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Sensory and chemical analyses of spontaneously fermented coffees

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ABSTRACT

Coffee fermentation has emerged as an innovative post-harvest process in recent years. The effectiveness of this process is in conferring sensory notes to the coffee that would please the consumer and allow for the positive differentiation of the coffees. This is achieved by monitoring the different parameters of the procedure of coffee fermentation. In this regard, the present study aimed to conduct a natural fermentation of the coffee fruit for up to 96 hours followed by the sensory (sensory characteristics for aroma, flavor, acidity, body and overall score of the 0 to 100) and chemical analyses (bioactive compounds, volatiles, organics, and fatty acids) to evaluate the influence of fermentation on sensory quality and on chemical compounds. The coffee (*Coffeea arabica* L.) samples for the study were collected from Presidente Olegário – MG (Harvest 2018/19) in the cerrado of Minas Gerais. The effects of fermentation and fermentation duration on the sensory quality and chemistry of coffee were evaluated. It was observed that after 48 h of fermentation, significant sensorial differentiation occurs, including an increase of the approximately two points in the final grade. In addition, time was revealed as the most relevant factor in the chemical analyses for the categorization of samples into different groups. Accordingly, it was concluded since coffees with subjected to 48, 72, and 96 h of fermentation got higher final grades, higher levels of palmitic fatty acid and characteristic compounds of fermented coffees (1,3-dioxolane 2,4,5-trimethyl, 2-furan methanol acetate, and butanoic acid) checking out the coffees wine-like notes and sweet notes to the beverage.

Key words: *Coffeea arabica* L.; sensory evaluation; volatile composition.

1 INTRODUCTION

Food quality improvement has been an important field of research for years. Continuous attempts at developing innovative processes for such improvement are reported for various foods, including coffee, as for example, use of microencapsulated starter cultures by spray drying coffee for fermentation (Martins et al., 2023), application of enzymes in coffee processing (Corrêa; Penha; Freitas-Silva, 2021), etc. The post-harvest phases of the coffee play a fundamental role in defining its final chemical composition by adding sensorial notes to the coffee that would please the consumer and also determining whether it would be possible to preserve the coffee. As it is one of the techniques most used by the industry in defining the chemical composition of raw beans, coffee processing also reflects on the sensory perception of the coffee drink (Duarte et al., 2010; Ribeiro et al., 2016; Saath et al., 2012). And fermentation, a process currently widely used in post-harvest coffee, can promote an increase in sensory quality in addition to being an economically viable alternative (Evangelista et al., 2014). This process uses a combination of biological and chemical reactions in which complex molecules in the pulp and mucilage around the seeds are degraded by the extracellular enzymes and organic acids produced by the action of microorganisms, which are present naturally in the coffee. The reactions generate a wide range of metabolites, including organic acids, higher alcohols, and esters, which add complexity to the flavor and aroma of the coffee, facilitating the production of beverages with distinct sensory characteristics (Bastian et al., 2021; Evangelista et al., 2014; Nigam; Singh, 2014; Bressani et al., 2018; Panji; Priyono; Zainuddin, 2018; Haile; Kang, 2019).

Coffee fruits provide the substrates for the development of microorganisms, such as bacteria, yeasts, and filamentous fungi, and these substrates as the sources of carbon and nitrogen for the microbes (Pereira et al., 2017). Coffee beans provide a large variety of substrates, including sugars, lipids, caffeine, chlorogenic acid, etc., which allows for the recurrent development of diverse and complex microbiota (Bastian et al., 2021). It should be noted that even with the increased use of spontaneous fermentation in coffee, there is a great lack of scientific work that seeks to understand the change in chemical compounds and in the sensory quality of this product after fermented.

Sensory analysis is the most widely used method for the quantitative characterization of coffee. The aroma, acidity, bitterness, body, flavor, sweetness, and overall impression of the beverage are the main sensory attributes that are analyzed, and coffee quality is defined based on the intensity and balance of these attributes (Tolessa et al., 2016). A consumer considers a coffee special when the coffee is perceived and valued according to a set of unique characteristics that differentiate it from other conventional coffees (Center for the Promotion of Imports - CBI, 2014). Therefore, sensory evaluations of coffee include determining the distinguishing characteristics of different samples as well as assigning attribute scores and accordingly determining the possible preference among different samples (Bouillé et al.,

2016; Maurice, 2017). According to the SCA (Special Coffee Association) methodology, trained tasters with Q-grader certification are eligible to conduct the sensory analysis of coffee (Special Coffee Association - SCA, 2009).

Therefore, understanding the mechanism of action of microorganisms in the spontaneous fermentation of coffee and the consequent transformations would facilitate producing a beverage with distinct sensory characteristics, which would fulfill the taste requirements of the most demanding consumers with refined palates. In this context, the present study aimed to follow a process for the spontaneous fermentation of Arabica coffee beans by evaluating the metabolites produced chemically and analyzing the differentiated flavor profiles thus produced.

2 MATERIAL AND METHODS

2.1 Characterization experiments

The fruits of *Coffea arabica* var. Mundo Novo were harvested and used in the present study. Among the fruits that were harvested and collected, ripe fruits had a higher proportion. The collected fruits were placed in a washer followed by the segregation of fruits with different densities. The denser fruits were separated manually from the less dense ones by spreading all fruits on a hanging bed and selecting only the ripe fruits. The coffee fruit was allowed to ferment naturally in an anaerobic environment without yeast inoculation. The fermentation process was conducted in a hermetically-sealed cylindrical container with a capacity of 100 L, and sampling was performed at different intervals of 0, 24, 48, 72, and 96 h after fermentation. At the desired time point (the desired fermentation duration), the coffee fruit was moved to the next stage of the experiment. In the present study, 5 treatments were performed and three replicates each. Totaling 15 samples in the experiment.

The fermented coffee beans were shifted to African beds. The fruits were distributed across the bed in a thin layer (14 $L.m^{-2}$) to allow for drying. This drying method is referred to as the natural coffee methodology (drying process). When the coffee beans reached a water content of 10.8% to 11.2% w.b., the coffee bean samples were collected and stored inside cold storage for the next 30 days (Borém, 2023). Afterward, the coffee beans were processed and sieved, followed by defect removal. Finally, the coffee beans were ready for use in the sensory and chemical analyses.

2.2 Sensory analysis

The sensory evaluation of the coffee samples was conducted by a sensory analysis panel comprising five trained tasters with a Q-grader international certification. All sample preparation and roasting procedures were conducted following the recommendations of the Specialty Coffee Association (SCA) Protocols – Cupping Specialty Coffee (Lingle, 2011).

The evaluation form used in this analysis comprised a predefined list of sensory characteristics for aroma, flavor, acidity, and body, which were based on the Check All That Apply (CATA) technique. The aroma and flavor attributes were evaluated based on a table containing 51 sensory descriptors. The acidity attribute was evaluated based on six sensory descriptors. The body attribute was evaluated based on five descriptors. The taster selected at least one descriptor for each attribute, which best represented the perception of the coffee being evaluated, although he/she could also include other descriptors. These sensory descriptors were determined by a panel of judges during a session for developing the descriptive terminology.

The sensory attributes, namely, sweetness, acidity, body, bitterness, astringency, and aftertaste, were evaluated using a linear scale of intensity with scores ranging from 0 [representing the absence of the attribute] to 10 [representing the maximum intensity of the attribute] (Meilgaard; Civille; Carr, 2006). The reference standards for each sensory attribute were established according to the Sensory Lexicon – World Coffee Research (Chambers et al., 2016).

The overall score achieved on the scale ranged from 0 to 100, according to the Specialty Coffee Association (SCA) Protocols – Cupping Specialty Coffee (Lingle, 2011) methodology.

2.3 Chemical composition analysis of the grains

The green coffee beans were evaluated for the following chemical components: fatty acids, organic acids, and bio-actives (caffeine, trigonelline, and chlorogenic acid). In addition, volatile compounds in the roasted beans used in the sensory analysis were characterized.

The coffee beans were first ground in a basic 11A grinder (IKA, Brazil) for approximately 1 min. Liquid nitrogen was added to facilitate grinding and prevent oxidation in the samples. After grinding, the samples were packaged in Falcon® tubes and stored in a freezer at –85 °C until analysis.

2.3.1 Organic Acids Profile

The organic acids profile was obtained using High-Performance Liquid Chromatography (HPLC) performed according to the methodology reported by Jham et al. (2002). The HPLC was performed using a C610H chromatography column at 50 °C, 10 µM of perchloric acid injected at a constant flow rate of 0.6 $mL.min^{-1}$ as the mobile phase, and a UV spectrophotometer operated at 210 nm for monitoring. The organic acids were extracted for subsequent analysis. Briefly, 250 mg of ground green coffee was placed in a falcon tube, to which 25 mL of 0.23% perchloric acid was added. The mixture

was stirred for 10 min in a mechanical shaker, following which the extract was filtered using an ordinary filter paper and then a membrane filter with a porosity of 0.45 µM.

Standard solutions of citric, malic, tartaric, succinic, lactic, quinic, and acetic acids were from Sigma–Aldrich (St. Louis, MO, USA) and were used for the identification of the chromatogram peaks, comparison of the retention times, and calculation of the respective concentrations of the detected compounds in the samples. The final levels of organic acids were expressed as percent dry matter (% D.M.).

2.3.2 Fatty Acids Profile

In order to obtain the fatty acid profile, 250 mg of ground green coffee beans were placed in 2.0 mL centrifuge tubes, to which 1.0 mL of hexane was added. The tubes were then placed in an ultrasonic bath at room temperature for 10 min. Afterward, the tubes were centrifuged for 2 min at 5,000 rpm and a 500 µL aliquot of the supernatant was transferred to 2.0 mL cryogenic tubes. The residual hexane present in the aliquot was evaporated inside a fume hood. Finally, the fatty acid content was determined based on lipid hydrolysis (Christie, 1989, adapted).

In the lipid hydrolysis step, approximately 10 mg of the extracted oil was diluted in 100 µL of 95% ethanol solution and 1 mol/L of 5% potassium hydroxide. After 10 s of mechanical stirring, the oil was allowed to hydrolyze for 5 min inside a Panasonic® 80 W conventional microwave oven. After cooling, 400 µL of 20% hydrochloric acid, a small amount of NaCl, and 600 µL of ethyl acetate were mixed through 10 s of stirring followed by a rest period of 5 min. Afterward, 300 µL of the organic layer was removed and placed in microcentrifuge tubes, following which it was subjected to evaporation to obtain the free fatty acids (Christie, 1989, adapted).

The free fatty acids were methylated using 100 µL of BF3/methanol (14%) and then heated in a water bath at 80 °C for 10 min. Subsequently, they were diluted with 300 µL of hexane and finally analyzed using gas chromatography.

The Shimadz GCMS-QP2010SE gas chromatograph with a mass spectrometer detector was employed for fatty acids determination. An SP-2560 column (Supelco) with a size of 100 m × 0.25 mm was employed. The following temperature gradient was adopted: 140 °C for 5 min, then incremental increase in the temperature at the rate of 4 °C/min up to 240 °C, and remaining at this temperature for the next 30 min. The temperature of the injector (1/20 split) was 240 °C and the injection volume was 2 µL. The detector temperature was 240 °C. Helium was used as the carrier gas (2 mL/min). The peaks corresponding to every fatty acid were determined through comparison with the respective Supelco37 methylated fatty acid standards. The final contents of the fatty acids were expressed as the percent relative area.

2.3.3 Bioactive Compounds

The non-volatile compounds such as caffeine, trigonelline, and chlorogenic acid were characterized using high-performance liquid chromatography (HPLC), and the methodology was adapted from Vitorino et al. (2001). Briefly, 0.5 g of ground green coffee was extracted in 50 mL of distilled water at boiling point and then placed in a boiling water bath for 3 min. The extract was filtered through ordinary filter paper and then through a 0.45 µm membrane.

The Agilent liquid chromatograph with a UV-vis detection system (model 1260 infinit II) was employed for bioactive compound determination. Discovery C18 chromatographic column (250 × 4.6 mm, 5 µm), operated at a wavelength of 272 nm, was used. The mobile phase comprised methanol: water: acetic acid (20:80:1) and was used at a flow rate of 1 $mL.min^{-1}$. A calibration curve for the identification and quantitative analysis was prepared using caffeine, trigonelline, and 5-caffeeolquinic acid (5-ACQ) standards. The final levels of the bioactive compounds were expressed in percent dry matter (% D.M.).

2.3.4 Volatile Compounds Profile

In order to obtain the volatile compounds profile, 2 g of roasted ground coffee was placed in hermetically-sealed vials and evaluated for its volatile compound composition using the method reported by Rabelo et al. (2020). The volatile compounds were extracted using the static headspace of a gas chromatograph/mass spectrometer model GC-MS QP-2010 SE (Shimadzu) equipped with the NST-100 column (30 m × 0.25 mm × 0.25 µm) with a Carbowax® type polyethylene glycol phase.

Data analysis and compound identification were performed using the GCMS solution software (version 4.4, Shimadzu Corporation, Japan) and the NIST NIST/EPA/NIH 2014 database. Next, the chemical identification was performed by comparing the MS spectra with those available in the database. The results were expressed as the relative percentage area corresponding to the peak area for each identified compound in the total chromatogram area.

2.4 Statistical Analysis

The statistical design of the present study was a Completely Randomized Design with 5 fermentation duration and 3 replicates for each duration. Multiple factor analysis (MFA) was then performed for the scale data obtained for the frequency of descriptors for the attributes of aroma, flavor, acidity, and body. The analysis was performed according to the methodology described by Ossani et al. (2017) using the R software version 4.1.0 (R Core Team, 2021).

Analysis of variance (ANOVA) was performed to analyze the result data obtained from the intensity scale for the attributes of sweetness, acidity, bitterness, astringency,

and aftertaste. The overall score and final score were obtained by employing the Sisvar software to perform the Scott-Knott test. In addition, the principal component analysis (PCA) plots were prepared using the software Chemoface to analyze the result data obtained from the chemical analyses.

The profiles of organic acids, fatty acids, volatile compounds, and bioactive compounds in the samples of different groups were subjected to principal component analysis (PCA) using the statistical software Chemoface version 1.64 (Nunes et al., 2012).

3 RESULTS

The results of the sensory analysis are presented in Table 1. It was observed that the intensity of sweetness did not vary with the fermentation duration. The fermentation duration, however, exerted a significant influence on the intensity of the acidity and body attributes and the final score of the coffee beverage. Significant differences in the attributes were observed particularly after 48 h of fermentation, including increased intensities of acidity and body attributes and higher scores of the coffee beverage.

The sensory attribute descriptors were evaluated using multiple-factor analysis, and the results are presented in Figure 1 [1a for aroma and 1b for flavor]. The correspondence plot (Figure 1aI and 1bI) illustrates the effect of fermentation duration on the frequency of the attribute descriptors. The correlation circle (Figure 1aII and 1bII) indicates the main sensory descriptors in each treatment. The position of the centroids of each fermentation duration in the correspondence plot is related to the vectors referring to the aroma/flavor descriptors in the correlation circle (Figure 1aII and 1bII).

The MFA results confirmed the results of the analysis of variance presented in Table 1. The differentiation of the samples over the fermentation duration and their separation into two predominant groups were observed. Chocolate and almond aroma notes were assigned to the samples fermented for 0, 24, and 48 h, while the samples fermented for 72 and 96 h were characterized by wine, coffee pulp, and spice aromas.

No differences in the flavor were observed between the samples fermented for 0 h and those fermented for 24 h. However, after the time point of 48 h, significant separation of the centroids related to the fermentation durations of 48, 72, and 96 h was observed (Figure 1bI). These results indicated that the fermentation duration influenced the flavor of the samples. The correlation circle of the flavor characteristics (Figure 1bII) revealed that chocolate and nut (almond) notes were predominant in the samples fermented for 0 and 24 h. In the spontaneous fermentation of natural cherry coffee for 48 h and 72 h, other flavors, such as floral and fruity ones, were detected. After 96 h of fermentation, the fermented flavor notes, such as winey and spicy notes, were predominant.

Overall, acidity could be described as the citric kind for all fermentation durations. However, with increasing fermentation duration, malic acidity emerged as the descriptor of acidity (Table 2). In general, the body of the beverage was creamy and viscous, with no difference observed among the samples collected at different fermentation durations. Therefore, based on the above sensory characteristics, the coffee had a predominantly citric acidity and a creamy body. In the present study, for the acidity and body attributes, it was not possible to construct graphs using the multiple factor analysis (MFA) as only a few descriptive variables were obtained for just one type of fermentation.

The results of the organic acid analysis of the coffee samples that were subjected to natural fermentation for different durations are presented in Figure 2.

The principal component analysis (Figure 2) presents the treatments as centroids and the organic acids as vectors. The dimension and direction of the vectors show the relationship of the compounds with the treatments, represented by the centroids. Thus, it is observed that acetic and citric organic acids were the main responsible for the dispersion of centroids. In which, acetic acid motivated the separation in the vertical direction while citric acid motivated the positioning of treatments in the horizontal direction. In addition, it is observed that succinic and lactic organic acids present vectors overlapping, and close to acetic acid, indicating a relationship between these organic acids. Similarly, quinic and malic acids are related to citric acid due to the low angle between them.

Table 1: The intensity scale means (0–10 scale) and the final scores (0–100) for the naturally fermented coffee samples.

Fermentation time (h)	Sweetness	Acidity	Body	Astringency	Aftertaste	Final Score
0	7.33[a]	6.36[b]	6.92[b]	0.28[a]	6.75[a]	84.61[b]
24	7.39[a]	6.61[b]	6.89[b]	0.00[a]	5.75[b]	84.53[b]
48	7.75[a]	7.36[a]	7.44[a]	0.31[a]	7.36[a]	85.53[a]
72	7.42[a]	7.25[a]	7.28[a]	0.06[a]	6.92[a]	85.92[a]
96	7.67[a]	7.58[a]	7.89[a]	0.64[a]	7.47[a]	86.36[a]

*Means followed by the same lower-case letter were not statistically different according to the Scott-Knott test ($P \leq 0.05$).

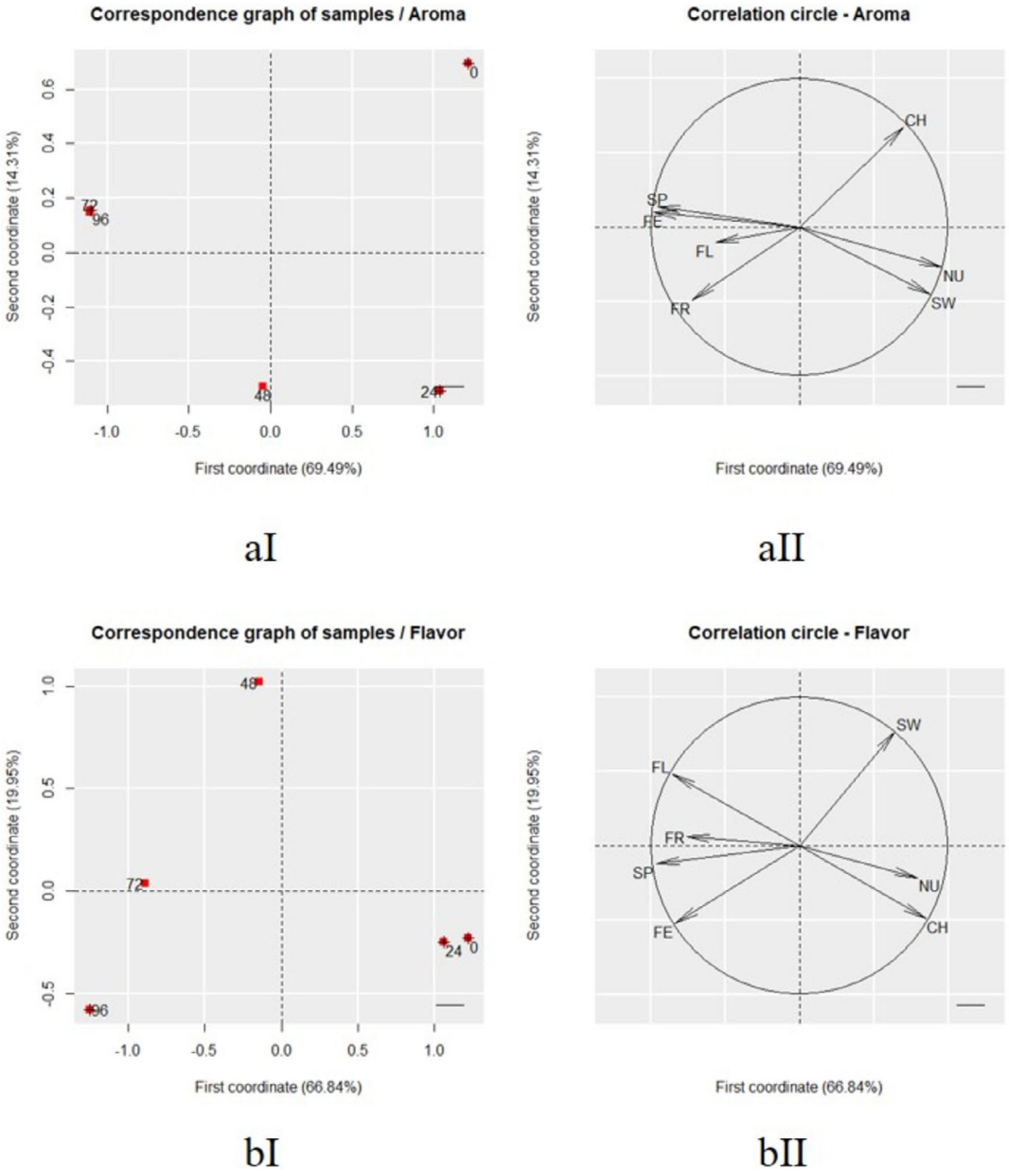

Figure 1: Multiple-Factor Analysis (MFA) of the aroma (A) and flavor (B) descriptors in the sensory analysis of the coffee samples subjected to natural fermentation.

Abbreviations: SW = Sweet; NU = Nuts; CH = Chocolate; SP = Spices; FE = Fermented; FL = Floral; FR = Fruity.

Table 2: Profile of organic acids (g.100g^{-1}) in green coffee beans from spontaneously fermented coffees.

Treatment	Oxalic	Citric	Malic	Quinic	Succinic	Lactic	Acetic
0	0.013	0.931	0.355	0.328	0.150^{c}	0.148^{b}	0.000^{d}
24	0.019	0.975	0.367	0.339	0.167^{d}	0.151^{b}	0.000^{d}
48	0.019	0.985	0.370	0.340	0.180^{c}	0.161^{b}	0.032^{c}
72	0.016	0.975	0.350	0.346	0.194^{b}	0.172^{b}	0.106^{b}
96	0.014	1.040	0.334	0.374	0.219^{a}	0.206^{a}	0.200^{a}

* Means followed by different letters in the columns differ significantly according to the Scott-Knot test ($P \leq 0.05$). Data are the average of three replicates. 0; 24; 48; 72 and 96 hours: time the fruits remained in the fermentation packaging.

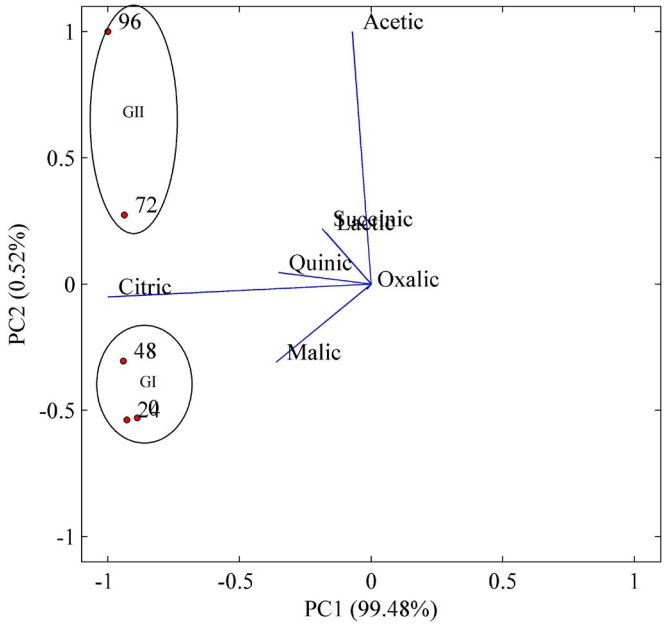

Figure 2: The Principal Component Analysis (PCA) of organic acids in the coffee beans subjected to natural fermentation.

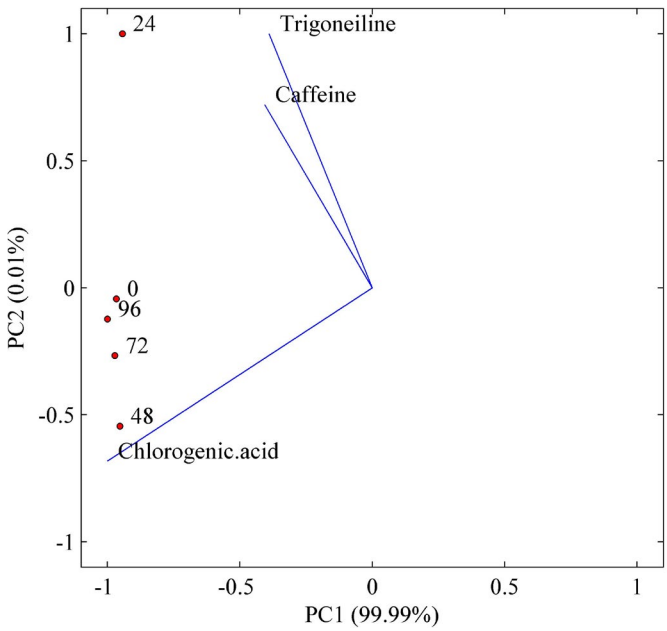

Figure 3: The principal component analysis (PCA) of the bioactive compounds in the coffee beans subjected to natural fermentation.

According to the direction and dimension of the vectors, the centroids (treatments) formed two clusters according to the duration of spontaneous fermentation. Group 1 (GI) comprised the samples that were fermented for zero, 24, and 48 h. Group 2 (GII) comprised the samples that were fermented for 72 and 96 h. The group GI samples had higher contents of citric and malic acids. The GII samples, which were fermented for 72 and 96 h, predominantly comprised acetic acid followed by lactic and succinic acids. The highest levels of acetic acid were detected in the 96 h fermentation groups. The concentrations of citric, quinic, and oxalic acids, as revealed in the chemical analyses, did not contribute to the differentiation of the two groups that had formed.

Furthermore, bioactive compounds, fatty acids, and volatile compounds in the samples were characterized. The results of the principal component analysis (PCA) of the bioactive compounds in coffee beans that were subjected to natural fermentation are presented in Figure 3 and Table 3.

According to the direction of the vectors, it can be observed that the concentration of caffeine and trigonelline present a strong relationship for the green coffee beans of the spontaneously fermented coffees. These compounds were the main responsible for the vertical direction of the graph. Whereas, chlorogenic acids had a greater influence on the horizontal separation of treatments. According to the positioning of the centroids, it is observed that 24h of spontaneous fermentation showed a higher concentration of caffeine and trigonelline than the other fermentation times.

Caffeine, trigonelline, and chlorogenic acid are common biochemical components of coffee. Analysis of these data revealed that these components did not differ according to the time of spontaneous fermentation of the natural coffee.

Table 3: Profile of bioactive compounds (g.100g^{-1}) in green coffee beans from spontaneously fermented coffees.

Treatament	Trigonelline	Caffeine	Chlorogenic acids
0	1.131	1.174	2.902
24	1.144	1.187	2.802
48	1.084	1.099	2.614
72	1.121	1.122	2.566
96	1.178	1.198	3.007

*Means followed by different letters in the columns differ significantly according to the Scott-Knot test (P ≤ 0.05). Data are the average of three replicates. 0; 24; 48; 72 and 96 hours: time the fruits remained in the fermentation packaging.

Nine fatty acids were identified (Figure 4; Table 4) in the evaluated samples analyzed, including saturated fatty acids [myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0), and behenic (C22:0)], monounsaturated fatty acids [oleic (C18:1) and gondoic (C20:1)], and polyunsaturated fatty acids [linoleic acid (C18:2) and linolenic acid (C18:3)].

Note in Figure 4 that the saturated fatty acid palmitic presented a prominent direction from the others. Differently, linoleic and behenic fatty acids are closely related to the polyunsaturated fatty acid linolenic, oriented to the negative region of the first principal component (PC1). Myristic and stearic fatty acids are closely related, as well as gondoic and oleic fatty acids, all related to arachidic fatty acid and directed to the negative region of the second principal component (PC2). According to the direction of the vectors, the centroids representing the treatments did not show clear grouping

according to the fermentation time. However, higher levels of saturated palmitic fatty acid were detected in the samples fermented for 72 h or 96 h, followed by the drying of the fruit. In the samples fermented for 24 h, the highest levels of arachidonic, oleic, and gondoic fatty acids were detected.

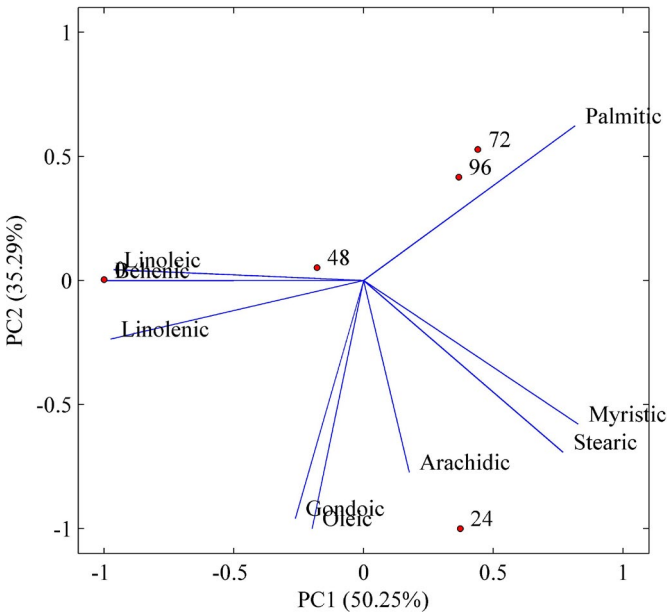

Figure 4: The principal component analysis (PCA) of the fatty acid profile of coffee beans subjected to natural fermentation.

Figure 5 presents the results of the volatile compound analysis of the fermented coffee beans subjected to natural fermentation followed by drying. The analysis revealed forty-four compounds belonging to the following 12 classes: furans (9), esters (3), aldehydes (4), dioxolane (1), ketones (6), amines (3), alcohols (2), pyrroles (3), phenol (1), pyridine (1), pyrazine (7), and acids (4) (Flamet, 2001).

Fermentation duration was revealed as the main factor that influenced the profile of volatile compounds. Fewer volatile compounds were detected in the samples that were fermented for longer durations. As visible in Figure 5, the distribution of volatile compounds exhibited a trend of less diversification in the samples that were subjected to longer total fermentation durations.

In the samples fermented for zero and 24 h, furan-2-methyl, furan-2-methoxymethyl, and furan-2,5-dimethyl were detected, all of which belong to the class of furans. The coffee samples subjected to spontaneous fermentation for 48 h exhibited a high concentration of butanal-2-methyl, while the samples fermented for 72 h contained buten-2-ol-2-methyl and 3-methyl-butanoic acid in high concentrations. In the samples fermented for 96 h, 1,3-dioxolane-2,4,5-trimethyl, 2-furan methanol acetate, and butanoic acid were present, which are the characteristic compounds of fermented coffees.

4 DISCUSSION

In natural coffee fermentation, the reduction of water activity as a result of drying results in the growth of bacteria, yeasts, and filamentous fungi (Pereira et al., 2021). The growth and development of these microorganisms lead to physicochemical changes in the coffee beans, water loss, and changes in the levels of organic compounds (acids, sugars, and volatile compounds), producing metabolites that confer additional flavors and aromas to the coffee, thereby enhancing the quality of the beverages (Evangelista et al., 2014). This development of microorganisms during the drying process could be the reason why even the control treatment group, in which samples were subjected to 0 h of anaerobic fermentation, exhibited physicochemical alterations.

An extended fermentation duration consequently increases the acidity of the beverage. This is because, as the fermentation duration increases, the pH of the mucilage decreases, and the acids generated from the degradation of pectin and coffee sugars continuously increase the acidity (Córdoba-Castro; Guerrero-Fajardo, 2016). Acidification is caused by fermentative bacteria, such as lactic and acetic bacteria, and also due to the alcohol acetification process (Puerta; Ríos-Arias, 2011). The intensification of acidity, which is a general consequence of the fermentative process, is enhanced further in aerobic environments (Dong et al., 2019; Galarza; Figueroa, 2022). This is because lactic acid and

Table 4: Relative area (%) of fatty acids in green coffee beans from spontaneously fermented coffees.

Tratamento	C14:0	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	C22:0	C22:1
0	0.38	35.35	7.71	8.66	43.33	2.03	1.06	0.71	0.77
24	0.93	36.69	8.26	9.06	40.55	2.32	0.73	0.34	1.12
48	0.60	36.81	7.80	8.24	42.39	2.08	0.71	0.41	0.96
72	0.59	39.98	7.91	8.00	39.96	2.15	0.64	0.37	0.40
96	0.77	38.42	7.99	8.32	41.36	1.82	0.62	0.29	0.41

*Means followed by different letters in the columns differ significantly according to the Scott-Knot test ($P \leq 0.05$). Data are the average of three replicates. 0; 24; 48; 72 and 96 hours: time the fruits remained in the fermentation packaging. Fatty acids: C14:0 - Myristic acid; C16:0 - Palmitic Acid; C18:0 - Stearic Acid; C18:1 - Oleic Acid; C18:2 - Linoleic acid; C20:0 - Arachidic Acid; C18:3 - Linolenic Acid; C22:0 - Behenic Acid; C22:1 - Gondoic Acid.

acetic acid bacteria are highly susceptible to the environment containing high CO_2 concentrations as CO_2 inhibits their development (Puerta; Ríos-Arias, 2011). Therefore, it was inferred that, since fermentation was conducted in a hermetic environment in the present study, the CO_2 concentration in the medium reduced the acidity of the coffee beverage after 48 h of fermentation, causing the intensity of the acidity attribute and the sensorial note to stabilize after this time point.

In a previous study, Jimenez et al. (2023) analyzed the influence of anaerobic fermentation on the chemical composition and quality of coffee and reported high concentrations of citric acid in natural coffee at the beginning of the process (0 h), in addition to certain amounts of malic and succinic acids. The production of organic acids during fermentation contributes to the creation of specialty coffees with differentiated and desired sensory characteristics (Cassimiro et al., 2022; Wang et al., 2019). The metabolic processes of acetic acid bacteria elevate the content of acetic acid in green coffee beans during fermentations conducted for long durations (Haile; Kang, 2019). Mota et al. (2020) also studied the impact of anaerobic fermentation on coffee quality and reported a predominance of acetic acid, in addition to citric and malic acids, at the end of the fermentation process.

Accordingly, the increased levels of acetic acid content observed in the liquid chromatography analysis conducted in the present study might be related to the increased intensity of the acidic taste noted by the evaluators after 48 h of fermentation, as the perceived acidity of coffee is a result of the donation of protons from acids to the receptors on the human tongue (Wu et al., 2013).

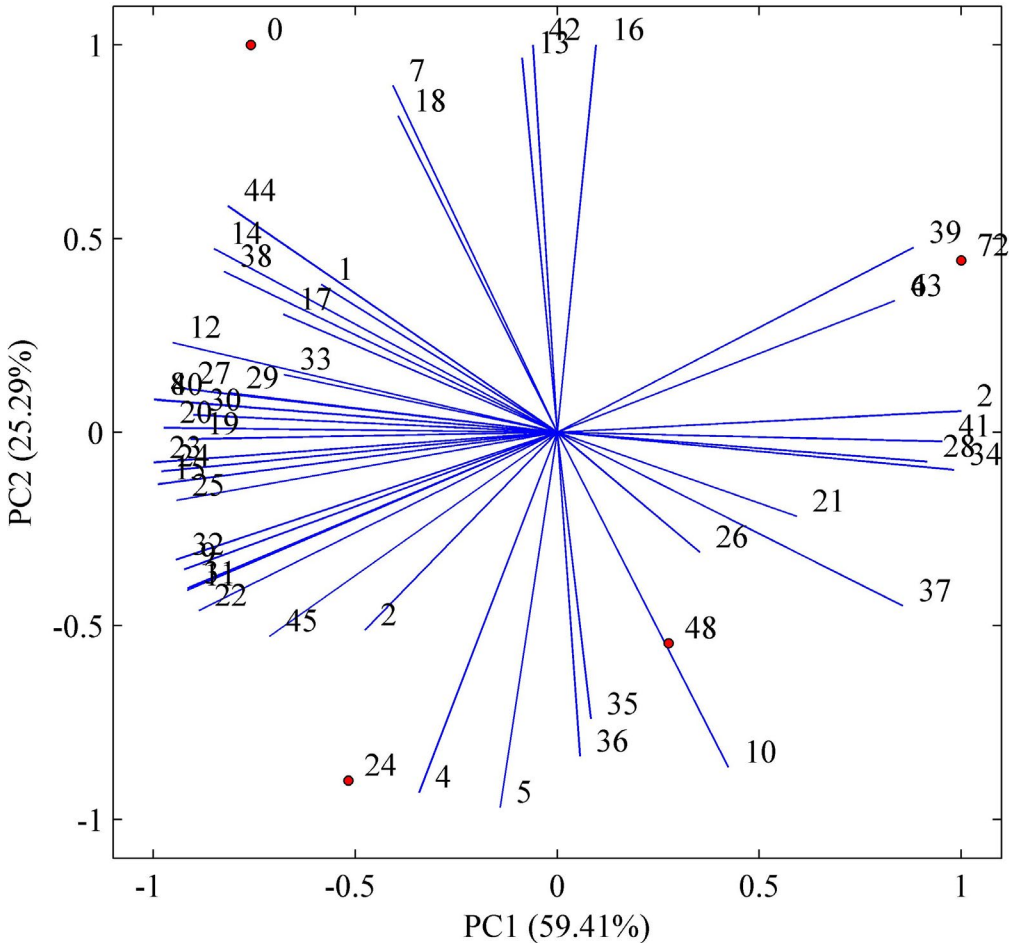

Figure 5: The principal component analysis (PCA) of volatile compounds in the coffee beans subjected to natural fermentation.

Legends: 1- Furan-2-methyl; 2- Ethyl acetate; 3- Butanal; 4- Butanal-2-methyl; 5- Butanal-3-methyl; 6- 1,3-Dioxolane-2,4,5-trimethyl; 7- Furan-2,5-dimethyl; 8- 3 Heptanone-5-ethyl-4-methyl; 9- 5-Nonylamine; 10- Buten-2-ol-2-methyl; 11- 2,3-Pentanedione; 12- 2-Vinylfuran; 13- 1H-Pyrrole-1-methyl; 14- 3,4-Hexanedione; 15- Phenol-3-methyl; 16- Pyridine; 17- Pyrazine; 18- Furan-2-methoxymethyl; 19- 3-Buten-1-ol-3-methyl; 20- Pyrazine-methyl; 21- Thietane-3-methyl; 22- N-nitrosodimethylamine; 23- Pyrazine-2,5-dimethyl; 24- Pyrazine-2,6-dimethyl; 25- Pyrazineethyl; 26- Pyrazine-2,3-dimethyl; 27- 4-Hydroxy-3-hexanone; 28- 2-Hydroxy-3-pentanone; 29- Pyrazine-2-ethyl-6-methyl; 30- 1-Hydroxy-2-butanone; 31- Maleic anhydride; 32- Furfural; 33- 3-Diethylamino-1,2-propanediol; 34- Acetic acid; 35- Furfuryl formate; 36- Ethanone-1,2-furanyl; 37- Pyrrole; 38- Propanoic acid ethenyl ester; 39- 2-furan methanolacetate; 40- 2-Furan carbox aldehyde-5-methyl; 41- Propanoic acid; 42- 1H-pyrrole-2-carboxaldehyde-1-methyl; 43- Butanoic acid-4-hydroxy; 44- 2-Furan methanol; 45- Butanoic acid-3-methyl.

In the present study, the concentrations of chlorogenic acids, caffeine, and trigonelline did not contribute to the differentiation of the coffee samples in terms of the fermentation process and the sensorial quality. According to Koskei et al. (2020), these compounds are not affected by the fermentation process, which was consistent with the results of the present study. The stability of these biochemical compounds during coffee processing is crucial to the quality of the coffee beverage. These compounds play important roles in influencing the aroma and flavor of coffee, as reported by Koskei et al. (2020). However, Haile and Kang (2019) reported an increase in the total polyphenol content of green coffee beans after fermentation using different yeast strains.

Koskei et al. (2020) reported that linoleic acid presented the highest concentration, followed by palmitic acid, stearic acid, oleic acid, and linolenic acid, in the green coffee samples subjected to different processing methods. Similar trends are reported for the concentration of fatty acids in coffee beans by other authors (Figueiredo et al., 2015; Hung; Chen; Chen, 2018). The present study verified certain differences in the fatty acids, which are attributable to several influencing factors, such as the fermentative process, microbial species, climate, geographical origin, and altitude, that affect the chemical matrix and, ultimately, the quality of the final product (Abreu et al., 2019, Figueiredo et al., 2019).

The volatile compound profile is reportedly associated strongly with the aroma and flavor perceptions of the coffee beverage. According to Almeida and Tarabal (2022), the authentic coffee from Cerrado Mineiro, the place where the authors conducted experiments, had intense and diverse flavors ranging from caramel to nutty notes, with delicately citrus acidity and long-lasting chocolaty flavor. This was consistent with the description of the sensory profile of the samples from the 0 and 24-h fermentation treatment groups in the present study. Sensory complexity increases with prolonged spontaneous fermentation. Jimenez et al. (2023) also described all naturally processed and fermented coffees with citrus, caramel, honey, chocolate, nutty, and fermentation (wine) notes.

The coffee samples with the highest sensory scores, i.e., those fermented spontaneously for 48, 72, and 96 h, exhibited the least number of volatile compounds. Toci and Farah (2014) and Toci, Azevedo and Farah (2020) also reported that better-quality coffees have fewer volatile compounds.

The compounds belonging to the class of furan and pyrazine chemical groups had the greatest number of representatives. Furan is a class of chemical compounds that are present in roasted coffee and closely related to its flavor. Furans are produced upon the degradation of carbohydrates during fermentation and confer herbal or fruity notes to the coffee (Liu et al., 2019; Elhalis et al., 2020). A study on naturally processed roasted coffee reported that furfural and 2-furan methanol (Bressani et al., 2018) were associated with fruity notes of coffee.

The compounds 1,3-dioxolane-2,4,5-trimethyl, 2-furan methanol acetate, and butanoic acid were detected in the samples of the 96-h spontaneous fermentation group in the present study. Martinez et al. (2021) also reported the presence of these compounds in the Catuai variety of coffee, in which these compounds conferred caramel, sweet, bitter, coffee, astringent, and burnt flavors, in addition to contributing to the antioxidant activity of the coffee (Bressani et al., 2021; Caporaso et al., 2018; Mota et al., 2022).

Pyrazines are one of the main compounds that impart nutty, earthy, roasted, and green aromas to coffee (Sunarharum; Williams; Smyth, 2014). The compounds pyrazine, 2,5-dimethyl, pyrazine, and 2,3-dimethyl are also considered critical volatile compound markers. In the present study as well, these three compounds were identified in the coffee samples upon natural fermentation.

Furthermore, as revealed in the MFA analysis, the samples fermented for 96 h were characterized by wine-like aromatic notes of coffee pulp and spices, the descriptors that correlated with the volatile compounds detected in these samples (1,3-dioxolane-2,4,5-trimethyl, 2-furan methanol acetate, and butanoic acid; the volatile compounds described as markers of fermented coffee). These results demonstrated the potential of using volatile compound analysis as a rapid method for the verification of the processing and sensory description of coffee post-harvest.

5 CONCLUSIONS

Higher final grades were obtained for the coffee samples subjected to 48, 72, and 96 h of fermentation. Natural fermentation did not modify the contents of trigonelline, caffeine, and chlorogenic acids in the coffee samples. Higher levels of acetic acid and palmitic fatty acid were detected in the 72-h and 96-h natural fermentation groups. The characteristic compounds of fermented coffees, namely, 1,3-dioxolane 2,4,5-trimethyl, 2-furan methanol acetate, and butanoic acid, were detected in the coffee samples fermented for longer durations and conferred wine-like notes and also, to a lesser extent, sweet notes to the beverage.

6 AUTHORS CONTRIBUTION

FMB supervised the experiment, APCA wrote the manuscript and performed the experiment, CMS wrote the manuscript, LH wrote the manuscript, LGAS wrote the manuscript and performed the experiment.

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