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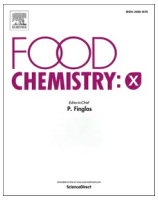

Optimizing commercial Arabica coffee quality by integrating flavor precursors with anaerobic germination strategy

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ABSTRACT

This study attempted to improve commercial Arabica coffee quality by integrating flavor precursors with anaerobic germination. Using raw coffee beans as materials, anaerobic germination was conducted with 5 g/100 g of flavor precursors (sucrose, glucose, fructose). The chemical composition and sensory quality of roasted coffee beans were analyzed. Results showed that adding flavor precursors facilitated the harmonization of water-soluble chemical components and altered aroma characteristics. Specifically, the inclusion of flavor precursors significantly increased the levels of 5-Hydroxymethylfurfural and volatile aldehydes. Principal component analysis (PCA) on chemical composition dataset revealed 48.7% variability. Sensory analysis, employing the Specialty Coffee Association (SCA) cupping protocol, demonstrated that combining flavor precursors with anaerobic germination transformed coffee flavor properties, enhanced quality, and substantially increased sensory scores ($p < 0.05$). Sucrose supplementation produced the highest sensory score and intensified fruity flavor attributes. Therefore, adding different flavor precursors forms distinct flavor characteristics, conducive to further improving the quality of germinated coffee.

1. Introduction

In recent years, there has been a rapid growth in consumer demand for high-quality and specialty coffee, leading to an increase in its niche market share (Cheng, Furtado, Smyth, & Henry, 2016; Wang et al., 2023). The trends of premiumization and continuous innovation in coffee products are increasingly embraced by consumers (Tang, Sun, Cornuz, Yu, & Lassabliere, 2021). The quality of coffee depends on various factors, including the complexity of growing factors such as species and origin, as well as post-harvest factors like processing, roasting, storage and brewing methods. These complexities pose challenges for producers aiming to enhance coffee quality (Cheng et al., 2016; Wang et al., 2022). The chemical composition of green coffee beans plays a crucial role in flavor formation (Hu et al., 2020). During the intense process of roasting coffee beans at high temperatures, numerous vigorous chemical reactions occur, including the Maillard reaction, Caramelization, and Strecker degradation (Lee, Cheong, Curran, Yu, & Liu, 2015; Tarigan, Wardiana, Hilmi, & Komarudin, 2022). The breakdown of various sugars and lipids along with amino acids and trigonelline contribute to the development of a unique aroma and special taste of coffee (Hu et al., 2020). Therefore, modifying the content and composition of flavor precursor compounds such as sugars and amino acids can significantly change both the aroma profile and the final taste of coffee (Liu et al., 2019).

Typically, the coffee trade is predominantly driven by green coffee beans. Prior to roasting, significant potential remains for exploring their processability. Effective pretreatment of green coffee beans can enhance the aroma of Robusta coffee (Wang et al., 2022). Liu et al. (Liu, Yang, Linforth, Fisk, & Yang, 2019; Liu, Yang, Yang, et al., 2019) manipulated flavor precursors (glucose, fructose, and sucrose) and employed chemical pretreatment (acetic acid) to reduce the perceived difference between Arabica and Robusta coffee while increasing the proportion of Robusta coffee in blend coffee. Additionally, starter or microbial fermentation has been applied to fermenting green coffee beans (Wang

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et al., 2022). Tang et al. (2021) evaluated the effects of *Aspergillus* spp. and *Mucor* spp. on Robusta coffee beans, proposing a novel approach for regulating and enhancing the coffee flavor profile. Pretreating coffee beans offers innovative strategies for improving coffee quality by altering various flavor precursors, resulting in distinctive aromas and flavors.

Seeds undergo significant physical and chemical transformations during germination, enhancing their nutritional and functional quality. Consequently, they have become a favored choice within the global food industry (Thakur et al., 2021;Wang, Wang, Hu, Al-Romaima, et al., 2023). Germination is known to alter the composition and increase levels of beneficial amino acids, such as gamma-aminobutyric acid (GABA), which increases during this stage (Kim, Han, Lim, & Cho, 2021). It also reduces aspartic acid levels, decreasing the formation of harmful acrylamide in baked goods (Kim et al., 2021; Wang, Wang, Hu, Al-Romaima, et al., 2023). In our previous study, anaerobic germination was employed as a novel strategy to enhance the quality of commercial Arabica coffee, yielding satisfactory outcomes (Wang, Wang, Hu, Al-Romaima, et al., 2023). However, this method led to a reduction in water-soluble and volatile compounds in roasted coffee, resulting in limited alterations in Specialty Coffee Association (2018) sensory scores (Wang, Wang, Hu, Al-Romaima, et al., 2023). Torrefacto is a distinctive type of roasted coffee wherein sucrose or glucose (up to about 15 g/100 g) is incorporated during the roasting process, forming a delicate sugar film on the coffee beans that safeguards them against oxidation and expedites the Maillard reaction (Liu, Yang, Yang, et al., 2019). Research has demonstrated that this particular roasting technique can release more antioxidants (Hasni, Safriani, Nilda, Rahmad, & Aneiza, 2021; López-Galilea, de Peña, & Cid, 2008; Ludwig, Bravo, De Pena, & Cid, 2013). Diverging from Torrefacto's addition of sugar during roasting, we introduce small molecular sugars during the germination process based on previous studies to modulate the concentration of flavor precursor sugars and facilitate the occurrence of Maillard chemical and caramelization reactions during roasting. Therefore, it is essential to investigate the impact of incorporating small molecular sugars on the quality of anaerobically germinated coffee.

This study aims to explore new ways to improve coffee quality by incorporating small molecule sugars (sucrose, glucose, and fructose) during the germination process, thereby innovating green coffee bean processing technology. The findings of this study can not only broaden our understanding of coffee flavor development, but also provide practical insights for coffee producers to meet consumers' growing demand for unique and high-quality coffee experiences.

2. Materials and methods

2.1. Sample preparation

Samples of green coffee beans (Catimor CIFC 7963, *Coffea arabica* L.) purchased in Baoshan City, Yunnan Province, China, were used. These beans were wet-processed in 2021/2022, sold as commercial coffee beans, and stored at room temperature before the experiment. Initially, a preliminary experiment was conducted to investigate the effect of adding flavor precursors at different concentrations on cupping scores (Data not shown); the maximum concentration (15 g/100 g) was determined based on the Torrefacto roasting addition (Liu, Yang, Yang, et al., 2019). However, the dosage of flavor precursors (5 g/100 g, 10 g/100 g, and 15 g/100 g) did not show significant differences in cupping scores. Considering factors such as the amount added, effect, and cost, a concentration of 5 g/100 g was selected for further study. Untreated coffee beans served as the control group (CB), while three additional groups were established: germination group (GCB), sucrose addition group (S-GCB), glucose addition group (G-GCB), and fructose addition group (F-GCB).

For the GCB, green coffee beans were soaked in water at a 1:2 g/mL ratio at 25 °C for four hours, followed by anaerobic germination at 35 °C for twelve hours (Wang et al., 2023). Beans were dried in a convection oven at 50 °C until reaching 10–12% moisture content. In the S-GCB, a sucrose solution equivalent to 5 g/100 g weight of coffee beans replaced water; all other operations remained consistent with the GCB. Similarly, for the G-GCB and F-GCB, solutions containing glucose or fructose, respectively, each equivalent to 5 g/100 g weight of green coffee beans, replaced water during the soaking process; all other operations were consistent with the GCB. These sugar solutions permeated the beans, influencing germination and sugar content, aiming to improve subsequent quality parameters. The samples were roasted using the same hot air method, with a higher flow of hot air at the bottom of the machine to agitate the beans, ensuring a more uniform and stable heating process that minimized variations between batches. Each sample (80 g) was roasted in an i-roast hot air bean roaster for 6 min, followed by a cooling time of 3 min to achieve a medium roast level. After two rounds of roasting, the beans were mixed and divided into two portions: one for chemical analysis and another for cupping evaluation. For chemical analysis, the samples were ground into powder, sieved through a 60-mesh screen, placed in airtight glass containers, and stored at −20 °C. The cupping samples were promptly transferred to check valve bags for sensory evaluation. All samples were prepared in triplicate, and samples of the same treatment were tested in parallel three times. All samples were prepared in triplicate, and then samples of the same treatment were mixed and tested in parallel three times.

2.2. NMR measurements of roasted beans

The water-soluble compounds in roasted coffee were characterized using ^{1}H NMR spectroscopy (Wang, Wang, Hu, Zhang, et al., 2023). A 40 mg sample was precisely weighed and placed into a 1.5 mL Eppendorf tube. Subsequently, 800 μL of D_2O (with TMSP-d4 as the internal standard at 0.03%) was added, and ultrasonic extraction was performed at 80 °C for 1 h. Following extraction, centrifugation was carried out at 5000*g* for 4 min. Finally, 450 μL of the supernatant was transferred to a 5 mm NMR tube for subsequent analysis.

All experiments were conducted at 25 °C using Bruker DRX-600 instruments (Bruker Biospin, Rheinstetten, Germany). The ^{1}H NMR spectra were obtained using the saturation method to suppress the water peak with the following parameters: sampling data point of 65,536, peak width of 12,019 Hz; acquisition time of 2.73 s, relaxation time of 10 s, and scans repeated for a total of 64 times.

The ^{1}H NMR data were processed using MestReNova (version 6.1.0–224; MestReC, Santiago de Compostela, Spain). Initially, the spectra were calibrated by referencing the TMSP-d4 signal at 0.0 ppm. A cut was then applied to remove the water peak between 5.0 and 4.5 ppm, followed by phase correction and baseline corrected operations. The identification of major chemical constituents was based on peak signals referenced from literature (Table S1) (dos Santos, Alvarenga, & Boffo, 2020; Hu et al., 2020; Wang, Wang, Hu, Zhang, et al., 2023).

The internal standard was normalized to a value of 1.000 for comparison purposes and the identified compounds were integrated to determine their relative peak areas. Integration of peak areas across different samples resulted in a dataset comprising 15 samples (rows) and 31 variables (columns).

2.3. Volatile characterization of roasted coffee

The volatile components in the roasted beans were adsorbed and concentrated using headspace solid-phase microextraction (HS-SPME) (Wang, Wang, Hu, Zhang, et al., 2023). Precisely weighed 0.5 g of the sample was placed into a 20 mL headspace vial. Subsequently, 100 μL of 0.01% 3-heptanone in methanol was added as an internal standard. The vial was pre-equilibrated at 60 °C for 20 min, then a 2 cm 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) StableFlex fiber (Supelco, Bellefonte, USA) was used for extraction over 30 min.

The adsorbed and enriched fibers were then desorbed by injecting them into a gas chromatograph 7890 A (Agilent Technologies Inc., Wilmington, DE, USA) coupled with a 5975C mass selective detector (Agilent Technologies Inc., Wilmington, DE, USA). Separation was achieved using a DB-WAX column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies Inc., Wilmington, DE, USA) (Wang, Wang, Hu, Zhang, et al., 2023). The inlet temperature was maintained at 250 °C, and helium was used as a carrier gas at a flow rate of 1.0 mL/min. The initial oven temperature was set at 60 °C for 3 min, followed by a gradual increase to 180 °C at a flow rate of 8 °C/min. Then it was held steady for 1 min before being further elevated to and maintained at 230 °C with an increment rate of 4 °C/min for a total period of 7 min. In the full scan mode, the electron ionization mode operated at an energy level of 70 eV, while the ion source temperature remained constant at 250 °C, and the scanning range encompassed *m/z* values ranging from 35 to 500 *amu*.

Compounds were identified by mass spectrometry, comparison with the NIST11 database, and linear retention indices (LRI) of volatiles under the experimental conditions were compared with the data in the literature for identification (Dong et al., 2019). Quantitative analysis of each component (μg/g) based on peak area and internal standard content resulted in a GC/MS dataset containing 15 coffee samples and 39 volatile compounds.

2.4. Sensory evaluation

The sensory evaluation of coffee was conducted according to the SCA Protocol (Specialty Coffee Association, 2018). All procedures and methodologies were approved by the Quality Assurance Department of the Yunnan International Coffee Exchange (YCE), adhering to its relevant guidelines and regulations. Additionally, informed consent was obtained from all panelists involved in the experiments. A total of ten positive attributes were specified in the protocol, including fragrance/aroma, uniformity, sweetness, flavor, acidity, body, aftertaste, balance, clean up, and overall. Each attribute was evaluated on a scale ranging from 6 to 10, with increments of 0.25. Defects were considered as negative scoring attributes. Three Q-Grader panelists, trained in the SCA cupping protocol, participated in the sensory analysis, scoring each attribute. After the evaluation, an overall score was assigned to each sample, from which the average score was calculated to represent its overall quality. Additionally, descriptive sensory attributes were assigned to each sample according to the Coffee Taster's Flavor Wheel (The Coffee Taster's Flavor Wheel, 2016).

2.5. Statistical analysis

Duncan's test of Analysis of Variance (ANOVA) was conducted using the SPSS 27.0 software package (IBM Corp, Armonk, America), with a significance level of $p < 0.05$ indicating statistical significance. Heat maps were generated using TBtools (version 1.120, China) (Chen et al., 2020). The datasets from the NMR and GC–MS platforms were integrated, and multivariate statistical analysis was performed on the combined data using SIMCA software (version 14.1, Umetrics, Sweden). Prior to formal analysis, the integrated dataset was normalized using UV scaling. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were employed to assess the heterogeneity among roasted coffee samples subjected to different treatments.

3. Result and discussion

3.1. Exploring water-soluble chemical composition changes based on ^1H NMR

The color formation of roasted coffee beans is primarily attributed to the Maillard reaction and sugar caramelization processes that occur during roasting (Tarigan et al., 2022). Studies show that adding flavor precursors such as sucrose, glucose, and fructose can modify the color of Robusta coffee beans, making them resemble the color to Arabica coffee beans more closely (Liu, Yang, Yang, et al., 2019). Under consistent roasting conditions, germinated coffee exhibits a brownish color due to germination consumption and precipitation of soluble components under anaerobic germination treatment (Wang, Wang, Hu, Al-Romaima, et al., 2023). Adding flavor precursors may increase chromatic aberration and darken germinated roasting beans to dark brown or black. These findings suggest that flavor precursors significantly influence the composition of germinated roasted coffee beans. Consequently, we utilized ^1H NMR spectroscopy to further investigate how flavor precursors affect the primary chemical components of these coffee beans.

The samples of CB, GCB, S-GCB, G-GCB, and F-GCB were analyzed using ^1H NMR to detect their chemical components, drawing on methodologies from previous studies (dos Santos et al., 2020; Wang, Wang, Hu, Al-Romaima, et al., 2023). A total of 31 compounds with characteristic signals were identified, as detailed in Table S1 and Fig. S1. The characteristic signals were integrated, and a corresponding NMR database was established. A heat map was generated to visualize the impact of adding flavor precursors on the main chemical components of germinated roasting coffee beans (Fig. 1). Furthermore, significant analysis of the relative content of the main compounds was conducted to compare the quality difference in germinated roasting coffee caused by the addition of flavor precursors (Table S2). As depicted in Fig. 1, anaerobic germination leads to significant changes in the chemical composition of coffee beans, consistent with prior research findings (Wang, Wang, Hu, Al-Romaima, et al., 2023). Additionally, different flavor precursors result in variations in the water-soluble chemical composition of the coffee, leading to flavors differences. The fructose addition group (F-GCB) exhibited greater similarity to the control group (CB) in terms of both composition and the concentration of compounds. The groups with added sucrose and glucose showed similar water-soluble chemical compositions but with higher content compared to single germination treatment.

Organic acids are the predominant compounds in coffee (Wang et al., 2021). Among them, CGAs are classified as phenolic acids and have the highest content, accounting for approximately 7–10% of green bean quality, which is significantly influenced by the degree of roasting (Hu et al., 2020). Germination treatment of green coffee beans can enhance the content of CGAs, a finding consistent with prior research (Wang, Wang, Hu, Al-Romaima, et al., 2023), and the addition of flavor precursors does not impact this enhancement. The flavor characteristics of CGAs are multifaceted as they contribute to acidity, acetic acid notes, astringency, and bitterness in coffee. Due to their significant antioxidant effects, CGAs have garnered increasing attention for their physiological activities (Hu, Wang, Zhang, & Qiu, 2019). The incorporation of different flavor precursors leads to variations in sugar content within green coffee beans, which subsequently influences acid formation. In contrast to previous findings (Wang, Wang, Hu, Al-Romaima, et al., 2023), lactic acid was reduced in germinated coffee during this study, possibly due to the present of microorganisms in green coffee beans. These variations in acid content are expected to reflect in sensory evaluations and influence the development of flavor characteristics.

Caffeine and trigonelline, each constituting approximately 1% of green beans, are the primary alkaloids present in coffee (Wang et al., 2021). They contribute to the bitterness and stimulating effects of coffee. Previous studies have shown that germination treatment can reduce the levels of these two alkaloids (Wang, Wang, Hu, Al-Romaima, et al., 2023). However, this study observed a less significant decrease in alkaloid content in germinated samples compared to previous research. This difference was attributed to variations in seed vitality. Adding three flavor precursors significantly raised the alkaloid content in germinated coffee, although levels still remained below those of pre-sprouting. Notably, S-GCB exhibited significantly higher concentrations of both alkaloids compared to G-GCB and F-GCB. Caffeine, making up about 10% of the bitterness, largely accounts for the stimulating effects of coffee, while trigonelline significantly influences its bitter flavor (Hu

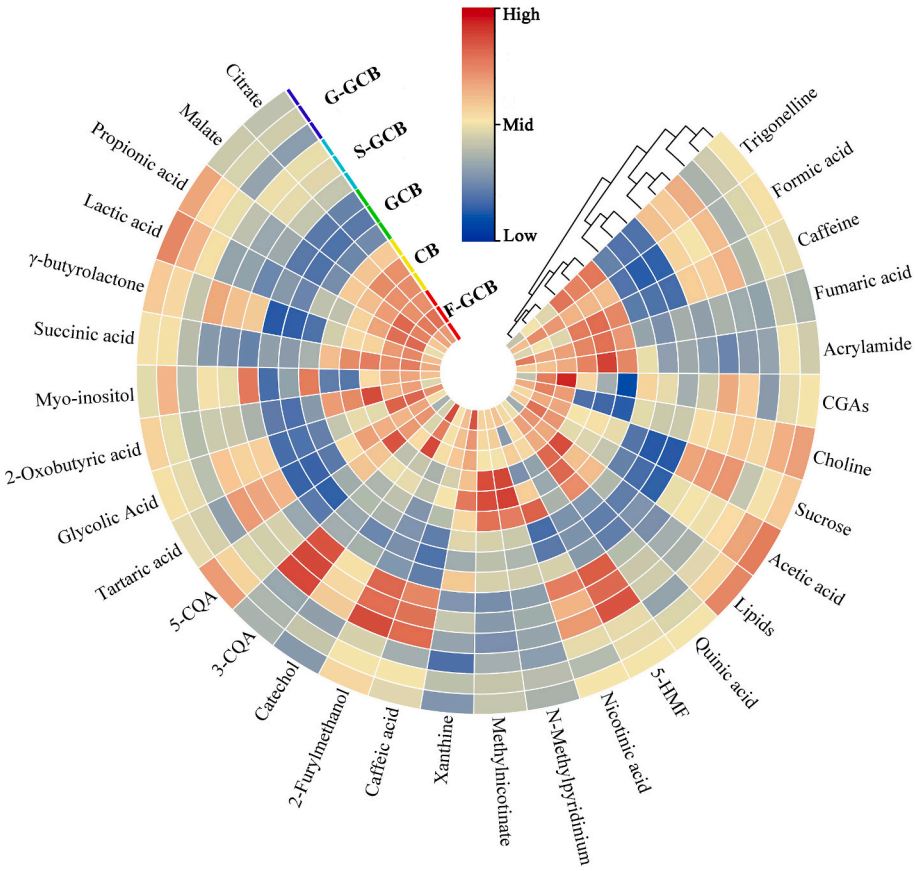

Fig. 1. Heatmap analysis of water-soluble compounds in roasted coffee samples. CB, non-treated green coffee beans; GCB, germinated green coffee beans for 12 h; S-GCB, germinated with 5 g/100 g sucrose added; G-GCB, germinated with 5 g/100 g glucose added; F-GCB, germinated with 5 g/100 g fructose added. 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.)

et al., 2019). Consequently, alterations in alkaloid content resulting from different flavor precursors may lead to variations in flavor characteristics.

Sucrose is the main small molecule carbohydrate in green coffee beans, with content that varies greatly depending on genetic characteristics and climatic conditions (Cheng et al., 2016; Wang et al., 2021). In addition to increasing sweetness, it also contributes to the formation of coffee color and aroma (Barbosa et al., 2019). During the roasting process, sucrose rapidly decreases due to its involvement in the Maillard and caramelization reaction. Consequently, only a small amount remains in medium roast coffee (Hu et al., 2020). By adding flavor precursors during germination, sugar consumption can be minimized. Roasted beans exhibit significantly higher levels of sucrose compared to green coffee beans (GCB), with S-GCB and F-GCB displaying the highest sucrose content that is notably greater than that found in CB. Furthermore, the chemical reaction of sucrose during roasting leads to acetic acid production, thereby influencing acidity development. Therefore, the addition of flavor precursors changes the sensory characteristics of germinated coffee.

The harmful compound acrylamide, which is generated during coffee roasting (Khezerolou et al., 2018), can be reduced through germination, consistent with previous studies (Wang, Wang, Hu, Al-Romaima, et al., 2023). The addition of sucrose and glucose does not significantly affect the formation of acrylamide in germinated coffee. However, while adding fructose slightly increases its formation rate, it still remains lower than that in the control sample (CB). Acrylamide primarily forms through the reaction between free asparagine and reducing sugars. Consequently, adding reducing sugars to coffee may elevate acrylamide content; however, germination reduces asparagine levels resulting in lower acrylamide content compared to CB. 5-Hydroxymethylfurfural (5-HMF) is an intermediate product of the Maillard reaction—a furanyl aldehyde produced during thermal decomposition of sugars and carbohydrates (Friedman, 1996; Park, Jo, & Lee, 2021). The type of sugar used can influence the rate at which 5-HMF forms (Toker, Dogan, Ersöz, & Yilmaz, 2013). Compared to CB, the levels of 5-HMF in S-GCB, G-GCB, and F-GCB increased by 2.61-fold, 1.50-fold, and 1.68-fold respectively. 5-HMF is considered acceptable in certain food products due to its association with sensory attributes (such as color, flavor, and odor) and quality indicators like thermal processing (Martins, Alcantara, Silva, Melchert, & Rocha, 2022). However, considering the potential toxicity of 5-HMF, elevated levels resulting from the addition of flavor precursors should also be taken into account; although safe thresholds for coffee have not yet been established (Martins et al., 2022). Furthermore, the concentration of 5-HMF is correlated with coffee quality and specialty coffee exhibits significantly higher levels compared to traditional blends (mix of Arabica and Robusta coffee), making it a useful marker for roasting purposes (Alcantara et al., 2021).

3.2. Characterization of volatile components

The addition of flavor precursors will change the concentration of corresponding flavor compounds, and a series of intricate reactions resulting from anaerobic germination treatment significantly influenced the volatile components in roasted coffee. A total of 39 volatile compounds were identified in the roasted samples, and these were classified into 11 groups based on their chemical properties, including two pyridines, 12 pyrazines, 12 acids, six aldehydes, one ester, four ketones, three alcohols, two pyrroles, three phenols, one furan, and three others

(Table S3). All samples contained these 11 compounds, with no significant differences observed in terms of qualitative comparison. Among them, pyrazines, acids, aldehydes, and alcohols exhibited the highest abundance, accounting for over 75% of the volatile components (Table S3). The heterogeneity of volatile compounds generated by different treatments contributes significantly to the specificity of aroma and flavor in coffee.

The study employed consistent roasting conditions. Fig. 2 illustrates the heat map depicting changes in content levels (μg/g) and relative content (%) of various volatile compound categories under different treatments. The total content of volatile compounds in CB was measured at 830.69 μg/g. This did not show any significant difference compared to S-GCB samples (842.64 μg/g) or G-GCB samples (836.17 μg/g); however, notable variations observed among individual components (Fig. 2A). In contrast to previous studies, a 12-h germination treatment resulted in a substantial reduction in overall volatile compound content (733.38 μg/g). This suggests that the decrease in flavor precursor compounds caused by germination leads to a decline in overall volatile compound content as well. F-GCB exhibited the highest total content of volatile compounds (980.68 μg/g). Therefore, the addition of flavor precursors can mitigate losses incurred due to germination-induced reductions in total volatile compounds. The changes in the relative content of volatile compounds in samples under different treatments are depicted in Fig. 2B, exhibiting significant variations. Consistent with previous studies (Wang, Wang, Hu, Al-Romaima, et al., 2023), germination treatment can significantly alter the relative content of volatile components, while the addition of flavor precursors also influences the composition and flavor characteristics of germinated roasting coffee.

Previous studies have demonstrated that anaerobic germination leads to a reduction in trigonelline, a flavor precursor, as well as pyridine compounds (Wang, Wang, Hu, Al-Romaima, et al., 2023). The findings of this study align with the notion that incorporating flavor precursors during anaerobic germination does not alter the characteristics of germinated coffee. The process of anaerobic germination may have enhanced the overall levels of reducing sugars and free amino acids, thereby increasing the content and relative proportion of pyrazine compounds in germinated roasting coffee, which is consistent with prior research (Wang, Wang, Hu, Al-Romaima, et al., 2023). Furthermore, the addition of two reducing sugars, glucose, and fructose, further augmented the concentration of pyrazines.

Moreover, the addition of flavor precursors during anaerobic germination results in an increase in both the quantity and relative content of aldehydes. Volatile aldehydes play a crucial role in the formation of coffee aroma as they are produced through Strecker degradation of amino acids (Diez-Simon, Mumm, & Hall, 2019; Lee et al., 2015). In this process, amino acids react with α-dicarbonyl compounds, leading to the release of one molecule of CO2 and subsequent degradation into aldehydes and amino ketones with reduced carbon atom count (Rizzi, 2008). Importantly, various unique aldehydes significantly contribute to the distinctive aromas exhibited of different food products (Rizzi, 1999). Additionally, these amino ketones undergo isomerization into enolamines, which then cyclize to form pyrazines. These pyrazines primarily impart aromatic flavors on food during roasting processes (Rizzi, 1999). Consequently, the alterations in aldehyde levels, induced by adding flavor precursors, can significantly enhance the aromatic characteristics of germinated coffee.

3.3. Multivariate analysis based on physicochemical indicators

PCA and HCA analyses were conducted on datasets of water-soluble and volatile components to assess the chemical composition differences resulting from the addition of flavor precursors in conjunction with anaerobic germination. The PCA score plot and loading plot (PC1 and PC2) are shown in Fig. 3A and Fig. 3B, respectively. Overall, the first two components (PC1 = 31.9% and PC2 = 16.8%) accounted for 48.7% of the variance, with no outliers observed among all samples. CB was positioned at the lower left side of the score plot, while GCB was located at the lower right side, indicating a clear separation between these two samples on PC1 due to germination. This separation suggests that germination significantly influenced the composition, consistent with previous studies (Wang, Wang, Hu, Al-Romaima, et al., 2023). Additionally, different flavor precursors had a significant impact on the composition of germinated roasting coffee, leading to distinct distributions on the PCA score plot. Notably, S-GCB appeared at the upper left side of the score plot and exhibited clear isolation from other groups on PC2. The primary contributors to this second component were identified

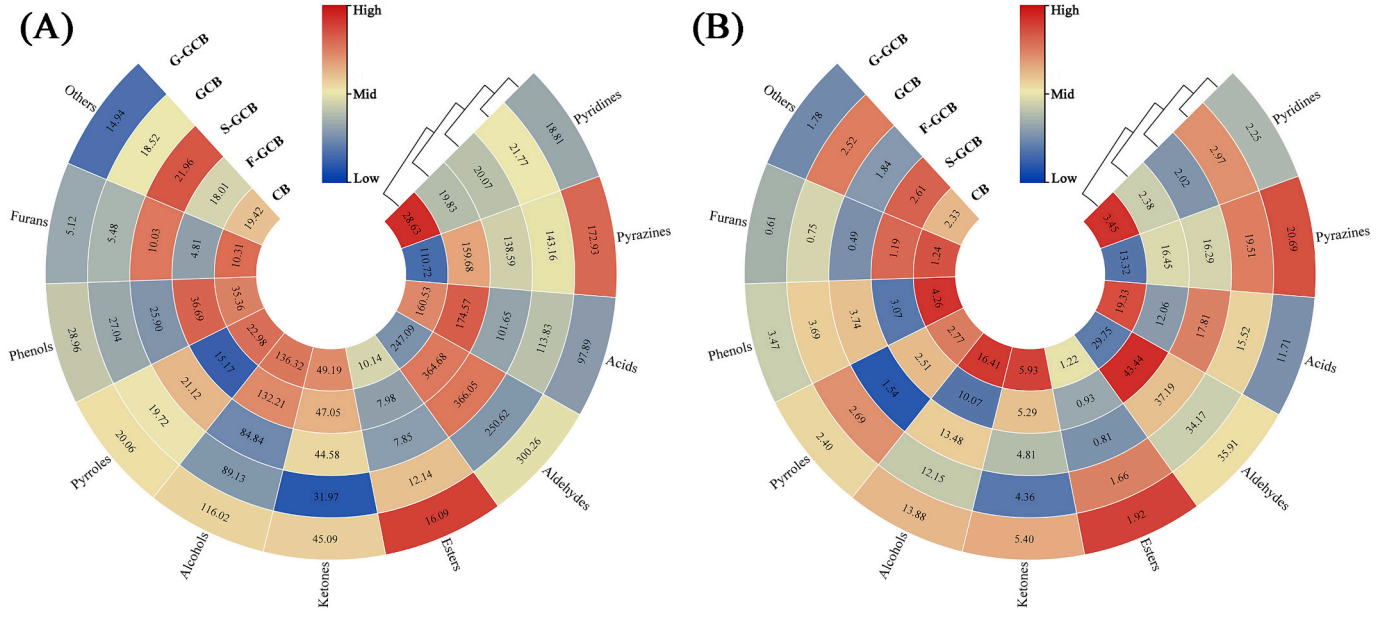

Fig. 2. Heatmap analysis of the contents (mg/g) (A) and relative contents (%) (B) of different volatile compounds in roasted coffee samples. CB, non-treated green coffee beans; GCB, germinated green coffee beans for 12 h; S-GCB, germinated with 5 g/100 g sucrose added; G-GCB, germinated with 5 g/100 g glucose added; F-GCB, germinated with 5 g/100 g fructose added. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

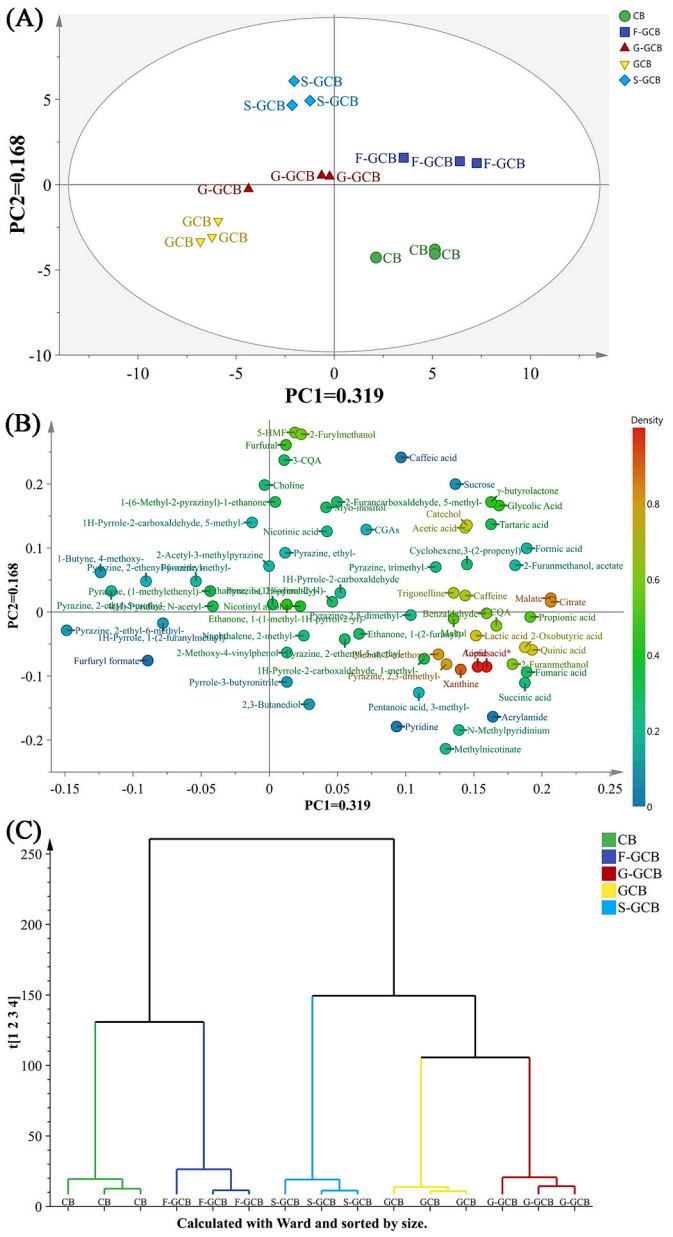

Fig. 3. The score plot (A), loading plot (B) and hierarchical cluster analysis (HCA) (C) of principal component analysis (PCA) based on chemical components in roasted coffee samples (n = 15). CB, non-treated green coffee beans; GCB, germinated green coffee beans for 12 h; S-GCB, germinated with 5 g/100 g sucrose added; G-GCB, germinated with 5 g/100 g glucose added; F-GCB, germinated with 5 g/100 g fructose added. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

as 5-HMF (p[2] = 0.2804), furfuryl alcohol (p[2] = 0.2778), and furfural (p[2] = 0.2612), suggesting that their high content in S-GCB primarily contributed to its distinct separation from other treatment groups.

HCA, based on the first four principal components (PC1 = 31.9%, PC2 = 16.8%, PC3 = 13.7%, and PC4 = 10.2%) as shown in Fig. 3C, effectively discriminates between different treatment groups and elucidate the relationship among their chemical compositions. Notably, there is a closer association between CB and F-GCB treatments. Accroding to Liu et al. (Liu, Yang, Yang, et al., 2019), adding 15 g/100 g of fructose enhance the aroma profile of Robusta coffee, making it more akin to Arabica coffee while maintaining stability during a 6-week accelerated shelf life. Consequently, the addition of fructose brings the flavor of germinated coffee closer to that of control coffee, with more pronounced flavor characteristics. Moreover, the metabolism of fructose does not promote an insulin response after absorption by the human body, alleviating concerns that sugar intake could cause blood sugar to rise (Campbell, Schlappal, Geller, & Castonguay, 2014). Considering that GCB and G-GCB cannot be distinguished based on their first two principal components, these two treatments show proximity on the score plot, indicating similarities in their composition and potentially comparable flavor quality traits. On the other hand, S-GCB stands apart from other treatments as show in Fig. 3A, and exhibits significant differences from other groups when considering their first four components (Fig. 3C). Sucrose, a major carbohydrate constituent in coffee beans, plays a crucial role as an aromatic precursor, generating volatile and non-volatile compounds such as furan, pyrazole, fatty acids, and hydroxymethylfurfural through Maillard reactions (Cheng et al., 2016). Therefore, incorporating sucrose can enrich the overall flavor complexity of coffee.

3.4. Sensory perception

The original composition of coffee and the multitude of compounds generated during roasting contribute to the diverse range of flavors exhibited by coffee, including bitterness, acidity, sweetness, aroma, and even alcoholic notes (Poisson, Blank, Dunkel, & Hofmann, 2017). The origin, primary processing methods, degree of roasting, and brewing techniques all play a role in shaping the flavor profile (Wang et al., 2022). Cupping is a means to assess the taste and characteristics of coffee and serves as an important tool for grading and purchasing decisions (Wang, Wang, Hu, Zhang, et al., 2023). Three experienced cuppers each evaluated 10 attribute of the samples according to the SCA cupping protocol (Specialty Coffee Association, 2018). Five groups of samples achieved perfect scores for uniformity, sweetness, and clean up, without any defect penalty points. The remaining seven attributes scored between 6.83 and 7.75 (Table S4; Fig. 4). The findings of this study demonstrate that the inclusion of flavor precursors can enhance body

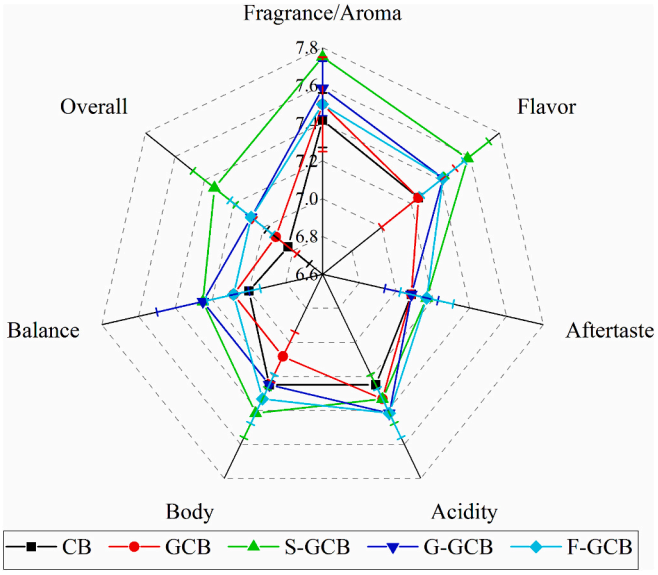

Fig. 4. The average score of attributes (excludes uniformity, sweetness, and clean cup, all treatments scored 10) of roasted coffee samples was assessed by protocol cupping SCA (3 Q-graders). CB, non-treated green coffee beans; GCB, germinated green coffee beans for 12 h; S-GCB, germinated with 5 g/100 g sucrose added; G-GCB, germinated with 5 g/100 g glucose added; F-GCB, germinated with 5 g/100 g fructose added. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and overall attribute evaluation, as well as improve the watery taste characteristic of germinated coffee. All samples achieved a total cupping score of 80 or higher, thus meeting the criteria for classification as 'specialty coffee' (Table S4). In recent years, Yunnan has witnessed a gradual standardization in coffee cultivation and processing techniques, leading to a steady improvement in coffee quality. The control sample used in this study was marketed as a premium-grade commodity bean in Yunnan, attaining the level of 'specialty coffee'. Consistent with previous research, germination treatment enhanced cupping performance without displaying significant differences (Wang, Wang, Hu, Al-Romaima, et al., 2023). However, compared to both control and germinated samples, the inclusion of flavor precursors significantly elevated cupping scores ($p < 0.05$), with sucrose supplementation yielding superior results compared to glucose and fructose supplementation ($p < 0.05$).

The sensory descriptors for five groups of samples were assessed by three tasters, following The Coffee Taster's Flavor Wheel (The Coffee Taster's Flavor Wheel, 2016) (Fig. 5). Nutty/cocoa descriptions were observed in all samples, with the control and germinated samples exhibiting pronounced nutty characteristics in further descriptors, while flavor precursors were added to enhance the cocoa characteristics. Descriptions related to sweet and brow sugar classes were consistently observed across all samples. Among deeper descriptors, honey was specifically identified in the fructose-added group. The germination treatment demonstrated a potential reduction in the acidity of roasted coffee; however, the inclusion of flavor precursors resulted in increased acidity levels, potentially attributed to acids generated from the sugar compounds during roasting. Fruity descriptors emerged in GCB, S-GCB, and G-GCB samples; among these deep descriptions, S-GCB stood out as distinct from the other two groups due to its more pronounced fruit flavors.

The addition or modification of flavor precursors may positively impact the flavor attributes of roasted coffee. However, it is crucial to pay attention to the corresponding compositional changes. Firstly, further exploration is needed regarding the health effects associated with the addition of sugar. Although the amount of sugar used in this method is lower compared to the traditional Torrefacto roasting method, which involves adding 15 g/100 g sugar (Liu, Yang, Yang, et al., 2019), significant differences can still be observed in cupping evaluations. Secondly, particular attention should be given to controversial byproducts generated during the roasting process due to flavor precursors, such as an increase in 5-HMF caused by sugar addition.

4. Conclusion

The content and composition of flavor precursors play a crucial role in determining the characteristics that contribute to coffee quality. In this study, roasted beans were prepared by incorporating 5 g/100 g flavor precursors (sucrose, glucose, and fructose) into cooperative anaerobic germination treatment. Essential chemical components of the roasted beans were analyzed using ^{1}H NMR and HS-SPME-GC–MS techniques, and sensory analysis was performed. The findings revealed that the addition of flavor precursors harmonized the water-soluble chemical composition and altered the aroma characteristics of roasted coffee. PCA based on chemical composition demonstrated distinct changes in compounds resulting from different flavor precursor additions. Based on SCA final scores, cupping evaluations indicated significant improvements in coffee quality when using added flavor precursors and anaerobic germination, with all samples classified as 'specialty coffee'. Notably, the S-GCB achieved significantly higher final scores compared to G-GCB and F-GCB ($p < 0.05$).

In summary, anaerobic germination combined with the addition of

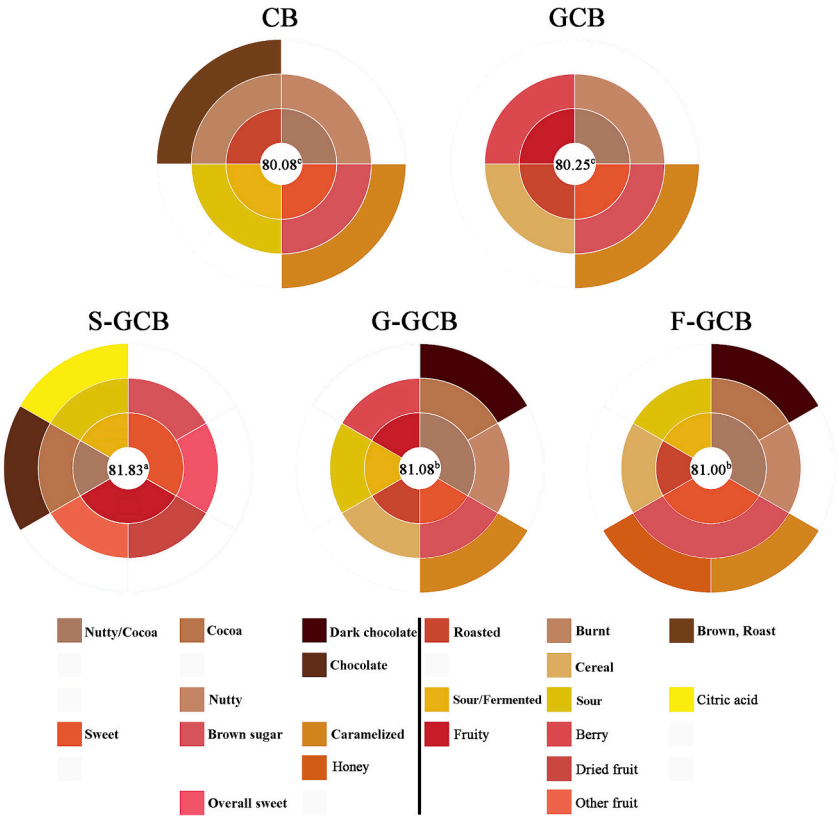

Fig. 5. Sensory descriptors and final score of roasted coffee samples. CB, non-treated green coffee beans; GCB, germinated green coffee beans for 12 h; S-GCB, germinated with 5 g/100 g sucrose added; G-GCB, germinated with 5 g/100 g glucose added; F-GCB, germinated with 5 g/100 g fructose added. Color 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.)

flavor precursors leads to substantial alterations in the chemical composition and sensory characteristics of coffee, ultimately enhancing cup quality. Further research is required to explore the underlying mechanisms behind this synergy and its impact on improving coffee quality. Additionally, optimizing commercial bean quality and determining the optimal amounts of added flavor precursors are necessary steps toward achieving the desired final flavors.

CRediT authorship contribution statement

Yanbing Wang: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Xiaoyuan Wang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Chenxi Quan:** Writing – review & editing, Supervision. **Abdulbaset Al-Romaima:** Writing – review & editing. **Guilin Hu:** Validation, Formal analysis. **Xingrong Peng:** 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.fochx.2024.101684.

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