., 2022). Unlike other green teas that include both harvested buds and leaves, Lu'an Guapian (LAGP) tea is made only from a single leaf using a unique processing technique. Although it has a characteristic shape and frost on the surface, its strong aroma and the low bitterness and astringency attract the most attention (Xia, 2016).

More than 700 volatile compounds can be detected in tea (Zhai, Zhang, Granvogl, Ho, & Wan, 2022). A variety of methods for extracting aroma compounds from tea have been developed through years of research. Headspace solid-phase microextraction (HS-SPME) is commonly used to analyze compounds with low molecular weight and high volatility in tea. However, this method is deficiencies for extracting molecular weight compounds. Instead, solvent-assisted flavor evaporation (SAFE) can be used to extract high molecular weight compounds with low volatility in tea (Engel, Bahr, & Schieberle, 1999; Hazel et al., 2018). Only a few compounds actually contribute to the flavor of tea (Zhai et al., 2022). Therefore, it is crucial to screen key aroma-active compounds in tea by methods such as calculation of calculate the odor activity value (OAV) (Ma et al., 2022) and modification frequency [MF (%)] (Cullere, San-Juan, & Cacho, 2011; Zhang et al., 2024).

The main components responsible for imparting flavor in green tea are tea polyphenols (TPs), free amino acids, alkaloids, chlorophyll, soluble sugars, etc. Polyphenols, the main component of tea, account for 18–36% of the dry weight of tea leaves and include compounds like catechins, flavonoids, flavonoid glycosides, anthocyanins, phenolic acids, etc. Catechins, as the main components of polyphenols, mainly impart bitter and astringent flavors and are the key flavor compounds that constitute the concentration and strength of tea infusions (Wan, 2003; Zhang, Cao, Granato, Xu, & Ho, 2020). Studies have shown that 70% of the umami taste intensity in green tea is related to amino acids (Wan, 2003). Additionally, the sweetness in green tea is mainly related

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https://doi.org/10.1016/j.fochx.2024.101534
Received 2 February 2024; Received in revised form 19 May 2024; Accepted 2 June 2024
Available online 4 June 2024

to soluble sugars. Metabolomics is the quantitative and qualitative analysis of metabolites, revealing the changing patterns of metabolites in organisms under different conditions (Wen et al., 2023). The commonly used analytical techniques for metabolite analysis include mass spectrometry (MS), high performance liquid chromatography (HPLC), and nuclear magnetic resonance spectroscopy (NMR) (Li et al., 2021). HPLC utilizes high pressure to separate compounds in a sample through a column for quantitative analysis. The method has the advantages of high sensitivity, high speed and high efficiency, which can realize the quantitative analysis of important compounds such as catechins and theaflavins in tea. (Jiang, Engelhardt, Thräne, Maiwald, & Stark, 2015). Ultraviolet (UV) spectrophotometry can be used for the quantitative analysis of compounds based on the selective absorption of light in the UV region (Cole & Levine, 2020; Wei et al., 2022). Additionally, electronic tongue (ET) as a kind of bionic sensing technology that can effectively avoid the errors caused by the interaction of gustatory substances or human subjective preference, has been applied in recent years to describe the overall profile of tea taste flavor (Huang, Lu, Deng, & Ning, 2022; Ren, Li, Wei, Ning, & Zhang, 2021; Zhang et al., 2024).

Studies have reported that the flavor profile of LAGP tea is affected not only by the growing environment and processing techniques but also by the tea tree variety (Xia, 2016; Zhang et al., 2024) reported that the drying method affected the aroma quality of LAGP tea significantly; in particular, pulley charcoal drying significantly enhanced the floral and fruity aroma of LAGP tea. However, research on the suitable varieties for LAGP tea is still lacking. Several tea varieties are now used to produce LAGP tea, resulting in uneven and unstable product quality in the market. Tea varieties like '*Anhui1*' (AH1), '*Quntizhong*' (QTZ), and '*Shuchazao*' (SCZ) are the most commonly processed varieties owing to their wide cultivation area. Therefore, in this study, the quality of LAGP teas produced from these three varieties was investigated using molecular sensory science and metabolomics techniques. The study aims to improve the understanding of the impact of tea varieties on the quality of LAGP tea. Additionally, it provides a theoretical basis for selecting different LAGP tea varieties in production and ensuring the stable development of its products. This study also provides a scientific basis for the directional development of LAGP tea of different qualities from the point of view of raw material production.

2. Materials and methods

2.1. Manufacturing process and collection of tea samples

Three tea varieties—*Camellia sinensis* cv. AH1 (large-leaf variety), *Camellia sinensis* cv. QTZ (medium-leaf variety), and *Camellia sinensis* cv. SCZ (medium-leaf variety)—were selected for this study. The selected tea plantations were located in Lu'an City, Jinzhai County, Anhui Province, China. The samples were collected in April 2022. The mean annual temperature of Lu'an City, Anhui Province, was 17.5 °C in 2021, the annual precipitation was 1173.2 mm, and the annual sunshine hours were 1954 h (NBS, 2021). The second tea leaf of each plant with the same growing period and growing conditions was used to produce the LAGP tea. A traditional processing method was used. Specifically, the tea was first fried in a pot at 110–120 °C for 85–100 s and immediately fried in another pot at 90–100 °C for 90–100 s. The tea was then dried for 15 min in a drying oven. After 24 h, the tea was redried for another 15 min in the same drying oven. Finally, all the tea samples were stored in a refrigerator at 4 °C.

2.2. Chemicals

Table S1 lists the details of the chemicals and materials used in the study. Dichloromethane was used after distillation.

2.3. Fresh leaf data collection

The thickness of the fresh leaves was measured using a digital percentage thickness gauge (BiaoKang, Shanghai, China). The leaf thickness was measured at 1.0 cm from the leaf margin, and measurements were repeated 20 times for each variety. The leaf-blade length and width were measured separately using a scale to calculate the leaf shape index R (Xia, 2016), where R = leaf length (mm) / leaf width (mm).

2.4. Quantitative analysis of volatile compounds

2.4.1. Quantitative descriptive analysis and aroma omission experiments

To accurately describe and analyze the aroma characteristics of the tea leaves, quantitative descriptive analysis (QDA) was conducted in an odorless room at 22 ± 1 °C (Cao et al., 2021). The sensory group comprised 12 panelists, with six men and six women aged 20–30 years, who were postgraduate students of the Tea Science Laboratory of Anhui Agricultural University. Prior to the formal experiment, the team members attended the "Tea Perception Assessment" training course. During the course, they were systematically introduced to the typical aromas of green tea and the appropriate terms to describe them. Each panelist received at least 40 h of sensory discrimination training for four weeks prior to the formal experiment. The trainees identified the odors of different food products and corresponded them with diluted aroma standard solutions. This sensory training enhanced the trainees' ability to perceive the smells and tastes associated with specific flavor and aroma standards. The food products used during the course were mainly the commercially available fruits and vegetables. The aroma standards were diluted as reported in the study by Zhang et al. (2023). Specifically, aroma standards (linalool, geraniol, hexanal, pyrazine, benzaldehyde, dimethyl sulfide, 1-octen-3-ol, etc.) were dissolved in ethanol and then diluted with water up to 100 times the common threshold for each substance.

The experiment consisted of two parts: in the first part, the panelists voted for eight aroma descriptors of tea broths, and those with the highest frequency descriptors of occurrence were retained. In the second part, the corresponding flavor and aroma criteria were developed in reference to these descriptors. The configuration of the aroma standard solution was consistent with that in the training method. Additionally, some of the substances for which it was difficult to find standards were replaced with the food. The eight descriptors and their corresponding criteria are described as follows: fruity (orange), green (hexanal), roasted (2-ethyl-3,5-dimethyl-pyrazine), floral (*trans-beta*-ionone), boiled bean-like (bean milk), malty (2-methyl-butanal), cooked corn-like (dimethyl sulfide), and sweet (coumarin). Each of the participants rated the intensity of each of the eight aroma descriptors. The selected aroma descriptors were rated on the following scales: 0–3.0 (weak or unperceived), 3.0–6.0 (moderate), and 6.0–9.0 (strongly perceived). For each experiment, each participant had to sniff thrice.

Tea infusions were prepared by weighing 8 g of the tea sample, followed by pouring 160 mL of boiling water over it, and finally steeping it for 4 min. Subsequently, 25 mL of the tea infusion was provided to the panelists in 50 mL brown sniff bottles.

Aroma omission experiments refer to Zhang et al. (2024). According to the quantitative results, the concentrations of the 12 detected odorants ($MF \geq 30$, $OAV \geq 1$) were used as the standard. All odorants were first dissolved in ethanol and the 12 key aroma compounds were added to deionized water at the existing concentrations of the samples. The 12 odorants with one component omitted from each and a control sample containing all odorants were prepared separately. Trained panelists assessed the differences between each omitted model and the full reconstructed model by triangulation tests. The significance level of the triangulation test was as follows: in a group of 12 people, the result was considered significant ($\alpha < 0.05$) if 8 people answered correctly. If 9 people answered correctly, the result is highly significant ($\alpha < 0.01$). If 10 people answered correctly, the result was considered significant ($\alpha <$

0.001).

2.4.2. Extraction of volatiles by HS-SPME and SAFE

For HS-SPME, volatile compounds were extracted using SPME fibers using the procedure reported by Wei et al. (2022). Briefly, tea samples (8 g) were weighed in a conical flask, added to 160 mL of deionized boiling water to brew, soaked for 4 min, and filtered into another conical flask using a funnel. Subsequently, 10 mL of the cooled tea infusion, 3 g NaCl, and 10 μL of 5 μg/L ethyl decanoate were taken in a 20 mL headspace bottle and immediately sealed. The mixture in the bottle was thoroughly homogenized using a vortex mixture, following which the bottle was placed in a water bath at 50 °C for 15 min. The SPME fibers were subsequently passed through the pores to enable volatile adsorption 1 cm above the liquid for 50 min. The fibers were then immediately inserted into a gas chromatography–mass spectroscopy (GC–MS) injector for thermal desorption (Zhang et al., 2023).

For SAFE, the tea broth was obtained under the same brewing conditions as those for HS-SPME. The 8-g tea samples were extracted for up to 4 min using 160 mL of deionized boiling water. After 4 min of brewing, the tea infusion was filtered and allowed to cool. Subsequently, 10 μL of ethyl decanoate internal standard was added at a concentration of 1000 μg/L, and the liquid was mixed thoroughly. Next, the tea was distilled in a vacuum environment at 10^{-2} Pa with a SAFE unit. The temperature of the thermostatic water bath in the sample distillation area and the circulating water bath in the SAFE unit are both 40 °C (Cui et al., 2021; Shen et al., 2023; Zhang et al., 2023). The distillate was extracted three times using 30 mL dichloromethane each time to obtain the final extract. The extracts were dried with anhydrous sodium sulfate and concentrated to 100 μL by purging with nitrogen at room temperature (25 °C) in a water bath.

2.4.3. GC–MS identification of aroma compounds

Volatile compounds were analyzed using a Model 8890-5977B GC–MS system (Agilent, Santa Clara, CA, USA). An HP-5MS column (30 m × 250 μm × 0.25 μm, J&W, Folsom, CA, USA) was used to separate the volatile compounds. The inlet temperature and injection method were 250 °C and non-split mode, respectively. For the SPME analysis, the column chamber was initially set to 40 °C. This temperature was maintained for 5 min before it was increased first to 160 °C at 4 °C/min and subsequently to 280 °C at 10 °C/min; this temperature was maintained for another 5 min. For SAFE, the column chamber was initially set to 40 °C and held at this temperature for 5 min. The temperature was then increased to 200 °C at 5 °C/min and held for 3 min, then to 230 °C at 3 °C/min and held for 2 min, and finally to 280 °C at 20 °C/min and held for 5 min. The helium carrier gas flow rate was 1.0 mL/min, and 1 μL of the sample was injected in the non-split mode. The mass-selective detector was operated in the positive electron ionization mode over a mass scan range from *m/z* 30 to 350 at 70 eV. A series of *n*-alkanes, C_6-C_{40}, were used to determine the linear retention indices.

2.4.4. Key aroma compounds obtained by GC-O

GC–MS olfactometric detection (GC-O) was performed using an Agilent 7890B gas chromatograph equipped with an MS detector and an olfactory detection port (ODP3 Gerstel, Germany). The effluent of the analytical column was divided into two equal parts and directed to the MS (250 °C) and sniffing port (230 °C). Helium, with a linear velocity of 40 cm/s, was used as the carrier gas. SPME and SAFE have different injection methods. At the beginning of the procedure, the SPME fiber was inserted into the GC injection port and resolved for 5 min, following which 2 μL of the SAFE-distillate was injected into the GC injection port. The splitless injection mode was used for the SPME and SAFE analyses. The chromatographic conditions for the GC-O were the same as those mentioned in Section 2.4.3.

GC-O was assisted by trained team members and the method was executed according to that reported by (Zhang et al., 2023). The specific sensory training methods are described in Section 2.4.1.

2.5. Quantitative analysis of nonvolatile compounds

2.5.1. ET measurements for taste strength

ET data acquisition was performed using the taste sensing system (SA402B, Insent Intelligent Sensor Technology, Inc., Kanagawa, Japan). The system consists of taste sensors and ceramic reference electrodes. The potential of the taste sensor film is detected based on the phase change with the reference electrode. The taste sensors (bitterness, astringency, umami, and sweetness) filled with internal solution (3.33 M KCl and saturated AgCl solution) were immersed in the reference solution (30 mM KCl and 0.3 mM TA), and the ceramic reference electrodes are activated in 3.33 M KCl solution filled with internal solution for 24 h. The sensors were tested before the experiments to ensure that their output voltages were within the normal range (Huang et al., 2022).

LAGP tea samples (8 g) were weighed and added to 160 mL of pure boiling water. After 4 min of brewing, the tea infusion was filtered through a 400-mesh gauze and cooled to room temperature, following which 35 mL of the tea infusion was pipetted into an ET measuring cup for measurement. Each sample was measured after a cleaning procedure (Ren et al., 2021).

2.5.2. Determination of catechins and caffeine by HPLC

The catechin and caffeine contents were measured using an Agilent 1260 HPLC system (Agilent, USA) equipped with the Waters Symmetry® C_{18} HPLC column (4.6 × 250 mm, 5 μm). For extraction, freeze-dried tea powder (0.2 g) and 70% methanol (5 mL) were mixed in a 10 mL centrifuge tube. Then, the tubes were placed in a thermostatic water bath at 70 °C for 10 min, shaking it once in the middle. After this, the tubes were taken out of the water bath and allowed to cool. The cooled extracts were centrifuged for 10 min at 3500 r/min and the supernatant was transferred to a 10 mL volumeter bottle. The residue was then extracted, and the procedure was repeated once. The two parts of the supernatant were combined, and the volume was made up to 10 mL using 70% methanol solution. Finally, 2 mL of the supernatant and 8 mL of a stable solution (250 mg EDTA-2Na, 250 mg ascorbic acid, 50 mL acetonitrile, and 0.5 L water) were mixed in a 10 mL volumetric bottle. The solution that was filtered through a 0.22-μm filter membrane was analyzed by HPLC. Based on the study by Fang et al. (2019), the elution conditions were maintained as follows: oven temperature: 35 °C; injection volume: 10 μL; flow rate: 1.0 mL/min; mobile phase A: 9% acetonitrile, 2% acetic acid, 0.2% EDTA-2Na solution (1.0 mg/mL), and 88.8% H_2O; mobile phase B: 80% acetonitrile, 2% acetic acid, 0.2% EDTA-2Na solution (1.0 mg/mL), and 17.8% H_2O; detection wavelength: 278 nm.

2.5.3. Analysis of free amino acids using high-speed amino acid analyzer

In accordance with the study by Lu et al. (2019), the free amino acid content was measured using a High-Speed Amino Acid Analyzer (L-8900, Hitachi, Tokyo, Japan). For extraction, the mixture of 20 mg freeze-dried tea powder and 4.0 mL 4% sulfosalicylic acid was extracted ultrasonically for 30 min and centrifuged at 12000 r/min for 30 min. The procedure was repeated twice. Following this, 1.0 mL of the supernatant was extracted, filtered through a 0.22-μm Millipore filter, and injected. The settings of the high-speed amino acid analyzer system were as follows: mobile phase: lithium citrate; UV–Vis detection wavelength: 570 and 440 nm; flow rates: 0.35 mL/min for mobile phase and 0.3 mL/min for derivatization reagent; column temperature: 38 °C; post-column reaction equipment temperature: 130 °C; autosampler temperature: 4 °C; injection volume: 20 μL. The peak areas of the compounds were compared to those of the amino acid standards. Free amino acid levels are expressed as μg/mL.

2.5.4. Analysis of soluble sugar and TPs by UV spectrophotometry

Based on the protocol adopted by (Haldar, Sen, & Gayen, 2017), the soluble sugar content was measured using an anthrone reagent. Briefly, 0.2 g freeze-dried tea powder, 0.8 mL water, and 4.0 mL fresh anthrone reagent were mixed. The mixture was placed in a boiling water bath and allowed to react for 10 min. The absorption of the cooled mixture was measured at 620 nm using a UV spectrophotometer (U-5100, Hitachi, Tokyo, Japan). The concentration of soluble sugar was calculated from the glucose standard curve (y = 6.7829 x + 0.0205, R^2 = 0.9933).

In accordance with ISO 14502–1 (ISO, 2005), Folin-Ciocalteu's phenol reagent was used to determine the TPs. For the extraction, a tea infusion sample was obtained by mixing 1.0 g of tea powder with 50 mL of boiling deionized water and keeping it in a boiling water bath for 5 min. Next, 1.0 mL of the sample was reacted with 5.0 mL of the freshly prepared 10% Folin-Ciocalteu's phenol reagent in a glass tube for 5 min. A 7.5% Na_2CO_3 reagent kept at room temperature was added to the resulting liquid, and the mixture was left for 1 h. Finally, the absorbance of the rested extract was measured at 760 nm. The TP content was expressed as a percentage of the dry weight (%, dw), and the TP concentration was calculated from the gallic acid standard curve (y = 0.0119 x + 0.0381, R^2 = 0.9991).

2.6. Calculate of OAV, MF, ratio of TPs to free amino acids, and statistical analysis

MF (%) was calculated as follows: MF (%) = $[F (\%) \times I (\%)]^{1/2}$. Here F, (%) is the detection frequency of the aroma-active compounds expressed as a percentage of the total number of judges and I (%) is the average intensity expressed as a percentage of the maximum intensity (Cullere et al., 2011).

The OAV value is an intuitive measurement of the composition and intensity of a sensory aroma. It is also an objective measurement for the contribution of each substance to the overall aroma of a sample (Ma et al., 2022). OAV = c (μg/L) / OT (μg/L), where OT (μg/L) is the threshold of the compound in water, and c (μg/L) is the concentration of the compound. The relative content of the volatile compounds was calculated from the peak area ratio of the target compound. For the aroma-active compounds with MF (%) > 30, a linear equation was used for absolute quantification. For other compounds, a known concentration of ethyl decanoate was used as an internal standard, and the relative amounts of the volatile compounds were calculated by calculating the peak ratios of the target compounds.

The ratio of TPs to free amino acids (TP/AA ratio) was calculated in accordance with the study by Wei et al. (2022). Specifically, TP/AA = TPs (%, dw) / Free AA (%, dw).

Statistical analyses were performed using Excel 2019. Analysis of variance (ANOVA) and least significance difference (LSD) calculations were performed using SPSS 27.0.

3. Results and discussion

3.1. Morphological characteristics of fresh leaves in different tea varieties

LAGP tea is a tea made only from a single leaf. The sunflower-seed-like shape of this leaf is its key characteristic. Therefore, images (Fig. 1) and fresh leaf characteristics (Table S2) of the second leaves of the three varieties at the same period of time were obtained. Significant difference in fresh leaf thickness among the three varieties ($p \leq 0.05$). The AH1 leaves were found to be the largest. These leaves were also the thinnest. The SCZ leaves were the thickest. They had a soft texture, and both sides

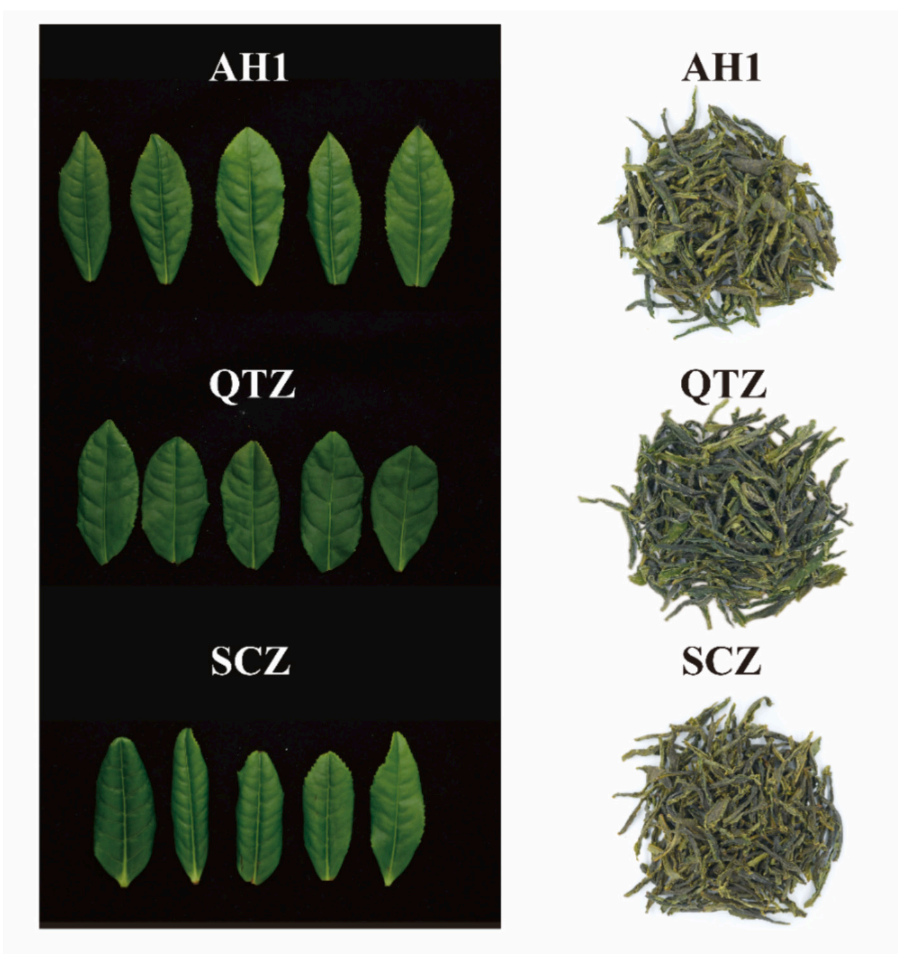

Fig. 1. Fresh leaves of *Anhui1* (AH1), *Quntizhong* (QTZ), and *Shuchazao* (SCZ), and the shape of dried LAGP tea made from three tea varieties.

of the leaves were clearly rolled upward. The blade thickness of QTZ is in the middle of the three varieties, and the sides of the leaf blade were also curled. According to Xia (2016), fresh leaves for preparing LAGP tea should have a leaf shape index (R) >2. The fresh leaves for all three varieties met these criteria. In addition, the shape of the leaf blade can be further determined by R. The AH1 and QTZ leaves were elliptical ($2.0 < R < 2.5$), and the SCZ leaves were long elliptical ($2.6 < R < 3$).

3.2. Effects of different tea varieties on the aroma and volatile compounds of LAGP

3.2.1. Aroma characteristics by QDA

Eight aroma attribute values were selected for the samples through professionals as fruity, green, roasted, floral, boiled bean-like, malty, cooked-corn like and sweet and then scored again for these attributes. The feature attribute scores were analyzed by ANOVA separately. Fig. 2 (A) shows that some aroma characteristics of the LAGP tea prepared from the three tea varieties using the same process were significantly different. No significant difference among the samples was observed for green, whereas floral, boiled bean-like, malty, boiled corn-like, sweet, and fruity were significantly different at the $p \leq 0.05$ level. AH1 had a significantly higher score for floral than for the other varieties, and the score for sweet was also relatively high. SCZ exhibited better performance for boiled corn-like. QTZ had more intense malty and roasted aroma, whereas fruity and sweet aromas were relatively weak. Overall, AH1 had a higher degree of aroma richness than SCZ and QTZ. However, the reason behind the difference in aroma quality is unknown. To investigate the origin of this difference, further tests and analyses were performed.

3.2.2. Effects of tea varieties on volatile compounds

A comprehensive separation and extraction of volatiles from tea was carried out using a combination of SPME and SAFE to analyze the composition of volatile components in LAGP tea (Yang, Baldermann, & Watanabe, 2013; Yin et al., 2022). A total of 132 volatile compounds were identified by GC–MS (https://webbook.nist.gov/chemistry/; Table S3), and the compound was quantified with reference to the ethyl decanoate internal standard. Based on their chemical structures, these 132 compounds were categorized into alcohols (21), aldehydes (22), ketones (22), heterocycles (21), acids (12), esters (17), and others (15 aromatics, 1 sulfide, and 1 olefin). Although the types of volatile compounds did not differ among the tea varieties, their contents differed significantly. The total contents of volatile compounds in QTZ, SCZ, and AH1 were 551.54, 409.94, and 763.35 μg/L, respectively. As evident, the volatile compound content was the highest in AH1, being almost twice that in SCZ. This is probably due to the different material bases in

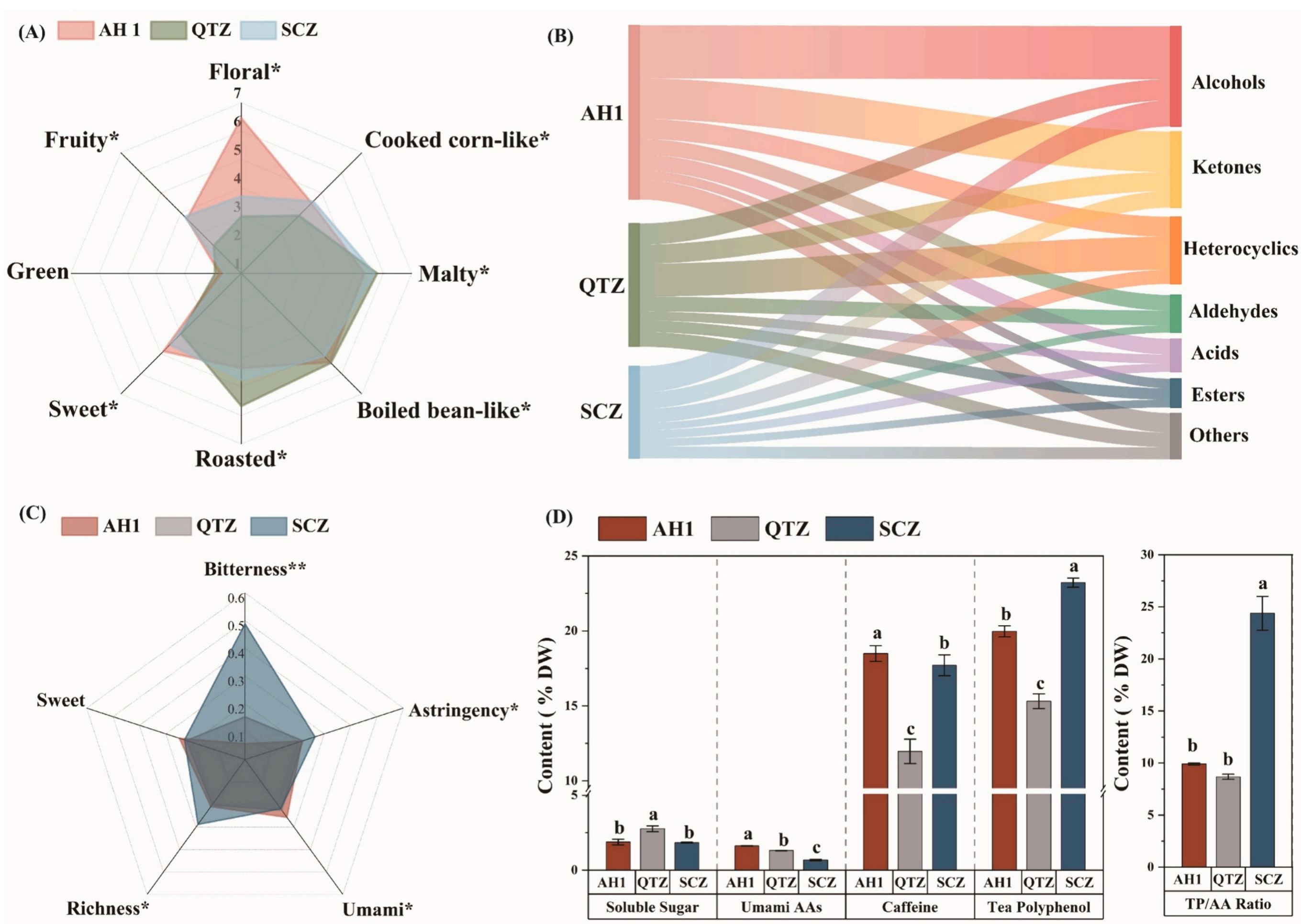

Fig. 2. (A) Quantitative descriptive analysis radar map of aroma of different varieties LAGP tea; "*", significant ($p < 0.05$); (B) Total volatile compounds and the content of each class of substance volatiles compounds in three varieties of LAGP tea. The bars on the left represent the total amount of volatile substances, and the right represents the content of different classes of compounds in the total substances in the sample; the width of a line indicates the amount of the corresponding volatile substance. (C) Radar map of taste scores using electronic tongue of different varieties LAGP tea. Taste score is processed by normalization, and "*" indicates $p < 0.05$, "**" indicates $p < 0.01$; (D) The contents of soluble sugar, tea polyphenols (TPs), caffeine, umami amino acids (aspartic acid, glutamic acid and theanine) and TP/AA ratio of samples, different alphabets indicate significant differences at the 0.05 level. Sample name *Anhui1* (AH1), *Quntizhong* (QTZ), and *Shuchazao* (SCZ).

the fresh leaves possessed by the different varieties (Fang et al., 2021). The types of volatile compounds that dominated each sample also differed significantly. Generally, alcohols and aldehydes accounted for the importance of volatile compounds in LAGP tea (Zhang et al., 2023). Fig. 2(B) shows that the total alcohol content in AH1 is relatively high, reaching a remarkable 31%, with a large contribution from phenylethyl alcohol and (*E*)-linalool oxide (pyranoid) (Fig. S1). The next highest content was that of ketones. SCZ also contained a high proportion of alcohols (28%); however, unlike the other two varieties, heterocyclics comprised 32% of the total volatile content in QTZ, whereas alcohols comprised only 15%. Most heterocyclics are reported to have a roasted odor (Zhai et al., 2023); hence, the high concentration of heterocyclics is probably responsible for the relatively high score for "roasted" in QTZ.

3.2.3. Screening and analysis of key different aroma actives compounds

The compounds with high content in different LAGP tea is different (Fig. S1); however, these high amounts of compounds do not necessarily have a significant impact on the aroma. A total of 51 aroma-active compounds in LAGP tea were screened by GC-O experiments in combination with the database (https://www.leibniz-lsb.de/en/databases) and the odor properties of these compounds in the samples were determined (Table S4). The compounds were classified in accordance with their odor characteristics (Fig. 3). In Fig. 3, the outer circle indicates each aroma-active compound smelled, and the inner circle indicates the different odor characteristics distinguished based on colors. The compounds with floral aroma characteristics accounted for a relatively large proportion. We screened 17 key aroma-active compounds under the screening conditions [MF (%) $>$ 30] employed by (Zhang et al., 2024), and calibrated the concentrations using linear equations (Table S5). Additionally, further screening was performed considering OAV $\geq$ 1. A total of 12 volatile components in the samples had OAV values greater than or equal to 1 (Table 1), namely, dimethyl sulfide (corn), 3-methyl-butanal (malty), 2-methyl-butanal (malty), (*E*,*E*)-2,4-heptadienal (green, fatty), phenylacetaldehyde (floral), linalool (floral,

Fig. 3. Aroma-active compounds detected using a gas chromatography–olfactometry (GC-O) sniffing port. The outermost indicates the aroma compounds sniffed by GC-O, the inner circle represents the set of the respective odor attributes of the compounds in the outer circle. The colors of the compound correspond to the color of the odor attributes.

Table 1
Concentrations, odor thresholds, corresponding linear equations and odor activity value of key aroma active compounds in samples.

CAS	Compounds [a]	Linear equations [b]	R^2	Linear range (μg/L)	OT (μg/L) [c]	OAV [d]		
						AH1	QTZ	SCZ
75-18-3	Dimethyl sulfide	$y = 0.6572\times - 2.8784$	0.9978	4.5–200	0.3	31.08	28.70	38.07
590-86-3	3-Methyl-butanal	$y = 0.5957\times - 0.2817$	0.9965	0.5–30	0.5	4.70	5.51	2.69
96-17-3	2-Methyl-butanal	$y = 0.9968\times - 0.4595$	0.9975	0.5–30	1.5	1.63	1.97	1.09
4313-03-5	(*E*,*E*)-2,4-Heptadienal	$y = 0.7103\times - 0.0664$	0.9947	0.4–15	0.032	13.18	24.69	5.95
122-78-1	Phenylacetaldehyde	$y = 0.7662\times - 0.9144$	0.9994	5–65	5.2	7.15	3.97	1.71
78-70-6	Linalool	$y = 0.3713\times - 0.2797$	0.9997	1–50	0.87	31.34	27.83	10.91
13,925-07-0	2-Ethyl-3,5-dimethyl-pyrazine	$y = 1.0269\times + 0.0707$	0.9998	0.1–12	0.28	5.25	17.54	4.42
106-24-1	Geraniol	$y = 0.4987\times - 1.1502$	0.9971	2.5–80	1.1	46.07	8.23	12.84
488-10-8	*cis*-Jasmone	$y = 0.2178\times - 0.2675$	0.9975	1.5–40	0.26	117.97	38.38	34.79
91-64-5	Coumarin	$y = 0.2692\times + 1.5345$	0.9962	20–675	11	9.16	3.28	2.64
5932-68-3	(*E*)-Isoeugenol	$y = 0.3233\times - 1.391$	0.9951	4.5–37.5	0.71	27.49	16.10	8.68
79-77-6	*trans-beta*-Ionone	$y = 6.3097\times + 0.1066$	0.9974	0.5–15	0.021	155.50	136.63	67.55

Note: [a] The retention index (RI) was computed by using n-alkanes (C_6-C_{40}) under the same chromatographic conditions with the detected volatile compounds by the HP-5MS capillary columns; [b] Linear equations were fitted by the peak area and corresponding concentration; [c] Odor threshold values in water from the Leibniz-LSB@TUM odorant database and literature; [d] Odor activity value, calculated as the ratio of odorant concentration in the LAGP tea infusion to odor thresholds in water. The samples were *Camellia sinensis* cv. *Anhui1* (AH1), *Camellia sinensis* cv. *Quntizhong* (QTZ), *Camellia sinensis* cv. *Shuchazao* (SCZ).

citrus-like), 2-ethyl-3,5-dimethyl-pyrazine (roasted), geraniol (floral), *cis*-jasmone (floral), coumarin (sweet), (*E*)-isoeugenol (floral), and *trans-beta*-ionone (floral).

The content of six key aroma-active compounds with floral flavors, namely, phenylacetaldehyde, linalool, geraniol, *cis*-jasmone, (*E*)-isoeugenol, and *trans-beta*-ionone, was significantly higher in AH1 than in the other two varieties (Fig. 4). This probably resulted in the stronger overall floral flavor of AH1 compared to that of the other two varieties in QDA. Linalool is one of the key aroma-active compounds in most teas, and its content is correlated to the leaf area of the tea plant—larger leaves have a poorer ability to convert linalool to linalool oxide, resulting in higher accumulation of linalool in the leaves (Zeng et al.,

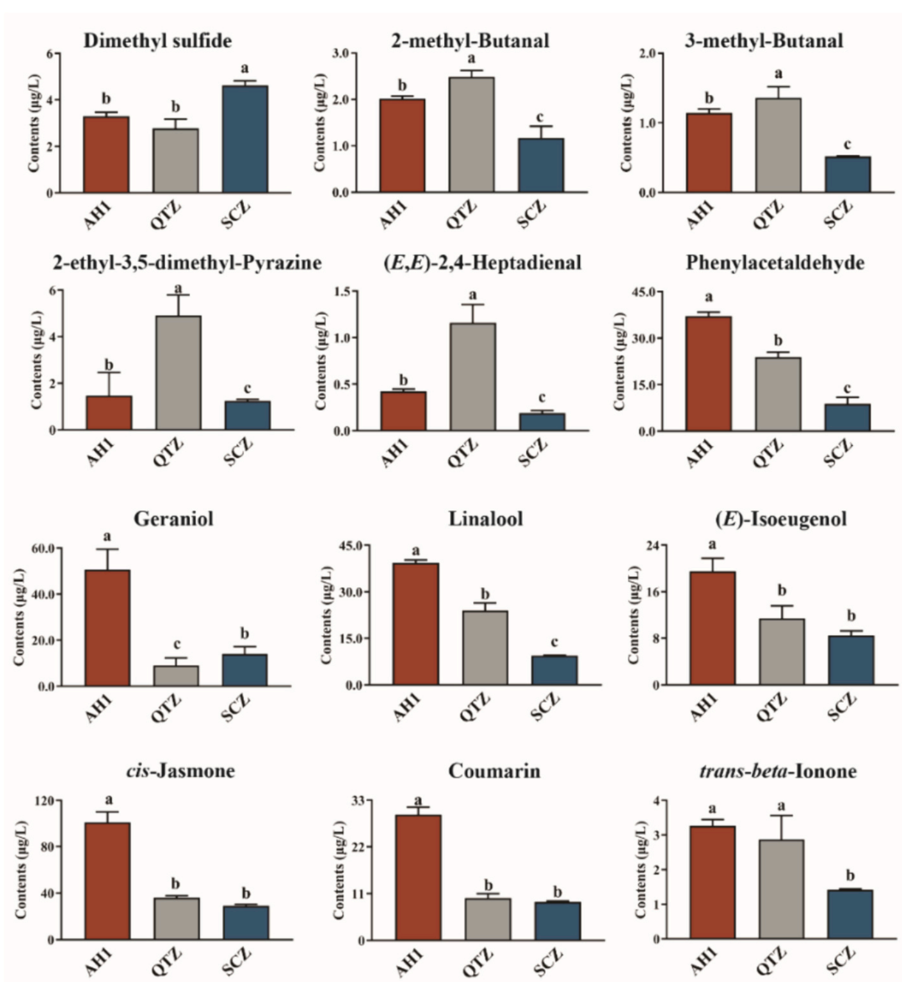

Fig. 4. The bar graph shows the key aroma-active compounds and their corresponding concentrations in *Anhui1* (AH1), *Quntizhong* (QTZ), and *Shuchazao* (SCZ), different alphabets indicate significant differences at the 0.05 level.

2021). AH1 is a large-leaf variety, while QTZ and SCZ are medium-leaf varieties. This might be the reason why its linalool content is higher than that of the other two varieties. *Trans-beta*-ionone exhibits an elegant violet floral aroma and has the highest OAV in LAGP tea, indicating that it plays the most important role in influencing the LAGP aroma (Zhang et al., 2024). Moreover, the content of coumarin, which is formed by the hydrolysis and lactonization of 2-coumaric acid and imparts a sweet flavor, is strikingly different in AH1, QTZ, and SCZ, being 100.78, 36.12, and 29.08 μg/L, respectively (Ho, Zheng, & Li, 2015). The cooked-corn flavor of SCZ was attributed to its high dimethyl sulfide content.

Similarly, the strong roasted flavor of QTZ can be attributed to its high 2-ethyl-3,5-dimethyl-pyrazine content. 2-ethyl-3,5-dimethyl-pyrazine has multiple biosynthetic pathways, such as 2,3-pentanedione and aminopropanone biosynthesis mechanisms or a cyclization of aminoacetone and acetaldehyde that is formed by the Strecker degradation of alanine or *L*-threonine with carbohydrates (Amrani-Hemaimi, Cerny, & Fay, 1995; Zhang, Zhang, Yu, & Xu, 2020) , has a roasted and earthy odor and is a key odorant in many teas (Xiao et al., 2017; Zhai et al., 2022). The higher alanine content in QTZ may account for its high pyrazine content (Table S7). Furthermore, 3-methyl-butanal and 2-methyl-butanal, which have a malty aroma, are the thermal degradation products of *L*-Isoleucine (Ile) and *L*-Leucine (Leu) (Ho et al., 2015). The strongest malty flavor in QTZ, might be attributed to the presence of more of the corresponding precursor compounds in the fresh leaves of QTZ.

To further confirm the contribution of 12 key aroma-active compounds, we performed aroma omission experiments. As shown in Table S8, *trans-beta*-ionone, (*E*)-isoeugenol, linalool, 2-ethyl-3,5-dimethyl-pyrazine, 3-methyl-butanal, 2-methyl-butanal and dimethyl sulfide has a high recognition accuracy. They contributed to the floral, sweet, roasted, malt and corn odors, respectively. The compounds with high recognition rates varied from sample to sample, suggesting that interactions between compounds influence our perception of flavor (Liao et al., 2020; Wei et al., 2024; Zhai et al., 2022). The odor omission experiments provide some basis for QDA results.

3.3. Effects of different tea varieties on the taste and nonvolatile compounds of LAGP

3.3.1. Taste characteristics of different tea varieties by ET

ET can quantify the taste strength through signals detected by taste sensors, thereby avoiding the influence of subjective factors from humans (Huang et al., 2022). The samples were analyzed using the sensor technique, and the results are shown in Fig. 2(C). The sweetness of the LAGP tea did not differ significantly; however, the bitterness, astringency, umami, and richness were significantly different among the varieties. AH1 had the lowest bitterness and astringency scores but the highest umami score. SCZ exhibited the strongest bitterness and astringency flavors and lower umami. The bitterness scores of QTZ lay between those of AH1 and SCZ, while the umami scores were not significantly different from those of SCZ. The response strength of ET is a result of the effect of the nonvolatile compound contents in the tea infusion. To further improve the study of the taste components of LAGP tea, the nonvolatile compounds in LAGP tea were examined to reveal the effect of different varieties on the taste quality of LAGP tea.

3.3.2. Difference in nonvolatile compounds in different tea varieties

Catechins and caffeine, which impart the puckering astringent and bitter taste, are important metabolites in tea and considered one of the important indexes for evaluating the quality of tea (Wan, 2003; Zhang, Cao, et al., 2020). Galloylated catechins are important polyphenols in tea and are responsible for the bitter and astringent taste of green tea (Wei et al., 2022). In contrast, non-galloylated catechins [(−)-epigallocatechin (EGC) and (−)-epicatechin (EC)] impart a sweet aftertaste to green tea (Zhang, Cao, et al., 2020). As shown in Table S6, the highest concentration of the six catechins detected was (−)-epigallocatechin gallate (EGCG), followed by (−)-epicatechin gallate (ECG) in all sample (Fang et al., 2021). As shown in Table 2, SCZ had the highest total catechin content, as evidenced by the higher EGC, EC, ECG, and (+)-catechin (C) contents compared to those in the other two varieties. The accumulation of catechins depends mainly on the genetic background of the variety, and their composition and content can be used as a chemical fingerprint to identify tea from different origins (Fang et al., 2021). Meanwhile, the caffeine content in SCZ was 1.49 times higher than that in QTZ. This may have influenced the pronounced bitter taste of SCZ. The contents of catechins and caffeine in AH1 are relatively high. Both caffeine and catechin contents were the lowest in QTZ.

Approximately 70% of the umami flavor intensity of green tea is attributable to amino acids, especially glutamic acid (Zhang et al., 2024). Based on the study by Wei et al. (2022), we classified the free amino acids as sweet, bitter, and umami (Table S7). The glutamic acid content in SCZ was significantly lower than those in AH1 and QTZ. Theanine is a special amino acid in tea, accounting for about 50% of the free amino acids, and has a umami flavor similar to that of monosodium glutamic acid (Wan, 2003). AH1 had the highest concentration of theanine, 301.83 μg/mL, which is 2.78 and 1.26 times higher than those in SCZ and QTZ, respectively. The relative aspartic acid contents exhibited the same trend as that of theanine. Interestingly, the trends of the total content of the three umami amino acids [Fig. 2(D)] were consistent with the sample scores for umami obtained using the ET.

The main sweet substance in tea is soluble sugar, which is formed during tea processing when part of the starch is hydrolyzed by

Table 2
Concentrations, relative content of taste compounds in samples.

Compound [x]	Concentration (μg/mL)		
	AH1	QTZ	SCZ
Umami			
Theanine	301.83 ± 1.12^a	240.31 ± 0.38^b	108.80 ± 0.42^c
Asp	44.55 ± 0.01^a	32.95 ± 0.01^b	23.88 ± 0.02^c
Glu	59.30 ± 0.01^a	44.93 ± 0.03^b	37.48 ± 0.51^c
Sweetness			
Pro	4.38 ± 0.35^a	3.00 ± 0.01^b	3.52 ± 0.50^b
Gly	0.98 ± 0.01^a	0.82 ± 0.01^b	0.92 ± 0.11^a
Ala	4.79 ± 0.59^a	4.31 ± 0.61^a	3.37 ± 0.17^b
Ser	10.70 ± 1.01^b	12.33 ± 1.27^a	5.86 ± 0.87^c
Thr	4.70 ± 0.13^a	3.47 ± 0.01^b	2.56 ± 0.05^c
Met	0.32 ± 0.02^a	0.27 ± 0.03^b	0.31 ± 0.02^a
Bitterness			
Caffeine	184.86 ± 2.52^a	119.68 ± 1.18^b	177.07 ± 1.33^c
EGCG	274.00 ± 12.66^a	142.90 ± 3.36^c	223.31 ± 12.95^b
EGC	20.56 ± 0.09^b	19.63 ± 0.22^c	22.03 ± 0.10^a
ECG	46.51 ± 2.47^b	31.47 ± 0.54^c	72.50 ± 4.42^a
EC	43.83 ± 6.83^b	49.73 ± 0.76^b	74.79 ± 2.53^a
Phe	4.81 ± 0.66^a	2.37 ± 0.02^c	3.17 ± 0.09^b
Arg	14.91 ± 1.03^b	15.17 ± 2.01^a	2.78 ± 0.09^c
Leu	1.19 ± 0.01^c	2.46 ± 0.12^a	1.53 ± 0.03^b
His	1.12 ± 0.05^b	95.71 ± 4.43^a	nd
Lys	3.24 ± 0.00^a	1.81 ± 0.00^b	1.73 ± 0.00^b
Tyr	5.00 ± 0.60^a	2.51 ± 0.40^c	3.36 ± 0.13^b
GABA	2.99 ± 0.05^b	2.03 ± 0.00^b	6.76 ± 0.01^a
Pro	4.38 ± 0.35^a	3.00 ± 0.01^b	3.52 ± 0.50^b
Astringency			
EGCG	274.00 ± 12.66^a	142.90 ± 3.36^c	223.31 ± 12.95^b
EGC	20.56 ± 0.09^b	19.63 ± 0.22^c	22.03 ± 0.10^a
ECG	46.51 ± 2.47^b	31.47 ± 0.54^c	72.50 ± 4.42^a
EC	43.83 ± 6.83^b	49.73 ± 0.76^b	74.79 ± 2.53^a
C	158.43 ± 2.29^c	181.54 ± 1.10^b	224.53 ± 3.95^a

Note: [x]: The compounds were identified through comparison with the standard. Leucine (Leu), lsoleucine (Ile), phenylalanine (Phe), tyrosine (Tyr), arginine (Arg), lysine (Lys), histidine (His), valine (Val), aspartic acid (Asp), glutamic acid (Glu), serine (Ser), proline (Pro), threonine (Thr), alanine (Ala), glycine (Gly), methionine (Met), aminobutyric acid (GABA), (−)-epigallocatechin (EGC), (+)-catechin (C), (−)-epigallocatechin gallate (EGCG), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG). nd, below the instrumentation detection line. Different alphabets indicate significant differences at the 0.05 level.

endogenous hydrolytic enzymes (Wan, 2003). In the present study, sweetness did not differ significantly among the three tea varieties in the ET tests. To validate this finding, the soluble sugar contents of the samples were measured through anthrone-sulfuric acid colorimetry. The soluble sugar content of QTZ (2.76%) was significantly higher than those of AH1 (1.88%) and SCZ (1.84%), but the difference between the contents of AH1 and SCZ was not significant. Therefore, we speculate that soluble sugar are not the main factor influencing the sweetness of LAGP tea infusion, but rather compounds such as TPs and catechins indirectly influence the strength of sweetness by affecting the degree of expression of bitterness and astringency (Huang et al., 2022; Wan, 2003; Zhang, Cao, et al., 2020).

However, AH1, which contains relatively high levels of catechins, exhibits lower bitterness in ET. The taste of a tea is the result of the combined effect of various flavor presenting substances dissolved in the tea broth. In addition to the catechins in tea that contribute to its bitterness, substances like flavonoids and flavonoid glycosides, anthocyanins and anthocyanidins, and phenolic and carboxyphenolic acids, which are all polyphenolic compounds, also contribute to the taste of tea. Reference to Yang (2014), the TP/AA ratio can provide a taste judgment of tea broth, and is an important indicator for assessing the potential of a tea variety. A lower TP/AA ratio indicates that the tea flavor will be mellower with more umami (Yang, 2014). Fig. 2(D) indicates that SCZ had a higher TP/AA ratio (24.5) than AH1 (9.90) and QTZ (8.68). SCZ had both the highest TP content and lowest amino acid content. AH1 had a higher TP content than did QTZ but also a higher free amino acid content, hence, there is not any significant difference in TP/AA ratio between AH1 and QTZ. This result suggests that SCZ might have a relatively heavier bitter taste, whereas AH1 and QTZ might have more umami and a mellower taste than SCZ (Wei et al., 2022).

In summary, the LAGP tea produced by AH1 had a better flavor quality and a stronger umami taste than did that produced from the other two varieties because of the highest glutamic acid and theanine contents and relatively low polyphenols. QTZ had a stronger umami taste than SCZ and a less bitter taste than SCZ because it had a higher umami amino acid content and the lowest polyphenol content. In contrast, SCZ had a prominent bitter and astringent taste because of its excessively high polyphenol content and its low amino acid content. Finally, SCZ had the poorest flavor quality among the three varieties.

4. Conclusion

Of the three tea varieties analyzed in this study, AH1 had the largest and thinnest fresh leaves, and the resulting LAGP tea had a distinctive floral aroma. Compared to QTZ and SCZ, AH1 had significantly higher levels of six floral key aroma-active compounds, namely, phenylacetaldehyde, linalool, geraniol, *cis*-jasmone, (*E*)-isoeugenol, and *trans-beta*-ionone. In addition, AH1, which is also rich in umami amino acids (theanine, glutamic acid, and aspartic acid), had high umami and low bitterness and astringency. In contrast, QTZ had oval-shaped leaves and had the highest 3-ethyl-2,5-dimethyl-pyrazine content when processed into LAGP tea, imparting it with a strong roasted aroma as well as a mellow flavor and relatively low bitterness. After preparing the LAGP tea, SCZ had the thickest leaves, a soft texture, and a cooked corn-like aroma. Moreover, it had the lowest content of volatiles, least umami amino acids, highest polyphenol content, and highest TP/AA ratio. Compared to those of AH1 and QTZ, the tea infusion of SCZ had more components contributing to the bitter and astringent taste, which is probably why it exhibited the strongest bitter and astringent taste. Overall, AH1 produced the best overall quality of LAGP tea whereas SCZ produced the lowest quality. Hence, AH1 can be considered the most suitable among the three varieties for LAGP tea production.

In addition, 12 different key aroma-active compounds were identified by molecular sensory science techniques. Among these compounds, *trans-beta*-ionone and 2-ethyl-3,5-dimethyl-pyrazine contributed the maximum to the floral and roasted aroma, respectively. The soluble sugar content was not a major factor affecting the sweetness of tea. In summary, exploring the effects of different tea varieties on the quality of LAGP tea can provide a theoretical basis and practical foundation for the selection of high-quality raw materials for LAGP tea production and thus, help in improving the quality of LAGP tea flavor.

Ethical statements

Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this sensory evaluation" where an affirmative reply was required to enter the sensory evaluation. They were able to withdraw from the sensory evaluation at any time without giving a reason. The tea products evaluated were safe for consumption.

CRediT authorship contribution statement

Wanzhen Feng: Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Huan Zhou:** Formal analysis, Methodology, Software. **Zhichao Xiong:** Resources, Methodology, Investigation, Data curation. **Caiyan Sheng:** Visualization, Supervision, Methodology. **Dongzhou Xia:** Visualization, Supervision, Investigation. **Jixin Zhang:** Data curation, Investigation, Supervision. **Tiehan Li:** Visualization, Supervision, Investigation. **Yuming Wei:** Visualization, Supervision, Investigation. **Wei-Wei Deng:** Writing – review & editing, Validation, Supervision. **Jingming Ning:** Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors acknowledge the financial support of the National Key Research and Development Program of China (2021YFD1601103).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101534.

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