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Investigation of the quality of *Lu'an Guapian* tea during Grain Rain period by sensory evaluation, objective quantitative indexes and metabolomics

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ABSTRACT

The harvest date is a crucial factor in determining tea quality. For *Lu'an Guapian* (LAGP) tea, Grain Rain period (GRP) represents a pivotal phase in the transformation of tea quality. The sensory evaluation, computer vision and *E*-tongue revealed that the liquor color score, B and G values of tea infusion were increased during GRP, while the astringency, bitterness intensities and the R value of the tea infusion were decreased. Consequently, the tea infusion exhibited a greener hue and the taste became appropriate during GRP. Non-targeted metabolomics revealed that the majority of amino acids and derivatives was reduced during GRP. Furthermore, flavonoids, in particular flavonol glycosides, exhibited considerable variation during GRP. Finally, nine metabolites were identified as markers for quality transformation during GRP by PLS and Random Forest. This study investigated the quality of LAGP teas during GRP and filled the gap in the variation of LAGP tea quality during GRP.

1. Introduction

Tea, as one of the three most popular non-alcoholic beverages in the world, is not only widely enjoyed and appreciated, but also has significant economic and cultural value (Cheng et al., 2023). However, there are many factors that affect the quality of tea. The specific varieties, harvest periods, origins, and processing techniques and other factors have a great impact on the quality of tea. Among these factors, harvest periods play a crucial role in determining the quality and characteristics of tea, with researchers claiming that the harvest period has a significant impact on tea quality (Xiao et al., 2023). The endogenous control of the tea plant, as well as dynamic environmental factors such as changes in temperature, rainfall, and night length with the harvest period, can affect the final quality of tea (Rakocevic & Martim, 2011). Yin et al. (2022) analyzed Xinyang Maojian green teas from spring and autumn harvests and observed significant variations in free amino acid and caffeine content, with notable differences within the spring season, emphasizing the importance of harvest timing on tea quality. Zeng et al. (2020) found that early spring tea had significantly higher levels of amino acids, organic acids, catechins, flavonol/flavone glycosides, and phenolic acids, while showing a decrease in proanthocyanidins compared to late spring tea. In conclusion, seasonal variations exert a significant influence on tea quality. Nevertheless, it is yet unclear whether tea quality changes from one date to the next over the course of a single harvest period.

Lu'an Guapian (LAGP) tea, a distinctive green tea made exclusively from tea leaves without buds or stems, is regarded as one of China's most prestigious green teas (Li, Liu, et al., 2022; Zhang et al., 2024). Unlike other green teas that are characterized by their light and subtle flavors, LAGP tea seeks a stronger flavor and advocates for 'robustness over delicacy' in its choice of tea leaves. Its main harvest period falls during Grain Rain period (GRP), one of the 24 solar terms in China, usually around the end of April. During this period, LAGP tea is mass-produced and sorted into different batches. Due to the complex environmental changes during GRP, the condition of tea trees may vary from day to day, leading to different qualities of fresh leaves and thus of LAGP tea. In recent years, a number of researchers have conducted studies on the quality of LAGP tea to varying degrees. In a recent study, Li, Liu, et al. (2022) provided a new grading strategy for LAGP tea and identified eight indicated grade-related biomarkers of LAGP tea. Yu et al. (2023) conducted a study on two grades of LAGP tea and found that the floral aroma was more pronounced in the premium LAGP tea. Moreover, LAGP

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tea with different over-fired drying methods exhibited significantly different quality characteristics, both in terms of taste and aroma (Zhang et al., 2023; Zhang et al., 2024). It could be found that metabolomics combined with other techniques was a common and effective way to investigate tea or LAGP tea quality.

Unfortunately, to date, there has been a paucity of research investigating the quality and mechanisms underlying the variation in LAGP teas during GRP. Therefore, the present study used targeted and non-targeted metabolomics as well as subjective and objective measurements to explore the quality differences and variations in LAGP teas during GRP in depth. Meanwhile, through multiple analytical techniques, the potential intricate relationships between metabolites and tea quality were analyzed and discussed to seek the quality indicators of LAGP teas during GRP. In summary, the present study aimed to fill the gap in the variation of LAGP tea quality during GRP and provide a quality basis for LAGP tea blending during GRP.

2. Materials and methods

2.1. Tea samples

Tea samples of LAGP teas (*Camellia sinensis*) were collected from Huiliu Tea Co., Ltd., located in Lu'an City, Anhui Province, China, harvested during GRP from April 14th to April 25th, 2023. All tea samples underwent standardized processing procedures, including withering, fixation, rolling, unblocking, sorting, drying, second-drying, and over-fired drying. Subsequently, the processed tea samples were airtight sealed in aluminum foil bags and stored at 4 °C for further analysis. Each sample was labeled with a unique designation based on its harvest date, namely GP14, GP15, GP16, GP17, GP18, GP19, GP20, GP21, GP22 and GP25.

2.2. Chemicals

Distilled water was purchased from Wahaha Group Company (Hangzhou, China). Deionised water was made by Milli-Q water purification system (Millipore, Billerica, MA, USA). 4-Chloro-DL-phenylalanine was purchased from MedChemExpress (Shanghai, China). Formic acid, acetic acid, acetonitrile and methanol were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Gallic acid (GA), caffeine, catechin (C), epicatechin (EC), epigallocatechin (EGC), gallocatechin gallate (GCG), epigallocatechin gallate (EGCG), epicatechin gallate (ECG) were purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). L-Ascorbic acid was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). EDTA-2Na was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China).

2.3. Sensory evaluation

The sensory evaluation panel was comprised twelve experienced tea evaluators, six men and six women, ranging in age from 22 to 45. Ethical permission, to conduct a human sensory study, was granted by our institution. 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. To maintain objectivity, the ten samples were anonymized with three-digit codes and randomly served for assessment. The evaluation criteria followed the Chinese national standard (GB/T23776–2018), which encompassed an examination of the appearance, liquor color, and taste attributes of LAGP teas during GRP. Each attribute was evaluated on a scale of 100, accompanied by professional commentary. The results, presented in Table 1, were shown as mean scores with standard deviations (SD).

Table 1
Results of the sensory experimental validation of the classification.

Samples	Appearance		Liquor color		Taste	
	Comment	Score	Comment	Score	Comment	Score
GP14	Deep green, straighter, silvery	92.0 ± 1.63	Greenish yellow, brighter	90.0 ± 0.00	Heavier and thicker with slightly bitter	89.0 ± 1.15
GP15	Deep green, straighter, silvery	91.0 ± 0.82	Greenish yellow, brighter	88.0 ± 1.41	Heavier and thicker with slightly bitter	87.0 ± 1.63
GP16	Deep green, straighter, silvery	92.0 ± 1.15	Greenish yellow, brighter	90.5 ± 0.58	Heavier and thicker	90.0 ± 0.82
GP17	Deep green, straighter, silvery	91.0 ± 0.00	Greenish yellow, brighter	89.0 ± 1.41	Mellow and thick with heavier	91.0 ± 0.82
GP18	Deep green, straighter, silvery	91.0 ± 0.71	Light yellowish green, brighter	92.0 ± 0.41	Mellow and thick	92.0 ± 0.71
GP19	Deep green with little yellow exposed, straight, silvery	89.0 ± 0.71	Yellowish green, brighter	91.0 ± 0.41	Heavier and thicker with slightly bitter	88.0 ± 1.63
GP20	Deep green with little yellow exposed, straighter, silvery	91.0 ± 1.41	Light yellowish green, brighter	93.0 ± 0.00	Mellow and thick	92.0 ± 0.71
GP21	Deep green with little yellow exposed, straight, silvery	90.0 ± 0.82	Yellowish green, brighter	92.0 ± 1.15	Heavier and thicker with slightly astringent	88.0 ± 0.82
GP22	Deep green with little yellow exposed, silvery	91.0 ± 0.71	Green, bright	94.0 ± 0.41	Mellower and thicker with slightly astringent	92.0 ± 1.15
GP25	Deep green with little yellow exposed, straight, silvery	90.0 ± 1.15	Green, bright	94.0 ± 0.71	Mellower and thicker	93.0 ± 0.41

Note: Data are presented as mean ± standard deviation ($n = 12$).

2.4. Color quantitative analysis

2.4.1. Measurements for dry tea

A total of 150 g of tea sample was evenly spread on a 30 cm by 20 cm white tea evaluating tray to ensure uniform coverage without any visible white surface. Subsequently, the *E*-eye system and methodology, as previously reported by Wei et al. (2021), were employed with partial modifications. Briefly, images were captured in JPG format at a resolution of 4000 × 6000 pixels using a Nikon D7100 camera (Nikon, Tokyo, Japan). Camera settings included no flash, a focal length of 24 mm, ISO sensitivity of 560, operation in manual mode, Av aperture of F/8.0, and a shutter speed of 1/160 s. White balance was set to a color temperature of 5560 K. Twelve images of dry tea were captured for each tea sample. Finally, these images were batch processed on computer to quantify the RGB values by RStudio (RStudio PBC, Boston, Massachusetts, USA).

2.4.2. Measurements for tea infusion

Based on the Chinese national standard (GB/T23776–2018), 3.0 g of LAGP tea sample was brewed with 150 mL of boiling pure water for 5 min, and then immediately separated. Subsequently, the tea infusion was transferred to a standard finished tea review bowl (volume: 240 mL) for photographing. The *E*-eye system and methodology remained consistent with the specifications outlined in section 2.4.1. A total of twelve images of the tea infusion were captured for each tea sample and then were batch processed on computer to quantify the RGB values by RStudio (RStudio PBC, Boston, Massachusetts, USA).

2.5. E-tongue measurements for the taste intensity

The *E*-tongue (TS-5000Z, INSENT, Tokyo, Japan) measurements used in this study were performed according to the methods previously described by Huang et al. (2022). *E*-tongue data were collected using the Taste Sensing System (SA402B, Insent Intelligent Sensor Technology, Inc., Kanagawa, Japan), which includes taste sensors and ceramic reference electrodes. Taste sensors detect changes in potential with reference electrodes containing an antifouling lipid membrane and an Ag/AgCl electrode. Sensors require preconditioning: taste sensors are immersed in a reference solution (30 mM KCl and 0.3 mM TA) and ceramic electrodes in 3.33 M KCl solution for 24 h.

A sensor check ensures correct output voltage. For sample preparation, tea infusions were prepared according to the specifications of GB/T23776–2018 and 35 mL of the infusion was used. Each sample was tested four times; the first measurement was discarded and the last three were retained. Results were converted to taste scores using Weber-Fechner's law, which states that perceived intensity is proportional to the logarithm of the stimulus intensity. All tests were performed in a 25 °C water bath.

2.6. Quantification of catechins, caffeine and gallic acid by high-performance liquid chromatography (HPLC)

The concentration of catechins, caffeine, and gallic acid was determined using an Agilent 1260 HPLC system (Agilent, USA) equipped with a Waters Symmetry® C18 HPLC column (4.6 × 250 mm, 5 μm). The HPLC (Agilent, USA) measurements employed in this study were conducted in accordance with the methods previously outlined by Zhang et al. (2024). Specifically, to prepare a sample, 0.2 g of lyophilised tea powder was mixed with 5 mL of 70% methanol in a 10 mL centrifuge tube. The tubes were heated in a 70 °C water bath for 10 min, shaking once during this time, and then cooled. The cooled extract was centrifuged at 3500 rpm for 10 min and the supernatant was transferred to a 10 mL volumetric flask. The residue was re-extracted using the same steps. The combined supernatants were made up to 10 mL with 70% methanol, then 2 mL of the supernatant was mixed with 8 mL of stabilizing solution (250 mg EDTA-2Na, 250 mg ascorbic acid, 50 mL acetonitrile, 500 mL water) in a 10 mL volumetric flask. This solution was filtered through a 0.22 μm membrane and analyzed by HPLC. The HPLC conditions were as follows: oven temperature of 35 °C, flow rate of 1.0 mL/min, injection volume of 10 μL and detection at 278 nm. The mobile phases were: Phase A: 9% acetonitrile, 2% acetic acid, 0.2% EDTA-2Na (1 mg/mL), 88.8% water. Phase B: 80% acetonitrile, 2% acetic acid, 0.2% EDTA-2Na (1 mg/mL), 17.8% water. The retention times and peak shapes of the practical samples were then compared with those of the standards to identify the metabolites.

2.7. Determination of metabolites by liquid chromatography–mass spectrometry (LC-MS)

The metabolite extraction procedure followed a modified protocol based on Xu et al. (2019). Specifically, 30.0 g of tea leaves were randomly selected from each sample in triplicate and ground into powder. Subsequently, 0.4000 ± 0.0010 g of each powdered tea sample was extracted using 8.0 mL of methanol/water (7/3, *v*/v) containing 4-chloro-DL-phenylalanine as the internal standard. The extracted samples were analyzed using an Ultra Performance Liquid Chromatography (UPLC) system (Ultimate 3000, Dionex, Sunnyvale, CA, USA) coupled to a mass spectrometer (Q-Exactive Focus, Thermo Fisher Scientific, Waltham, MA, USA). The LC-Orbitrap-MS analysis method is detailed in Table S1.

2.8. Data analysis

All experiments and sample analyses were performed in triplicate, except for color quantitative analysis which was twelve replicates. And the results are presented as mean values. The concentrations of nonvolatile compounds were analyzed using principal component analysis (PCA) and partial least-squares (PLS) regression via SIMCA-P (V14.1, Umetrics, Umea, Sweden). Analysis of variance (ANOVA) for statistical differences was performed using SPSS Statistics27 (SPSS Inc., Chicago, IL, USA). Additional plots were created using RStudio (RStudio PBC, Boston, Massachusetts, USA) and Adobe Illustrator 2021 (Adobe Systems Inc., California, USA).

3. Results and discussion

3.1. Sensory evaluation of LAGP teas during GRP

Firstly, a sensory evaluation was conducted to assess the overall sensory characteristics of LAGP teas during GRP. As shown in Table 1, in the term of appearance, the most notable variation was in the color of the dry tea. Comments varied from “deep green” to “Deep green with little yellow exposed”. This trend could be linked to the maturation of the tea leaves. On the contrary, the liquor color gradually turned green as the harvest date progressed (“greenish yellow” to “yellowish green” to “green”). The disparate color change patterns observed in dry tea and tea infusion were due to the distinct pigments involved: lipophilic pigments like chlorophylls dominated the dry tea color, while the tea infusion color was primarily influenced by the water-soluble pigments (Li et al., 2019; Li et al., 2021; Qiu et al., 2022). Meanwhile, The increase in liquor color scores during GRP suggested that these visual changes are appreciated by the evaluators and likely contribute to the perceived quality of the tea. In the term of taste, the most frequent comment was “thick”, indicating that the tea infusion is rich in contents and has a viscous feel. In early GRP, the descriptors of the LAGP tea infusion were accompanied by “heavy”, which means the tea infusion is rich in content and has a high level of astringency. However, in late GRP, the descriptors of the LAGP tea infusion were often accompanied by “mellow”, referring to the appropriate strength and soft taste. This shift in taste profile was reflected in the higher taste scores for teas harvested later in GRP. In conclusion, the sensory evaluation clearly demonstrated that the timing of harvest had a significant impact on the sensory quality of LAGP teas, affecting not only their appearance and liquor color but also their taste.

3.2. Color values and taste intensity of LAGP teas during GRP

In order to objectively analyze the quality changes of LAGP teas during GRP, we employed computer vision and *E*-tongue to obtain the color information and taste intensity (Fig. 1). And the raw pictures of dry tea and tea infusion of LAGP teas during GRP were shown in Fig.S1. It could be seen that the tea infusion of LAGP teas during GRP had a clear tendency to gradually turn green. By linear fitting, it revealed that the RGB values of dry tea were barely correlated with harvest period (Fig. 1A-C). Whereas the RGB values of tea infusion had a definite correlation with harvest period (Fig. 1D-F). Specifically, the R value (R^2 = 0.624) decreased with harvest date during GRP, while G value (R^2 = 0.228) and B value (R^2 = 0.465) increased. The trends were consistent with the sensory results, with the liquor color turning gradually green as

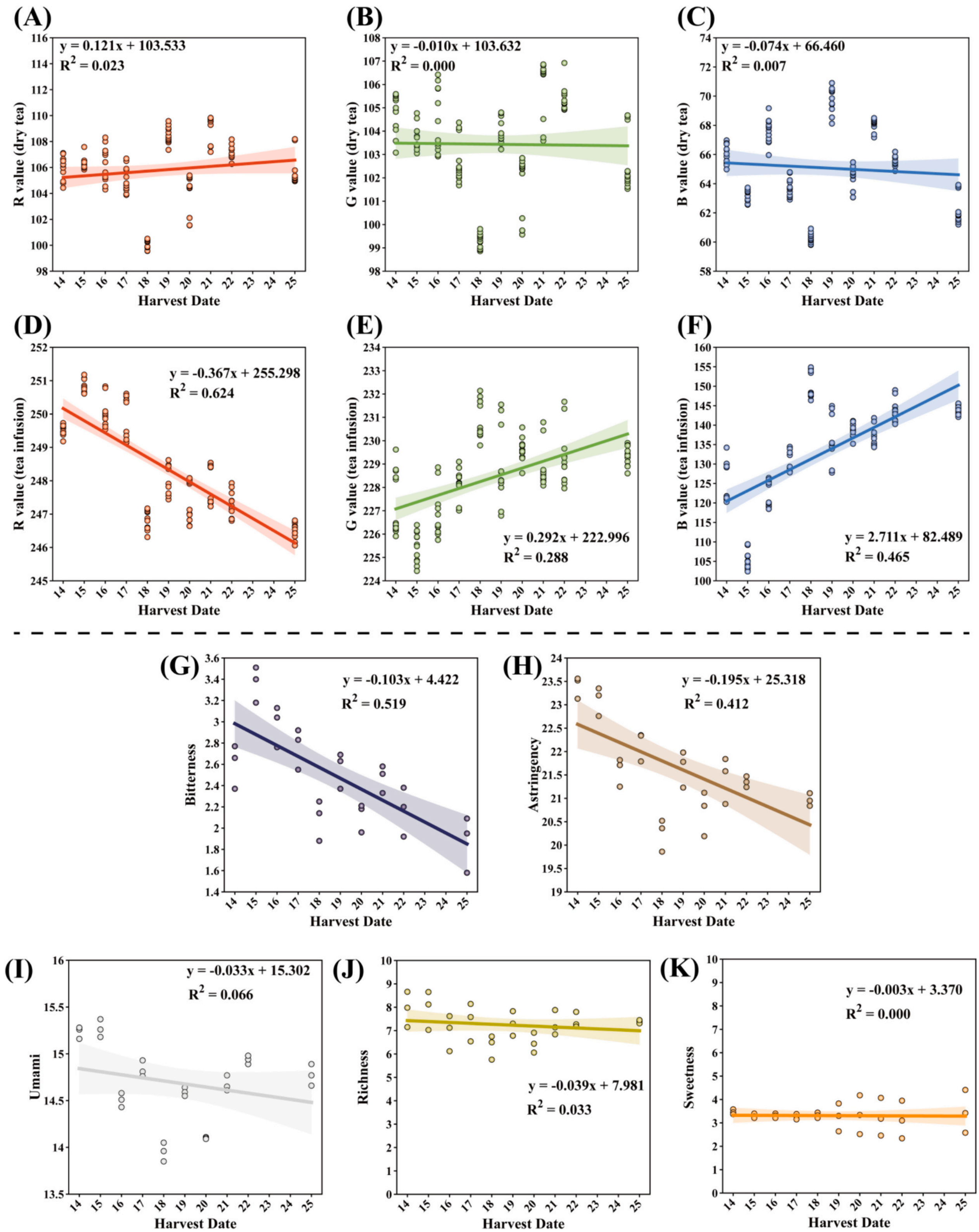

Fig. 1. The linear fitting of the RGB values of dry tea (A-C) and tea infusion (D—F) as well as taste intensities (G-K) with the harvest data.

the harvest date progressed. The differences in appearance and liquor color trends may be attributed to the fact that the compounds affecting appearance were not uniform to those affecting liquor color. A study claimed that lipid-soluble pigments played a significant role in determining the color of green tea appearance (Li et al., 2019). However, liquor color was evaluated based on the tea infusion, which was obtained by water brewing; hence, anthocyanins, the largest water-soluble pigment, might be the main contributor to liquor color (Zhao et al., 2022).

As a fundamental parameter, taste plays a pivotal role in the comprehensive assessment of tea quality (Zhang et al., 2020). In terms of taste intensity, the bitterness ($R^2 = 0.519$) and astringency ($R^2 = 0.412$)

intensities of LAGP teas exhibited a decreasing trend during GRP (Fig. 1G-H). However, the other three *E*-tongue information, umami, richness and sweetness, were barely correlated with the harvest date (Fig. 1I-K). These indicated that, during GRP, the main variations of taste of LAGP teas were bitterness and astringency. Ye et al. (2022) claimed that the bitter and astringent taste of tea, particularly in green tea, remains undesirable for many consumers. Therefore, the reduction in bitterness and astringency intensity might be responsible for the variation in taste scores of LAGP teas during GRP.

3.3. *Correlation analysis of color values, taste intensity and sensory scores*

To provide a further insight into the color values, taste intensity and sensory scores, a Pearson correlation analysis was performed between the three data terms and the harvest date (Fig. 2A). The results revealed that the liquor color score, taste score as well as B and G values of tea infusion were positively correlated with the harvest date. And the liquor color score, B and G values of tea infusion had a stronger correlation ($r > 0.6$). In addition, the richness, umami, appearance, astringency, bitterness intensities and the R value of tea infusion were negatively correlated with the harvest date. And the astringency, bitterness intensities and the R value of tea infusion had a stronger correlation ($r < -0.6$). The above situations suggested that these attributes were particularly sensitive to the timing of harvest.

Fig. 2B showed the relationships between the color values, taste intensity and sensory scores. The positive correlation among the *E*-tongue data terms—bitterness, astringency, umami, and richness intensity—indicated that these attributes might be interdependent during GRP, potentially influencing the overall sensory experience of LAGP tea. Moreover, the R value of tea infusion was negatively correlated with the G and B values of tea infusion, while it was positively correlated with bitterness, astringency, umami and richness intensity.On the contrary, the G and B values of tea infusion were negatively correlated with the four terms of the *E*-tongue data. The findings suggested an intrinsic linkage between infusion color and taste in LAGP tea during GRP, potentially influenced by variations in metabolites.

Mantel analysis specifically showed that during the GRP, the taste score of LAGP teas was primarily influenced by bitterness intensity. This finding underscored the critical role of bitterness in shaping the overall taste perception of these teas. As a matter of common sense, the liquor color score exhibited a strong correlation with the RGB values of tea infusion ($p < 0.01$; $r \geq 0.4$). However, the appearance score of LAGP teas during GRP didn't show any significant correlation with the above data. This situation might be due to that the appearance score is not only determined by the color but also by factors such as shape, streak, evenness, and clarity. In conclusion, this section revealed a robust correlation between the infusion color and taste intensity in LAGP tea during GRP, indicating that the timing of harvest significantly affects the sensory qualities. Importantly, bitterness intensity was identified as the key determinant of taste scores, exerting a substantial impact on the tea's flavor profile.

3.4. *Quantitative analysis of the major metabolites of LAGP teas during GRP*

In order to investigate the variation in the chemical constituents of LAGP teas during GRP, a quantitative analysis was conducted of eight major metabolites, including six individual catechin compounds (C, EC, ECG, EGC, GCG and EGCG), GA and CAF. Among the six individual catechin compounds, EGCG (90.35 mg/g to 95.92 mg/g), EGC (29.60 mg/g to 34.89 mg/g), and ECG (18.66 mg/g to 23.88 mg/g) exhibited higher levels of content (Table S2). As shown in Fig. 3A, the contents of C, EC, ECG, GA and CAF gradually decreased with harvest date during GRP. But there was no significant trend in the contents of the rest three compounds, EGC, GCG and EGCG. Performing a Pearson correlation analysis between the harvest date and the contents of the eight major metabolites, it could be also found that C ($r = -0.89$), ECG ($r = -0.88$), GA ($r = -0.70$) and CAF ($r = -0.75$) were negatively correlated with the harvest date (Fig. 3B). Besides, the content of EGC ($r = 0.59$) was positively correlated to the harvest date. In conclusion, with the exception of EGC, the contents of the five individual catechin compounds, GA and CAF, were reduced to varying degrees during GRP. The observed trends were consistent with those reported in previous studies investigating green teas at different harvest periods (Kim et al., 2016; Wakamatsu et al., 2019).

Subsequently, Pearson correlation analysis was performed between the taste intensity and the contents of the eight major metabolites to explore the associations (Fig. 3B). It could be seen that C, ECG and GA were positively correlated to bitterness, with GA having the strongest correlation ($r = 0.79$). Meanwhile, C, EC, ECG and GA were positively correlated to astringency, with ECG having the strongest correlation (r

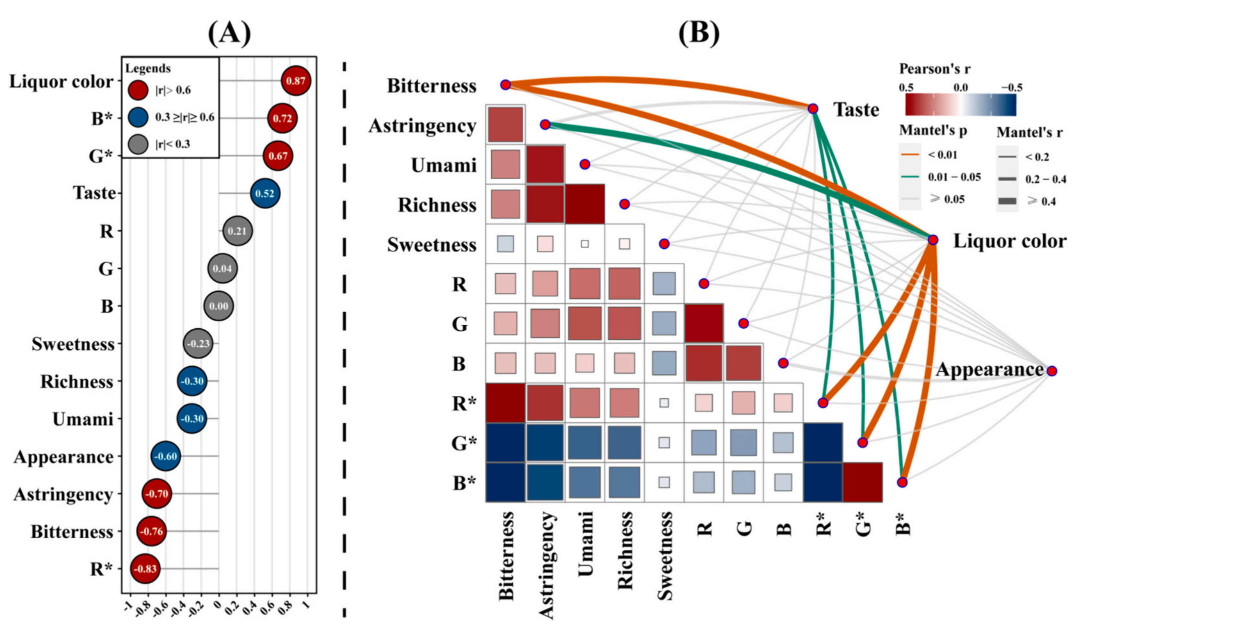

Fig. 2. The results of correlation analysis: RGB values of dry tea and tea infusion as well as taste intensities with harvest data (A) and sensory evaluation scores (B).

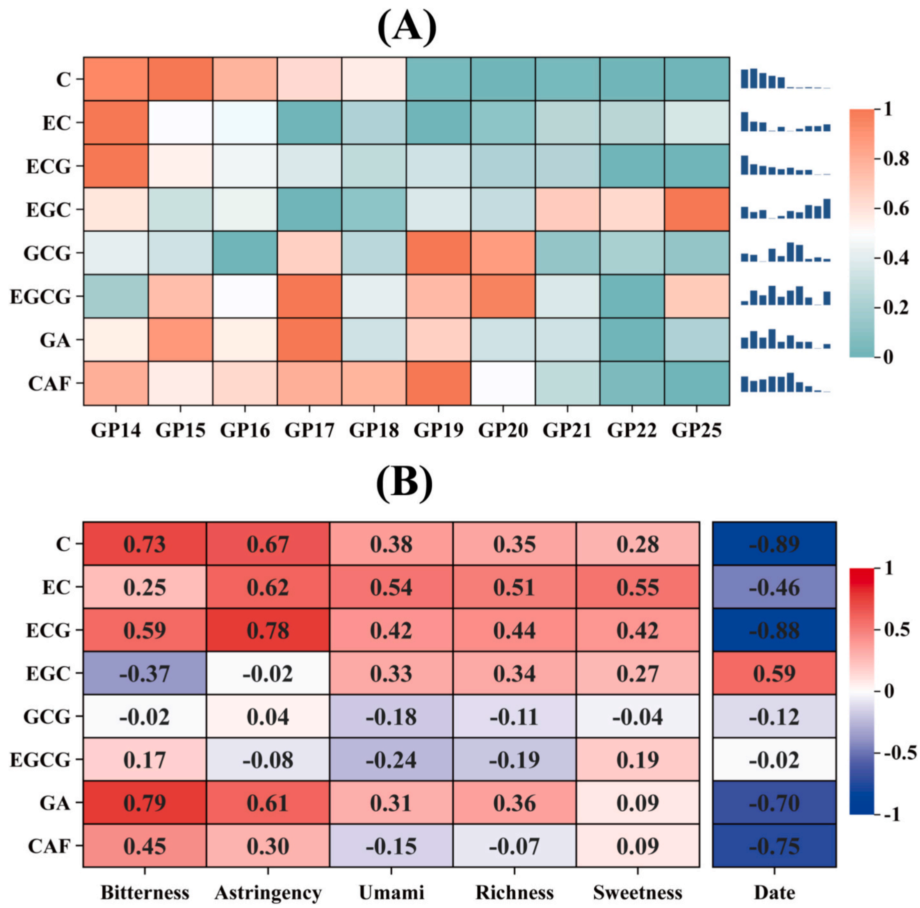

Fig. 3. The heat map of the concentrations of the major metabolites in LAGP teas during GRP (A) and the result of correlation analysis of the concentrations of the major metabolites with taste intensities and harvest date (B).

= 0.78). However, the eight major metabolites didn't show any significant correlation with umami, richness and sweetness. In summary, the contents of C, ECG, GA and CAF gradually decreased with harvest date during GRP. Among them, GA primarily affected the bitterness intensity of LAGP teas during GRP, while ECG primarily influenced the astringency intensity of LAGP teas during GRP.

3.5. Analysis of non-targeted metabolomics by LC-MS

Non-targeted metabolomics was performed on LAGP teas during GRP to comprehensively analyze the variation in its chemical constituents. A total of 2328 ion features of metabolites were obtained by processing Raw LC-MS datasets using the method described by Wei et al. (2021). Subsequently, the ion features were used to multivariate statistical analysis (Fig. S2). The score plot of the principal component analysis (PCA) illustrated that the QC samples were tightly clustered near the center, indicating excellent reproducibility in both the metabolite extraction process and subsequent metabolomics analysis (Chen et al., 2020). Meanwhile, the distribution path of the samples on the PCA score plot was approximately the same as the direction indicated by the blue arrow, indicating that the nonvolatile metabolites of LAGP teas during GRP were correlated with the harvest date.

3.5.1. Exploring the differential metabolites correlated with the harvest date by WGCNA

Next, to elucidate the correlation between the nonvolatile metabolites and the harvest date, a weighted gene co-expression network analysis (WGCNA) was conducted to establish a co-expression network (Fig. 4A&B). This analysis involved identifying modules of co-expressed metabolites and assessing their association with the harvest date during GRP. Based on dynamic tree cut and merged dynamic, the metabolites of LAGP teas during GRP were partitioned into six distinct modules. Three modules exhibited highly significant correlations with the harvest date. The brown and blue modules demonstrated positive correlations with the harvest date, while the turquoise module exhibited a negative correlation. Subsequently, according to MS^2 spectra, Tea Metabolomics Database (https://hmdb.ca/) and our previous study (Xu et al., 2019), a total of 215 metabolites were indentified from the three modules (Table S3). Specifically, there were 113 metabolites in MEturquoise, 52 metabolites in MEbrown and 50 metabolites in MEblue.

As shown in Fig. 4C, the 215 metabolites were classified into seven superclasses (phenylpropanoids and polyketides; lipids and lipid-like molecules; organic oxygen compounds; organic acids and derivatives; organoheterocyclic compounds; benzenoids; nucleosides, nucleotides, and analogues). The waffle chart showed in detail the quantities of the seven superclasses of metabolites in the three modules. The two pie charts showed the number of each of the seven superclasses of metabolites as a percentage of the positively correlated modules (MEturquoise) and negatively correlated modules (MEbrown and MEblue), respectively. The proportions of the seven superclasses of metabolites were found to be approximately equal in MEturquoise, MEbrown, and MEblue, indicating that the number of metabolites with increasing and decreasing levels in LAGP teas during GRP was approximately equal by superclass classification. Among the metabolites identified, phenylpropanoids and polyketides constituted the largest proportion (36% or 35%), indicating that these metabolites exhibited the most variation with the harvest period.

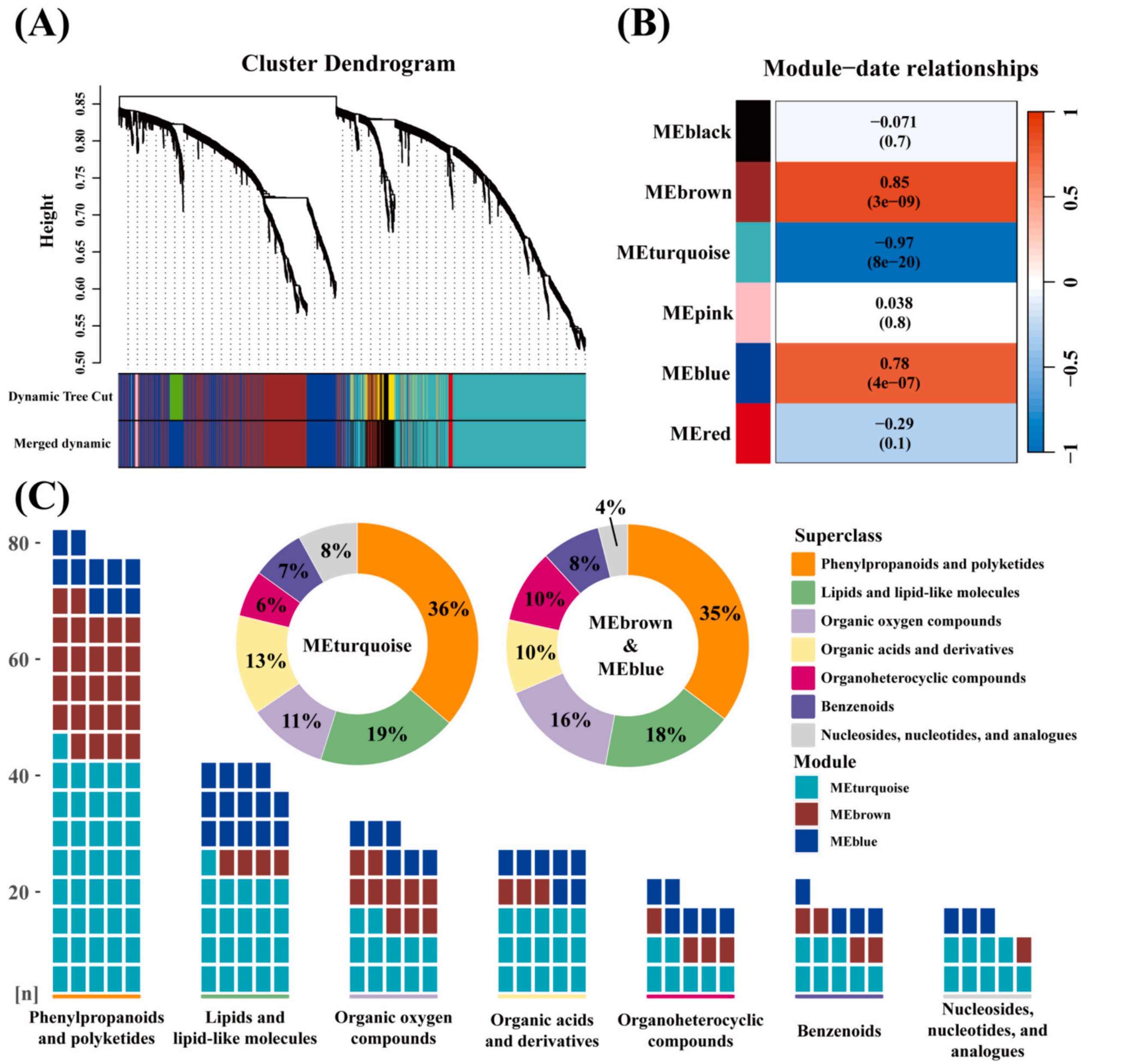

Fig. 4. Association of metabolites with the harvest date. (A) Hierarchical clustering dendrogram of WGCNA. (B) Module-date relationships. (C) The waffle chart and pie charts of metabolites in different modules.

3.5.2. The variation of amino acid and derivatives in LAGP teas during GRP

Amino acids are recognized as vital biomolecules in *Camellia sinensis* L. that influence the overall sensory quality of green tea and undergo dynamic fluctuations throughout its growth cycle (Sun et al., 2022).As shown in Fig. 5A&B, there were eight amino acids and derivatives, L-phenylalanine, L-tyrosine, L-arginine, L-histidine, L-theanine, L-glutamine, L-targinine, and L-glutamic acid, were correlated with the harvest date during GRP. Six of these amino acids and derivatives belonged to MEturquoise, indicating that most of the amino acids and derivatives in LAGP teas showed a decreasing trend in their concentration during GRP. And only L-targinine and L-glutamic acid belonged to MEblue, while L-targinine and L-glutamic acid did not exhibit a clear trend based on the heat map analysis.

Amino acids can be classified into five primary taste attributes: sweet, sour, bitter, salty, and umami. These taste attributes are determined by the chemical structure of amino acids and their interactions with taste receptor cells in the oral cavity. Among the eight amino acids and derivatives, L-phenylalanine, L-arginine and L-histidine were bitter amino acids according to the previous researches (Li, Luo, et al., 2022; Sforza et al., 2006). Meanwhile, the concentrations of the four bitter amino acids were gradually decreased during GRP, which might be responsible for the decrease in bitterness intensity of LAGP tea. In addition, L-theanine, an exclusive non-proteinaceous amino acid, is recognized as one of the most important components in tea. It imparts a distinctive flavor profile to tea, particularly contributing to the umami taste sensation (Li, Ma, et al., 2022; Wen et al., 2020). Narukawa et al. (2008) claimed that theanine has a complex taste profile, primarily umami, sweetness, and bitterness, with taste intensity escalating as theanine concentration increases. In conclusion, eight amino acids and derivatives were found to be correlated with harvest date during GRP. The majority of these showed a decreasing trend in their concentration during GRP. And the observed decrease in bitterness intensity of LAGP teas during GRP may be attributed to the aforementioned decreasing

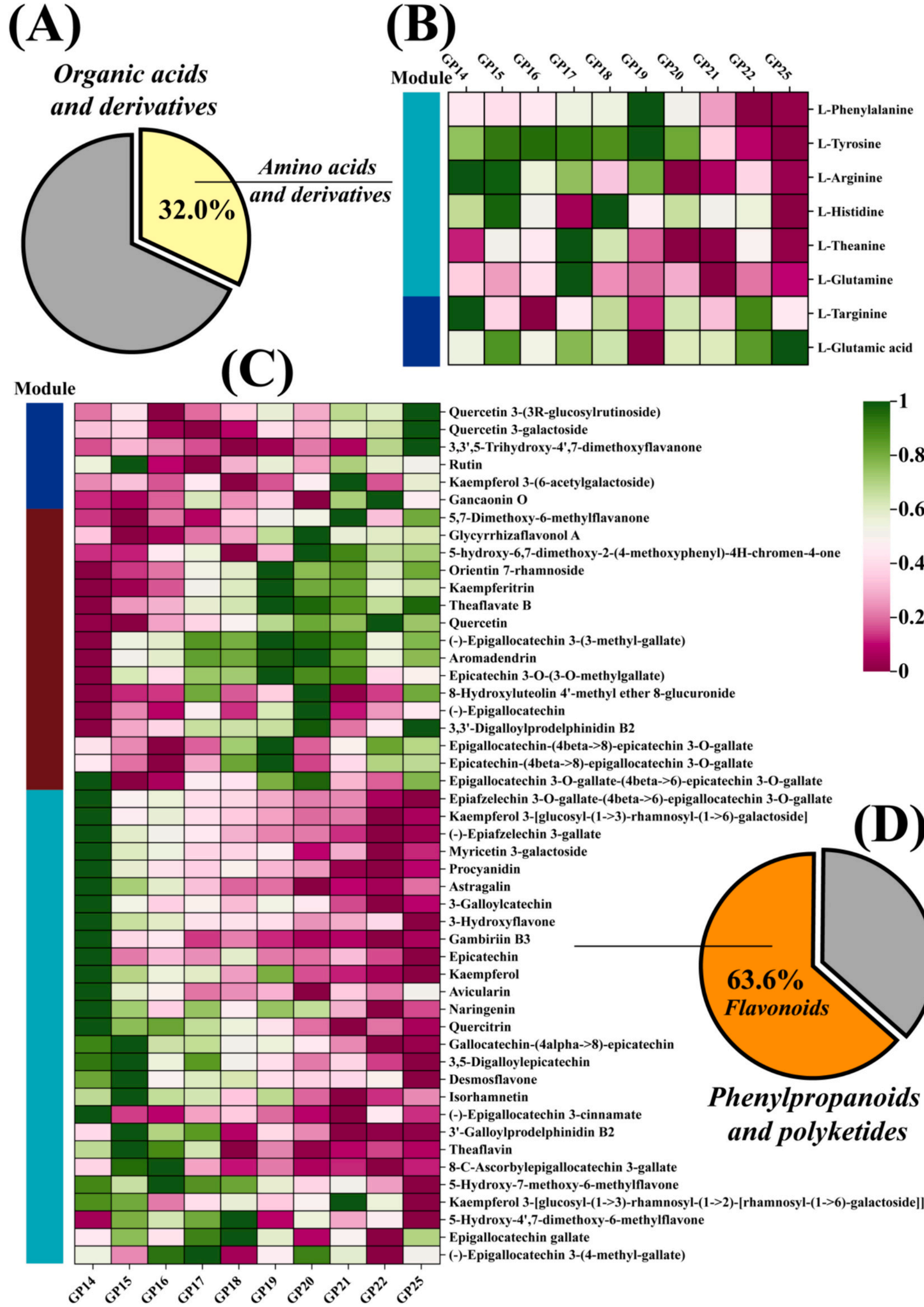

Fig. 5. The pie chart (A) and heat map (B) of amino acid and derivatives in organic acid and derivatives; the heat map (C) and pie chart (D) of flavonoids in phenylpropanoids and polyketides.

trend.

3.5.3. *The variation of flavonoids in LAGP teas during GRP*

Flavonoids are compounds based on 2-phenylbenzopyran and are classified into six classes: flavones, flavanones, isoflavones, flavonols, flavanols, and anthocyanins (Balentine et al., 1997). As the major secondary metabolites in plants, flavonoids are the primary compounds associated with quality. In addition, the flavonoids constituents play a pivotal role in shaping the color, flavor, and aroma attributes of brewed tea (Zhang et al., 2017). As shown in Fig. 5C&D, the amount of flavonoids was 63.6% of phenylpropanoids and polyketides in LAGP teas during GRP, indicating that flavonoids exhibited the greatest variation during GRP. Specifically, there were six flavonoids belonging to MEblue, 16 flavonoids belonging to MEbrown, and 27 flavonoids belonging to MEturquoise. The number of flavonoids (27) exhibiting a negative correlation with the harvest date was somewhat higher than the number of flavonoids (22) exhibiting a positive correlation with the harvest date.

Flavonol glycosides, the second largest subclass of flavonoids in tea leaves, contribute to the bitter and astringent taste of tea infusions along with catechins and caffeine. The impact of these compounds on tea flavor is twofold: first, their remarkably low taste thresholds (ranging from 0.001 to 19.80 μmol/L) exceed those of catechins (190–930 μmol/L) and theaflavins (13–26 μmol/L); second, they enhance the bitterness of caffeine (Scharbert et al., 2004; Scharbert & Hofmann, 2005; Ye et al., 2022). In this study, 13 flavonol glycosides were found to be associated with harvest date during GRP, e.g. rutin, avicularin, quercitrin, kaempferitrin, quercetin 3-galactoside, myricetin 3-galactoside etc. According to previous research, among these flavonol glycosides, rutin and myricetin-3-galactoside were found to be significantly correlated with the astringency and bitterness of LAGP tea (Zhang et al., 2024). And Sun et al. (2023) claimed that quercitin was responsible for the bitterness of Rizhao green tea. Furthermore, flavonoid glycosides play a significant role in imparting the yellow color and brightness to tea infusions due to their absorption band in the visible light spectrum, which ranges from approximately 360 to 380 nm (Agati et al., 2011; Zhang et al., 2017). In conclusion, flavonoids, particularly flavonol glycosides, exhibited considerable variation during GRP. These fluctuations could potentially impact the quality of LAGP tea, particularly with regard to bitterness, astringency, and the yellow color of tea infusion.

3.6. Exploring the differential metabolites correlated with LAGP tea quality during GRP

So far, it has been clearly known the quality variations of LAGP tea during the GRP by means of sensory evaluation, *E*-tongue and computer vision. Furthermore, based on target metabolomics and non-target metabolomics, the metabolites in LAGP tea during GRP and the variations were identified and analyzed in depth. Specifically, eight major metabolites (six individual catechin compounds, GA and CAF), which are considered as important quality indicators, were quantitatively detected by HPLC. And using LC-MS, a more comprehensive analysis was performed to identify and quantify a wide range of metabolites present in tea samples. Meanwhile, most amino acids and catechins were decreased in content, which caused the reduction of bitterness intensity. And catechins were belonged to flavonoids which varied significantly during GRP. Based on these findings, it was necessary to integrate the findings from both targeted and non-targeted metabolomics with sensory evaluation and objective quantitative indices to develop a holistic understanding of the quality attributes of LAGP tea during GRP. This integration will pave the way for a more precise characterization of the metabolic changes that drive the sensory perception and ultimately contribute to the tea's unique profile.

Random Forest (RF), a machine learning, was used to explore the differential metabolites correlated with LAGP tea quality (Fig. 6). The initial step involved the construction of an RF model based on the aforementioned metabolite data. Subsequently, the top 20 significant metabolites were screened based on the increase in Mean Squared Error Percentage (MSE%). Increase in MSE% is a metric used to assess the importance of variables in a RF model. It represents the increase in the mean squared error when a given variable is randomly permuted, indicating the extent to which that variable contributes to the model's predictive performance. Whereafter, a correlation analysis was performed between the top 20 significant metabolites and the characteristic data of LAGP teas (taste intensity and color values). The results demonstrated that nine metabolites (five flavonoids, two tannins, one amino acid, and GA) were significantly correlated with LAGP tea quality, particularly with regard to bitterness, astringency, and the color of the tea infusion. And the correlations with bitterness, astringency and R value of tea infusion were positive, while the correlations with G and B values of tea infusion were negative. As mentioned earlier, flavonoids

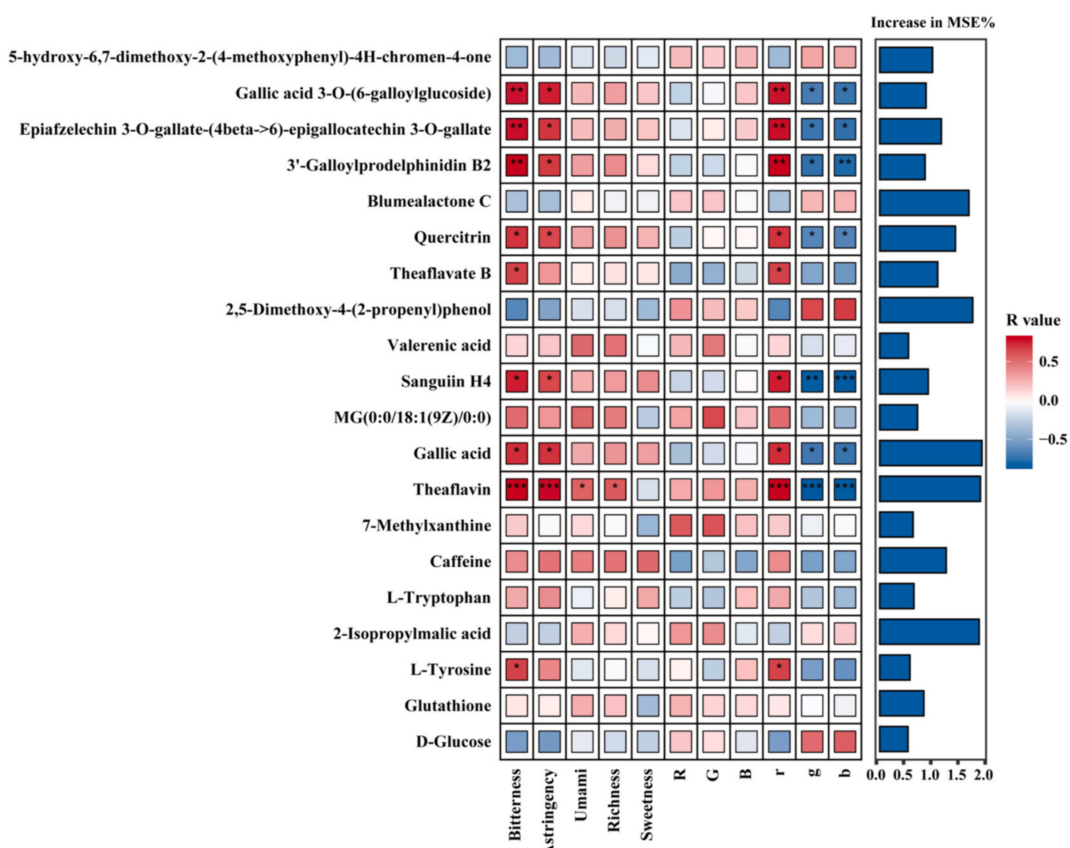

Fig. 6. The differential metabolites correlated with LAGP tea quality screened by random forest model and correlation analysis.

are important compounds that affect the flavor and color of tea. Notably, theaflavin was the most significant metabolite correlated with bitterness, astringency and color of tea infusion ($p < 0.001$). Theaflavins, which belong to a class of biflavonoids characterized by a benzotropolone backbone, comprise approximately 2% of dried tea leaves, with theaflavin representing a specific type within this class. Some research has reported that theaflavin contributes to the bitterness and astringency of tea, as well as its color (Takemoto & Takemoto, 2018; Yamazaki et al., 2014). Meanwhile, the human bitter taste receptor hTAS2R39 has been identified as the primary receptor for the bitterness of theaflavins.

Among the numerous families of phenolic compounds, tannins stand out as the major ones associated with food astringency (Soares et al., 2020). Gallic acid 3-O-(6-galloylglucoside) and sanguiin H4, the two tannins correlated with LAGP tea quality, are also classified as hydrolysable tannins. Han et al. (2022) claimed that hydrolysable tannins not only affect the quality of tea, but also show a significant correlation with the grade of Huangshan Maofeng green tea. And L-tyrosine showed a significant positive association with bitterness (Li, Ma, et al., 2022). Additionally, it was discovered that gallic acid has a positive correlation with bitterness and astringency. Gallic acid, as a benzoic acid derivative, is one of the major phenolic acids in teas. Due to its high aqueous solubility, phenolic acids, including gallic acid, are considered important flavor compounds in various teas, associated with the astringency and sourness of tea infusion (Ye et al., 2022). Furthermore, Chen et al. (2022) demonstrated that phenolic acids are associated with the bitter and astringent tastes of green tea infusion, with gallic acid exhibiting the strongest association with the astringent taste.

Subsequently, the PLS was performed based on the nine quality differential metabolites (X) and the harvest date (Y) of LAGP teas during GRP (Fig. 7). The score scatter plot showed a left-to-right distribution of samples with harvest date in the t1 direction, which is consistent with the previous PCA results (Fig. 7A). And the cross-validation with 200 permutation tests demonstrated the reliability of this PLS model (with intercepts of R2 and Q2 equal to 0.117 and − 0.392, respectively; Fig. 7B). The coefficients quantify the strength of the correlation

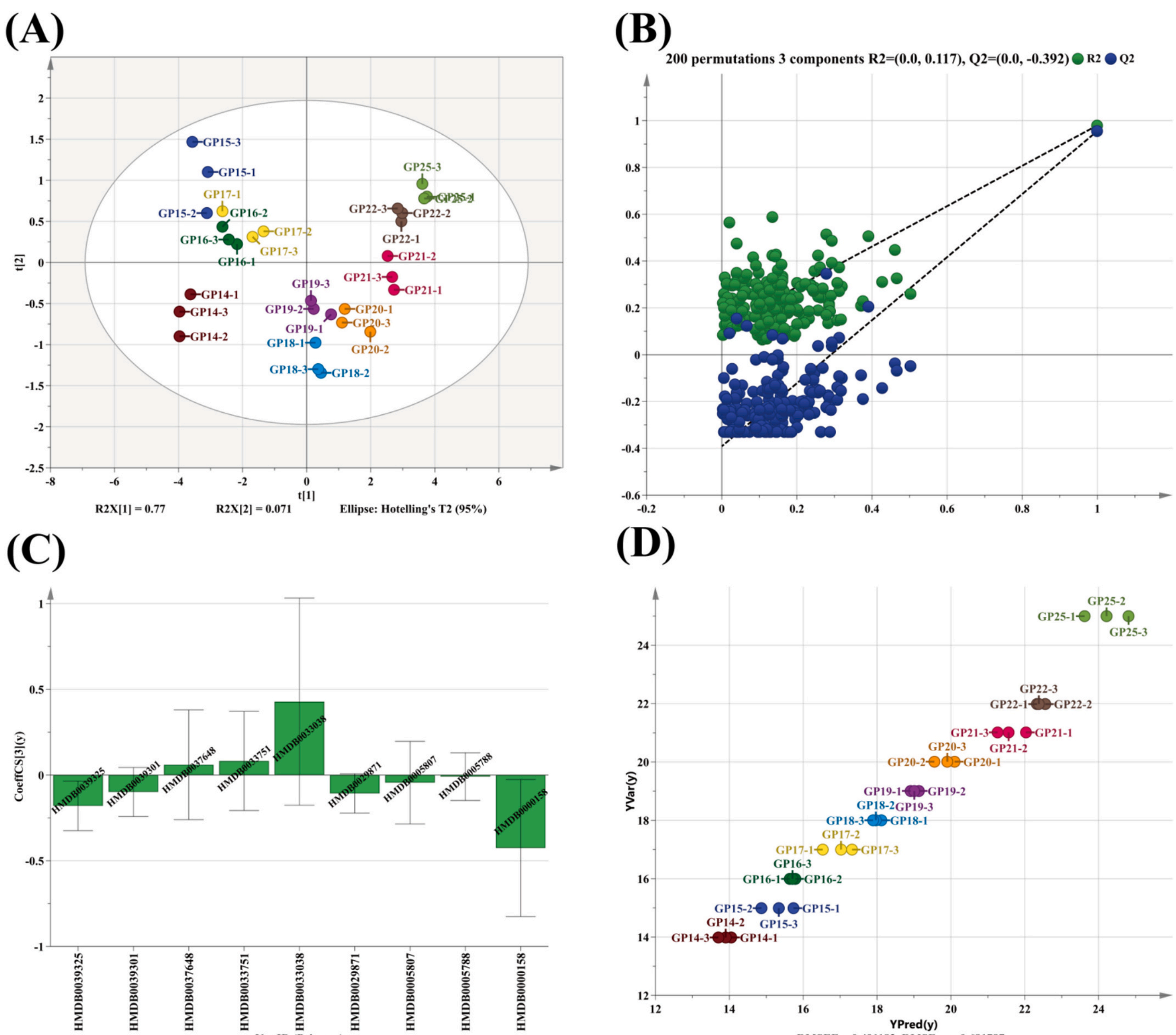

Fig. 7. Analysis of the nine quality differential metabolites. (A) OPLS-DA score plot. (B) Permutation test plot. (C) Histogram of regression coefficients. (D) Predicted results of the the equation of regression.

between Y and the systematic component of each X variable, and the equation of regression was constructed based on the coefficients plot (Fig. 7C), i.e.

$Y = 5.70–0.18 \times X1–0.10 \times X2 + 0.06 \times X3 + 0.01 \times X4 + 0.43 \times X5–0.11 \times X6–0.04 \times X7–0.01 \times X8–0.43 \times X9$.

In order to facilitate the expression of the equation, we employed the *X1* to *X9* range to represent the nine metabolites. The specific metabolites are listed in Table S4. Among the nine quality differential metabolites, theaflavate B (coefficients = 0.43) and L-tyrosine (coefficients = −0.43) contributed the most to the modeling results. Finally, we applied the equation of regression to predict the harvest period using real data and compared it to the true values (Fig. 7D). The plot showed that the distribution between true and predicted values was close to diagonal, and the values of RMSEE and RMSEcv were <1. These indicated that the nine quality differential metabolites combined with PLS were feasible for harvest date prediction and the nine quality differential metabolites were able to represent the differences among LAGP teas during GRP.

4. Conclusion

This study aimed to assess the quality evolution of LAGP teas during GRP by targeted and non-targeted metabolomics as well as subjective and objective measurements. The sensory evaluation revealed that as the harvest date advanced, the dry tea's color shifted from green to yellow, while the infusion's hue intensified towards green. And the taste score of LAGP tea was higher in late GRP than in early GRP. Through objective measurements, computer vision and *E*-tongue, it was found that the liquor color score, B and G values of tea infusion were increased during GRP, while the astringency, bitterness intensities and the R value of tea infusion were decreased. Besides bitterness intensity was the main influencing factor for the taste score of LAGP teas during GRP, and GA was the main influencing factor for the bitterness intensity of LAGP teas during GRP. Non-targeted metabolomics revealed a decrease in amino acid concentrations, which may correspond to the reduced bitterness observed. Furthermore, flavonoids, particularly flavonol glycosides, exhibited considerable variation during GRP. These fluctuations could potentially impact the quality of LAGP tea, particularly with regard to bitterness, astringency, and the yellow color of tea infusion. Finally, nine metabolites (5 flavonoids, 2 tannins, 1 amino acid and GA) correlated with LAGP tea quality (mainly bitterness, astringency and color of tea infusion) were identified using random forest and PLS. In summary, this study elucidated the quality characteristics and variations in LAGP teas during GRP in depth. Furthermore, it analyzed and discussed the possible complex relationships among the sensory indexes, metabolites, and the harvest date. These insights not only fill a critical knowledge gap but also inform tea blending strategies to enhance quality throughout the GRP.

Ethical statements

Ethical permission, to conduct a human sensory study, was granted by our institution. 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

Yida Wu: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tiehan Li:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Wenjing Huang:** Writing – review & editing, Validation, Supervision, Formal analysis, Conceptualization. **Jixin Zhang:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Yuming Wei:** Writing – review & editing, Supervision, Conceptualization. **Yujie Wang:** Writing – review & editing, Supervision. **Luqing Li:** Supervision, Resources, Funding acquisition. **Jingming Ning:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

All authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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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.2024.101595.

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