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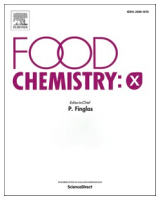

Integration of widely targeted metabolomics and the e-tongue reveals the chemical variation and taste quality of Yunnan Arabica coffee prepared using different primary processing methods

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ABSTRACT

UPLC-Q-TOF-MS and electronic tongue analysis were applied to analyse the metabolic profile and taste quality of Yunnan Arabica coffee under seven primary processing methods. The total phenolic content ranged from 34.44 to 44.42 mg/g DW, the e-tongue results revealed the strongest umami sensor response value in the sample prepared with traditional dry processing, while the samples prepared via honey processing II had the strongest astringency sensor response value. Metabolomics analysis identified 221 differential metabolites, with higher contents of amino acids and derivatives within dry processing II sample, and increased contents of lipids and phenolic acids in the honey processing III sample. The astringency and aftertaste-astringency of the coffee samples positively correlated with the trigonelline, 3,5-di-caffeoylquinic acid and 4-caffeoylquinic acid content. The results contributed to a better understanding of how the primary processing process affects coffee quality, and supply useful information for the enrichment of coffee biochemistry theory.

Introduction

Coffee, which shows the typical features of pleasant taste including the balanced mixture of sourness, bitterness, astringency and nuttiness, is the beverage with the third highest consumption amount worldwide. It is also a commercial product with the highest popularity, with an estimated 400 billion cups consumed annually (Spence and Carvalho, 2020). The three primary species of coffee grown worldwide are Arabica, Robusta, and Liberica, with Arabica widely known for its desirable characteristics, flavour, and aroma (Cong et al., 2020). Coffee is mostly distributed in Yunnan and Hainan provinces of China; over 99 % of coffee grown in Yunnan Province is of the Arabica species. Coffee consumption is closely related to human health and includes various compounds with biological activities, such as alkaloids, polyphenols, amino acids, carbohydrates, lipids and proteins (Hu et al., 2019). To take an example, caffeine is a major alkaloid in coffee granting the stimulant nature of coffee, and it possesses antioxidant properties (Miłek, Młodecki, and Dżugan, 2021). Active coffee compounds have been recognized as its flavor precursors. Processing initiates at once following the harvesting of fresh coffee fruit for avoiding unexpected fermentation or fruit spoilage that seriously influences coffee beans' physicochemical composition, reducing the commercial value (Barrios-Rodríguez et al., 2021). Consequently, exploring impacts of primary processing methods on chemical composition in roasted coffee beans is of great significance,

Abbreviations: CG, Traditional dry processing; CGA, Chlorogenic acid; CQA, Caffeoylquinic acid; CS, Traditional wet processing; diCQA, Di-caffeoylquinic acid; FC, Fold change; FQA, Feruloylquinic acid; GA, Dry processing I; GB, Dry processing II; HPLC, High-performance liquid chromatography; KEGG, Kyoto Encyclopedia of Genes and Genomes; MA, Honey processing I; MB, Honey processing II; MC, Honey processing III; MRM, Multiple reaction monitoring; OPLS-DA, Orthogonal partial least squares discriminant analysis; PCA, Principal component analysis; Q TRAP, Quadrupole/linear ion trap; TFC, Total flavone content; TPC, Total phenolic content; UPLC-ESI-MS/MS, Ultra-performance liquid chromatography-electrospray ionisation-tandem mass spectrometry; UPLC-Q-TOF-MS, Ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry; VIP, Variable importance of projection.

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since green coffee beans' biochemical composition determines flavor quality by means of the roasting-mediated chemical reactions.

Notably, for this complicated operation chain, its eventual product is a cup of coffee. Technologies that improve coffee quality can be implemented at different stages of production, from coffee planting to storage. Primary processing of coffee involves peeling, de-gumming, fermentation, and drying. With the development of coffee industry, problems regarding coffee yield and quality associated with conventional processing methods are becoming more and more pronounced. Different primary processing approaches are used for obtaining green coffee beans — a product commercialized in international market for transformation to coffee drinks - for roasting. Previous studies have examined how primary processing approaches affected coffee bean and related product quality. Cortés-Macías et al. (2022) reported impacts of dry, wet, as well as semi-dry processing on coffee chemical composition within Huila region; they reported that coffee processed using dry approach exhibited increased caffeine contents. Pereira et al. (2020) investigated the effects of four types of wet processing on coffee quality at six different altitudes, focusing on improving the drink final quality; according to their results, spontaneous fermentation showed an increased potential for sensorial result $>$ 900 m. Chen et al. (2019) examined crude fat, crude protein, together with caffeine levels in raw coffee beans processed through pectin non-immersion fermentation, observing that their levels increased relative to those in coffee beans processed through immersion fermentation. However, no studies have investigated different primary processing methods or alterations of the Arabica coffee bean chemical composition in Yunnan Province. Thus, traditional drying processing, traditional wet processing, and honey processing methods were applied to treat fresh coffee fruits. In addition, anaerobic fermentation can improve the flavour quality of coffee samples, so anaerobic fermentation (different times) combined with traditional drying processing were also used in this study. Honey processing combined with heat pump drying (different times) was also applied to compare with other processing methods. Therefore, seven different primary processing methods based on above combinations were used to compare and elucidate chemical profiles and taste quality of Yunnan Arabica coffee beans.

Widely-targeted metabolomics analysis comprises advantages of non-targeted and targeted metabolite detection technique to achieve high throughput, high sensitivity, and wide coverage (Zhang, Lu, & Liu, 2022; Wang et al., 2023). This helps to effectively, qualitatively and quantitatively determine pathways related to the stress responsive mechanisms of plants (Yang et al., 2019). Metabolomics is adopted in food safety inspections, raw material or product quality determination, and main chemical compound identification across different species in the food industry (Sidwick et al., 2017). Xu et al. (2019) performed ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS) to identify 5 novel compounds (pyrimethanil, 1-palmitoyllysophos-phatidylcholine, harman, norharman, 4-hydroxy-3-methoxycinna-maldehyde); these compounds were depicted to be the typical markers for discriminating coffee samples processed through cold brew, pour-over, and boiling approaches. Similarly, by means of targeted metabolomics, 146 differential metabolites were screened in four green teas of an identical tea variety - Longjing 43 (*Camellia sinensis* var. *sinensis*) (Shi et al., 2022). By characterising the metabolic profiles of coffee prepared using different primary processing methods, a mechanistic association of alterations of coffee metabolism with taste characteristics can be provided, which resembles the function of storage age in taste quality and metabolic characteristics of Qingzhuan tea (Cheng et al. 2021b).

Changes in coffee metabolites may offer important data for understanding mechanisms related to alteration of coffee quality in primary processing. The electronic tongue (e-tongue) has been developed as the instrument to stimulate the human tongue, which can more sensitively, digitally and objectively describe taste than a human taster. Such sensory technology is used for profiling fruit juice and infusion taste (Banerjee et al., 2019). Dong et al. (2019) reported coffee bean taste characteristics obtained by different drying methods through HS-SPME/GC–MS and the e-tongue. Many studies are conducted to examine the coffee chemical composition and sensory quality, but little is known about changes of coffee taste quality and metabolic composition chemical profiles prepared using different primary processing methods.

In this study, the e-tongue was applied in comparing coffee sensory quality differences prepared using diverse primary processing methods. Differences in critical metabolites of seven coffee samples were analyzed by ultra-performance liquid chromatography-electrospray ionisation-tandem mass spectrometry (UPLC-ESI-MS/MS). Widely-targeted metabolomics was integrated with the e-tongue and multivariate regression to illustrate changes in chemical profiles and taste quality of Yunnan Arabica coffee beans, and elucidate relation of taste quality with chemical composition. We aim to provide comprehensive insight into the roles of primary processing approaches in the coffee bean chemical composition and sensory quality for improving their flavour quality suitable for different consumer markets.

Materials and methods

Chemicals and reagents

Methanol, acetonitrile, acetic acid and formic acid of high-performance liquid chromatography (HPLC) grade were provided by Merck (Darmstadt, Germany). Caffeine, trigonelline, oxalic, tartaric, quinic, malic, acetic, citric, and succinic acid standards were provided by Sigma-Aldrich (St. Louis, MO, USA). The 3-caffeoylquinic acid (3-CQA), 4-CQA, 5-CQA, 3,4-di-caffeoylquinic acid (3,4-diCQA), 3,5-diCQA, 4,5-diCQA, 4-feruloylquinic acid (4-FQA), and 5-FQA standards were provided by Victory Biological Technology Co., Ltd. (Sichuan, China). We utilized ultrapure water obtained using the Maters-plus UVF system (Hetai, Shanghai, China) during all experimental processes, and filtered them using the 0.22 mm membrane at once prior to use.

Sample preparation and primary processing

Fresh coffee fruits (Yunnan Arabica coffee, Katim P7963) were harvested in March 2022 at the Aini Coffee Estate, Pu'er City, Yunnan Province. Fruits in the mature stage with the same color (red) and size were harvested. Fresh coffee fruits were chosen for eliminating impurities or defected beans, and were later classified as seven batches with different primary processing methods: traditional dry processing (CG), fresh coffee fruits were directly dried under the sun without any treatment; dry processing I (GA), coffee fruits were subjected to sun-drying after 72 h of anaerobic fermentation; dry processing II (GB), coffee berries were subjected to sun-drying after 150 h of anaerobic fermentation; traditional wet processing (CS), the fresh coffee fruit was depulped and demucilaged, then dried in the sun; honey processing I (MA), the coffee fruit was dried in the sun after removing the pulp; honey processing II (MB) the fresh coffee fruit was stripped of its pulp without demucilage, dried by heat pump at 40 °C for 30 h to a moisture content of 25.0 %, then sun-dried to a moisture content of 11.0 $\pm$ 1.0 g/100 g DW; and honey processing III (MC), the fresh coffee fruit was stripped of its pulp without demucilage, air-dried at 25 °C room temperature for 10 h, dried by heat pump at 30 °C for 18 h to a moisture content of 25.0 %, and finally, sun-dried to a moisture content of 11.0 $\pm$ 1.0 g/100 g DW. The primary processing approach was used to process freshly harvested coffee beans, followed by drying utill reaching the moisture content of 11.0 $\pm$ 1.0 g/100 g DW. The moisture content of coffee samples prepared by various primary processing methods were determined by the Agricultural Industry Standard of China (NY/T 604–2020), ensuring a moisture content of 11.0 $\pm$ 1.0 g/100 g DW to prevent deterioration. Finally, a huller was used to remove the shells and acquire green coffee beans in further analysis.

Coffee sample roasting processes

We used dried green coffee beans in the roasting step. After collecting samples (100.0 g) in every fraction, the PRE1Z drum coffee roaster (Emmerich am Rhein, Germany) was employed for roasting at the initial roasting temperature of 150 °C; thereafter, it varied to a medium degree (150–180 °C) for 8 min. The electronic coffee grinder (Mahlkönig, Hamburg, Germany) was utilized to grind samples, followed by sieving of grinded coffee beans with the standard sieve for excluding great particles. The grinded, medium-roast coffee beans under the same roasting conditions were analytically determined in triplicate.

Color measurement of coffee infusion

Coffee infusions were prepared in line with the Specialty Coffee Association of America cupping method. First, coffee powder (8.25 g) was dissolved in boiling ultrapure water (150.0 mL), and the mixture was later subjected to stirring and 5.0 min incubation within the boiling water bath. After 1-h cooling to ambient temperature, coffee was filtered with Whatman No. 1 filter paper (Sigma-Aldrich, St. Louis, MO, USA) before colour determination (Stanek et al., 2021). The color of coffee infusions processed by diverse primary processing approaches was analyzed by the colorimeter (SP62; X-rite, Grand Rapids, MI, USA) with CIELAB system. Colour parameters were L* (L* = 0 and 100 indicate black and white, separately), a* [red (+), green (–)], while b* [yellow (+), blue (–)]. We determined the colour coordinates within CIE L* a * b* space by using illuminant system D65/10°. Besides, equations (1), (2), and (3) were utilized to determine total colour differences (ΔE), chroma (C*), together with hue (H*), respectively. The measurements were conducted in triplicate to obtain the means with standard deviations of color parameters.

$$\Delta E = \sqrt{\left(L_0^* - L^*\right)^2 + \left(a_0^* - a^*\right)^2 + \left(b_0^* - b^*\right)^2} \quad (1)$$

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (2)$$

$$H^* = \tan^{-1}\left(b^*/a^*\right) \quad (3)$$

E-tongue-assisted taste assessment

Coffee taste quality prepared using the seven primary processing methods was analyzed by the e-tongue (SA402B; Intelligent Sensor Technology, Inc., Tokyo, Japan) (Gao et al., 2022). Briefly, coffee powders (1.0 g) were added into 100.0 mL boiling ultrapure water, followed by 5-min standing in a boiling water bath. After cooling to ambient temperature, the coffee infusions were sieved using Whatman No. 1 filter paper for taste evaluation. In taste measurements, we used AAE (umami), CAO (sourness), CTO (saltiness), COO (bitterness), and AE1 (astringency) sensor probes. Typically, the e-tongue both evaluates the above-mentioned fundamental coffee infusion tastes, and analyses the aftertastes (such as aftertaste-bitterness, richness, or aftertaste-astringency) (Zhu et al., 2020). We cleaned and calibrated this e-tongue prior to analysis under the following conditions: room temperature, 25 °C; sampling time, 30 s; aftertaste collecting time, 30 s; and cleaning time, 180 s. E-tongue measurement was performed four times per sample. Results acquired from those last three procedures were used in later analyses.

Total phenolic content (TPC) and total flavone content (TFC) measurements

To measure the TPC, coffee powders (0.20 g) were added into the 70 % (v/v) methanol (5.0 mL) for 15.0 min ultrasonic extraction (SB-5200DTD; Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) twice under ambient temperature. The samples were later centrifuged for a 15.0 min period at 5000 rpm and pooled, to obtain coffee extract. Gallic acid standard solutions (0.00, 0.02, 0.04, 0.06, 0.08, 0.10 mg/mL) were utilized for constructing the calibration curve, whereas distilled water was used for preparing a blank. The standard or filtrate was then introduced with 0.5 mL Folin–Ciocalteu reagent for 5.0 min mixing, followed by addition of saturated sodium carbonate (1.0 mL) and reaction with samples in dark for a 30.0 min period. TPC was determined using the UV–Vis spectrophotometer (Shimadzu, Kyoto, Japan) at 765 nm (Qin et al., 2023).

TFC was determined by preparing the extracted coffee as for TPC. A calibration curve was then constructed using the rutin standard solutions (0.00, 0.02, 0.04, 0.06, 0.08, 0.10 mg/mL). The 60 % (v/v) ethanol (3.0 mL) and 50.0 mg/mL $NaNO_2$ solution (10.0 mL) were added to 2.0 mL of the standard and samples, the mixtures were subjected to 30.0 s vortexing and 6-min standing. Thereafter, $Al(NO_3)_3$ solution (1.5 mL) was introduced to the resultant mixture, followed by 30.0 s vortexing and 15-min standing. TFC was measured with the UV–Vis spectrophotometer (Shimadzu) at 510 nm (Qin et al., 2023).

HPLC analysis of major secondary metabolites

Determination of alkaloids and chlorogenic acids

For alkaloid and chlorogenic acid (CGA) analyses, an aliquot of 0.5 g grinded coffee samples were placed into the centrifuge tube with 50.0 mL methanol (70 %, v/v). After 10-min centrifugation at 5000 rpm, supernatants were obtained. The caffeine, trigonelline, 3-CQA, 4-CQA, 5-CQA, 4-FQA, 5-FQA, 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA contents within coffee extract were then analyzed through HPLC according to description of Smrke et al. (2015) after mild modification, with the Agilent 1290 instrument (Agilent Technologies Inc., Santa Clara, CA, USA). The reverse-phase C18 column (inner diameter, 100 mm × 2.1 mm; particle size, 1.7 mm) was utilized to separate the coffee bean extracts. The mobile phase included methanol (A) and the 0.1 % (v/v) glacial acetic acid aqueous solution (B). The gradient elution procedure was shown below, 0–30 min, 5–100 % A; 30–32 min, 100–100 % A. The injection volume, column temperature and mobile phase flow rate were 1.5 μL, 25 °C and 0.2 mL/min, separately. Detection wavelengths were 254 nm and 325 nm for alkaloids and CGAs, respectively. All samples were analysed in triplicate.

Organic acid measurement

The oxalic, tartaric, quinic, malic, acetic, citric, and succinic acid contents in coffee samples were determined by HPLC analysis with diode-array detection HPLC-DAD. Briefly, 5.0 g grinded coffee samples were added into the 100-mL round-bottomed flask, followed by addition of 60.0 mL de-ionised water. Thereafter, the reflux extraction mode was applied in extracting the resultant reaction mixture for a 2.5 h period under 70 °C. After centrifugation (4000 rpm for 10 min), supernatants were diluted using ultrapure water to 100.0 mL. Later, 100.0 mL extract was sieved with the 0.22 mm regenerated cellulose membrane again before analyzing organic acid content.

Organic acid content was analyzed as described by Bressani et al. (2020) after mild modifications through using the Zorbox SB-Aq (4.6 mm × 250 mm, 5 mm particle) at 210 nm. The mobile phase comprised methanol and 0.01 mol/L NaH_2PO_4 buffer solution (pH = 2.00 ± 0.02 after adjustment using o-phosphoric acid). Besides, we conducted an isocratic elution procedure using the mobile phase at the 0.5 mL/min flow rate. A standard curve was utilized to quantify organic acid content.

UPLC analysis

A UPLC-ESI-MS/MS system (ExionLC™ AD, 4500 Q TRAP; Applied Biosystems, Waltham, MA, USA) equipped with the Agilent SB-C18 column (1.8 μm, 2.1 mm × 100 mm) was employed for analyzing coffee sample extracts. Its mobile phase comprised solvent A (pure water containing 0.1 % formic acid) and solvent B (acetonitrile contained

within 0.1 % formic acid). Samples were measured using the following gradient procedure, 5 % solvent B at 0.0 min; linear increase to 95 % in 9.0 min, maintenance at 95 % for 1.0 min, then decrease to 5 % in 1.1 min, and maintenance at 5 % for 2.9 min. The injection volume, column oven and flow velocity were 4.0 μL, 40 °C and 0.35 mL/min, separately. We alternately connected the effluent to the ESI-triple quadrupole/linear ion trap (Q TRAP)-mass spectrometer.

ESI-Q TRAP-MS/MS conditions

Linear ion trap (LIT) and triple quadrupole (QQQ) scans were acquired on a Q TRAP-MS, AB4500 Q TRAP UPLC/MS/MS system, equipped with an ESI turbo ion-spray interface, operating in positive and negative ion modes and controlled by Analyst 1.6.3 software (AB Sciex). The following ESI source operation parameters were used: source temperature, 550 °C; ion spray voltages, 5500 and 4500 V in the positive and negative ion modes, separately. We set the ion source gas I, gas II, and curtain gas at 50, 60, and 25 psi, separately, with high collision-activated dissociation. In addition, this study obtained QQQ scans as multiple reaction monitoring (MRM) experiments, and set a collision gas (nitrogen) to medium. We determined the de-clustering potential and collision energy of the MRM transitions by de-clustering potential and collision energy optimisation. A typical set of MRM transitions was observed in every period in line with metabolites eluted in the above process. In this study, the metabolites were identified by matching the retention time, fragmentation patterns, and accurate *m/z* values to the standards in the self-constructed metabolite database (Wuhan MetWare Biotechnology Co. Ltd., Wuhan, China). MultiaQuant software was used to integrate and calibrate chromatographic peaks, the area of each peak represents the relative metabolite content of each sample.

Multivariate statistical analyses and network visualisation

Multivariate statistical analyses included principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), together with hierarchical cluster analysis (HCA). First, we performed PCA using datasets from every sample for elucidating total metabolic differences and variation degree of different types of coffee samples. Pairwise comparison was then performed through OPLS-DA, while differential metabolites were acquired according to the threshold of the variable importance of projection (VIP) at 1.0 in OPLS-DA model. Besides, we additionally selected differential metabolites using fold changes from the univariate analyses. Differential metabolites were also enriched using Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Results and discussion

Colour parameters for coffee infusion

Colour has a critical effect on assessing food quality by consumers. As shown in Fig. 1A, the colour of the coffee infusions varied significantly. The colour parameters for coffee infusions processed by diverse primary processing approaches can be summarised in Table S1. For analytical purposes, we chose CG to be a reference sample. The maximum colour lightness was found in the MB sample (7.31 ± 0.02), followed by the MA (6.63 ± 0.03) and CS (6.05 ± 0.05) samples; an increase of L* value indicates coffee infusion brightening. The infusion colour of coffee intuitively indicates the level of melanoidins, the main pigments in coffee beans (Gigl et al., 2022); this phenomenon demonstrates that the MB sample contains a lower melanoidin content. For a*, CG (9.89 ± 0.04) and CS (2.41 ± 0.09) samples had the greatest and smallest values, separately. The blue-yellow coordinate parameter value was between 0.76 ± 0.04 (CS) and 4.92 ± 0.02 (CG), and alterations of b* value were predicted based on sample loss and increased yellowness. The fermentation time was extended, causing a decrease in the a*, b*, and C* values relative to CG sample. These variations of L*, a*, and b* values are associated with pigment degradation, or the nonenzymatic browning Maillard reaction during primary processing processes (Zhang et al., 2021). The greatest ΔE value could be obtained from CS samples, while

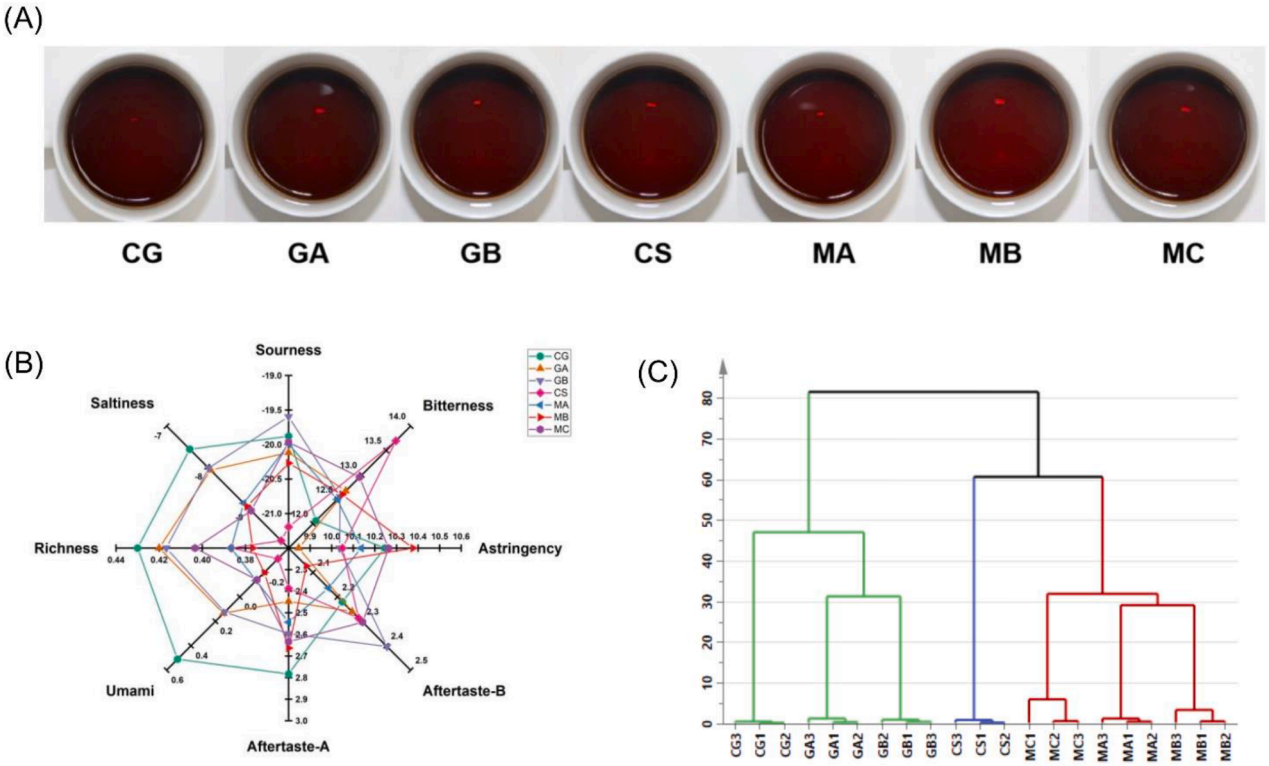

Fig. 1. Taste evaluation of seven kinds of coffee. (**A**) Infusion colour of coffee, (**B**) Spider plot of taste characters, (**C**) Dendrogram of taste scores as input matrix.

the smallest ΔE value was observed from CG samples, demonstrating that the darker the coffee infusion, the more red and yellow the colouration. By contrast, relatively lighter coffee infusions were obtained when prepared using the MA or MB methods.

E-tongue response to coffee sample taste profile

Coffee sample taste quality was detected using the e-tongue, the intelligent recognition system stimulating human tongue and more sensitively, digitally and objectively describing tastes than human taster (Jiang et al., 2018). A radar plot was used to depict responses of diverse sensors to coffee taste quality (Fig. 1B). Sourness and saltiness scores of all coffee infusions were below the threshold and undetectable in humans (Zhu et al., 2020). The critical point of umami was zero in the e-tongue analysis; responses of umami sensors for the CG, GA, and GB samples were all > zero. The lowest and highest response values of the umami sensor were found in the GB (0.12) and CG (0.51) samples, respectively; with the increase in fermentation time, response value of the umami sensor to the coffee infusion weakened, and umami was undetectable by humans in the CS, MA, MB, and MC coffee samples. The coffee infusions prepared using the CS (13.69) and CG (12.06) methods exhibited the highest and lowest response values to the bitterness sensor, respectively, indicating that the coffee samples prepared using the wet processing method had stronger bitterness than those prepared using the dry processing method. The CG sample exhibited greatest aftertaste astringency, whereas GB sample exhibited greatest aftertaste bitterness across all coffee samples. A dendrogram was created to demonstrate relations and distributions of those roasted coffee samples subjected to diverse primary processing approaches (Fig. 1C). As illustrated in the figure, taste phenotypes of the coffee samples could be classified into three clusters: CG-GA-GB, CS, and MC-MA-MB. The distinctive taste phenotype of the CS sample may be attributed to its unique processing methods; wet treatment for peeling and de-gumming endowed this sample with a unique flavour and the strongest bitterness. The clustering of MA, MB, and MC samples indicated a high similarity in their taste characteristics; thus, the change in overall taste quality of coffee prepared by de-pulping without de-gumming under different drying conditions was insignificant.

Comparison of the TPC and TFC of coffee samples

Currently, coffee has been recognized to be the functional beverage as it possesses abundant bioactive substances, such as alkaloids, phenolic compounds, or additional new compounds with activities of antioxidation and anti-inflammation (Wu et al., 2021). The TPC of coffee beans varies greatly owing to the regulation of a range of factors, including origin, variety, treatment, and bean properties. As indicated from Table 1, TPC was highest in the CG (44.42 mg/g DW), while MA (41.89 mg/g DW) sample ranked the second place. Such results are consistent with findings obtained by Contreras-Calderón et al., 2016, who indicated that the TPC ranged from 12.50 to 49.10 mg/g DW in 58 coffee samples; the TPC was found to be the lowest in GB samples (33.18 mg/g DW), as polyphenols undergo microbial degradation and gradually decrease during fermentation (Cheng et al. 2021a). The TPC exhibited a significant decline as the fermentation time increased, and the relatively high TPC in CG and MA may be attributed to the absence of fermentation. The TPC was also affected by various drying methods, as evidenced in the MA, MB, and MC samples.

Flavonoids are a group of polyphenolic compounds whose molecular structure is C_6-C_3-C_6 that directly affect the colour and flavour of plants. The TFC in was highest in the GB sample (159.38 mg/g DW); TFC of GA sample (132.29 mg/g DW) was similar to MB sample (133.54 mg/g DW), but apparently increased relative to MA sample (108.96 mg/g DW). Impacts of fermentation on TPC and TFC exhibited divergent trends, whereby an increase in fermentation time resulted in a decrease in TPC levels, but increase in TFC levels.

Analysis of main secondary metabolites within coffee samples

Caffeine accounts for a main alkaloid component within coffee beans, which is responsible for bitterness of coffee (van Dam,Hu,and Willett, 2020). The caffeine and trigonelline contents of coffee beans processed by diverse primary processing approaches can be observed from Table 1. GA (12.32 ± 0.03 mg/g DW) and MC (11.12 ± 0.02 mg/g DW) samples had the greatest and smallest caffeine contents, respectively. Caffeine contents across GB (12.24 ± 0.02 mg/g DW) and CS (12.22 ± 0.04 mg/g DW) samples were similar, while caffeine levels of the beans after the seven preparation processes varied between 11.12 ± 0.02 and 12.32 ± 0.03 mg/g DW; which is because that caffeine has a stable structure. Trigonelline ranks the second place among alkaloids obtained through enzymatic methylation of nicotinic acid (Heo et al., 2020). Trigonelline levels followed the order below, MA > MB > MC > CG > GB > GA > CS; the trigonelline contents of samples prepared via the MA, CG, and CS methods were 3.71 ± 0.05, 3.1 ± 0.05, and 2.09 ±

Table 1
Variation in major secondary metabolites among coffee beans prepared by seven different primary processing methods.

NO.	Content(mg/g)	CG	GA	GB	CS	MA	MB	MC
	Active compounds							
1	TPC	44.42 ± 5.76^{a}	40.91 ± 4.28^{abc}	33.18 ± 3.11^{d}	37.11 ± 2.53^{bcd}	41.89 ± 3.16^{ab}	34.86 ± 2.12 ^{cd}	34.44 ± 2.32 ^{cd}
2	TFC	128.54 ± 3.82^{bc}	132.29 ± 1.91^{b}	159.38 ± 1.25^{a}	125.63 ± 5.45^{c}	108.96 ± 2.60^{d}	133.54 ± 4.02^{b}	122.71 ± 3.82^{c}
	Alkaloid							
3	Trigonelline	3.10 ± 0.05^{d}	2.51 ± 0.01^{f}	2.74 ± 0.01^{e}	2.09 ± 0.01 ^{g}	3.71 ± 0.05^{a}	3.58 ± 0.06^{b}	3.28 ± 0.05^{c}
4	Caffeine	11.63 ± 0.02^{d}	12.32 ± 0.03^{a}	12.24 ± 0.02^{b}	12.22 ± 0.04^{b}	11.65 ± 0.01^{d}	12.05 ± 0.02^{c}	11.12 ± 0.02^{e}
	Chlorogenic acids							
5	3-CQA	3.53 ± 0.02^{a}	2.64 ± 0.02^{d}	2.97 ± 0.02^{c}	2.03 ± 0.01^{e}	2.97 ± 0.01^{c}	3.18 ± 0.01^{b}	2.62 ± 0.02^{d}
6	5-CQA	9.05 ± 0.01^{a}	6.77 ± 0.03^{f}	7.79 ± 0.03^{d}	5.2 ± 0.04 ^{g}	7.9 ± 0.02^{c}	8.61 ± 0.01^{b}	7.19 ± 0.04^{e}
7	4-CQA	3.86 ± 0.04^{c}	3.23 ± 0.02^{e}	3.75 ± 0.02^{d}	2.75 ± 0.01^{f}	4.05 ± 0.01^{b}	4.39 ± 0.01^{a}	3.87 ± 0.03^{c}
8	5-FQA	1.89 ± 0.04^{e}	1.72 ± 0.02^{f}	2.01 ± 0.03^{d}	1.92 ± 0.01^{e}	2.42 ± 0.01^{b}	2.58 ± 0.01^{a}	2.32 ± 0.02^{c}
9	4-FQA	0.72 ± 0.04^{a}	0.47 ± 0.02^{b}	0.37 ± 0.02^{c}	0.25 ± 0.01^{d}	0.24 ± 0.01^{d}	0.19 ± 0.01^{e}	0.04 ± 0.00^{f}
10	3,4-diCQA	0.45 ± 0.01^{a}	0.31 ± 0.00^{d}	0.34 ± 0.01^{c}	0.27 ± 0.01^{f}	0.37 ± 0.01^{b}	0.44 ± 0.01^{a}	0.30 ± 0.01^{e}
11	4,5-diCQA	0.28 ± 0.01^{a}	0.19 ± 0.01^{c}	0.21 ± 0.01^{b}	0.15 ± 0.01^{e}	0.22 ± 0.01^{b}	0.28 ± 0.01^{a}	0.18 ± 0.01^{c}
12	3,5-diCQA	0.51 ± 0.01^{b}	0.35 ± 0.01^{f}	0.41 ± 0.01^{d}	0.32 ± 0.01 ^{g}	0.47 ± 0.01^{c}	0.57 ± 0.01^{a}	0.37 ± 0.01^{e}
	Organic acids							
13	Oxalic acid	0.84 ± 0.01^{c}	3.09 ± 0.02^{a}	2.85 ± 0.02^{b}	0.10 ± 0.00 ^{g}	0.70 ± 0.02^{d}	0.26 ± 0.01^{f}	0.34 ± 0.01^{e}
14	Tartaric acid	1.26 ± 0.04^{b}	2.55 ± 0.05^{a}	1.08 ± 0.04^{c}	0.51 ± 0.01^{e}	0.62 ± 0.02^{d}	0.33 ± 0.03^{f}	0.18 ± 0.02 ^{g}
15	Quinic acid	3.27 ± 0.03^{b}	4.57 ± 0.07^{a}	1.26 ± 0.06^{f}	2.94 ± 0.03^{d}	3.1 ± 0.08^{c}	2.96 ± 0.01^{d}	2.60 ± 0.04^{e}
16	Malic acid	10.51 ± 1.29^{c}	5.13 ± 0.08^{d}	6.56 ± 0.33^{d}	22.31 ± 1.81^{a}	23.37 ± 0.25^{a}	19.05 ± 0.05^{b}	18.38 ± 0.59^{b}
17	Acetic acid	14.46 ± 1.50^{d}	19.41 ± 0.08^{b}	20.88 ± 0.08^{a}	18.84 ± 1.44^{b}	18.11 ± 0.37^{b}	16.53 ± 0.53^{c}	7.10 ± 0.03^{e}
18	Citric acid	14.03 ± 1.10^{a}	8.68 ± 0.38^{b}	2.53 ± 0.02^{c}	2.48 ± 0.13^{c}	1.50 ± 0.01^{d}	1.17 ± 0.03^{d}	1.06 ± 0.02^{d}
19	Succinic acid	3.64 ± 0.36^{f}	7.95 ± 0.59^{a}	4.67 ± 0.03^{de}	6.81 ± 0.03^{b}	5.22 ± 0.03^{c}	4.26 ± 0.01^{e}	5.02 ± 0.05 ^{cd}

0.01 mg/g DW, respectively, and trigonelline levels within beans prepared with honey-processing approach increased relative to those prepared by the wet approach.

CGAs are main phenolic compounds in coffee and have a critical effect on forming the flavour of roasted coffee; they contribute to final bitter and astringent flavour, and significantly affect coffee quality (Kulapichitr et al., 2022). In coffee, the main CGAs classes include CQA, FQA, and diCQA. Eight CGA isomers were quantified to determine the impacts of diverse primary processing approaches on CGA isomer profiles of Arabica coffee beans (Table 1). Both 3-CQAs (2.03–3.53 mg/g DW) and 5-CQAs (5.20–9.05 mg/g DW) were highest in CG and lowest in CS beans; these results demonstrate that the 3-CQA and 5-CQA contents were significantly affected by peeling and de-gumming, and the contents were higher in the whole treated beans. The highest and lowest 4-FQA contents (0.04–0.72 mg/g DW) could be detected from CG and MC samples, respectively. Additionally, 3,4-diCQAs (0.27–0.45 mg/g DW) and 4,5-diCQAs (0.15–0.28 mg/g DW) were highest within the CG samples and lowest in the CS samples, and 3,5-diCQAs (0.35–0.57 mg/g DW) had greatest levels within MB samples whereas smallest levels within CS samples. These heterogeneities are possibly associated with different primary processing methods that affect metabolic activities in coffee seeds, causing stress metabolite loss or accumulation, such as phenolic substances (Cheng et al., 2019). CGAs are related to acidity, astringency, and bitterness of coffee beverages; however, the greater CGA content indicates better coffee sensory quality benefits (Angeloni et al., 2019).

In coffee, its acidity may be mostly associated with organic acids, and they significantly affect flavour balance. There were 7 organic acids measured within the coffee beans (Table 1); coffee beans processed through CS method exhibited greatest total organic acid content (53.99 mg/g DW), whereas samples processed through MC approach displayed the lowest content (34.68 mg/g DW). Similar to Elhalis et al. (2021), wet processing of coffee beans increased organic acid level and significantly improved the quality, flavour, aroma, and sensuality of coffee beverages. Organic acids with the highest abundance included malic acid, acetic and citric acids, while tartaric acid was least abundant. Oxalic acid and acetic acid contents in samples that underwent the GA and GB processing markedly increased relative to other treatments, demonstrating that dry anaerobic fermentation was beneficial for the accumulation of oxalic and acetic acid. Citric acid leads to tartness, berry and fruity flavours (Martinez et al., 2019). The citric acid content (1.06–14.03 mg/g DW) was highest in CG and lowest in MC samples, and was significantly higher in dry-treated than in wet and honey-treated coffee beans.

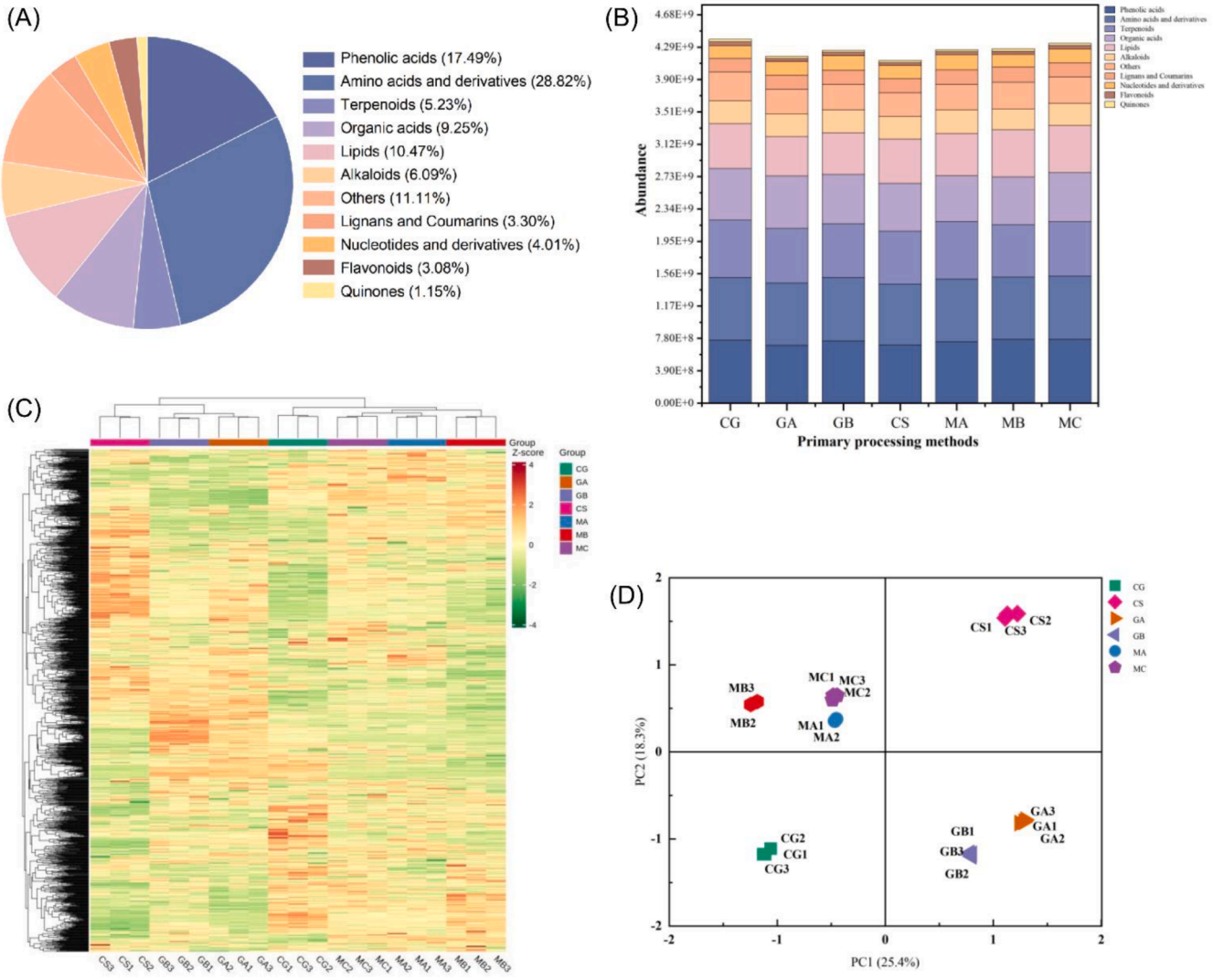

Fig. 2. Metabonomic analysis of coffee prepared using the seven primary processing methods. (**A**) Classification of metabolites based on the HMDB (Human Metabolome Database) 4.0 database, (**B**) Comparison of metabolite categories, (**C**) Heatmap analysis, (**D**) Distribution of coffee samples in the two-dimensional score plot of principal component analysis.

Metabolomic analyses on coffee beans

Overview on the coffee bean metabolites

In total, 1395 metabolites were found in the GC-, GA-, GB-, CS-, MA-, MB-, and MC-treated coffee samples. The most diverse were amino acids and derivatives (402 species), phenolic acids (244 species), lipids (146 species), and organic acids (136 species) (Fig. 2A); 73 terpenoids, 85 alkaloids, 155 others, 46 lignans and coumarins, 56 nucleotides as well as corresponding derivatives, 43 flavonoids, and 16 quinones were also identified. The multiple reaction monitoring mode was employed for quantifying metabolites, with abundances being determined based on mass spectral signal strength. After quantitative comparison of metabolites through category, the CG sample had the highest abundance of metabolites compared with other processing methods, and was about 1.34-times richer in flavonoids than the MA sample (Fig. 2B). The application of CG had a negligible impact on the loss of metabolite abundance, while exhibiting the greatest potential to preserve flavonoid metabolites. The GA sample contained high levels of organic acids and a low abundance of lipids. The GB sample contained more amino acids and derivatives than the other treatments; anaerobic fermentation may have resulted in their release. The MC sample contained higher levels of phenolic acids and lipids than the other treatments, with a 19.41 % increase in lipid content compared with the GA sample. These results suggest that different primary treatments affect the amounts of flavonoids, lipids, nucleotides, phenolic acids, and amino acids in coffee.

We conducted heatmap hierarchical clustering analysis for analyzing metabolite accumulation in coffee samples from the seven primary processes (Fig. 2C). The heat map revealed distinct up- and down-regulation degrees of coffee fraction metabolites after different primary processings, indicating substantial changes in the metabolic processes of coffee beans. Those three biological replicates for every group were grouped together, demonstrating great data dependability and strong homogeneity across the replicates. PCA was also applied to explore variations in metabolites of the coffee samples. The PCA bio-plot showed clustering of the three replicates within groups, although the seven groups were isolated from each other (Fig. 2D); 25.4 % and 18.3 % of the original dataset's characteristics differentiated by first and second principal components (PC1 and PC2, separately). In accordance with findings of heatmap analysis, the two-dimensional distribution of the CG, MA, MB, and MC samples was on left side of PC1, whereas CS, GA, and GB samples were on its right side; this suggested substantial metabolite variations across the seven groups. The MC and MA samples were dispersed more closely on the PCA plane than the other groups, indicating the small heterogeneities among the metabolite profiles of the MC and MA samples.

Differential metabolites identified among coffee beans

OPLS-DA has been the multivariate statistical analytical approach with supervised pattern recognition which maximises group differentiation and helps identify differential metabolites. OPLS-DA was first conducted for pairwise comparisons (Fig. S1), which suggested that the two types of samples were on positive and negative sides of PC1, demonstrating that these samples had significantly different chemical compositions. R^2X and R^2Y of the model stand for interpretation rates of X and Y matrices, separately; Q^2 represents prediction capacity. As revealed by pairwise comparisons, R^2Y and Q^2 values were > 0.90, revealing the suitability of this model. We also validated OPLS-DA model by 200 alignment experiments, confirming the significance of this model (Fig. S2).

The volcano plot was drawn to visualize differences in metabolite levels between the two sample groups (Fig. S3). According to OPLS-DA model analysis, we identified significantly different metabolites between the two groups with a VIP ≥ 1, fold change (FC) ≥ 2, or FC ≥ 0.5; additionally, there were 211 differential metabolites obtained from seven kinds of coffee beans (Table S2). Amounts of differential metabolites found in various groups varied greatly, and we utilized a Venn diagram for depicting crossover and differentiation of various metabolites in various differential control groups (Fig. S4A); the down-regulated metabolite number increased relative to up-regulated ones in pairwise comparisons (Fig. S4B).

Between the CG and CS samples, 133 differential metabolites were selected, of which 96 and 37 were more abundant in the CG and CS samples, respectively. This result was consistent with the distribution between the CG and CS samples in the PCA plot, which indicated a significant difference in their metabolic activity. Between the CG and GA samples, we selected 56 differential metabolites, including 20 with up-regulation whereas 36 with down-regulation. Between the CG and GB samples, 74 differential metabolites were selected; 38 were up-regulated, indicating that the physiological metabolic activity of several key metabolites affecting coffee taste quality was attributed to the different anaerobic fermentation times. The lowest number of differential metabolites was screened between the CG and MC samples, identifying 42 down-regulated and 10 up-regulated metabolites. When compared with the other comparison groups, the variations of metabolite levels detected between CG and MC samples differed the least.

The 20 most significant metabolites exhibiting greatest fold-changes in coffee were selected (Fig. S5). When compared with the CG samples, the up-regulated metabolites in the GA, GB, and MC samples were mainly amino acids and derivatives, phenolic acids, organic acids, and lipids. There were two main organic acids, dl-3-phenyllactic acid and dl-glyceraldehyde-3-phosphate; the main phenolic acids were 2-hydroxy-3-phenylpropanoic acid, 2,6-dimethoxybenzaldehyde, and 3-(4-hydroxyphenyl)-propionic acid. Organic and phenolic acids were up-regulated, providing a rich substrate for enzymatic activity and contributing to the formation of coffee flavours (Prakash et al., 2022). Furthermore, the lipids 1-linoleoyl-*sn*-glycerol-diglucoside, 1-linoleoyl-glycerol-2,3-di-O-glucoside, and 2-linoleoylglycerol-1,3-di-O-glucoside were up-regulated in the MC sample. Lipids participate in Strecker degradation reactions as important food ingredients to create new flavour molecules. Down-regulated metabolites are also abundant, with amino acids and derivatives, organic acids, flavonoids, terpenoids, phenolic acids, and other substances predominating. This is because coffee beans with comparatively un-altered plant physiology have their cellular and tissue structures affected after a series of treatments; physiological activities are thus substantially disrupted, and many metabolites are down-regulated. More amino acids and derivatives had decreased levels within GA, GB, CS, MA, MB, and MC samples than in the CG samples, indicating their dramatic transformation to coffee-specific flavours with both production and depletion.

Crucial differential altered metabolites in coffee beans

A total of 211 metabolites from the coffee samples prepared using different primary processing methods were categorized and compared (Table S2). Such differential metabolites were categorized as 11 classes, including amino acids and derivatives, phenolic acids, organic acids, and lipids. To better visualise concentration trends of differential metabolites among different samples, heat maps were utilised to analyse 67 amino acids and derivatives, 30 phenolic acids, 23 organic acids, along with 21 lipids (Fig. 3). The four major metabolite classes can be obtained below.

a. Amino acids and derivatives

The levels of thr-lys-phe-ser-val, glu-thr-asp-arg, ala-lys-asn-glu, and ala-leu-val-lys-ala were higher than those of additional amino acids and derivatives (Fig. 3A). The most significant elevation of ala-lys-asn-glu was observed in the CS sample, exhibiting a 4.75-fold increase compared with the CG sample. Ile-phe-ala-gly-lys was up-regulated in the GA (2.32-fold) and GB (2.21-fold) samples; this may be attributed to protein breakdown during primary processing due to microbial fermentation, leading to an increase in amino acids and their derivatives (Chan et al., 2021). For the honey-processed samples (i.e., the MA, MB, and MC samples), amino acids and derivatives mostly had decreased

Fig. 3. Heatmap using the differentiate metabolites from targeted metabolomic analysis as the input matrix. (**A**) Amino acids and their derivatives (n = 67), (**B**) Phenolic acids (n = 30), (**C**) Organic acids (n = 23). (**D**) Lipids (n = 21).

levels compared with CG treatment of intact coffee beans; only some of these amino acids, including glu-thr-asp-arg and ile-asp-leu-arg, were up-regulated in the MA sample, while gly-lys-leu and ile-asp-leu-arg were up-regulated in the MB sample. In the MC sample, only lys-ser-leu-ala-met and ala-asp-his were up-regulated.

Coffee beans have metabolic activities in post-harvest processing, which are responsive to different abiotic stresses, like de-pulping immediately prior to processing, drought stress in drying, or acidic and anoxic stresses in underwater fermentation (Shavrukov and Hirai, 2016). Such stress-associated metabolic responses alter coffee beans' metabolic profile. Amino acids account for critical factor regarding taste; diverse amino acids like L-valine, L-phenylalanine (bitter-tasting), L-serine, L-proline, and L-ornithine — possess various taste properties that impart a sweet taste to coffee infusions (Shi et al., 2022). The taste of coffee may be affected by variations in the amino acid concentrations caused by the various primary processing techniques.

b. Phenolic acids

In total, 21 phenolic acids were identified among the differential metabolites. Ten differential phenolic acid metabolites were found in the GB sample; nine were up-regulated and one was down-regulation compared with the CG sample. Among them, 2,6-dimethoxybenzaldehyde, 3-(4-hydroxyphenyl)-propionic acid*, and 2-hydroxy-3-phenylpropanoic acid were significantly up-regulated by 3.57-fold, 3.02-fold, and 4.31-fold, respectively, compared with the CG sample. These findings suggest that fermentation stimulates the synthesis of phenolic acids, and that the microorganisms involved in anaerobic fermentation further promote the conversion of phenolic acids through the secretion of various extracellular enzymes. Eleven differential metabolites were identified in the CS sample; four were up-regulated and seven were down-regulated, and 1,3-o-di-p-coumaroylglycerol, 2-naphthol*, and coniferaldehyde were only up-regulated in the CS sample. All distinct metabolites discovered in the MA, MB, and MC samples were down-regulated (Fig. 3B). After a series of treatments, the cellular and tissue structures were damaged and their physiological functions were significantly disrupted; this may be a reason for the down-regulation of phenolic acid metabolites in coffee beans with relatively intact plant physiological functions.

c. Organic acids

Organic acids have critical effect on taste quality, contributing to sourness and fruity taste of coffee; they are important intermediates of carbohydrate catabolism and inhibit bitterness (Wang et al., 2021). Seven organic acids — namely, 2-hydroxy-4-methylpentanoic acid, dl-glyceraldehyde-3-phosphate (3.30-fold), oxalic acid (2.77-fold), 2-hydroxyisocaproic acid, 5-acetamidopentanoic acid, l-lactic acid, and

dl-3-phenyllactic acid* (3.44-fold) — were significantly up-regulated in the GB sample. The expression of organic acids was up-regulated, providing a rich substrate for enzymatic activity and contributing to the formation of coffee flavour (Prakash et al., 2022). The MB coffee samples exhibited the highest content of jasmonic acid, a crucial stress hormone in plants (Fig. 3C). Mao et al. (2021) demonstrated that jasmonic acid is closely linked to plant stress resistance, as it regulates genes associated with stress responses, thereby mitigating biological or abiotic stresses.

d. Lipids

The various lipid differential metabolites detected in the seven types of primary processed coffee samples included fatty acid, glycerol esters, and lysophosphatidylethanolamine. By contrast, hydroxy ricinoleic acid; 9-hydroxy-12-oxo-15(Z)-octadecenoic acid; hydroperoxylinoleic acid; 13-kode; (9Z,11E)-13-oxooctadeca-9,11-dienoic acid; 12,13-epoxy-9-octadecenoic acid; 9(10)-epome; (9R,10S)-(12Z)-9, 10-epoxyoctadecenoic acid; together with 9-hydroxy-10,12,15-octadecatrienoic acid increased by 2.61-, 2.31-, 2.19-, 2.50-, 2.68-, 2.26-, and 2.47-fold in the GB compared with CG sample, respectively (Fig. 3D). Lipids are the main food ingredients, which are involved in Strecker degradation reactions for producing new flavor compounds (Li, oey, and Kebede, 2022). Several free fatty acids have been identified to be the important aroma precursors of coffee, like 13-kode; (9Z,11E)-13-oxooctadeca-9,11-dienoic acid; and 9-hydroxy-10,12,15-octadecatrienoic acid.

Among all coffee samples, 13-kode, (9Z,11E)-13-oxooctadeca-9,11-dienoic acid and 9-hydroxy-10,12,15-octadecatrienoic acid were the most abundant in the GB sample and least abundant in the CS sample. These metabolites correlate with forming unsaturated C6-C9 aldehydes together with volatile alcoholic compounds (Yang, Baldermann and Watanabe, 2013). Three lipid metabolites (1-linoleoyl-*sn*-glycerol-diglucoside*, 1-linoleoylglycerol-2,3-di-o-glucoside*, and 2-linoleoylglycerol-1,3-di-o-glucoside*) had significantly increased concentrations within the MC sample compared with others ($p < 0.05$). Additionally, 1-(2,3-dihydroxypropoxy)-3-(((2-(dimethylamino)ethoxy)(hydroxy)phosphoryl)oxy) propan-2-yl (8E,11Z,14Z)-octadeca-8,11,14-trienoate* was significantly down-regulated in the GA sample.

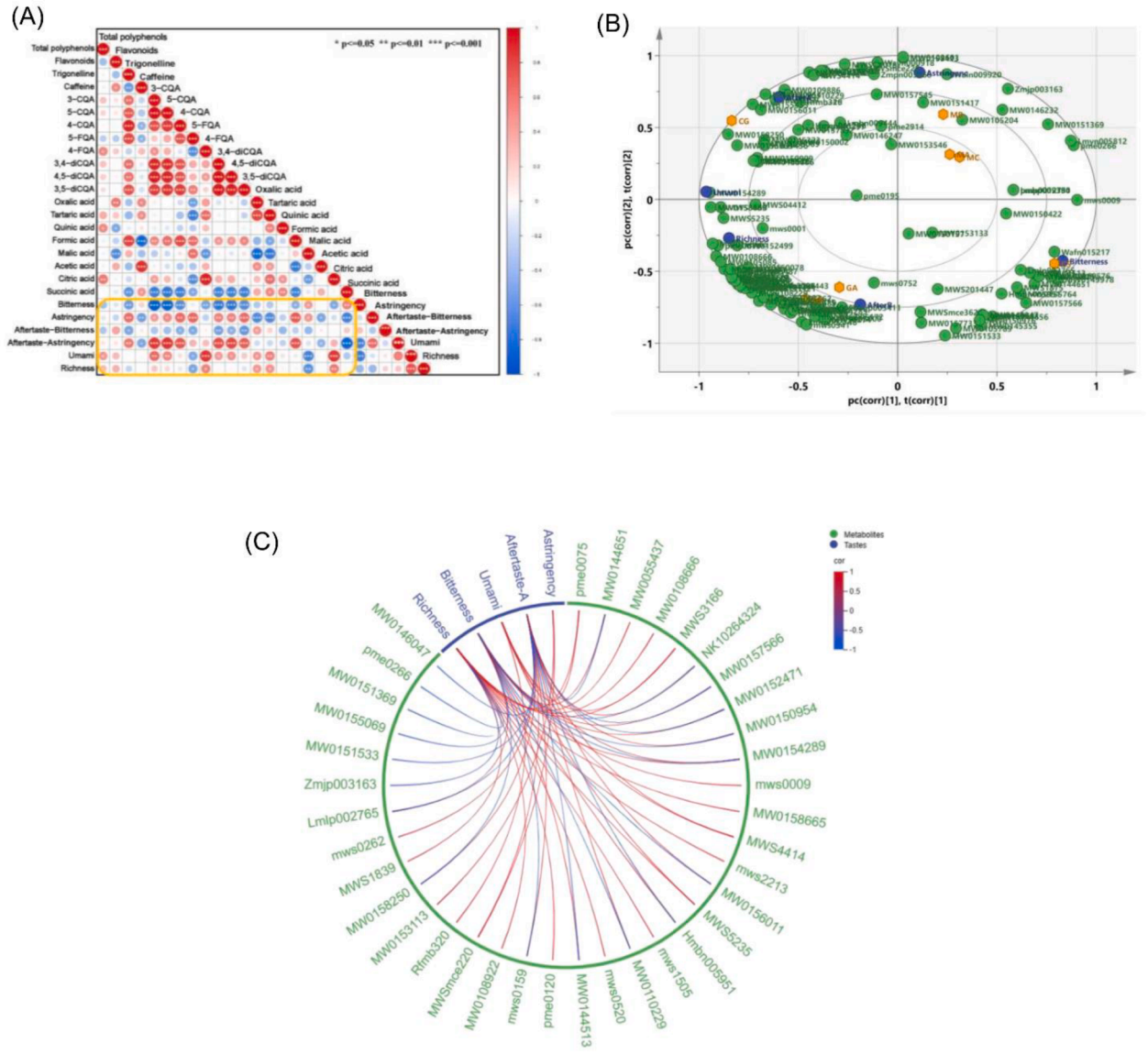

Fig. 4. Taste-metabolite association in the seven kinds of coffee beans under different primary processing methods. (**A**) Heatmap representing the person correlation analysis between their taste scores and main active compounds/secondary metabolites. (**B**) Score plot of the orthogonal partial least squares discriminant analysis; R^2X [1] = 38.2 %, R^2X [2] = 39.1 %. (**C**) Chord plot representing the Pearson correlation analysis between taste scores and metabolites.

Coffee bean taste quality is related to corresponding metabolites

Coffee quality can be accessed via a combination of physical, chemical, and organoleptic perspectives. Physical analysis involves assessing coffee bean characters, such as shape, size, and defects. Chemical analysis involves the identifying and quantifying different compounds, like caffeine, CGAs and trigonelline, which are precursors to forming volatile compounds in roasting (Tolessa et al., 2017). For understanding the relation of taste quality with major effective compounds/secondary metabolites, PCA was performed for determining relation of six taste properties with 19 compounds (Fig. 4A); red and blue stand for positive and negative correlation, separately, with darker colours indicating the greater correlation. Astringency and aftertaste-astringency of coffee were positively related to trigonelline, 4-CQA, and 3,5-diCQA, conforming to those observed by Córdoba et al. (2021); they stated that certain sensory features like astringent flavour and aftertaste, are extensively related to caffeine, CGAs, and trigonelline contents within coffee beverages. Furthermore, malic acid was identified an aliphatic acid that positively correlates with bitterness; a certain bitterness is perceived when its concentration is excessively high (Fujimoto et al., 2021). Additionally, oxalic acid was found to positively correlate with a bitter aftertaste. TPC positively correlated with umami taste, while the CGAs 3-CQA and 4-FQA strongly correlated with umami taste. The present work comprehensively analyzes chemical profiles and organoleptic qualities of Yunnan Arabica coffee.

The unique taste of coffee reflects numerous phytochemicals, but not some taste-active compounds. An OPLS-DA model according to changes of metabolites and tastes across the seven types of typically processed coffee samples was built for exploring the taste-metabolite relation. For the 211 different metabolites, their mass intensities were utilized to be X variables, while six taste intensities were adopted to be Y variables. Consistent with those above results, the CG sample exhibited the greatest aftertaste-astringency and umami. MB sample demonstrated the greatest astringency, whereas GB sample exhibited greatest aftertaste bitterness among all coffee samples prepared using the seven primary processing methods (Fig. 4B). Critical compounds related to coffee taste quality were identified using three criteria, including VIP-value > 1.0, correlation coefficient $r > 0.7$, and $p < 0.05$. By applying these criteria, we identified 37 critical taste-active compounds (Fig. 4C).

The astringency of coffee positively associated with d-glucono-1,5-lactone and lys-pro-cys; its aftertaste-astringency was negatively related to ala-leu-val-lys-ala, thr-asn-asp-lys, 1,3-o-di-p-coumaroylglycerol, and stilbostemin B, and positively associated with his-his-gln, *trans*-2-butene-1,4-dicarboxylic acid, 12-hydroxyoctadecanoic acid, and h-tyr-tyr-tyr-oh. Coffee bitterness negatively correlated with his-his-gln, nap-met-oh, pro-thr-val-asn-cys, h-tyr-tyr-tyr-oh, phenylpyruvic acid, and trp-thr-gln, and positively correlated with ala-leu-val-lys-ala, thr-asn-asp-lys, leu-pro-phe, coniferaldehyde, 1,3-o-di-p-coumaroylglycerol, and ala-asp-his. The umami taste of coffee positively correlated with *n*-acetyl-l-glutamic acid, *n*-palmitoylglycine, nap-met-oh, *trans*-2-butene-1,4-dicarboxylic acid, 12-hydroxyoctadecanoic acid, and 1-methylpiperidine-2-carboxylic acid, whereas richness was positively associated with 5-aminovaleric acid, tiglylglycine, and ethylparaben.

Conclusion

We conducted e-tongue analysis and UPLC-ESI-MS/MS in coffee samples for investigating roles of primary processing methods in the metabolic profiles and taste quality, and exploring the relation of taste quality with chemical composition. E-tongue analysis classified the coffee taste phenotypes into three clusters: CG-GA-GB, CS, and MC-MA-MB. The CG sample had the strongest response value for the umami sensor, the MB sample had the strongest response value for the astringency sensor, while GB sample displayed greatest aftertaste bitterness in all coffee samples. Diverse primary approaches were compared, as a result, coffee beans had different quality. Differences in TPC, TFC, and organic acid contents were significantly different among the different primary processing methods; but the impact of processing methods on additional factors, like caffeine and trigonelline levels, could be negligible.

Metabolomic analysis classified the chemical phenotypes of coffee into CS, GA-GB, CG, and MA-MB-MC clusters. There were 221 compounds discovered to be differential metabolites, and amino acids and derivatives were enriched in the GB sample, while phenolic acid and lipid contents were enriched in the MC sample. The astringency and aftertaste-astringency of coffee positively correlated with trigonelline, 4-CQA, and 3,5-diCQA, and these chemical components exerted a significant influence on the coffee flavour profile. By investigating the chemical characteristics of coffee beans prepared using different primary processing methods, the quality attributes of coffee beverages can be adjusted, thereby optimising primary processing techniques and enhancing the product quality.

CRediT authorship contribution statement

Huinan Zhai: Data curation, Methodology, Software, Validation, Writing – original draft. **Wenjiang Dong:** Funding acquisition, Investigation, Writing – original draft, Writing – review & editing. **Xingfei Fu:** Formal analysis, Supervision. **Guiping Li:** Methodology. **Faguang Hu:** Formal analysis, Investigation, Supervision.

Declaration of competing interest

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

Data availability

The data that has been used is confidential.

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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.2024.101286.

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