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Anaerobic germination of green coffee beans: A novel strategy to improve the quality of commercial Arabica coffee

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ABSTRACT

This study aimed to improve the brewing quality of commercial Arabica coffee through anaerobic germination. Changes in important compounds and cupping scores of germination roasting coffee with different germination degrees were investigated by ^{1}H NMR, HS-SPME-GC-MS and sensory analysis. Statistical analysis of multivariate analysis results indicated that 6 water-soluble chemical components and 8 volatile chemical components have the potential to be markers of germinated roasting coffee. In addition, germination significantly reduced caffeine content and acrylamide formation in roasted coffee. Sensory analysis according to the Specialty Coffee Association (SCA) cupping protocol demonstrated that anaerobic germination modified flavor attributes, improved the quality, and increased sensory scores. Furthermore, anaerobic sprouting increased fruity descriptors, but oversprouting did not improve overall attributes while producing both fermentative and vegetable descriptors. Therefore, suitable anaerobic germination of green coffee beans can be used as a new strategy to improve the flavor of commercial Arabica coffee.

1. Introduction

Coffee is the second-largest traded commodity after petroleum, and it is popular worldwide as a brewed beverage (Saberian et al., 2021; Wang et al., 2022). Coffee beans are rich in caffeine, chlorogenic acids, crude polysaccharides, organic acids, coffee oils and other ingredients (Hu et al., 2019). The balanced combination of bitterness, sourness, nutty and astringency gives the coffee a unique mellow taste and refreshing effect (Dong et al., 2019). The formation of coffee quality is a complex process that is influenced by species/cultivars, agronomic measures, the growing environment, and the processing process (Haile and Kang, 2019; Muschler, 2001; Wang et al., 2021). Hence, there are plenty of studies on a single-factor or multi-factor combination of planting and primary processing on improving coffee quality. Nevertheless, high-quality coffee production is not enough to meet the needs of consumers (Wang et al., 2021). Improving quality is a complementary strategy to increase productivity, allowing coffee to move into high-value areas where coffee growers can earn higher incomes. The coffee trade is dominated by commercial green coffee beans (Broissin-Vargas et al., 2018). At present, selecting suitable starters or microorganisms to ferment green coffee beans provides a new idea for improving coffee flavor (Wang et al., 2020, 2022). However, compared with the origin, altitude, depth and other factors, as the final product before roasting, the processibility of green coffee beans needs to be further enriched. Therefore, it is necessary to explore some new green coffee bean processing methods that can improve the quality of coffee beverages.

The germination and malting of grains, pseudo-grains and oilseeds can cause tremendous changes in the physical and chemical composition, which help improve related nutritional and functional qualities

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(Farinon et al., 2022; Thakur et al., 2021). This makes seeds in the germination stage a popular choice for the world food industry (Praphutphitthaya et al., 2016). Nevertheless, coffee seeds (moisture contents between 10 and 12%) are classified as intermediate between orthodox seeds (long-lived seeds and can be successfully dried to moisture contents as low as 5% without injury) and recalcitrant seeds (short-lived which cannot be dried to moisture content below 20–30% without injury and are unable to tolerate freezing) (Selmar et al., 2006). It was reported that a series of metabolic reactions related to germination is induced in the harvested coffee cherries, and the subsequent processing methods largely determine the evolution of germination (Kleinwächter et al., 2015; Selmar et al., 2006). The uneven initial metabolic state in turn affects the properties of cupping, which is also the main reason for the difference in flavor caused by different processing methods (Kleinwächter et al., 2015). The green coffee beans obtained after primary processing and storage have weakened vitality and cannot be used for seedling cultivation (Eira et al., 2006; Waters et al., 2017). Furthermore, at the optimal temperature of 30–32 °C, Arabica coffee bean germination takes approximately three weeks, whereas at 17 °C, it takes three months (Waters et al., 2017). In comparison to the entire germination cycle, Arabica coffee seeds began to produce radicle protrusion between the fifth and sixth day of swelling at 30 °C in the dark (da Silva et al., 2019; Eira et al., 2006). Longer germination durations can lead to the development of harmful microorganisms and off-flavors in coffee (Bourneow and Toontam, 2019). Consequently, the quality-enhancing methods of aerobic germination of conventional grain and oil crops are not suitable for coffee.

Soaking green coffee beans in water at 28–32 °C for 12–36 h, and then sowing after the radicle of the seeds grows can shorten the germination cycle and has a certain application value in coffee seedlings breeding (Lin et al., 2021). This is also a common method for producing sprouted coffee beans in the food industry (Geun and Hui, 2017). Shortening the germination time can reduce microbial growth or off-flavor formation and ensure a good flavor and safety level of coffee (Park, 2015). However, research on the flavor and quality of sprouted coffee, particularly germinated roasting coffee, is still lacking. In this context, we employ anaerobic germination as a technique for innovation in the coffee germination process and investigate its effect on coffee quality and sensory qualities. The purpose of this study was to investigate the water-soluble and volatile components of roast anaerobic sprouted coffee beans at different stages and to understand the effect of sprouting on coffee brewing quality combined with cupping.

2. Materials and methods

2.1. Chemicals and standards

Deuterated water (D_2O, 99.9%) for nuclear magnetic resonance (NMR) detection was produced by Energy Chemical (Shanghai, China). External standards were used for NMR quantification: sodium-3-trimethylsilylpropanoate (TMSP-D4, CIL, Cambridge, America). 3-Heptanone (98%) was purchased from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Methanol (99.9%) for internal standard preparation was purchased from Shanghai Xingke High Purity Solvent Co., Ltd.

2.2. Sample preparation

The green coffee beans (CB, Catimor 7963, *Coffea arabica* L.) by wet processing were harvested in 2020/2021 as commercial beans were collected from Baoshan of Yunnan province in China, and stored at room temperature approximately eight months before germination. Before the formal germination experiment, the soaking time and germination temperature were optimized, respectively (Fig. S1). CB (200g) was soaked in distilled water (400 mL) for 4h at room temperature (25 °C). After that, add 50 mL of distilled water was added to submerge the CB completely, and then germinated at 35 °C for 12 or 24 h. To prevent the loss of some water-soluble chemical compositions from CB, the amount of water used has just overflowed the coffee beans. Soaked green coffee beans (SCB) and germinated green coffee beans (GCB) were dried in a convection oven at 50 °C to a moisture content of around 12% (Figs. S2 and S3).

The beans (150 g) were roasted in a Kaleido coffee roaster (Wuhan Kaweher Electrical Appliance Co., Ltd. Models: Sniper M2, Kaleido, Wuhan, China) at 200 °C. The roasting time was 8–10 min; comparing the Specialty Coffee Association (SCA) standard color chart, it was determined that the roasting endpoint reaches the medium roasting degree of SCA grade (Specialty Coffee Association, 2018), then samples were immediately air-cooled for 3 min. The beans were divided into two parts, one for chemical analysis and the other for cupping. The samples for chemical analysis were ground into powder, sieved (60 mesh), placed in a sealed glass container and stored in a freezer (−20 °C). The cupping samples were transferred to the one-way valve bag for sensory evaluation as soon as possible. The experiment consisted of three treatments (SCB, GCB-12 and GCB-24) and a control (CB). All samples were prepared in triplicate, and then samples of the same treatment were mixed and tested in parallel three times.

2.3. Water-soluble chemical analysis by ^1H NMR

Water-soluble compounds in roasted coffee were analyzed by ^1H NMR according to the method of Wang et al. (2021). 40 mg of powdered sample was poured into a 1.5 ml Eppendorf tube, then D_2O containing TMSP–D4 at a concentration of 0.03% was added. TMSP–D4 was used as an internal standard for chemical shift calibration and quantitative analysis. After ultrasonication (80 °C, 1 h) and centrifugation (5000 g, 4 min), 450 μL of the supernatant was transferred to a 5 mm NMR tube for analysis.

All spectra were obtained by using Bruker DRX-600 instruments (Bruker Biospin, Rheinstetten, Germany) at 25 °C. The ^1H NMR spectra were recorded at the acquisition parameters including sampling data point = 65536, peak width = 12,019 HZ, acquisition time = 2.73 s, relaxation time = 10 s and scans = 128 times.

^1H NMR data was imported into MestReNova (version 6.1.0–224; MestReC, Santiago de Compostela, Spain). After the spectra were calibrated (TMSP-d4 signal at 0.0 ppm), cut (water peak at 5.0–4.5 ppm), phased corrected and baseline corrected, the main chemical components were identified based on previous studies (dos Santos et al., 2020; Hu et al., 2020; Wang et al., 2021).

All ^1H NMR resonances in the samples were presented in the chemical range between 0.50 and 9.50 ppm. The identified compounds were integrated and acquired their relative peak areas (integration of internal standard, normalized to 1.000). Subsequently, a dataset was built in which each row represented a coffee sample, and each column contained the relative peak areas of the compounds. The dataset consisted of 12 samples (rows) and 31 variables (columns).

2.4. Volatile composition analysis

The volatile compounds of roasted coffee samples were determined using headspace solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS). Weighed 0.5 g of samples into headspace vials (20 mL) and equilibrated for 20 min at 60 °C (Caprioli et al., 2012). Subsequently, the volatiles were extracted using a 2 cm 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) StableFlex fiber (Supelco, Bellefonte, USA) for 30 min (Caporaso et al., 2018). This type of fiber was one of the most commonly used and most effective in coffee analysis (Caporaso et al., 2018). As an internal standard, 100 μL of a 0.01% 3-Heptanone methanol solution was added (Caporaso et al., 2018).

After HS-SPME, the fiber-containing headspace volatile compounds were directly injected into a gas chromatograph 7890A (Agilent Technologies Inc., Wilmington, DE, USA) joined together with a 5975C mass

selective detector (Agilent Technologies Inc., Wilmington, DE, USA). A DB-WAX chromatographic column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies Inc., Wilmington, DE, USA) was used (Dong et al., 2019). GC conditions were chosen according to Caporaso et al. (2018), slightly adapted to available column types. The injection system was set to splitless, the injector temperature was maintained at 250 °C and the carrier gas helium flow rate was 1.0 mL/min. The column oven was programmed as follows: from 60 °C to 180 °C (hold it for 1 min) at 8 °C/min, then to 230 °C (hold it for 7 min) at 4 °C/min. The mass spectrometer operated conditions: EI model, 70 eV; ion source temperature, 250 °C; transmission line temperature, 290 °C; acquisition mode, full-scan; scan range: *m/z* 35–500 *amu*.

The identification of volatile compounds was performed by comparing their mass spectra and retention indices (retention indices calculated by retention times of C_8–C_{26} alkane standard mixtures) with the NIST11 database. In addition, the identification was performed by comparing the linear retention index (LRI) of volatiles with the literature data. The relative contents of compounds ($\mu g/g$) were calculated based on the peak area and internal standard. On this basis, a gas chromatography-mass spectrometry (GC-MS) dataset comprising 12 coffee samples and 39 volatile compound contents was established.

2.5. Sensory analysis

The evaluation of coffee brews was conducted following the SCA protocol (Specialty Coffee Association, 2018). All experiments followed the relevant guidelines and regulations of Yunnan International Coffee Exchange (YCE). The Quality Assurance Department of YCE approved the procedures and methodologies. All panelists had provided informed consent. Three Q-Graders trained on the SCA cupping protocol scored 10 attributes, including fragrance/aroma, uniformity, sweetness, flavor, acidity, body, aftertaste, balance, clean up and overall. Sensory attributes were evaluated on a scale of 6–10, with a score of 0.25 for each attribute. In addition, defects were considered negative scores attributes. After evaluating all attributes, tasters assigned descriptive sensory attributes and an overall score to each sample (The Coffee Taster's Flavor Wheel, 2016). The team's average scores represent the overall quality of the coffee brew.

2.6. Statistical analysis

SPSS 13.0 software package was used for Duncan's test and one-way ANOVA between different treatments. Data are presented as the mean value ± standard deviation. P-values of less than 0.05 were considered statistically significant. The clustering heat map analysis was accomplished by the TBtools software (Chen et al., 2020)

Multivariate statistical analyses of the datasets were performed using SIMCA 14.1 software (Umetrics, Sweden). The datasets were normalized by UV scaling for the ^{1}H NMR dataset and the GC-MS dataset, and then the effect of germination degree on the water-soluble and volatile compounds of germinated roasting coffee was explored by principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA).

3. Results and discussion

3.1. Changes of water-soluble components in germinated roasting coffee

3.1.1. Exploring changes in the germination process based on ^{1}H NMR

NMR technology can quickly identify the main chemical components of roasted coffee beans and has been used in variety identification, origin traceability, authenticity identification, etc. (Consonni et al., 2012; Defernez et al., 2017; Toci et al., 2018). Considering that the composition of green beans will change during the soaking and germination, which will inevitably affect the composition of roasted beans, we further reveal the changes of soaking and germination on the main chemical components of roasted coffee beans by ^{1}H NMR.

Performing ^{1}H NMR detection on CB, SCB, and GCB samples, and identifying the chemical constituents of the spectra according to kinds of literature (dos Santos et al., 2020). Table S1 shows the signal assignments of the recognized metabolites, and a total of 31 compounds with characteristic signals were identified. Fig. 1 shows the characteristic ^{1}H NMR spectrum of germinated roasting coffee. At the same time, based on the NMR database, heatmap analysis was applied to visualize the relative changes of compounds during germination (Fig. S4).

Organic acids are the most abundant class of compounds in light and medium roasted coffee beans, and they are responsible for coffee's sour taste (Wang et al., 2021). Soaking is a water-absorbing stage, with less precipitation of soluble acidic compounds, and insignificant changes in acidic compounds in CB and SCB samples. Compared with CB, the content of lactic acid increased significantly after soaking and germination treatment, which may be due to the spontaneous fermentation of green beans with the participation of lactic acid bacterium under an anaerobic environment (Wang et al., 2020). The organic acid components in green coffee beans, such as citric acid, malic acid, quinic acid and other organic acids, were leached or metabolized during the germination process and decreased to a certain extent. In addition, depletion of flavor precursor compounds during sprouting can also reduce flavor content in roasted beans, such as oligosaccharide consumption during sprouting reduces formic and acetic acid levels. Organic acids are a crucial factor affecting the sensory properties of coffee (Ribeiro et al., 2018), so the changes in their content due to germination can alter the flavor profile.

CGAs are phenolic acids, mainly including caffeoylquinic acids (CQAs), *di*-caffeoylquinic acids (*di*CQAs) and feruloyl quinic acids (FQAs) (Hu et al., 2020). With the increase in roasting degree, the content of CGAs decreased significantly, and the degradation of CGAs could be declined by about 50% under moderate roasting conditions. The maximum amount of CGAs was observed in roasted beans after soaking, which was 33.30% greater than before soaking. After 24 h of anaerobic germination in coffee beans, the CGAs in them were reduced to the level of ungerminated roasted coffee. In two previous studies, an increase in CGAs in germination coffee beans was observed (Kim et al., 2018; Praphutphitthaya et al., 2016). CGAs could be beneficial to health due to their potential to reduce the risk of cardiovascular disease, type 2 diabetes and Alzheimer's disease, as well as their antibacterial and anti-inflammatory activities (Hu et al., 2019). Therefore, the utilization of germination technology in the production of coffee beverages may have a positive impact on the coffee industry.

Caffeine, an abundant alkaloid in coffee, is responsible for the bitter taste of coffee and is the source of its stimulating effect (Hu et al., 2019; Wang et al., 2021). After the soaking and germination, the caffeine content gradually declined (Fig. 1, S2). The caffeine content in SCB decreased by 19.88% compared with the initial value (CB). Furthermore, germination further reduced the caffeine content. After 12 h and 24 h of anaerobic germination, the caffeine content decreased by 49.17% and 62.25%, respectively. These results are consistent with the findings of Praphutphitthaya et al. (2016), who reported a gradual decrease in caffeine content to 54% over the 8-day germination time. Caffeine impairs embryonic growth and differentiation in the earliest stages of coffee germination; hence, a large quantity of caffeine may be transported and degraded, which is the main reason for the substantial drop in caffeine levels throughout germination (Friedman and Waller, 1983).

Trigonelline, another abundant alkaloid in coffee, also contributes to coffee's bitter taste (Wang et al., 2021). During the roasting process of coffee beans, trigonelline is partially decomposed to produce volatile pyridine and non-volatile compounds such as N-methylpyridinium ions, nicotinic acid, and methyl nicotinate (Stadler et al., 2002). Its transformations resemble those of caffeine, and its concentration declines gradually after soaking and germination. Trigonelline works as a precursor of nicotinamide adenine dinucleotide (NAD) and is converted to

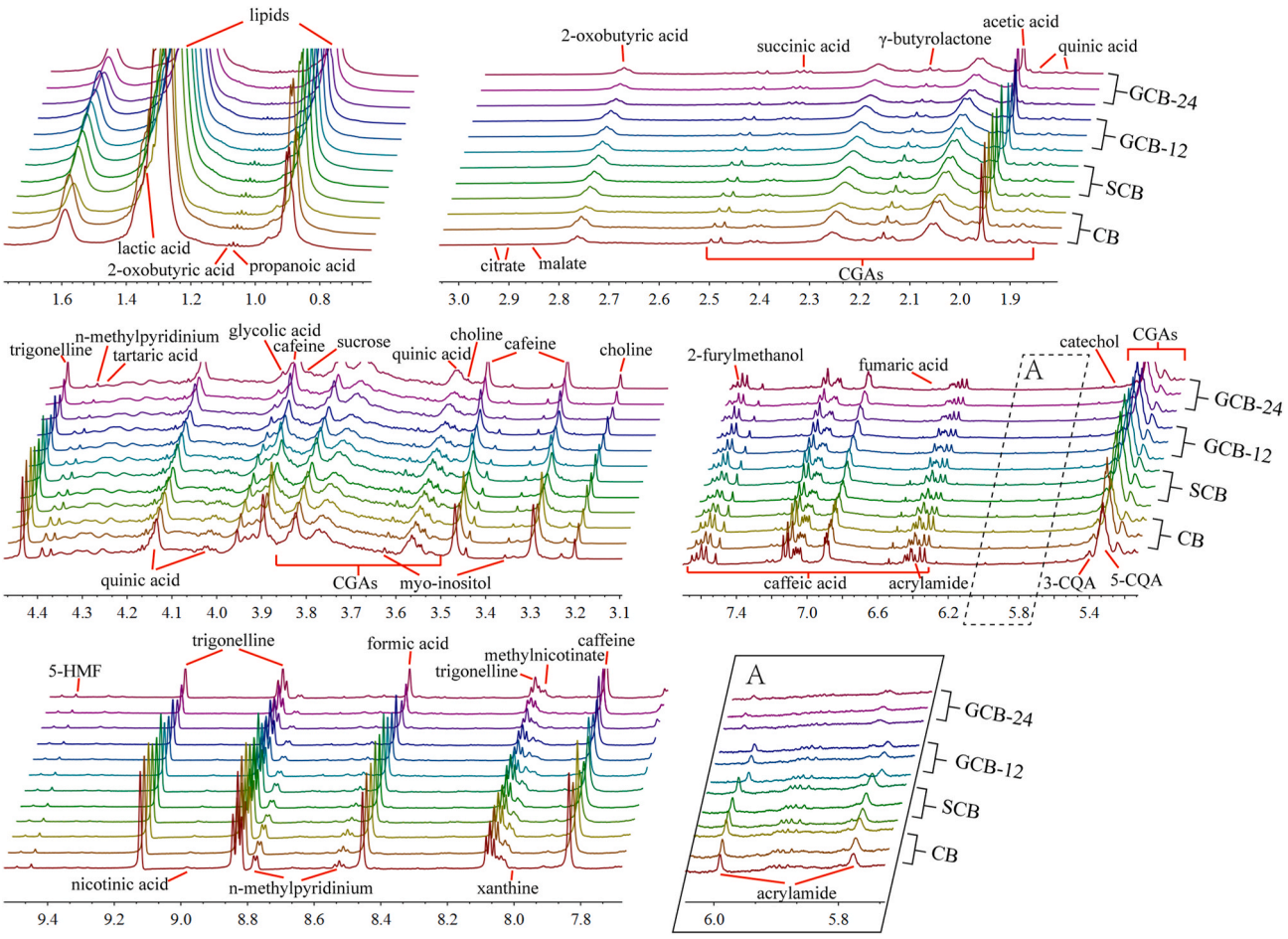

Fig. 1. Characteristic ^{1}H NMR spectrum of germinated roasting coffee (δ_H 0.70–9.50 ppm). CB, green coffee beans; SCB, soaked green coffee beans; GCB-12, germinated green coffee beans for 12 h; GCB-24, germinated green coffee beans for 24 h ^{1}H NMR spectra are arranged according to the degree of germination, with CB, SCB, GCB-12 and GCB-24 are arranged in order from front to back. CGAs, chlorogenic acids; 3-CQA, 3-caffeoylquinic acid; 5-CQA, 5-caffeoylquinic acid; 5-HMF, 5-hydroxymethylfurfural. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

pyridine nucleotides during the germination and early growth of certain plant seeds (Mazzafera, 1991). Consequently, a drop in trigonelline concentration during soaking implies that "germination" has commenced.

Acrylamide was identified by numerous researchers as a hazardous compound formed during the thermal processing of food (baking, roasting and frying) (Khezerolou et al., 2018). In coffee products, the Maillard reaction that occurs during roasting is one of the main pathways for the formation of acrylamide (Schouten et al., 2020). Consistent with the literature (dos Santos et al., 2020), we observed acrylamide-specific peaks (δ_H 6.39, 5.99, 5.78 ppm) in the ^{1}H NMR spectrum. Soaking and germination can reduce the acrylamide content in roasting coffee. The content of acrylamide in SCB was more than 40% lower than in CB. The content of acrylamide in GCB was a substantial decrease, reaching more than 70%, and there was no significant difference in the content of the two groups of germination samples (Table S2). The formation of acrylamide is mainly due to the binding of free asparagine to reducing sugars. A previous study has reported that asparagine levels decreased in brown rice after soaking and germination (Kim et al., 2021), while studies on these changes in coffee are lacking. The reduction of acrylamide in roasting coffee may be related to the degradation of precursor compound asparagine during germination. Therefore, anaerobic germination is expected to be a new method to reduce the production of acrylamide in coffee. 5-Hydroxymethylfurfural (5-HMF) is another contaminant that was also detected in various heat-treated foods, reacting with asparagine during heating to form acrylamide (Hamzalıoğlu and Gökmen, 2020). Soaking and malting had no significant effect on the content of 5-HMF in roasting coffee.

3.1.2. *Identification of water-soluble component markers during germination*

To accurately describe the effect of germination on the content of the main components of coffee, PCA and PLS-DA analyses were performed on the ^{1}H-NMR database. The variance explained rates of PC1 and PC2 of the PCA analysis model were 62.7% and 27.2%, respectively (Fig. S5). The results showed that the score of PC1 for CB and SCB was positive while the PC1 score of GCB was negative, and there were no outliers in all samples. These data were further analyzed using PLS-DA according to the germination stage. The PLS-DA model was fitted with four principal components ($p < 0.05$), and the model fit was $R^2X = 0.964$, $R^2Y = 0.965$, and a good model could be established ($Q^2 = 0.879$). To further verify the validity of the model, 200 cross-validations were employed to evaluate its stability and predictive ability. The results show that the PLS-DA model has high reliability ($R^2 = 0.9902$) and predictive ability ($Q^2 = 0.8828$), and there is no overfitting problem. The score plot of the PLS-DA model shows that GCB can be well differentiated from CB and SCB (Fig. 2A). The corresponding loading plots showed that lactate was highest in 12-h GCB and xanthine was highest in 24-h GCB (Fig. 2B). Additionally, soaking has changed the chemical composition of coffee, increasing the levels of CGAs, sucrose, 2-isobutyric acid, choline, etc.

Hierarchical cluster analysis (HCA; Fig. 2C) was used to reveal the pre- and post-sprouting relationships in coffee beans. The process of seed

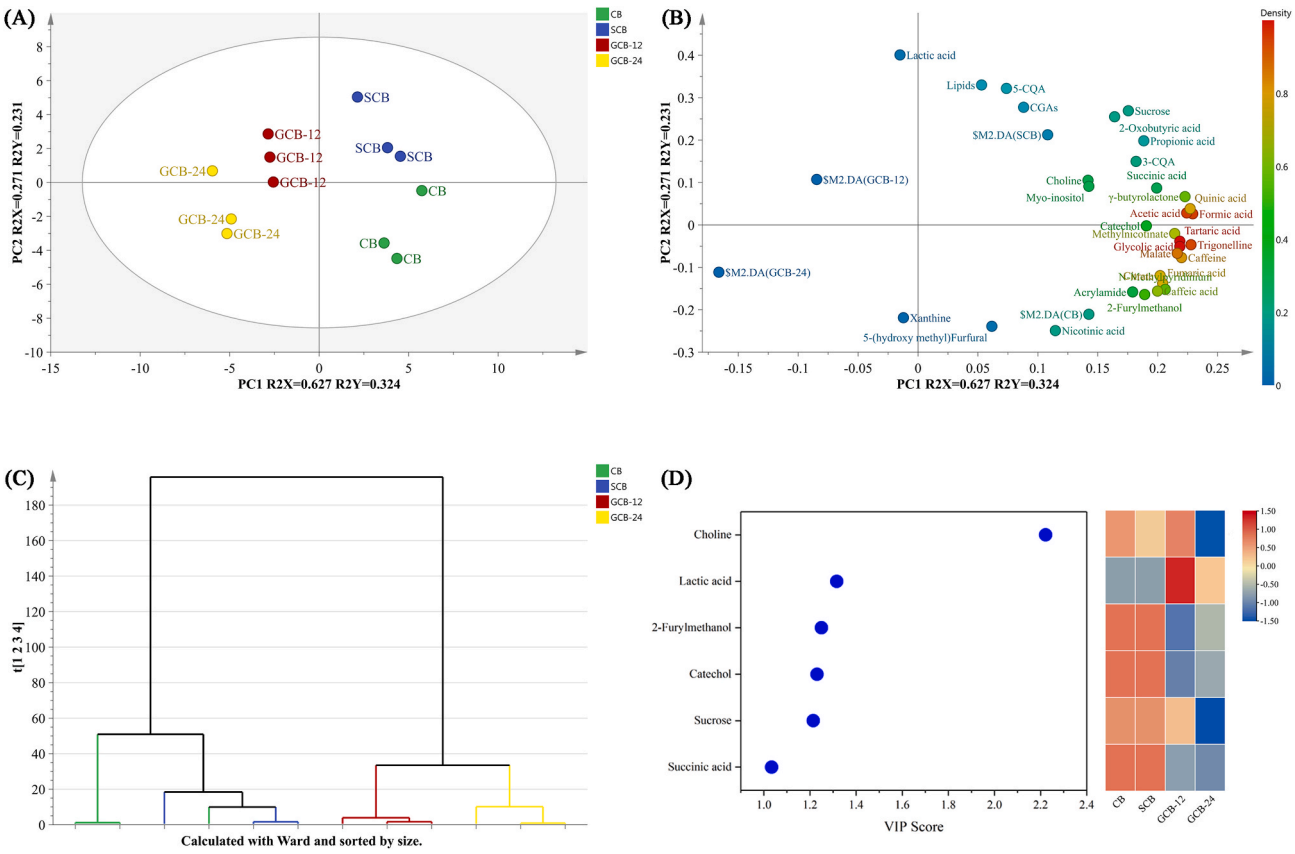

Fig. 2. The score plot (A), loading plot (B) and hierarchical cluster analysis (HCA) (C) of PLS-DA analysis based on the ^{1}H NMR data of samples. (n = 12). (D) Water-soluble component markers during germination (VIP >1 and $p < 0.05$). The colored boxes on the right indicate the relative concentrations of the corresponding compounds. CB, green coffee beans; SCB, soaked green coffee beans; GCB-12, germinated green coffee beans for 12 h; GCB-24, germinated green coffee beans for 24 h. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

imbibition helps to break dormancy, which can lead to structural disorder and cell membrane damage, thereby accelerating germination by secreting low molecular weight solutes (amino acids, ions, sugars, etc.) and germination inhibitors (such as abscisic acid) (Matilla et al., 2005). After the green coffee beans were soaked, the water-soluble compounds of the roasted beans changed to some extent and were distributed in different quadrants of the score plot, but this change was not enough to form a clear separation in the HCA. A clear separation was formed between the different treatments after germination, indicating that germination significantly changed the coffee bean composition, which was also well represented in the loading plot.

The variable importance in projection (VIP) values of compounds in PLS-DA were a parameter for screening biomarkers in the metabolome, and a VIP value greater than 1 can be considered to significantly contribute to the separation (Wang et al., 2020). Different germination stages were considered significantly different when VIP >1 (Fig. S6) and $P < 0.05$. Overall, 6 significantly different water-soluble compounds were identified (Fig. 2D), including choline, lactic acid, 2-*furylmethanol*, catechol, sucrose and succinic acid. The results indicated that these 6 compounds play an important role in differentiating the degree of germination and can serve as potential candidate markers for germinated roasting coffee.

3.2. Analysis of fragrance compounds in germinated roasting coffee

3.2.1. Volatile compounds

The volatility profile of coffee is an essential organic characteristic of high-quality coffee, which determines consumer acceptance and preference (Dong et al., 2019). To evaluate the germination's effect on the flavor of roasted coffee beans, HS-SPME-GC-MS was used to analyze the volatile compounds of samples obtained from different degrees of germination. A total of 39 volatile compounds were detected in the roasted samples. Volatile compounds are classified into 11 classes based on chemical properties, including two pyridines, 12 pyrazines, two acids, six aldehydes, one ester, four ketones, four alcohols, two pyrroles, three phenols, one furnans and three others (Table S3). In addition to furans (only 2-acetyl-5-methylfuran was detected), 10 other chemical families were present in all samples. *N*-acetyl-4(H)-pyridine (fatty, dusty, nutty notes) and 2-methyl-naphthalene were detected only in CB, while 2,3-butanediol (natural odor of cocoa butter, sweet notes) was only recognized in GCB. Therefore, germination can produce some differences in volatiles.

CB had the highest volatile content (479.20 μg/g); by contrast, GCB with 24 h had the lowest volatile content (353.28 μg/g). Soaking and 12-h germination treatments (423.60 μg/g and 470.52 μg/g) had little effect on total volatile content, but the chemical class content varied significantly. The heat map of the relative content changes of different classes of volatile compounds in roasted coffee beans with different germination treatments is shown in Fig. 3. The main chemical classes found in CB were aldehydes (34.84%) and alcohols (22.50%). Furfural, 5-methyl-2-furan carboxaldehyde and 2-furan methanol (8.93, 19.03, and 19.03%, respectively) were the most abundant compounds identified in CB.

After soaking, the chemical groups of volatile components changed, with the major alterations occurring in acids, lipids, and pyridines groups. In the process of water absorption, the dissolution of carbohydrates in the precursor flavor substances was conducive to the formation of organic acids, while the structural disorder increased the lipids content. Soaking and germination both reduced the content of pyridines, mainly due to the lower content of the flavor precursor trigonelline.

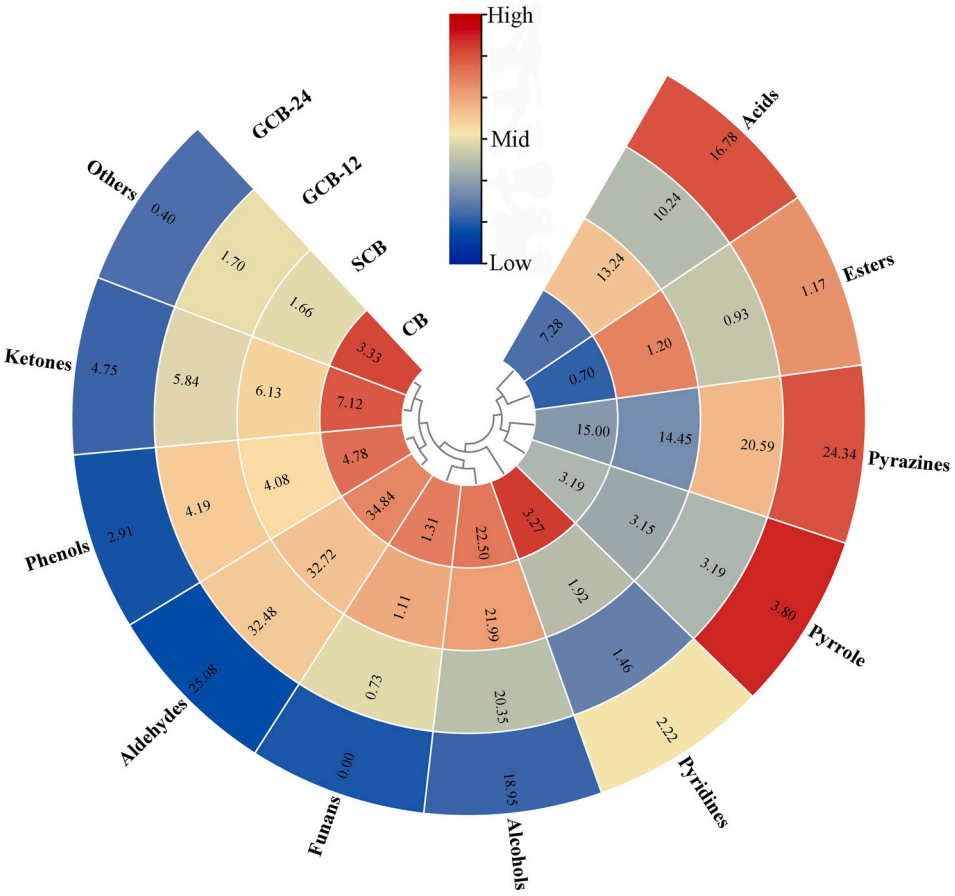

Fig. 3. Heatmap analysis of relative content (%) of different volatile compounds among different classes in germinated roasting coffee. CB, green coffee beans; SCB, soaked green coffee beans; GCB-12, germinated green coffee beans for 12 h; GCB-24, germinated green coffee beans for 24 h. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The composition of volatile compounds at different germination times varied greatly. On the one hand, anaerobic germination consumed some nutrients, and the content of flavor precursors varies significantly with the germination process. On the other hand, sprouting promotes the formation of some new substances, such as γ-aminobutyric acid (GABA) (Gong et al., 2016), but prolonged hypoxia can lead to cell toxicity, which was harmful to positive flavor formation. The high content of pyrazines in germinated roasting coffee may be related to the formation of reducing sugars and the rise in total free amino acids during germination.

3.2.2. *Identification of volatile constituent markers during germination*

Visualized analysis of differences in volatile components during coffee germination was conducted by PCA and PLS-DA. The variance-explained rates of PC1 and PC2 of the PCA analysis model were 42.9% and 16.5%, respectively, and no outliers appeared (Fig. S7). Except for the sample group with 24-h germination, other sample groups could not be separated. PLS-DA score plot based on 4 principal component fit shows a clear separation between all sample groups and quality parameters were statistically acceptable ($R^2X = 0.754$, $R^2Y = 0.927$, and $Q^2 = 0.654$) (Fig. 4A and C). In addition, 200 cross-validations results showed the model has high stability and predictive power ($R^2 = 0.9101$ and $Q^2 = 0.6577$) without overfitting problem. The corresponding loading plots showed that pyrazine compounds have a very large correlation term with the germination sample group (Fig. 4B). Soaking had a significant effect on volatile components, a result similar to Kim et al. (2021) on GC-MS metabolic profiling of brown rice.

In the VIP value of the GC-MS discriminant model, there were 14 compounds with VIP values greater than 1 (Fig. S8). At the same time, combined with $p < 0.05$, volatile compounds are considered significant differences. Overall, 8 significantly different volatile compounds were identified, including 2-methyl-naphthalene, N-acetyl-4(H)-pyridine, 2-ethyl-5-methyl-pyrazine, 2-ethenyl-6-methyl-pyrazine, trimethyl-pyrazine, 2,3-butanediol, 2-acetyl-5-methylfuran, furfural (Fig. 4D), of which three belonged to pyrazines. Pyrazine were present at higher levels in germination roasting coffee (Fig. 4D) and was mainly formed by the Maillard reaction (Adams et al., 2008), which indicates that the germination process promotes the formation of precursor flavor compounds sugars and amino acids. Higher levels of amino acids have also been reported in germinated rice (Kim et al., 2021).

3.3. *Sensory evaluation and flavor characteristics*

High-quality coffee as a specialty product is increasingly in demand (Wulandari et al., 2022). Table S4 shows the attribute scores of the 3 Q-graders according to the protocol cupping SCA assessment. All treatments scored 10 for uniformity, clean up, and sweetness. The scores for other attributes ranged from 6.83 to 7.33 and were visualized in Fig. 5. SCB and GCB scored significantly higher on acidity compared to CB. In the 24-h germination samples, the body attribute score was reduced but not statistically significant. Soaking and germination treatments can improve cupping scores but with some statistical differences ($p < 0.1$). Due to the excellent performance of numerous attributes, SCB (80.08) was designated as a specialty coffee. Small differences at the end of cupping were beneficial because producers can sell the beans at a higher price (Martinez et al., 2021).

According to The Coffee Taster's Flavor Wheel (2016), the sensory descriptors were defined by Q-Graders, and the sensory descriptions are

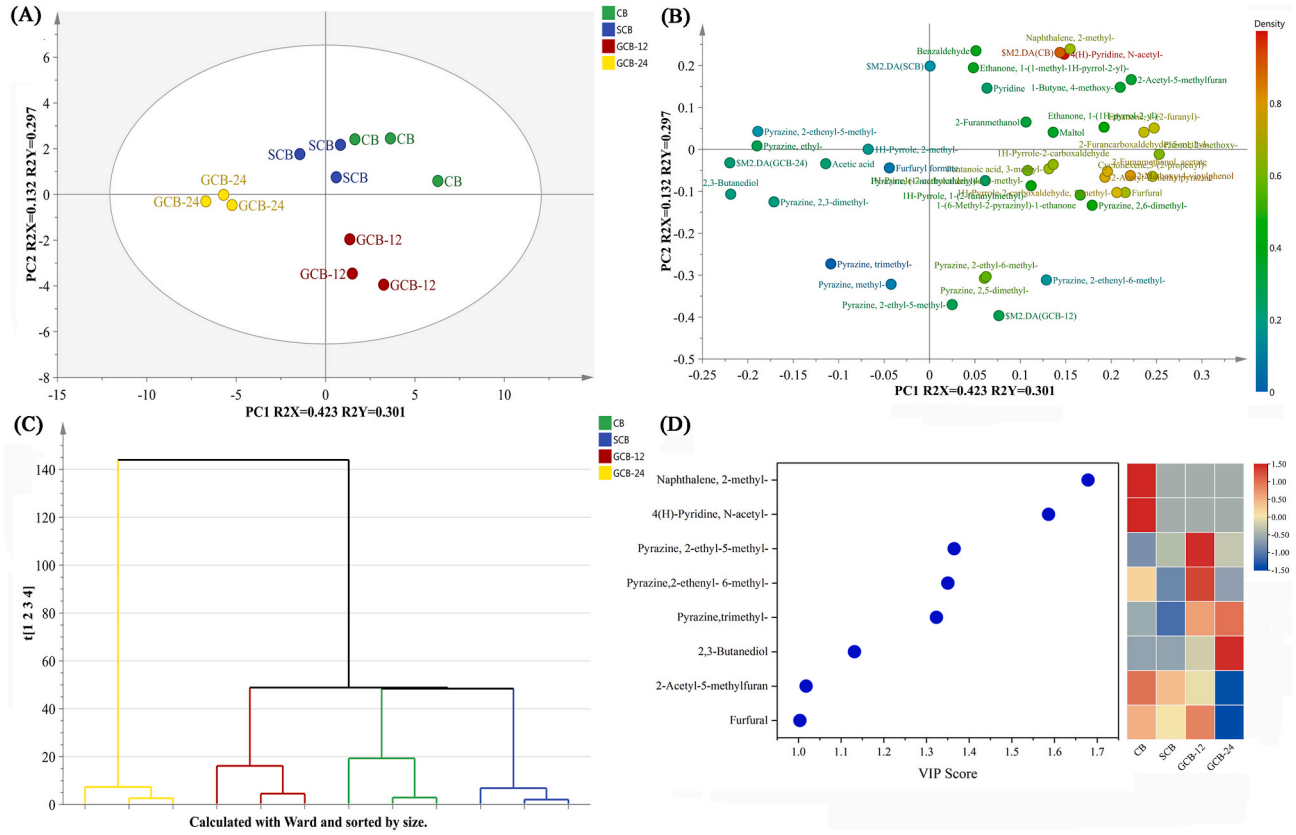

Fig. 4. The score plot (A), loading plot (B) and HCA (C) of PLS-DA analysis based on the GC/MS data of samples. (n = 12). (D) Volatile constituent markers during germination (VIP >1 and p < 0.05). The colored boxes on the right indicate the relative concentrations of the corresponding compounds. CB, green coffee beans; SCB, soaked green coffee beans; GCB-12, germinated green coffee beans for 12 h; GCB-24, germinated green coffee beans for 24 h. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

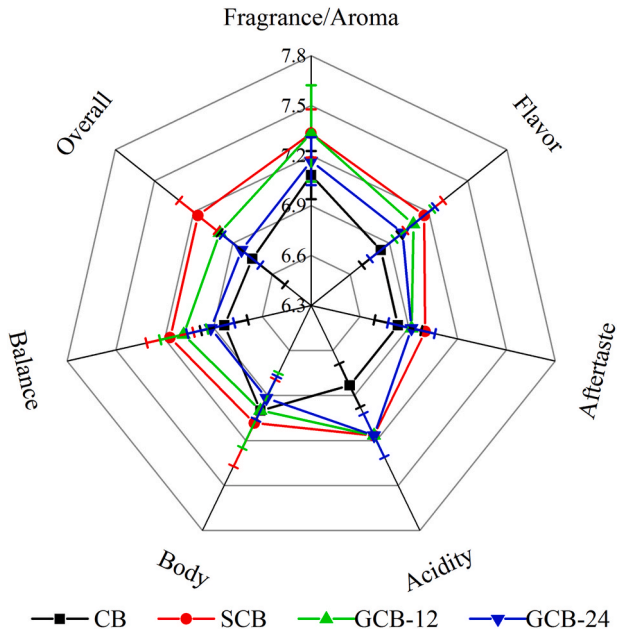

Fig. 5. The average score of attributes (excludes uniformity, sweetness, and clean cup, all treatments scored 10) was assessed by protocol cupping SCA (3 Q-graders). CB, green coffee beans; SCB, soaked green coffee beans; GCB-12, germinated green coffee beans for 12 h; GCB-24, germinated green coffee beans for 24 h. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

shown in Fig. 6. All samples presented nutty/cocoa and roasted descriptors, but there were some differences in further descriptors. Soaking and germination reduced burnt and cocoa flavors while leading to cereal and nutty flavors. The descriptor sweet/brown sugar/caramelized was perceived in all samples. The fruity descriptor was present in SCB and GCB samples compared to CB, while spices/brown spice and chemical/bitter flavor disappeared. Given the health benefits of germination coffee and the positive change in flavor profile, germination coffee has the potential to create new niche markets.

Notably, the scores of these two sprouted roasted coffees were not significantly different, but 24-h germination samples contained alcohol and vegetative descriptors. Over-sprouting may result in loss or changes in flavor or taste compounds. Furthermore, long-term anaerobic conditions can lead to a certain degree of fermentation and risk of microbial contamination in green coffee beans. The overall attribute of GCB-24 was lower than those of SCB or GCB-12 ($p < 0.1$), which was considered watery by the cuppers. Overall data showed that sensory characteristics and acceptability were influenced by germination time. Consequently, an appropriate degree of anaerobic germination can effectively improve the overall sensory quality and acceptability of coffee.

4. Conclusion

Micro-sprouting technique (soaking and anaerobic germination) was a novel method that has the potential to improve commercial coffee beans. ^{1}H NMR spectroscopy and HS-SPME-GC-MS were necessary to determine the formation/loss of metabolites in roasted coffee with different degrees of germination. At the same time, combining with multivariate analysis was an effective strategy to monitor the changes in

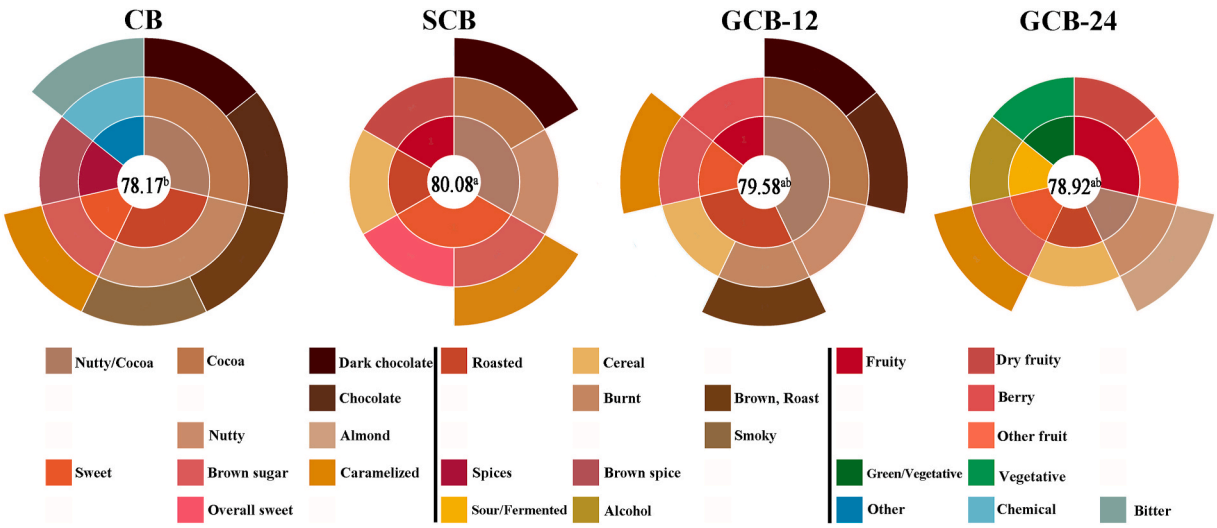

Fig. 6. Sensory descriptors and final score of gemination roasting coffee. CB, green coffee beans; SCB, soaked green coffee beans; GCB-12, germinated green coffee beans for 12 h; GCB-24, germinated green coffee beans for 24 h. Color reference and hierarchy division from the Coffee Taster's Flavor Wheel. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the composition of germinated roasting coffee as a result of varying germination degrees. These results showed that 6 water-soluble compounds and 8 volatile compounds were the major contributors to the changes in the compounds' profiles. Additionally, germination significantly reduced caffeine and acrylamide levels in roasted coffee, resulting in the production of new coffee products with reduced caffeine and low acrylamide levels.

Sensory evaluation revealed that nutty/cocoa, roasted, and sweetness descriptors for all treatments, with the micro-sprouting adding fruity descriptors, resulted in unique flavor attributes. Micro-sprouting increased SCA scores and improved quality. But over-sprouting rendered the coffee bland while producing fermentation and vegetable descriptors.

In conclusion, proper anaerobic germination of green coffee beans can be employed as a novel strategy to improve the flavor of commercial Arabica coffee and thus develop new niche markets. Micro-sprouting will affect the changes in the microstructure of coffee beans and the occurrence of chemical reactions, thereby affecting the production and release of flavor compounds, and its mechanism needs to be further explored. In addition, the quality of commercial coffee beans is affected by many factors, further studies are needed on the anaerobic germination of coffee with different varieties, different processing techniques, and various storage times.

CRediT authorship contribution statement

Yanbing Wang: Methodology, Validation, Formal analysis, Writing – original draft. **Xiaoyuan Wang:** Methodology, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Guilin Hu:** Supervision, Writing – review & editing. **Abdulbaset Al-Romaima:** Writing – review & editing. **Xingrong Peng:** Validation, Formal analysis. **Jinhong Li:** Writing – review & editing, Project administration, Funding acquisition. **Xuehui Bai:** Validation, Formal analysis. **Zhongrong Li:** Validation, Formal analysis. **Minghua Qiu:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100461.

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