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Quality analysis and characteristic difference identification of organic tea and conventional planting tea based on ICP, HPLC and machine algorithm

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ABSTRACT

With the continuous expansion of the organic tea industry, distinguishing the authenticity of organic tea is crucial to maintain the market's stability. Thus, organic and conventional planting teas (green teas) of Dajianshan were selected as the objects in this study. The components (water extract, polyphenols, mineral element, etc.) were compared by high-performance liquid chromatography, inductively coupled plasma spectrometry, and mass spectrometry. The main difference substances were screened by multivariate statistical analysis methods (PCA, OPLS-DA, and LDA). Results showed significant differences in 37 of 51 components ($P < 0.05$). Statistical analysis showed 15 components (including amino acids, mineral elements, and catechins, VIP >1) that can be used as the characteristic differences between organic and conventional planting teas. The accuracy of the identification reached 93.9 %, providing a reference for the quality evaluation and identification of organic and conventional planting teas.

1. Introduction

Tea originates in China and is made from the tender leaves or buds of Camellia, a plant in the Camellia family. Tea has become one of the three most popular non-alcoholic beverages in the world. It is rich in a variety of chemical components and trace elements, with significant medicinal and health benefits, such as excitatory central nervous system, antioxidant, antitumor, antibacterial, and blood-pressure lowering (Li et al., 2024; Ponder & Hallmann, 2019). According to the planting mode, it can be divided into organic and conventional planting teas.

Organic tea does not use synthetic pesticides, fertilizers, and growth regulators during production, does not use synthetic food additives during processing, and has been approved by a certification body (Manzoor, & Ni, & Ruan, 2024). Conventional planting tea can use pesticides, fertilizers, growth regulators, etc. in production. Harmful substances such as agricultural residues and heavy metals that can potentially harm our bodies may exist (Giampieri et al., 2022).

Therefore, compared with conventional planting tea, the market for organic tea market is better than that for conventional planting tea, and the organic tea output increased from 17.3 ten thousand tons in 2017 to 29.4 ten thousand tons in 2021, with a growth rate of 69.9 %. In 2022, the certified area of organic tea gardens in Yunnan Province reached 11.2 hm^2, accounting for 22.5 % of the total area of tea gardens in the province. Meanwhile, the production of organic tea is also increases yearly, with excellent market potential. Currently, many illegal traders sell conventional planting tea as organic tea, often adulterated. Such fake products are difficult to distinguish visually. It brings huge economic losses to consumers, disrupts the market, and affects consumers' trust in the quality of organic tea products. Thus, an effective strategy must be identified to distinguish organic tea from conventional planting

Abbreviations: Abbreviations, Full names; GC, Gallocatechin; EGC, Epigallocatechin; EC, Epicatechin; C, Catechin; EGCG, Epigallocatechin gallate; ECG, Epicatechin gallate; INQ, Index of nutritional quality; PCA, Principal component analysis; OPLS-DA, Orthogonal partial least squares-discriminant analysis; LDA, Linear discriminant analysis; ORG, Organic tea; CK, Control check; HPLC, High performance liquid chromatography; ICP-MS, Inductively coupled plasma-mass spectrometry; ICP-OES, Inductively coupled plasma-optical emission spectrometer.

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tea.

Due to the different cultivation conditions of organic and conventional planting teas, their quality also differ (Kim, Kim, Kim, & Kim, 2015). Differences exist among tea polyphenols, caffeine, free amino acids, flavonoids, mineral elements, and other active ingredients. These differences can be used as characteristic markers to distinguish organic tea from conventional planting tea. Meanwhile, the combination of machine-learning algorithms to screen feature markers and establish the discriminant model for identifying the agricultural products' authenticity has achieved good results. Commonly used methods are principal component analysis (PCA), linear discriminant analysis (LDA), orthogonal partial least square analysis (OPLS-DA), and other methods of authenticity identification. Such identification has an important contribution to the detection of chemical markers. Liu et al. (2019) established a discriminant model using PCA and LDA to identify the origin of green tea from Zhejiang and Shandong provinces. They found discriminant accuracy rates of 92.30 % and 87.80 %, respectively. Wang (2018) used rare-earth element fingerprint combined with PCA-LDA, partial least squares–LDA analysis, neural network model, and LDA to identify tea from different provinces of Shandong, Sichuan, Guizhou, and Zhejiang. The accuracy rates were 80.80 %, 87.90 %, 92.93 %, and 95.05 %, respectively.

In the present study, we proposed a model to distinguish organic tea from conventional planting tea. First, the main characteristic components of organic and conventional planting teas were determined, including water extracts, free amino acids, tea polyphenols, caffeine, flavonoids, catechins, mineral elements, and heavy-metal elements. The substances that differed between organic tea and conventional planting tea were further identified. Then, through correlation analysis and combined with PCA, OPLS-DA, and LDA linear discriminant multivariate statistical analysis methods, the characteristic difference substances of organic and conventional planting teas were identified to provide a reference for the quality evaluation and discrimination of organic and conventional planting teas.

2. Materials and methods

2.1. Plant materials

Tea samples were collected from Dajianshan (23°3′42″N, 101°2′43″E) organic ecological and conventional planting tea gardens in Puer City, Yunnan Province of China. The annual rainfall in the tea garden is about 1600 mm, and the average yearly temperature is 18.2 °C. The soil is primarily yellow-red with pH 5.8 and altitude of 1800–2000 m. Organic tea was certified by China's and EU organic certifications, whereas conventional planting tea was not certified. All samples were gathered from the same tea variety (*Camellia sinensis* var. Islamic cv. Yunkang No.10, "Yunkang 10") simultaneously, and the same processing technology was utilized to manufacture sun-dried green tea. Fifteen organic tea samples and conventional planting tea were selected. The harvested tea leaves were crushed, screened, dried, and kept in tight bags away from light.

2.2. Chemical reagents

Potassium dihydrogen phosphate, anhydrous sodium carbonate, basic lead acetate, trisodium citrate dihydrate, citric acid monohydrate, ninhydrin, stannous chloride, and caprylic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium hydrogen phosphate dodecahydrate, and glacial acetic acid were from Tianjin Fengchuan Chemical Reagent Technology Co., Ltd. (Tianjin, China). Nitric acid was from Suzhou Jingrui Chemical Co., Ltd. (Suzhou, China). The standard chemicals, namely, gallocatechin (GC), epigallocatechin (EGC), catechin (C), epicatechin (EC), epigallocatechin gallate (EGCE), rutin, epicatechin gallate (EGC), hyperoside, kaempferol-3-O-rutinoside, quercetin, and kaempferol were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). The purity of these standards was over 98 %. Chromatographic-grade acetonitrile, methanol, and formic acid were purchased from Tedia Co., Inc. (Fairfield, OH, USA). All other chemicals were of the highest analytical grade.

2.3. Determination of water-extract content

The dry weight of the extract was determined using the evaporation and oven methods (He, Liu, & Huang, 2011). About 2.00 g tea powder was weighed in a 500 mL conical bottle, to which 300 mL of boiling distilled water was added. The mixture was placed in a water bath at 100 °C for 45 min and filtered. The filter paper was placed in a continuous-temperature drying oven at 120 °C and weighed after cooling.

2.4. Determination of tea polyphenol content

The Folin–Ciocalteu method (Margraf, Karnopp, Rosso, & Granato, 2015) was used to determine the amount of tea polyphenols. Tea powder was weighed at 0.20 g, and 5 mL of 70 % methanol aqueous solution was added. After extracting twice in a water bath at 70 °C, the volume was adjusted with extraction solvent to 10 mL. Following filtering, 2 mL of extraction solution was added to 8 mL of 70 % methanol aqueous solution, followed by 5.0 mL of Folin–Ciocalteu phenol reagent. After 3–8 min reaction, 4 mL of 7.5 % sodium carbonate solution was added to 25 mL. After incubating at room temperature for 1 h, the absorbance at 765 nm was measured, and a blank sample was created by replacing diluted tea extract with water. A gallic acid calibration curve [$Y = 0.0103\ X + 0.016$ ($R^2 = 0.9984$)] was plotted at different concentrations (30–70 μg/mL) to measure the quantity of tea polyphenols.

2.5. Determination of caffeine content

Caffeine content was determined following the methodology outlined by Han, Wang, Fu, and Ahmed (2018). Initially, 1.50 g of tea powder was weighed and extracted in a boiling water bath with 225 mL of distilled water for 45 min. Subsequently, the filtrate was diluted to a final volume of 250 mL, and then 10 mL of the solution was pipetted into a 100 mL volumetric flask. Into this flask, 4 mL of diluted hydrochloric acid and 1 mL of basic lead acetate were added. After being brought to volume and filtered, 25 mL of the resulting filtrate was transferred into a 50 mL volumetric flask, and 0.1 mL of sulfuric acid solution was added for further dilution and volume adjustment. The solution was filtered, and the absorbance was measured at 274 nm. For comparison, a blank sample was prepared using water instead of the diluted tea extract. Caffeine content was evaluated using the calibration curve $Y = 47.65\ X - 0.0075$ ($R^2 = 0.9998$) and produced with various values (8–16 μg/mL).

2.6. Determination of free amino acids

2.6.1. Determination of total free amino acids

Total free amino acids were measured by ninhydrin colorimetry (Stauß et al., 2024). About 1.50 g of tea powder was weighed and extracted in a hot water bath, followed by dilution to a final volume of 250 mL. Subsequently, 0.5 mL of phosphate buffer solution and ninhydrin were added to the extract. The absorbance of the resulting solution was measured at 570 nm, with a corresponding blank test performed for accurate readings. The standard curve for determining total free amino acids was $Y = 3.3369\ X - 0.3655$ ($R^2 = 0.9982$).

2.6.2. Determination of theanine content

Theanine was detected with a high-performance liquid chromatography system (Waters, USA) (GB/T 23193–2017). We steeped 1.00 g of tea powder sample into 100 mL of boiling distilled water for 30 min. It was filtered to a set volume via a 0.45 μm microporous filter membrane and stored at 4 °C for the subsequent experiment. Theanine content was evaluated using the calibration curve Y = 47.65 X − 0.0075 (R^2 = 0.9998) produced at various concentrations (0.01–0.20 mg/mL). The chromatographic analysis used a Waters C18 column (4.6 mm × 250 mm, 5 μm). The mobile phase was formed by solvent H_2O (A) and acetonitrile (B). The elution was isocratic, comprising 95 % A and 5 % B. The column temperature was 35 °C. The detection wavelength was 210 nm with 0.7 mL/min flow rate and 10 μL of injection volume. The compounds were identified based on retention time and quantified by peak area using the external standard method.

2.6.3. Determination of free amino acid monomers

Following the method of Huang et al. (2024) with slight modifications, 2.00 g of tea powder was added into 200 mL of boiling water for 10 min in a hot water bath. After filtering the extract and diluting to 250 mL, the diluent was passed through a 0.45 μm water filter membrane for analysis. The standard analysis column of the automatic amino acid analyzer (SYKAM, Munich, Germany) used was 4.6 mm × 150 mm. The temperature program for the reaction column was as follows: start (58 °C), 19 min (58 °C), 24 min (74 °C), 47 min (74 °C), 51 min (58 °C), and 59 min (58 °C). The reactor temperature was set to 130 °C, with an injection volume of 50 μL. The flow rates were adjusted to 0.45 mL/min for the elution pump and 0.25 mL/min for the derivatization pump. The system was operated under a maximum pressure of 35 bar, and dual-channel UV detection was conducted at 570 and 440 nm.

2.7. Determination of mineral-element content

The sample treatment method by reference Ye et al. (2017) was performed with slight modification. About 0.50 g of tea powder sample was precisely weighed and dissolved in a polytetrafluoroethylene tube. Then, 10 mL of nitric acid and 1 mL of perchloric acid were introduced into the graphite digester. Digestion was performed according to the temperature-gradient protocol specified in Suppl. Table S1. After digestion, 0.65 mL of 1:1 nitric acid was added, and the volume was repeatedly cleaned to 25 mL using ultrapure water. A blank control was established. The glassware was immersed in 20 % nitric acid solution for at least 24 h and then washed with ultrapure water.

Element contents were determined using the method outlined by Ye et al. (2017). The parameters of the ICP-OES (PerkinElmer, USA) instrument were set as follows: plasma gas-flow rate at 12 L/min, auxiliary gas at 0.3 L/min, nebulizer gas-flow rate at 0.65 L/min, radiofrequency power at 1300 W, and pump flow rate at 1.5 L/min. The mixed-element standard curve was prepared using serial solutions of 0.02, 0.10, 1.00, 2.00, 10.00, and 100.00 mg/L. The analytical wavelength for each element and the corresponding standard curve details are presented in Suppl. Table S2.

2.8. Determination of catechins and flavonoids

With slight modifications to the method described by Najman, Sadowska, Wolińska, Starczewska, and Buczak (2023), 1 g of tea powder was added into 80 % methanol solution. After incubation in an ultrasonic bath for 30 min before centrifuging at 5000 r/min for 5 min, the recovered supernatant was filled with 25 mL of extraction solvent. Then, 1 mL of supernatant was passed through a 0.45 μm organic filter membrane. The mobile phase comprised H_2O (A) and acetonitrile (B). The mobile phases were programmed as follows: 0–20 min, 98 %–85 % B, 20–35 min, 85 %–80 % B, 35–45 min, 80 %–70 % B, 45–50 min, 70 %–55 % B, 50–60 min, 55 % B, 60–61 min, 55 %–5 % B, and 61–67 min, 5 % B. The injection volume was 10 μL, the column temperature was maintained at 30 °C, and the flow rate was set to 0.6 mL/min. Detection was performed at wavelengths of 280 and 360 nm to accurately quantify the catechins and flavonoids in the samples by comparing with standard references.

2.9. Determination of heavy-metal content

Referring to 2.7 for tea sample preparation, the analytical curve was established for Cd, Cr, and Pb by using a series of dilutions of 100 mg/L multi-element mixed-ion standard solution in 1 % (*v/v*) nitric acid ranging within 0.20–50.00 mg/L. A separate 100 mg/L Hg standard solution was prepared to avoid Hg contamination, and an analytical curve was generated for Hg, also in 1 % (v/v) nitric acid, with dilutions ranging within 0.20–50.00 mg/L. Additionally, 10 mg/L certified standard solution was diluted in 1 % (v/v) nitric acid to obtain an internal standard solution containing 50 μg/L of Rh, In, and Cd.

We referred to the methodology of Li, Li, Chen, and Meng (2018) with slight modification to the instrument parameters for the ICP-MS (X Series II, Thermo Scientific, US). The detailed parameter settings were as follows: plasma gas-flow rate of 15 L/min, carrier gas-flow rate of 1.0 L/min, helium gas-flow rate of 4.5 mL/min, auxiliary gas-flow rate of 0.8 L/min, radiofrequency power of 1550 W, sampling depth of 8.0 mm, platinum cone for the sampling cone, and nickel cone for the intercept cone. The analysis mode was set to standard mode.

2.10. Sensory evaluation

According to the Chinese national standard GB/T 23776–2018, sensory evaluation was conducted by a panel of three professional appraisers with certificates in tea sensory evaluation. Using the ratio of 1:50, 3 g of tea was weighed and quickly infused with boiling water in a standard vessel for sensory assessment. The evaluation panel conducted three separate assessments of the tea at infusion times of 2, 3, and 5 min. The sensory-evaluation score was comprehensively weighted and averaged based on the shape and color of dry tea (25 %), the color of tea soup (10 %), aroma (25 %), taste (30 %), and the appearance of waste tea (10 %). Each panel member completed the assessment process independently, with each sample evaluated three times.

The color of the tea infusion was measured using an NR10QC colorimeter. Before taking readings of the samples, the instrument was subjected to a blank calibration, followed by the determination of L* (lightness), a* (red+, green−), and b* (yellow+, blue−) values. These measurements were repeated three times for consistency.

2.11. Evaluation of the antioxidant activity of tea

The antioxidant capacity of organic and conventional planting teas was investigated using $ABTS^+$ and DPPH free-radical scavenging ability detection kits (Boxbio Science & Technology Co., Ltd., Beijing, China), which were utilized to measure the relative free-radical scavenging abilities. The kit instructions were followed for the primary determination methods and processes. Formula (1) was used to compute the free-radical scavenging.

$$\text{Free radical scavenging } (\%) = 1 - \frac{A_{determination-}\ A_{control}}{A_{blank}} \times 100\% \tag{1}$$

2.12. Data processing and analysis

Every experimental group was subjected to three parallel measurements, with the mean ± standard deviation representing the results. All data were processed using Microsoft Excel 2016 and statistically analyzed ($P < 0.05$) using SPSS 27.0. OPLS-DA was performed using SIMCA 14.1 software. Permutation test and cross-validation were performed to prevent model overfitting.

3. Results and discussion

3.1. Comparison of water extract, tea polyphenols, caffeine, and free amino acids in tea samples

The physicochemical components of organic and conventional planting teas were measured, and the results are shown in Fig. 1A. No significant differences existed in the contents of water extract, tea polyphenols, and caffeine. The total free amino acid content of organic tea was significantly higher than that of conventional planting tea ($P <$ 0.05). The active ingredients in tea were primarily dissolved after brewing in hot water because the human body absorbs and utilizes them in the form of tea soup (Kozłowska et al., 2022). Tea polyphenols are some of the important indices to evaluate the quality of green tea. Our study found that the content of tea polyphenols in conventional planting tea was higher than that in organic tea. This result was similar to Ponder's study on organic and conventional raspberries, which may be related to the types of fertilizers applied to conventional planting tea (Ponder & Hallmann, 2019). Tseng and Lai (2022) also showed that an appropriate zinc fertilizer can promote the synthesis of polyphenols in tea plants. Qiu et al. (2024) researched green tea and further confirmed that nitrogen application also significantly increases the total polyphenol content of tea.

The caffeine content of tea typically ranges from 2 % to 4 %, imparting a bitter taste (Cui et al., 2023). This component stimulates the central nervous system, contributing to its energizing and anti-fatigue effects. However, an excessively high caffeine content causes the tea to be bitter. Organic tea has been found to contain lower caffeine levels than conventional planting tea, suggesting a potentially milder bitter taste. The content of free amino acids in tea affects the sweetness of tea soup and alleviates its bitter taste. Another study has further confirmed that organic tea is less bitter and more drinkable (Ying, Qi, Junrong, Xueyuan, & Qiong, 2015).

3.2. Comparison of free amino acid monomers in tea

Theanine is the most abundant amino acid in tea. It endows tea with the characteristics of moisturizing and sweetening and alleviates the bitterness. The astringent taste is brought by tea polyphenols and caffeine, the primary source of the umami taste (Zhong, Lu, & Lv, 2023). A total of 17 free amino acids were identified using the automatic amino acid analyzer (Suppl. Fig. S1), as shown in Table 1. A total of 14 amino acids were successfully isolated from organic tea and conventional planting tea, including 6 essential (5.59 $mg \cdot g^{-1}$) and 9 medicinal (8.41 $mg \cdot g^{-1}$) amino acids. Among them, tyrosine, phenylalanine, and arginine had the highest content, accounting for 5.06 %, 6.80 %, and 4.47 % of free amino acids, respectively. As shown in Table 3, the mean theanine of organic tea was 12.25 $mg \cdot g^{-1}$, which was significantly higher than that of conventional planting tea (9.24 $mg \cdot g^{-1}$, $P < 0.01$). The content of other amino acids was also higher than that of conventional planting tea, indicating that organic tea had high nutritional value.

Free amino acids are also related to the taste of tea. Liu et al. (2023) showed that aspartic acid, theanine, and glutamic acid, which are umami amino acids, can all suppress bitterness. This indicated that

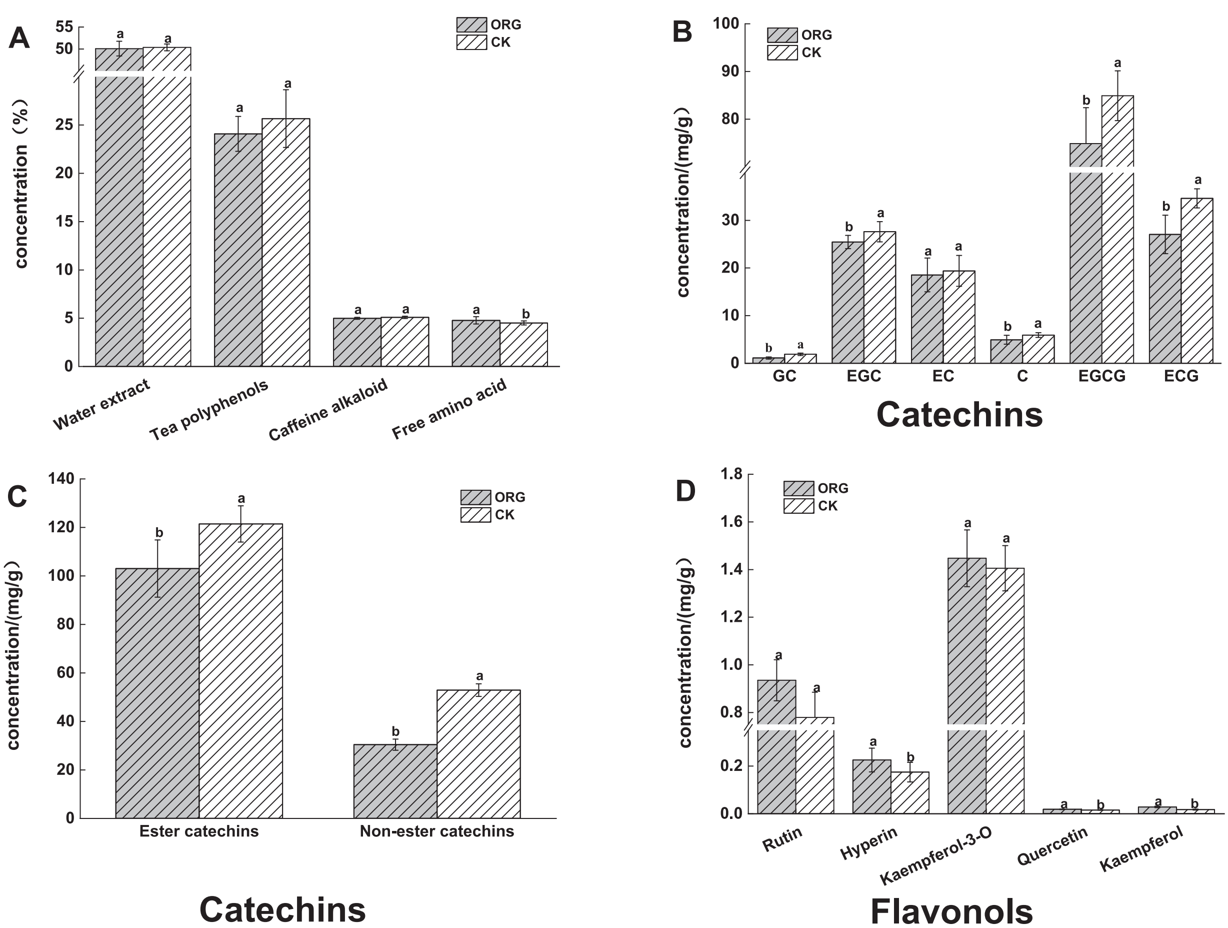

Fig. 1. Comparison of characteristic components between organic and conventional planting tea.
(A) Comparison on general physicochemical composition content of organic and conventional planting tea. (B) Comparison of catechins content between organic and conventional planting tea. (C) Proportion of catechins compositions in organic and conventional planting tea. (D) Comparison of flavonoid content between organic and conventional planting tea.

Table 1
Comparison on general physicochemical composition content of organic and conventional planting tea.

Amino acid species	Organic tea content(mg·g^{-1})	Conventional planting tea content (mg·g^{-1})	Relish
Aspartic acid	0.80 ± 0.10^{a}	0.65 ± 0.15^{b}	Umami
Glutamic acid	0.90 ± 0.12^{a}	0.85 ± 0.18^{a}	Umami
Glycine	0.43 ± 0.12^{a}	0.11 ± 0.04^{b}	Sweet taste
Cystine	0.74 ± 0.05^{a}	0.54 ± 0.10^{b}	–
Valine	0.70 ± 0.16^{a}	0.11 ± 0.02^{b}	Sweet taste
Methionine	0.91 ± 0.20^{a}	0.20 ± 0.04^{b}	–
Isoleucine	0.83 ± 0.23^{a}	0.14 ± 0.04^{b}	Bitter taste
Leucine	0.76 ± 0.14^{a}	0.35 ± 0.07^{b}	Bitter taste
Tyrosine	1.18 ± 0.39^{a}	0.05 ± 0.01^{b}	Bitter taste
Phenylalanine	1.58 ± 0.95^{a}	0.43 ± 0.19^{b}	Bitter taste
Histidine	0.09 ± 0.01^{a}	0.07 ± 0.01^{b}	Bitter taste
Lysine	0.81 ± 0.19^{a}	0.15 ± 0.03^{b}	–
Arginine	1.04 ± 0.32^{a}	0.05 ± 0.03^{b}	Bitter taste
Proline	0.21 ± 0.02^{a}	0.15 ± 0.04^{b}	Sweet taste
Theanine	12.25 ± 2.64^{a}	9.24 ± 1.32^{b}	Fresh and Refreshing
Sweet amino acid	15.28	11.11	
Bitter amino acid	5.48	1.10	
TAA	23.23 ± 0.67	13.10 ± 0.33	
EAA	5.59 ± 0.33	1.38 ± 0.13	
NEAA	5.39 ± 0.39	2.47 ± 0.32	
MAA	8.41 ± 0.32	2.84 ± 0.28	

Note: "E" stands for essential amino acid (EAA); "N" stands for non-essential amino acid (NEAA); "M" stands for medicinal amino acid (MAA); TAA is the sum of all free amino acids. Different lowercase letters in each line indicate significant difference, $P < 0.01$.

umami amino acids can effectively improve the astringent sensation of tea.

3.3. Comparison of macroelement and microelement in tea

Mineral elements are crucial components for maintaining human health and can be obtained only through food intake, playing a significant role in sustaining the health of the body. They are also important factors affecting the growth of tea plants and the quality of tea leaves (Yang et al., 2022). The present study compared 7 macroelements and 14 microelements in organic and conventional planting teas, and the results are presented in Tables 2 and 3. In organic tea, the highest content of significant elements was K (15,644.51 mg·kg^{-1}), and the lowest was Na (46.77 mg·kg^{-1}); the highest content of trace elements was P (4765.29 mg·kg^{-1}), and the lowest was V (0.09 mg·kg^{-1}). In conventional planting tea, the highest content of significant elements was K (9700.71 mg·kg^{-1}), and the lowest was Na (38.54 mg·kg^{-1}); the highest content of trace elements was Ti (9656.73 mg·kg^{-1}), and the lowest was V (0.05 mg·kg^{-1}). These results showed that the contents of Al, Ca, Fe, K, Mg, Na, Co, and Cu in organic tea were significantly higher than those in conventional planting tea. By comparison, the contents of S, B, Si, and Ti in organic tea were significantly lower than those in conventional planting tea ($P < 0.05$).

Table 2
Comparison on macroelement content between organic tea and conventional planting tea.

Type of element	Organic tea(mg·kg^{-1})	Conventional planting tea(mg·kg^{-1})
Al	463.85 ± 108.88^{a}	303.37 ± 52.86^{b}
Ca	3993.98 ± 690.61^{a}	3510.67 ± 324.99^{b}
Fe	62.82 ± 12.91^{a}	53.12 ± 8.35^{b}
K	$15,644.51 \pm 7961.68^{a}$	9700.71 ± 2830.65^{b}
Mg	2831.53 ± 498.95^{a}	2268.84 ± 205.85^{b}
Na	46.77 ± 10.71^{a}	38.54 ± 10.62^{b}
S	4897.76 ± 1850.83^{b}	7072.27 ± 2743.17^{a}

Note: Different lowercase letters in each line indicate significant difference, $P < 0.05$.

Table 3
Comparison on microelement content between organic tea and conventional planting tea.

Type of element	Organic tea(mg·kg^{-1})	Conventional planting tea(mg·kg^{-1})
B	17.78 ± 3.81^{b}	22.82 ± 7.96^{a}
Ba	12.16 ± 3.18^{a}	10.56 ± 2.11^{a}
Co	0.40 ± 0.27^{a}	0.15 ± 0.12^{b}
Cu	12.14 ± 5.14^{a}	6.53 ± 4.92^{b}
Mn	728.91 ± 220.18^{a}	1015.280 ± 758.471^{a}
Ni	5.57 ± 1.17^{a}	4.87 ± 0.63^{a}
P	4765.29 ± 656.96^{a}	4995.02 ± 362.25^{a}
Se	37.43 ± 9.12^{a}	39.08 ± 17.95^{a}
Si	14.00 ± 3.28^{b}	19.65 ± 3.71^{a}
Sr	3.99 ± 0.74^{a}	3.75 ± 0.90^{a}
Ti	2798.75 ± 1119.84^{b}	9656.73 ± 3054.83^{a}
V	0.09 ± 0.05^{a}	0.05 ± 0.06^{a}
Zn	23.70 ± 6.83^{a}	22.96 ± 9.07^{a}

Note: Different lowercase letters in each line indicate significant difference, $P < 0.05$.

The reasons for the differences in elemental content may be the tea plant's specific absorption of various elements. Tea plants reportedly accumulate Mn and Al (Diniz, Pistonesi, Alvarez, Band, & de Araujo, 2015). Mn is closely associated with photosynthesis and can increase the activity of polyphenol oxidase in tea, as well as promote the synthesis of amino acids (Huang, Wang, et al., 2024). The intake of Al may potentially harm our health. Second, the differences in elemental content may be related to the cultivation modes, fertilizers, and climate. Organic tea uses only organic fertilizers and does not use other fertilizers and pesticides, making the root of the tea tree more biodiverse. Conversely, conventional planting tea uses various fertilizers and pesticides, decreasing the root biodiversity (such as fungi and earthworms) and thus the absorption of major and trace elements (Czech, Szmigielski, & Sembratowicz, 2022).

3.4. Mineral-nutrition evaluation

The index of nutritional quality (INQ) serves as an international dietary evaluation metric for assessing the extent to which various nutrients fulfill human energy requirements (Jalali et al., 2022). Based on an adult male's daily energy requirement of 9410 kJ, the INQ values were calculated using the standard of 1372 kJ contained in 100 g of green tea, alongside the recommended intake of mineral elements outlined in the "Chinese Dietary Reference Intakes." INQ stands for the ratio of nutrient density in food to the caloric density per 100 g of that food. Specifically, nutrient density was defined as the amount of a particular nutrient in 100 g of food divided by the recommended daily intake of that nutrient for a specific individual. Meanwhile, caloric density is defined as the calories contained in 100 g of food divided by the total daily calorie requirement for the same specific individual. According to the data presented in Tables 4 and 5, except for Na, the INQ values for mineral elements in organic and conventional planting teas exceed 1. Organic tea exhibits higher nutritional indices for Ca, K, Mg, Zn, and Cu than conventional planting tea, underscoring its superior nutritional value and ability to satisfy human demands for mineral nutrients better.

3.5. Comparison of catechins and flavonoids in tea

The catechins and flavonoids in organic and conventional planting teas were determined, and the chromatogram of the determination standard is shown in Suppl. Fig. S2.

3.5.1. Comparison of catechins in tea

Catechins are the main polyphenols in tea, with EGCG being the most abundant and considered the primary antioxidant active substance (Khalatbary & Khademi, 2020). As illustrated in Fig. 1B, organic tea

Table 4
INQ values of mineral elements in organic tea.

Mineral element	Ca	P	K	Na	Mg	Fe	Zn	Cu	Mn
Human needs(mg/d)	800	720	2000	1500	330	16	12.5	0.8	4.5
Tea content(mg/100 g)	399.4	476.5	1564.5	4.7	283.2	6.3	2.4	1.2	72.9
Nutrient density	0.499	0.662	0.782	0.003	0.858	0.394	0.192	1.5	16.2
Heat density	0.146	0.146	0.146	0.146	0.146	0.146	0.146	0.146	0.146
INQ	3.42	4.54	5.37	0.02	5.89	2.70	1.32	10.30	111.11

Note: INQ $<$ 1, cannot meet the needs of the human body; When INQ $=$ 1, the demand of the human body is reached; When INQ $>$ 1, it indicates that the nutritional quality of food is good, the same below.

Table 5
INQ value of mineral elements in conventional planting tea.

Mineral element	Ca	P	K	Na	Mg	Fe	Zn	Cu	Mn
Human needs(mg/d)	800	720	2000	1500	330	16	12.5	0.8	4.5
Tea content(mg/100 g)	351.0	499.5	970.1	3.9	226.9	5.3	2.3	0.7	101.5
Nutrient density	0.439	0.693	0.485	0.003	0.688	0.331	0.184	0.875	22.5
Heat density	0.146	0.146	0.146	0.146	0.146	0.146	0.146	0.146	0.146
INQ	3.00	4.75	3.32	0.02	4.71	2.70	1.26	5.99	154.10

contained the highest amount of EGCG (74.85 mg·g^{-1}) and the lowest amount of GC (1.11 mg·g^{-1}). Conventional planting tea had the highest concentration of EGCG (84.90 mg·g^{-1}) and the lowest concentration of GC (1.89 mg·g^{-1}). Furthermore, conventional planting tea accumulated considerably higher levels of EGCG, ECG, EGC, C, and GC than organic tea ($P < 0.01$). These results were similar to those obtained by Hu et al. (2016) on the catechin content of tea trees in Yunnan province.

The composition and proportion of catechins have an important influence on tea quality. Catechins can be divided into ester-type and non-ester-type catechins. EGCG, ECG, and GC are ester-type catechins, which make the tea taste strong and bitter. C, EC, and EGC are non-ester catechins, which make tea bitter and light in taste. As shown in Fig. 1C, the contents of ester and non-ester catechins in organic tea were significantly lower than those in conventional planting tea ($P < 0.05$), indicating that organic tea may be less bitter than conventional planting tea. Liu et al. (2023) found that the bitterness and astringency of catechin monomer is enhanced with increased concentration, and the bitterness of ester catechin is higher than that of non-ester catechin, suggesting that organic tea may have a better taste. We can also use the catechin quality index [(EGCG + ECG) / EGC × 100] to reflect the quality and tenderness of green tea (Deng et al., 2022). As shown in Fig. 1C, the catechin index of organic tea was higher than conventional planting tea, indicating that organic tea had a moderate tenderness and better quality. This result was consistent with the research findings of Li, Wang, Xiao, and Xi (2015) on organic green tea and conventional green tea from eight tea-producing regions in China. They found that the average catechin index of organic tea (744.98) is higher than that of the conventional planting tea average (617.29).

3.5.2. Comparison of flavonoids in tea

Tea is rich in flavonoids, which have substantial antioxidant properties and are readily soluble in water, creating tea soup that is yellowish green. Flavonoids are also the primary component of green tea soup color (Chen, Han, & Tong, 2024). Flavonoids are the primary source of bitterness and astringency of tea and significantly affect its quality. As depicted in Fig. 1D, among flavonoids in organic and conventional planting teas, kaempferol-3-O-rutinoside was the most abundant, whereas quercetin was the least. Organic tea had significantly higher levels of hyperoside, quercetin, and kaempferol than conventional planting tea ($P < 0.01$). Mitchell's research results on organic and conventional tomatoes are similar to those in the current experiment, which can be attributed to the low availability of nitrogen. This low availability affects the expression of nitrogen-related structural and regulatory genes, so the plant flavonoid accumulation is regulated (Mitchell, Hong, & Koh, 2007).

3.6. Comparison of heavy-metal content in tea

Heavy-metal elements in tea leaves are typically absorbed through soil and accumulate within the plant. Organic tea is a natural product, and although the production process of tea garden environment and soil requirements are high, its safety assessment is also critical. As shown in Table 6, the Pb, Cd, and Cr concentrations in organic and conventional planting teas remain below the regulatory limits, and no element was found. Organic tea exhibited lower Pb and Cd levels than conventional planting tea. Conversely, the Cr element was higher possibly due to chromium's high absorption and accumulation capacity in tea soil (Yan et al., 2016). However, the contents were still within Chinese safety standards. Overall, organic tea had a better planting and growing environment than conventional planting tea, making it more secure.

3.7. Comparison of sensory-quality characteristics of tea

3.7.1. Comparison of tea sensory-score results

To compare the difference in sensory quality between organic and conventional planting teas, three samples were selected from organic tea (ORG1, ORG2, and ORG3) and conventional planting tea (CK1, CK2, and CK3) for sensory evaluation. The sensory quality of organic and conventional planting teas was compared, and results are shown in Suppl. Table S3 and Fig. 2A, B. The results of the sensory evaluation showed that the color of organic tea was yellower and brighter than conventional planting tea, the aroma was rich and fragrant, and the taste was fresh and long lasting. The color of conventional planting tea soup was

Table 6
Comparison of heavy metal content between organic and conventional planting tea.

Heavy metal element	Organic tea (mg·kg^{-1})	Conventional planting tea (mg·kg^{-1})
Cd	0.02 ± 0.01^{b}	0.03 ± 0.01^{a}
Hg	ND	ND
Cr	1.77 ± 0.46^{a}	1.07 ± 0.43^{b}
Pb	ND	0.12 ± 0.07
As	ND	ND

Note: "ND" means not detected; Different lowercase letters in each line indicate significant difference, $P < 0.01$.

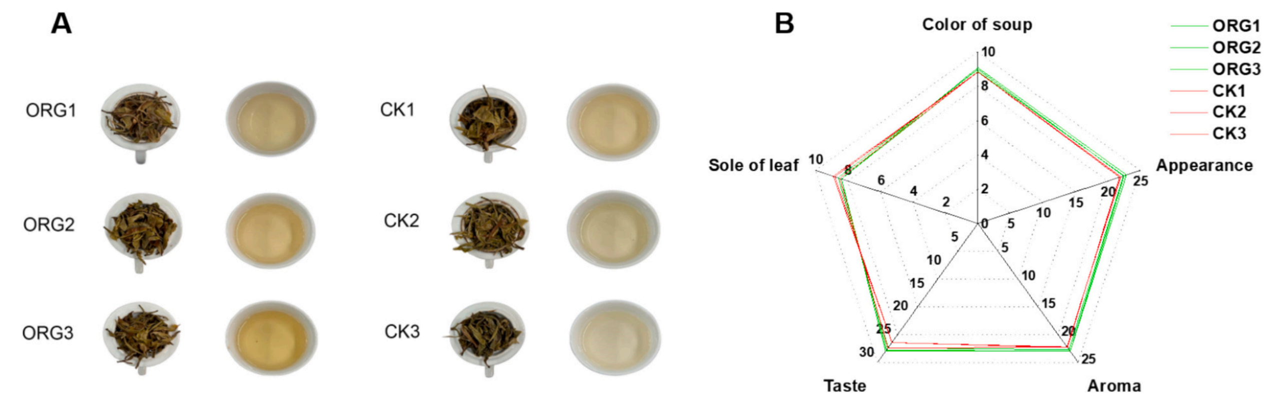

Fig. 2. Sensory evaluation results of organic and conventional planting tea.
(A) Comparison of sensory evaluation between organic and conventional planting tea. (B) Radar chart of sensory evaluation scores for organic and conventional planting tea.

light yellow; the aroma was primarily mild, and the taste was fresh, strong, durable, and slightly astringent. The color of organic tea was brighter than that of conventional planting tea, which may be related to the higher content of flavonoids in organic tea than in conventional planting tea. Organic tea tasted fresher and less astringent than conventional planting tea, which may be due to the higher content of free amino acids in organic tea. The lower ratio of tea polyphenols to amino acids endowed tea with fresher and refreshing taste substances. The content of caffeine, tea polyphenols, and ester catechins was lower, so the tea soup was less bitter and astringent.

Both organic and conventional planting tea had lower leaf-base quality scores. Red leaves and red stems on the leaf base may be caused by no drying and stacking in time or too long browning time during processing (Zhang et al., 2023). In the comprehensive sensory-evaluation score, sample ORG1 had the best appearance, color, aroma, and taste, followed by ORG2, ORG3, CK2, and CK3, whereas sample CK1 scored the lowest.

3.7.2. Comparison of the color difference of tea soup

Fig. 3 shows the color difference between organic and conventional planting teas. Organic tea had considerably greater a* (-1.17 ± 0.28) and b* (7.66 ± 1.53) values than conventional planting tea a* (-0.83 ± 0.14) and b* value (4.24 ± 0.37) ($P < 0.01$), but no significant difference in L* value. Results showed that the color of organic tea soup was more red than yellow, consistent with the result that organic tea had a higher score of light yellowish green in the sensory evaluation and conclusion of yellow-green leaf bottom.

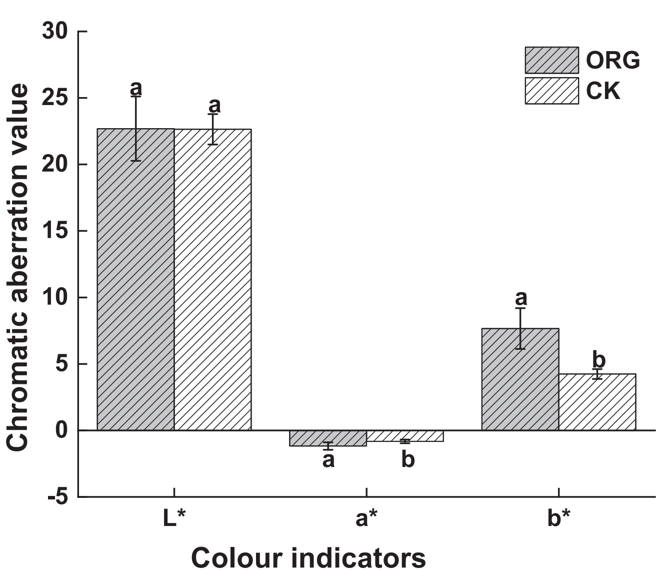

Fig. 3. Comparison of the difference in tea color between organic and conventional planting tea.

3.8. Comparison of antioxidant activity

The antioxidant activity of tea is primarily attributed to phenolic compounds present in its leaves. Fig. 4 shows that the scavenging ability of DPPH and $ABTS^+$ free radicals increased linearly when the concentration of tea increased. When the concentration of tea reached 50 $\mu g \cdot mL^{-1}$, the scavenging rates of free radicals were the highest. DPPH was 74.24 % and 75.97 %, and $ABTS^+$ was 85.41 % and 87.28 %, respectively. These results showed that both kinds of tea had strong scavenging ability on DPPH and $ABTS^+$ free radicals, and no significant difference existed between organic and conventional planting teas regarding the antioxidant activity of DPPH and $ABTS^+$. Conversely, conventional planting tea had slightly higher antioxidant capacity. The reason may be the significant role of tea polyphenols in the antioxidant activity of tea extracts, consistent with the result that the content of tea polyphenols in conventional planting tea was slightly higher than that in organic tea in result 1 ($P > 0.05$). Reche et al. (2019) showed no significant difference in antioxidant activity between organic and conventional planting jujube, which was also attributed to the fact that no significant difference existed in the content of polyphenols, similar to our results.

3.9. Multivariate statistical analysis of biochemical quality of organic and conventional planting tea

3.9.1. Correlation analysis

The premise of PCA is to assume a strong linear correlation between the original data variables, so the correlation between the measured quality components was analyzed. A Pearson correlation heat map analysis was established on the leading internal-quality indicators of 51 organic and conventional planting teas. Due to the extensive data, the P-value was set to 0.001 to ensure highly significant results. As depicted in Fig. 5, most amino acids were positively correlated with one another, whereas most amino acids were negatively correlated with Se, Ti, heavy metals, and catechins. Mineral elements can increase the concentration of free amino acids, especially the application of magnesium and potassium, which can significantly increase the content of free amino acids in tea. The highest content of free amino acids was found in tea applied with magnesium and potassium (Piyasena & Hettiarachchi, 2023). Result 3 showed that organic tea had a higher content of K and Mg than conventional planting tea, and result 2 showed that organic tea had a higher content of free amino acids than conventional planting tea,

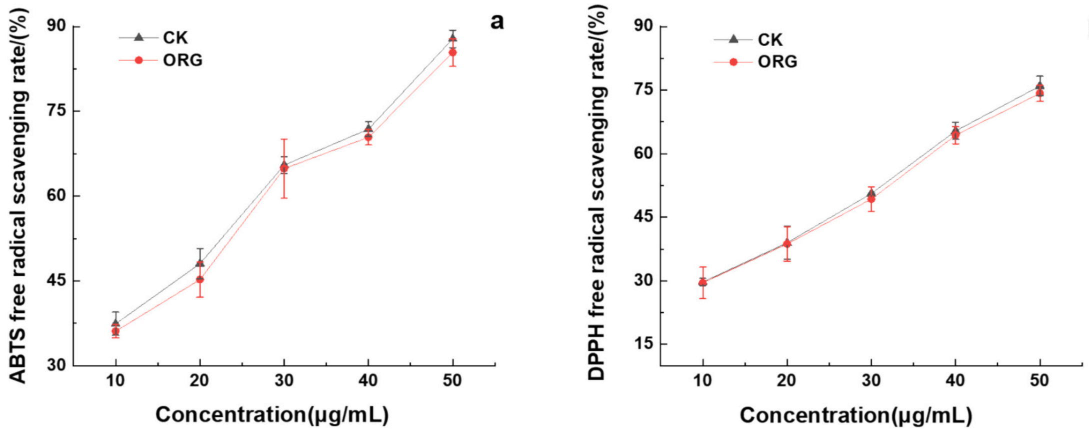

Fig. 4. Scavenging ABTS^{+} and DPPH radicals' effect on different concentrations of organic and conventional planting tea. (a) Effect on ABTS^{+} free radical scavenging rate. (b) Effect on DPPH free radical scavenging rate.

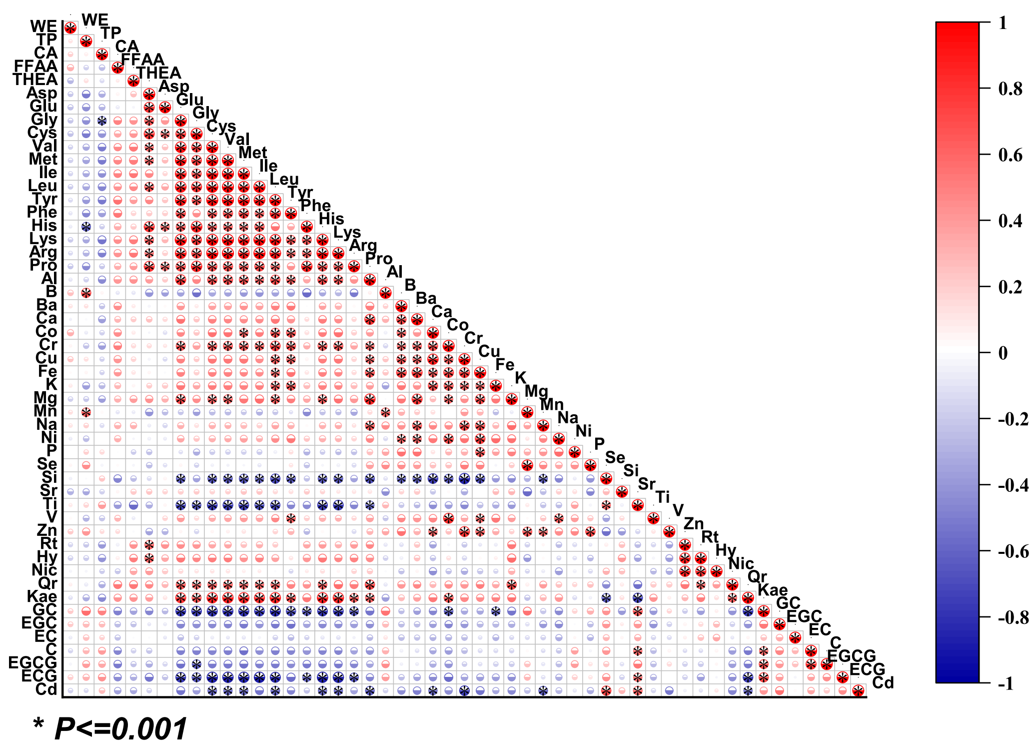

Fig. 5. Heatmap for correlation analysis of substances in tea.

consistent with the results of correlation analysis. However, the correlation among quality components had many overlaps, so further PCA was necessary.

3.9.2. Analysis of principal components (PCs) of organic and conventional planting tea

We extracted the first nine PC indicators with a cumulative variance rate > 80 % according to the PC load coefficient to identify the indicators of difference between organic and conventional planting teas. We identified the index represented by each PC by the absolute value of the load coefficient > 0.4 (Suppl. Table S4). As shown in Suppl. Table S5 shows the eigenvalues and variance contribution rates for the first nine significant components, with a cumulative contribution rate of 87.325 %. Results suggested that these nine key components encompassed most of the information from 51 indicators and can better reflect the quality components of organic tea than conventional planting tea.

The differences in organic and conventional planting teas composition were shown in the scatter diagram of the extracted PC (Fig. 6A). The PCA score plot revealed that although a preliminary classification between organic and conventional planting teas was achieved to a certain extent, the cumulative variance contribution of the first two PCs (PC1 and PC2) fell short of 80 %. This suggested that the PCA model lost a significant amount of information when processing the raw data, resulting in poor separation, low clustering within and between groups, and numerous interfering factors, ultimately affecting classification efficacy. Therefore, further optimization analysis was required to use the supervised discriminant model.

3.9.3. Orthogonal partial least square analysis of organic and conventional planting tea

To identify the substances that caused the difference between organic and conventional planting teas, a detailed data analysis was conducted through OPLS-DA to model the predictor changes associated with and orthogonal to the dependent variable, respectively (Yan et al.,

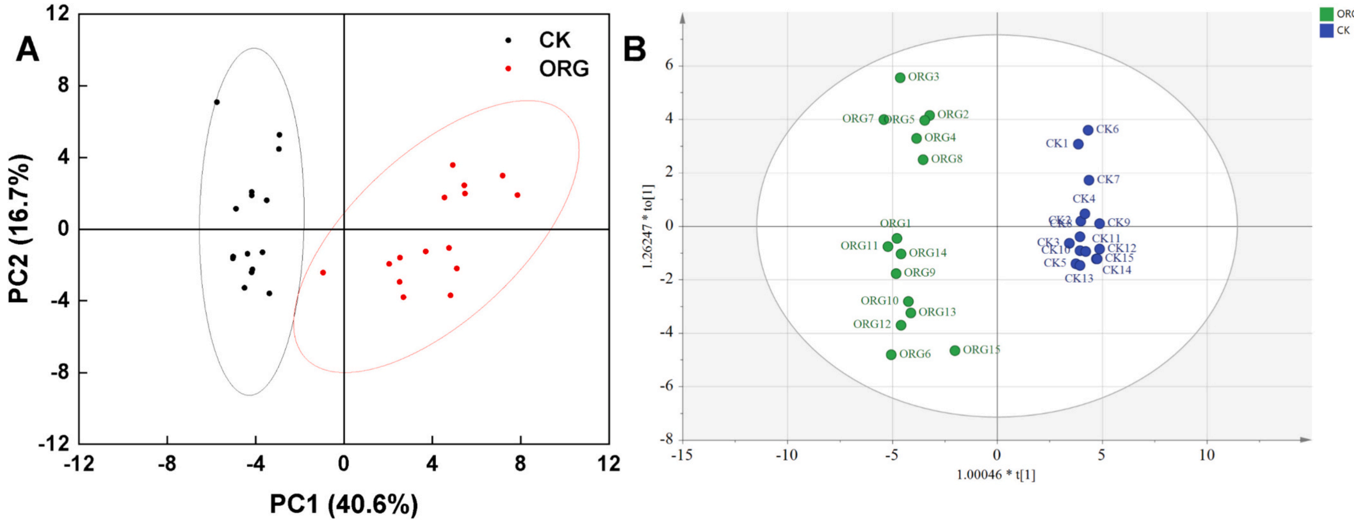

Fig. 6. Principal component analysis and orthogonal partial least squares analysis of organic and conventional planting tea.
(A) PCA scores of components in organic tea and conventional planting tea. (B) Plot of OPLS-DA scores for components in organic and conventional planting tea.

2024). Among the 51 measured components, 37 with significant differences ($P < 0.05$) were introduced into the OPLS-DA model. R^2X, R^2Y, and Q^2 were the three key metrics for evaluating the model's performance, where R^2X represented the interpretation rate of the X matrix, R^2Y indicated the interpretation rate of the Y matrix, and Q^2 assessed the model's predictive capability. Values greater than 0.5 for these metrics signified an acceptable model, with closer values to 1 indicating more substantial predictive power. In this study, the model achieved $R^2X = 0.706$, $R^2Y = 0.979$, and $Q^2 = 0.913$, all exceeding 0.5, suggesting that the model was good.

Furthermore, the analysis results revealed that 30 samples of organic and conventional planting teas were grouped on the positive and negative axes of the X axis, respectively, with no overlap, and the difference was significant. (Fig. 6B). All samples fell within the 95 % confidence interval, indicating no significant outliers and confirming that the experimental data were within normal ranges. As evident from the variable importance in projection (VIP) values (Fig. 7) comparing organic and conventional planting teas, 15 indicators with VIP values greater than 1 were identified as the primary marker components contributing to the differences between the two tea types.

To explore whether the OPLS-DA model had an overfitting phenomenon, we conducted 200 cross-permutation tests on the model, and the results are shown in Suppl. Fig. S3. The intercept between the Q^2 and Y-axis was less than 0, indicating no model overfitting, and the verification result was valid.

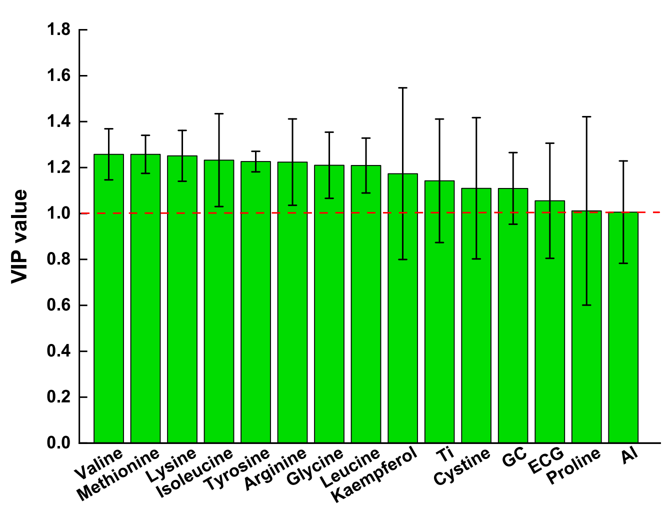

Fig. 7. Chart of variable importance in projection.

3.9.4. *LDA analysis of organic and conventional planting tea*

An LDA model was established to verify the consistency of the analysis results among different models and the discriminant effect of other substances based on the differentiated substances screened by VIP values (Ma et al., 2016). The "leave one method" for cross-validation was used to avoid model overfitting. Additionally, a portion of the data was randomly selected to remove its classification information and construct the discriminant function. At the same time, the remaining known sample data were utilized as a training set to assess the model's correctness. Finally, the equation was established in the model, which can capture all variance information. The established criterion discriminant function equation was given below:

$$\begin{aligned} F(X) = & -8.315 - 9.252\,X_{Gly} + 4.565\,X_{Cys} + 56.599\,X_{Val} - 49.200\,X_{Met} \\ & + 7.100\,X_{Ile} - 11.248\,X_{Leu} + 2.034\,X_{Tyr} + 17.212\,X_{Lys} \\ & - 3.774\,X_{Arg} + 26.672\,X_{Pro} + 0.001\,X_{Al} + 0.002\,X_{Ti} \\ & + 214.017\,X_{Kae} - 2.918\,X_{GC} + 0.118\,X_{ECG}. \end{aligned}$$

The cross-validation results for all samples are presented in Suppl. Table S6, with an accuracy rate of 93.3 %. Additionally, a test set comprising four organic and five conventional planting tea samples was selected, with their group information removed. Conversely, the remaining 21 samples served as the training set for model development. The test set was then introduced into the training set through cross-validation. The model correctly identified 9 out of 11 organic tea samples and misclassified 2. Conversely, for the 10 conventional planting tea samples, nine were correctly identified and one was misclassified. The model achieved an accuracy rate of 85.7 % on the training set and a perfect accuracy rate of 100 % on the test set. This finding suggested that reducing the sample size may affect the model's accuracy.

Through the combination of univariate significance analysis, OPLS-DA, and LDA, we identified valine, methionine, lysine, isoleucine, tyrosine, arginine, glycine, leucine, kaempferol, Ti, cystine, GC, ECG, proline, and Al as discriminatory indicators. These indicators achieved a discrimination accuracy of 93.3 %, demonstrating their high stability and reliability in differentiating organic and conventional planting teas regarding their unique chemical constituents. Therefore, they can be selected as marker substances.

4. Conclusion

This study compared organic green tea with conventional planting tea planted in Dajianshan, Yunnan province of China to investigate the variations in sensory quality and distinctive components. Results showed that organic tea had higher ratings in sensory evaluation, appearance, taste, and aroma than conventional planting tea. The soup's color was yellow and green, the taste was fresh and sweet, and the aroma was rich and lingering in organic tea. Organic tea also contained significantly more free amino acids, theanine, quercetin, kaempferol, and minerals (such as Al, Ca, Fe, K, Mg, Na, Co, and Cu) than conventional planting tea. At the same time, the content of EGCG, ECG, EGC, C, GC, and Cd was significantly lower. Organic tea contained fewer heavy-metal components and more essential minerals, making it safer for human health.

Furthermore, the LDA of PCA-OPLS-DA combined with LDA confirmed that valine, methionine, lysine, isoleucine, tyrosine, arginine, glycine, leucine, kaempferol, Ti, cystine, GC, ECG, proline, and Al can be selected as markers to differentiate organic tea from conventional planting tea. This study can serve as a valuable reference for objectively evaluating the quality of organic tea.

CRediT authorship contribution statement

De Zhou: Writing – original draft, Methodology, Formal analysis, Data curation. **Yunfei Hu:** Visualization, Investigation, Data curation. **Xi He:** Supervision, Software, Methodology. **Lijuan Du:** Data curation. **Lian Bao:** Software. **Ming Zhao:** Supervision. **Jinliang Shao:** Project administration, Funding acquisition. **Qingyan Tang:** Writing – review & editing, Supervision, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102299.

Data availability

Data will be made available on request.

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