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Widely targeted metabolomics analysis of different Wuyi Shuixian teas and association with taste attributes

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A R T I C L E I N F O

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A B S T R A C T

There are two major types of Wuyi Shuixian teas with distinct flavor properties in the market: regular Shuixian, and Laocong Shuixian that is produced from old tea plants with higher sale price. However, the chemical composition difference between these two types of Shuixian teas is still unclear. In this study, the widely targeted metabonomics and sensory evaluation were carried out to investigate the metabolite profiles and the flavor properties of Laocong and regular Shuixian tea samples. The results of organoleptic evaluation showed the Laocong Shuixian teas achieved dramatically higher total scores of sensory quality than that of regular Shuixian, and the sour palate of Laocong Shuixian tea was much lower than that of regular Shuxian. A total of 692 metabolites were identified by using metabolic determination, 43 of which were different metabolites in Laocong Shuixian teas discriminated from regular Shuixian. The contents of caffeic acid, kaemperfin, genistin, quercetin 3-glycosides and *p*-coumaric acid-*O*-glycoside were abundantly present in regular Shuixian tea. The analysis on different metabolites and taste attributes showed that phenolic acidic compounds were the major contributors to the sour taste of regular Shuixian. This study interpreted the chemicals underlying the different taste properties of Laocong and regular Shuixian teas.

1. Introduction

Tea plant (*Camellia sinensis* (L.)) is a highly valuable crop used for beverage production. Tea can be sorted into six types based on the processing method, namely black tea, white tea, green tea, yellow tea, oolong tea, and dark green tea, with increasing oxidation degree [1]. Fujian province is renowned for its production of semi-fermented oolong tea [2]. Wuyi Shuixian tea, a subtype of oolong tea, originates from Jianyang County, Nanping City, Fujian Province. It is made from the banjhi shoots, consisting of two or three leave and a banjhi bud harvested from *Camellia sinensis* cv. Fujian Shuixian. Wuyi Shuixian tea is known for its woody and flowery fragrance, smooth taste, and nutty flavor [3]. There are different types of Wuyi Shuixian teas with their very own characteristic flavor, which are highly associated with their raw materials. The age of the tea plants is considered as an crucial factor in determining the sensory properties of Wuyi Shuixian teas. The Shuixian tea produced using raw materials harvested from tea plants aged over ten years is referred to as "Laocong Shuixian", which is believed to have superior quality and commands a hight price.

The distinct sensory quality of "Laocong Shuixian" can be attributed to its metabolite profile, which differs from regular Shuixian tea [4]. However, the previous studies on Shuixian teas mainly focoused on the metabolism pathways, chemical characterization or

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healthy benefits, the specific metabolites that contribute to the different flavor between "Laocong Shuixian" and regular Shuixian teas remain unclear. The study on different Shuixian tea varieties showed that the major discrimination among the wildtype Shuixian genotype and its albino mutant was that the former had higher contents of chlorophyll and carotenoids [5]. The roasting degrees was a major contributor to the distinction of volatile profiles between Shuixian and other oolong teas [6]. The phenolic-enriched extracts from Shuixian tea were able to inhibit the growth and metastatic capacity of breast cancer cells, showing a potential application as antioxidant and anticancer candidates in food and pharmaceutical industry [7].

In recent years, due to the sharp increase in demand for Laocong Shuixian tea, adulteration of the expensive tea with cheap teas (such as regular Shuixian teas) may take place. The appearance and color of these cheap teas are similar to Laocong Shuixian, therefore it is difficult to distinguish the authenticity of Laocong Shuixian tea. Identification and quantification the distinct metabolites among Laocong Shuixian and regular Shuixian teas is crucial in order to assess the adulteration of teas.

Recent advancements in analytical technologies, such as liquid chromatography and mass spectrometry combined with high-throughput techniques, have been applied in metabolomic studies [8,9]. These advanced analytical methods provide valuable insights into the comprehensive metabolite profiles that control or influence biochemical processes or metabolites accmulation [6], determine germplasm diversity [10], or detecting the dynamic changes during the manufacturing, contibuting to the sensory characteristics [11,12]. The in-depth and robust datasets obtained from metabolomic analyses show the potential to uncovered metabolites responsible for specific features.

To explore the metabolites contributing to the taste variation of Laocong Shuixian and regular Shuixian teas, metabolomic analysis was conducted using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to profile the metabolite abundance of various Wuyi Shuixian teas, including Laocong Shuixian and regular Shuixian The comprehensive sensory evaluation and quantitative descriptive analyses were carried out to provide an overall assessment and taste-specific sensory evaluation of different Wuyi Shuixian teas. A co-expression network constructed based on the metabolic dataset and sensory property scores of different Shuxian teas by using weighted gene co-expression network analysis (WGCNA) was applied to further identify the metabolites that are highly correlated with taste attributes. The flow chart of the experimental procedure is presented in Fig. S1.

2. Materials and methods

2.1. Materials and reagents

The Wuyi Shuixian tea samples were obtained from the local market in Wuyi Mountain area. The regular Shuixian tea is commonly characterized by its floral fragrance, fresh and brisk tastes according to the national standard of oolong tea (GB/T 30357.4–2015 Oolong tea-Part 4 Shuixian). Laocong Shuixian teas are characterized by woody, green-moss, or bamboo-leaf with charcoal-baked aroma respctively apart from the attrbutes of regular Shuixian [13]. Thus, three representative kinds of Laocong Shuixian tea with typical featrues of aroma, as well as one regular Shuixian tea with ordinary attbutes of Shuixian teas, were collected from the local market of Wuyi Mountain area and used in this study. The three Laocong Shuixian teas were termed LCSX1, LCSX2, and LCSX3, the regular Shuixian tea was termed SXCK accordingly. All samples were stored at −25 °C prior to further analysis.

Methanol and acetonitrile of the chromatographical grade were obtained from Sigma-Aldrich (Beijing, China), ultrapure Milli-Q water was supplied by Millipore Elix advantage 5 (Billerica, MA, USA).

2.2. Sensory evaluation and quantitative descriptive analysis of tea samples on tea samples

Two sensory evaluation methods were carried out to give a full concept of the sensory attributes of different Wuyi Shuixian tea samples. The sensory evaluation panel are consisted of 7 professional engaged in tea production and organoleptic assessment. The members of panel are composed of 4 males and 3 females ranged from 30 to 50 years old. The preparing procedures of tea infusions were conducted according to Chinese national standard GB/T 23776-2018. In brief, the tea brewing method was to weigh 5 g for each sample into the cup set (pot and lid), then pour a quantity of 110 mL freshly boiled water (100 °C) to pot for the brewing. Then the tea infusion was transferred to bowls to appraise the aroma and taste of tea infusions. The dry tea score, tea infusion aroma score, tea infusion taste score, tea infusion color and infused leaf score were evaluated by panelists, and the total score was calculated. In addition, a quantitative descriptive analysis was employed to assess the taste attributes of Wuyi Shuixian teas, including smoothness, sourness, astringency, bitterness, sweetness and umami taste according to the previous study [14]. The panelists rated the intensity of taste attributes of tea samples based on a nine-point scale ranging from 0 (absent) to 9 (extremely strong). The average score of each attribute was used for plotting diagrams. All panelists were informed and the informed consent was obtained from all participants for our experiments.

2.3. Metabolomic analysis on tea samples

The metabolite extraction from each Shuixian sample and metabolomic analysis were performed by aMetware Biotechnology (Wuhan, China). In brief, the tea sample was ground by a miller (MM 500, Retsch Company, Haan, Germany) with zirconia beads for 3 min at 20 Hz. Then, 100 mg of tea powder was extracted with 0.6 mL of 70% methanol containing 0.1 mg/L lidocaine for internal standard at 4 °C for 12 h. After centrifugation at 12, 000 g for 12 min, the supernatants were collected and filtered through 0.22 μm filter (Anpel, Shanghai, China) prior to LC-MS/MS analysis. A quality control (QC) sample was prepared by mixing 15 g of each sample in order to unravel the repeatability and reliability of the analytical results.

The extracts were submitted to an UPLC coupled with an electrospray ionization tandem mass spectrometry (CBM30A system, Applied Biosystems, Waltham, USA), using the following UPLC conditions: mobile phase A = 0.04% acetic acid + 99.96% water (v/v), mobile phase B = 0.04% acetic acid + 99.96% acetonitrile (v/v); linear gradient elution: from 95.0% (v) A/5.0% (v) B to 5.0% (v) A/95.0% (v) B within 10 min, then remaining at 5.0% (v) A/95.0% (v) B for 1 min, followed by 4 min re-equilibrium; column temperature 38 °C; injection volume 5 μL. An ESI-triple quadrupole-linear ion trap mass spectrometer (API 4500 Q UPLC/MS/MS System) was employed for linear ion trap (LIT) and triple quadrupole (QQQ) scans in both positive and negative ion modes. Multiple reaction monitoring was employed for identification and quantification of individual metabolites. Analyst software (AB SCIEX Pte. Ltd,

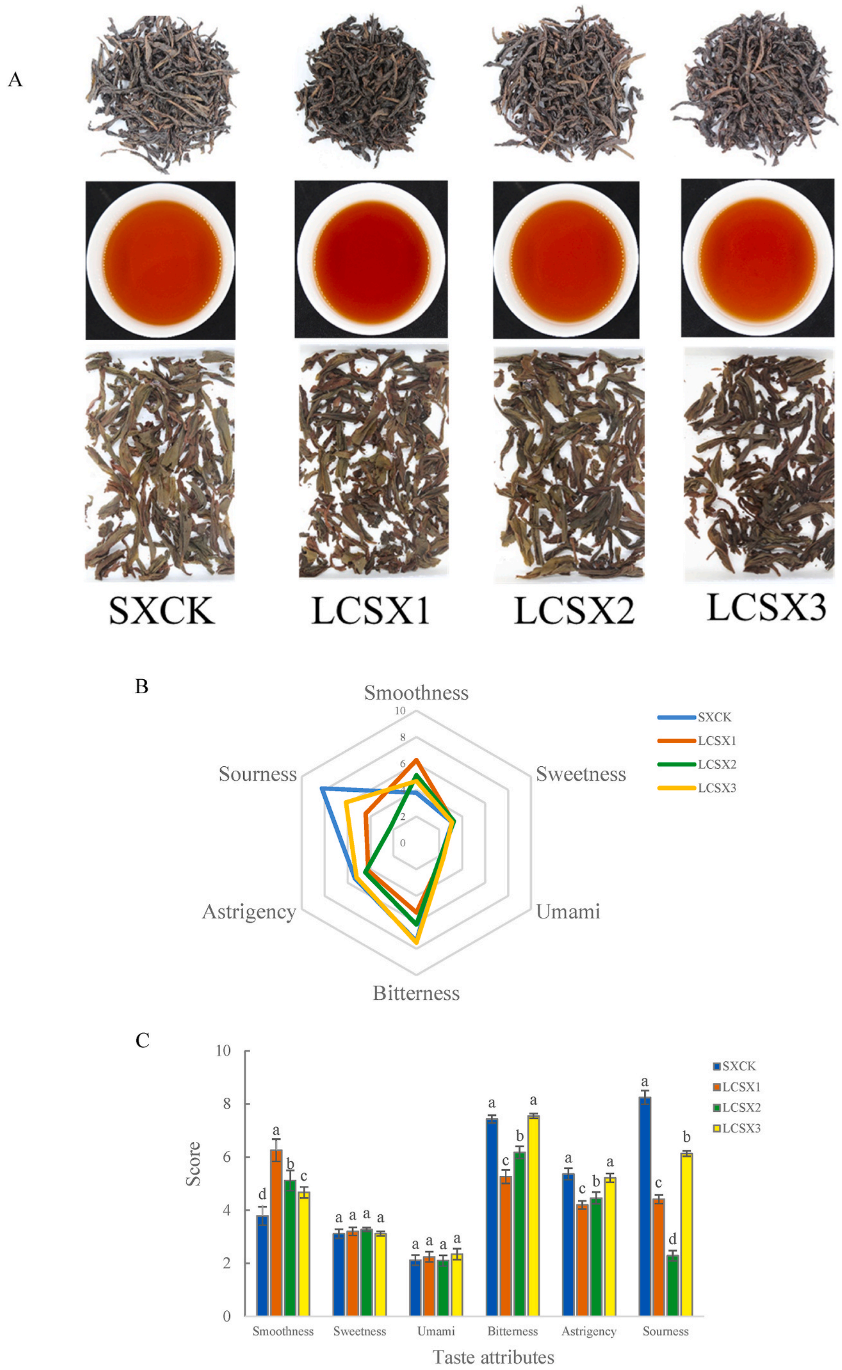

Fig. 1. The profile of different Shuixian tea samples used in this study. (A): The appearance of different Shuixian tea samples. The dry tea, infusion and infused tea of each Shuxian tea sample were preseented from top to bottom, respectively. (B):The radar plot of the taste features of different Shuixian teas. (C):The scores of taste attributes of different Shuixian tea samples.

Framingham, USA) was used for data filtration and calculation. The identification of metabolites were carried out by comparing the retention times, mass-to-charge (*m/z*) values, and the fragmentation patterns with the authentic standards or by searching against the public databases (MassBank, KNApSAcK, HMDB, and METLIN).

2.4. Differential metabolites analysis

Orthogonal projection to latent structures-discriminant analysis (OPLS-DA) were used to visualize the dataset into the components, and the variable importance projection (VIP) values were calculated. The criteria of VIP values $\geq$ 1.0 and $|Log_2$ (Fold change)$|\geq 1$ was used to selected different metabolites for each paired-comparison.

2.5. Association analysis on non-volatiles and taste attributes

The construction of co-expression network was performed based on the metabolic profiles and sensory property scores of different Shuxian teas using R package WGCNA. This analysis were carried out with a correlation soft-thresholding power ($\beta = 8$ in this study). After calculating the topological overlap measure, a dendrogram was plotted and the highly correlated metabolites were clustered into modules according to the DynamicTreeCut algorithm, then the clustering algorithm was used to enrich highly related metabolites in the network into modules to illustated the flavor associated metabolites.

2.6. Data analysis

Three replicates were randomly collected from each tea sample and used for determination. Principal component analysis (PCA) was performed on SIMCA 14.1 (Umetrics Company, Malmö, Sweden). To assess the homogeneous clusters of samples, the log_2-transformed dataset were used to conduct the heatmap and hierarchical clustering analysis (HCA) using R package.

3. Results and discussion

3.1. Sensory evaluation on different types of Shuixian teas

Fig. 1A shows the appearance features of various Shuixian teas, including dry tea leaves, infusion color, and infused tea leaves. It is not easy for an ordinary consumer to discriminate regular "Wuyi Shuixian"from "Laocong Shuixian" teas only based on the appearance of dry tea leaves and tea infusion. Thus, a comprehensive sensory evaluation was performed to assess the overall sensory properties of different types of Shuixian teas, and the results were displayed in Table 1, including the scores of dry tea, infusion aroma, infusion taste, infusion color, infused leaf and the calculated total score based on the weighted score of all the five individual factors. As presented in Table 1, LCSX teas achieved higher total scores related with sensory quality, which was consistent with the market recognition. Taste is an important index to rank the quality of different Shuixian teas, with LCSXs achieving higher taste scores than SXCK (Table 1).

To elaborate the taste difference between "Laocong Shuixian" and regular Shuixian tea, a quantitative descriptive analysis was carried out. The radar plot of the taste features of different Shuixian teas is presented in Fig. 1B. The result showed different types of Wuyi Shuixian teas had similar flavor features, with relatively lower intensities of sweetness and umami taste, moderate intensities of smoothness and astringency but distinct bitterness and sourness (Fig. 1B). Notably, the sourness of SXCK was much stronger than that of LCSXs (Fig. 1C), which could be the most significant sensory quality differnet from Laocong Shuixian teas.

Table 1
Comprehensive sensory evaluation result of Wuyi Shuixian tea samples[a].

Tea sample	Dry tea	Aroma	Taste	Infusion color	Infused leaf	Total score
SXCK	Burly, tight, curly, slight red edge (87.9 ± 1.6)	Obvious charcoal-baked aroma (85.4 ± 2.1)	Harsh, sour, comparatively mellow (85.1 ± 2.2)	Deep and bright orange yellow (89.7 ± 1.4)	Burly, soft and bright (89.6 ± 1.7)	86.4
LCSX1	Bold, tight, curly, glossy with obvious red edge (95.1 ± 2.0)	Obvious roast and woody fragrance (92.9 ± 2.3)	Mellow, thick, smooth, with a little sour (95.3 ± 1.4)	Clear and bright orange red (90.0 ± 0.8)	Burly, soft bright with obvious red edge (95.1 ± 1.3)	94.3
LCSX2	Bold, tight, curly, glossy with obvious red edge (92.5 ± 2.8)	Strongly sweet bouquet, with slight green-moss fragrance (95.1 ± 0.9)	Mellow, thick and smooth, with a little sour (91.9 ± 1.3)	Deep and bright orange yellow (89.7 ± 1.5)	Burly, soft and bright with even appearance and red edge (95.1 ± 0.7)	93.2
LCSX3	Tight, curly, glossy, comparatively burly, with obvious red edge (89.8 ± 1.1)	Steamed bamboo-leaf with charcoal-baked aroma (89.6 ± 0.8)	Brisk, comparatively sour (90.1 ± 0.7)	Deep and bright orange yellow (90.3 ± 0.8)	Burly, soft and bright with even appearance and red edge (94.7 ± 0.8)	90.4

[a] The individual score of each index was labeled in the parenthesis. The total score = (dry tea score × 20%) + (aroma score × 30%) + (taste score × 35%) + (infusion color score × 5%) + (infused tea score × 10%) based on the evaluation standard of GB/T 23776-2018.

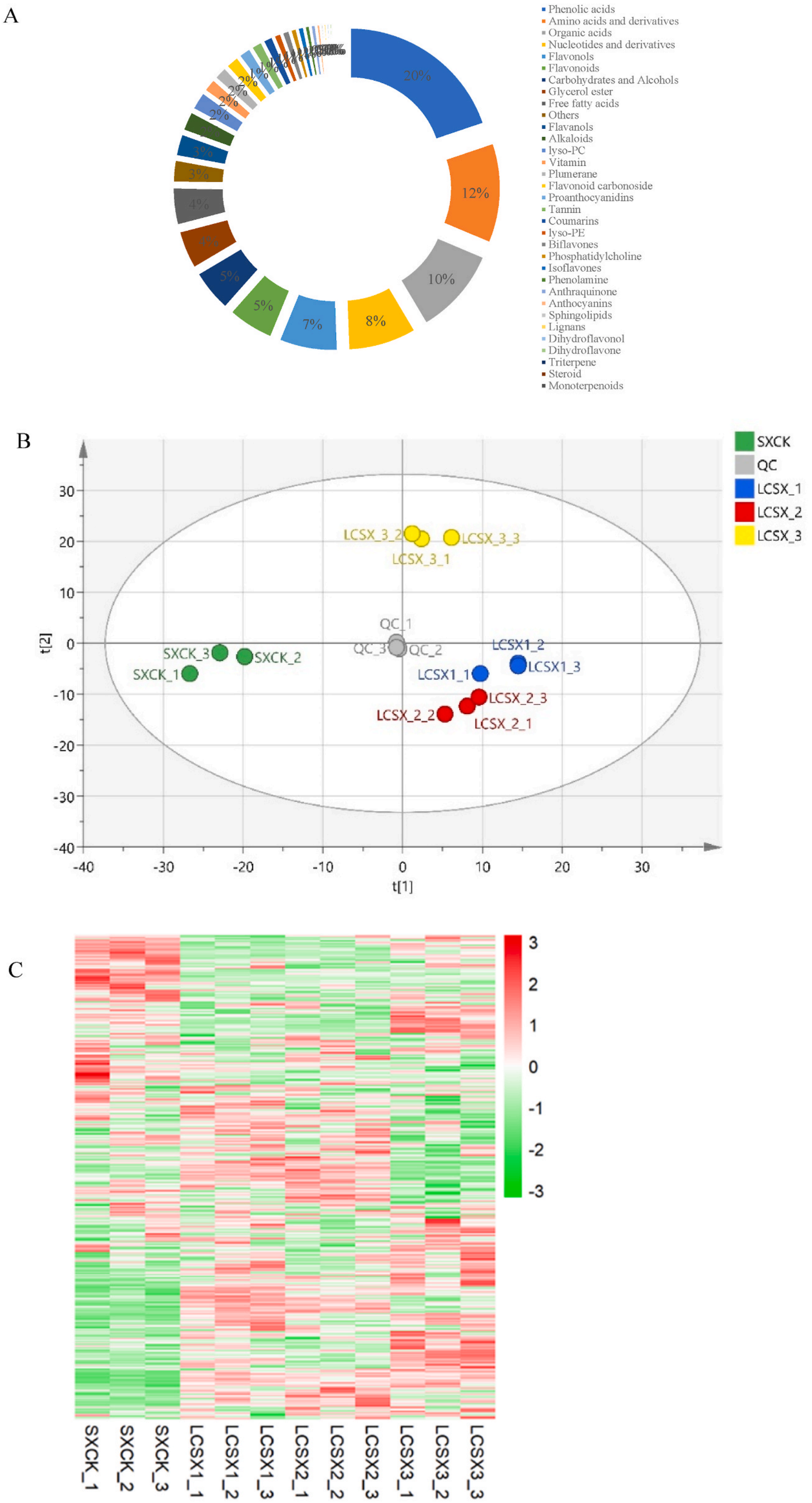

Fig. 2. The non-volatile metabolite profiles of Wuyi Shuixian teas. (A): Pie chart of biochemical categories of differential metabolites. (B): The PCA plot of different tea samples. (C) The heatmap of different Shuixian teas.

3.2. The non-volatile metabolic profiles of Shuixian teas

A total of 692 metabolites were identified and quantified by widely-targeted metabolomic determination, which were categorized into 33 classes, including 137 phenolic acids, 80 amino acids derivatives, 70 organic acids *etc* (Table S1). Fig. 2A shows the distribution

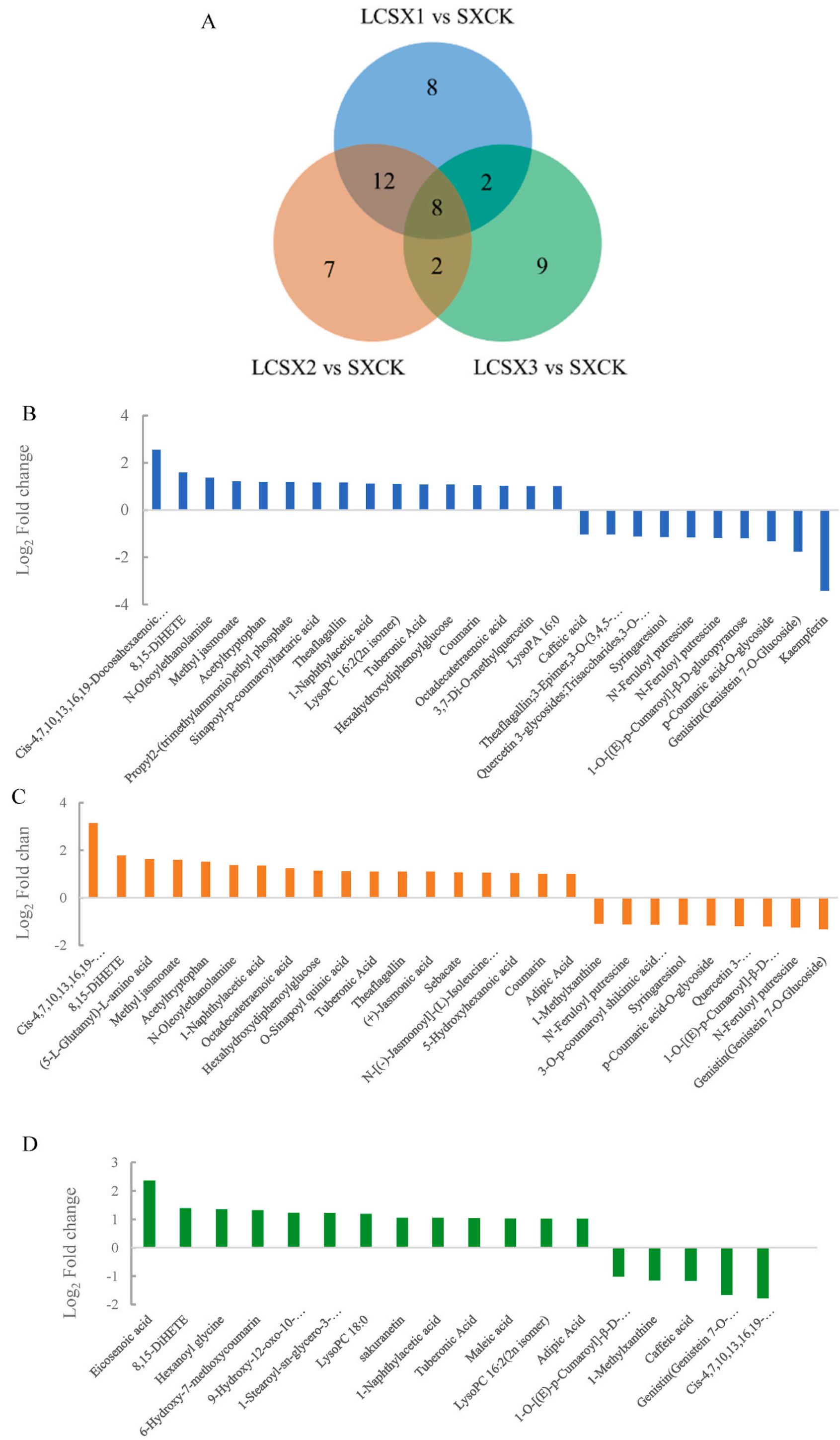

Fig. 3. The different metabolites based on paired-comparison. (A): the Venn plots of different metabolites. (B), (C), (D): the significant different metabolites for the comparison pairs of LCSX1/SXCK, LCSX 2/SXCK and LCSX3/SXCK, respectively. (E): The heatmap and HCA clustering results of 43 significant different metabolites.

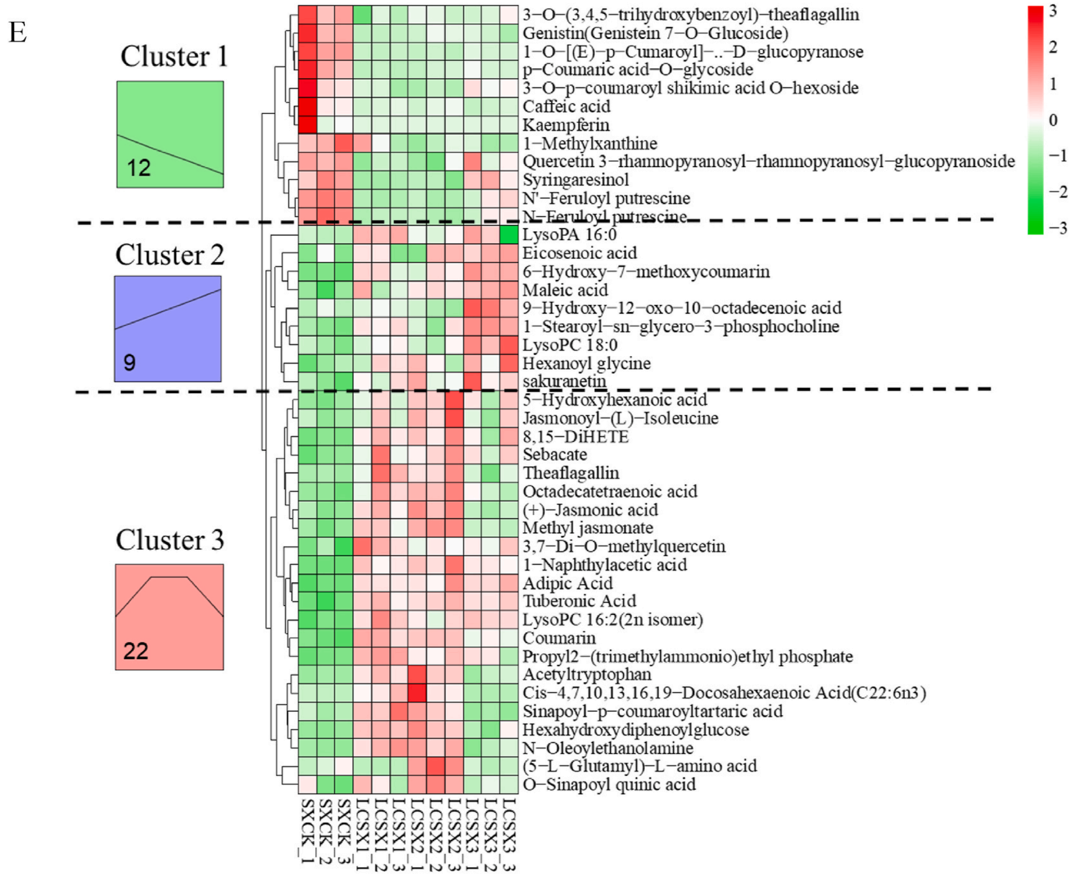

Fig. 3. (*continued*).

ratios of different compound classes based on the number of compounds.

PCA was used to discriminate the metabolic profiles of different Wuyi Shuixian teas. Clearly, the triplicates of each sample were clustered together, and separated from other samples (Fig. 2B). QC group was closely located around the origin of orthogonal coordinate, indicating analytical results is reliable and repeatable. The first two of PCs accounted for 53.9% of total variance (PC1 = 34.5%, and PC2 = 19.4%). The triplicates of SXCK sample were distributed in negative direction of PC1, whereas the triplicates of LCSX1, LCSX2 and LCSX3 were located in positive direction of PC1, indicating SXCK was distinguished from LCSXs based on PC1. Moreover, LCSX1 and LCSX2 had the shortest distance in between (Fig. 2B), suggesting a similar metabolic profiles in these two samples. Fig. 2C shows the heatmap of the metabolites in these four Shuixian teas. Apparently, the feature pattern of SXCK was different from those of LCSXs. Among Laocong Shuixian samples, the feature patterns of LCSX1 and LCSX2 showed the highest similarity, which was consistent with the PCA result.

3.3. *The significant different metabolites between Laocong and regular Shuixian teas*

To explore the biomarkers for discriminating of LCSXs for SXCK, the paired-comparisons were conducted to obtain the significant different components using OPLS-DA. The result showed that dozons of components were screened out in the comparison of LCSX1/SXCK, LCSX2/SXCK and LCSX3/SXCK, respectively (Fig. 3A, Table S2). After filtering out the inaccurately identified substances, the rest compounds were further applied to refine the significant different metabolites between the Laocong and regular Shuixian teas. Both LCSX1 and LCSX2 contained much higher content of *cis*-4,7,10,13,16,19-docosahexaenoic acid, which were above 2.5 folds of those in SXCK, followed by 8,15-DiHETE, with the fold change being above 1.5 (Fig. 3 B & C). Lower contents of genistin and *p*-coumaric acid-*O*-glycoside were observed in both LCSX1 and LCSX2 compared with SXCK (Fig. 3 B & C). For LCSX3, two lipid derivatives, namely eicosenoic acid (2.4 folds) and 8,15-DiHETE (1.4 folds), were abundantly present in LCSX3, while the contents of genistin and *cis*-4,7,10,13,16,19-docosahexaenoic acid were lower than SXCK (Fig. 3D).

A total of 43 different metabolites compared with SXCK were summarized and hierarchically clustered (Fig. 3E). The significant variation in metabolite profiles over the four Shuixian teas could be roughly divided into three major clusters as demonstrated in Fig. 3E. For the sourness and astringency-related compounds, phenolic derivatives, such as caffeic acid, kaemperfin, genistin, quercetin 3-glycosides and *p*-coumaric acid-*O*-glycoside were abundant in SXCK (Cluster 1, Fig. 3E). Phenolic acids, exhibit lingering-tartness flavor, which enhance the acidity of tea infusion [15], and contribute to astringent and bitter tastes [16], collectively resulted in the dramatically sour, astringent and bitter palates in SXCK (Fig. 1 B & C). Fatty acids, such as *cis*-4,7,10,13,16,19-docosahexaenoic acid, 8,15-DiHETE, were higher in LCSX1 and LCSX2 compared with LCSX3 and SXCK (Cluster 3, Fig. 3E). The lipids in food matrix play an important role as a barrier to bitter, astringent or sour tastes by decreasing aqueous volume and contact surface area between aqueous phase and mouth [17], resulting in the less bitterness or astringency of LCSX1 and LCSX2 as aforementioned in the sensory evaluation (Fig. 1 B & C).

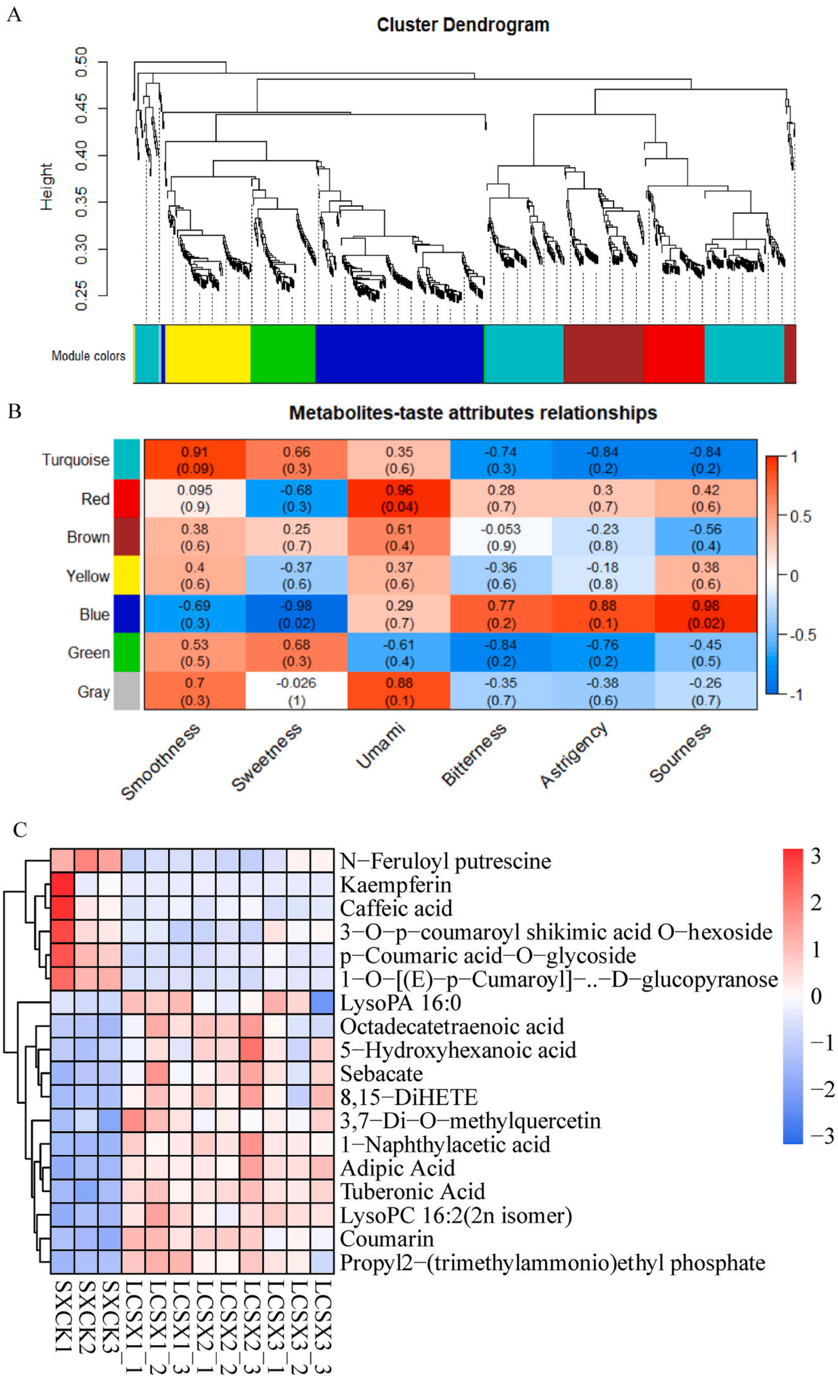

Fig. 4. Correlations of the non-volatiles in Wuyi Shuixian teas and the taste attributes. (A): The hierarchical clustering dendrogram for co-expression modules. (B): The achieved 7 modules. The upper and lower numbers represented the correlation coefficients and *P*-values, respectively. (C): The heatmaps of the significant different metabolites in the key module.

3.4. Association analysis on metabolites and taste features via WGCNA

In this study, WGCNA was used to correlate the quantified metabolites with the scores of taste features in various Shuixian teas. As a result, seven modules achieved via WGCNA, which were presented in Fig. 4A, and the Pearson's coefficients were exhibited in the cells of Fig. 4B (upper). Based on the quantitative descriptive analysis result of taste, sourness is the most distinguishable attribute for discriminating various Shuixian teas, while sweetness and umami taste were weak and negligible (Fig. 1C). Accordingly, a key module (blue module) presented in Fig. 4B was achieved with a positive correlation with the attribute of sourness ($P < 0.05$). This suggests the compounds clustered in blue module might be related with the sourness of Shuixian teas. Then, the compounds clustered in blue module were blasted against with the significant different metabolites clustered in Fig. 3E, and a total of 18 metabolites were obtained as shown in Fig. 4C. Notably, six compounds were highly enriched in SXCK, including 5 phenolic acids (caffeic acid, N-feruloyl putrescine, *p*-coumaric acid-*O*-glycoside, 1-*O*-[(E)-*p*-Cumaroyl]-β-D-glucopyranose and 3-*O*-*p*-coumaroyl shikimic acid O-hexoside), and 1 flavonoid (kaempferin), as shown in Fig. 3E. It was reported that the intensity of sour palate is directly ascribed to the total molar concentration of acids that have one or more protonated carboxyl groups [18]. In the present study, the phenolic acids contained one or more carboxyl groups that could dissociation hydrogen ions in tea infusion, were abundant in SXCK (Fig. 4C), suggesting that these acidic components predominantly contribute to the sourness of regular Shuixian tea. This is congruous with the previous study that sour perception is mainly associated with acids [19]. Additionally, astringency that elicited by flavonoid phenols (such like kaempferin and 3,7-di-*O*-methylquercetin in Fig. 4C), is thought to be responsible for the precipitation of salivary proteins and flavonoid compounds [20]. The precipitation of salivary protein would be strengthened when acids is presented in food matrix [21], accounting for the stronger astringency in SXCK (Fig. 1C). This result is consisted with the previous study on the flavor perception of sorghum food that the caffeic acid enhanced the sour, astringent and bitter tastes of wholegrain sorghum bread [22]. Although the abundance of 5-hydroxyhexanoic acid, adipic acid, and sebacate, were higher in LCSXs than in SXCK, these fatty acids were likely to contribute negligibly to the sourness of LCSXs infusion, as the fatty acids were not the dominant contributors of sour intensity in food matrix [16]. In this study, the rich abundance of phenolic acids in SXCK and the interaction with flavonoid could be dominant factors to intensify the perception of sourness, bitterness and astringency tastes of SXCK.

Plant age impacts the physicochemical properties and functional quality of fruits, and less volatile acids or ascorbic acid were found in plants with older ages [23]. One of the plant age related compound is coumarin, which was predominant in LCSXs than in SXCK in this study (Fig. 4C). The content of coumarin in leaves increased with the age of the plants, which plays a crucial role in inter-species competition or regualtion plant senescence [24], which could be a promising biomarker for the discrimination of Laocong Shuixian and regular Shuixian teas. Collectively, the significant different contents of phenolics and fatty acids potencially contributed to the flavor discrimination between Laocong and regular Shuixian teas, which could be used to detect or quantify the levels of Shuixian tea adulteration. Nevertheless, further investigations on the contents of biomarker compounds in tea plants with various ages, or accurate assess authentication of teas are needed in future study.

4. Conclusion

The metabolite profiles and flavor properties of Laocong and regular Shuixian tea samples obtained from the local market were investigated by a widely targeted metabonomic and quantitative descriptive analyses. The regular Shuixian tea showed a higher intentsity of sour palate than Laocong Shuixian teas, and the later demonstrated a better comprehensive quality in this study. A total of 692 compounds were identified, of which 43 metabolites were identfied as the significant different compounds discriminating Laocong Shuixian and regular Shuixian teas. Furthermore, 18 biomarkers, including caffeic acid, 3-*O*-*p*-coumaroyl shikimic acid O-hexoside, 1-*O*-[(E)-*p*-cumaroyl]-β-D-glucopyranose, N-feruloyl putrescine, *p*-Coumaric acid-*O*-glycoside, 3,7-di-*O*-ethylquercetin, and kaempferin were proposed as the key different metabolites between Laocong and regular Shuixian teas, which are feasible chemical descriptors to characterize and authenticate Shuixian tea samples.

Funding

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Ethics statement

The sensory evaluation on tea samples were conducted according to established ethical guidelines, and informed consent was obtained from the participants.

Author contribution statement

Hu Shang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Chensong Zhu: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
Weijiang Sun: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18891.

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