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CHEMICAL SCIENCES

Influence of Fermentation Time and Inoculation of Starter Culture on the Chemical Composition of Fermented Natural Coffee Followed by Depulping

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Abstract: Fermentation using starter cultures has been considered an alternative and economically viable technology for the production of specialty coffees. This type of technology promotes several benefits, such as increased sensory quality, control over the fermentation process, predictability of the final product and added value. Coffee (*Coffea arabica* L.) samples for this study were collected in Presidente Olegário - MG (2018/19 crop year) in the Cerrado region of Minas Gerais. The effects of natural fermentation and inoculation of the yeast *Torulaspora delbrueckii* and duration of fermentation (0, 24, 48, 72 and 96 hours) on the sensory and chemical quality (analysis of bioactive, volatile, and organic compounds and fatty acids) of coffee were evaluated. The objective of this study was to determine the effect of fermentation time and starter culture inoculation on the chemical composition of fermented coffees. Fermentation time significantly influenced the sensory description of the coffee beverage, with notes of honey, brown sugar and almond predominating up to 48 hours, for coffees fermented for 72 and 96 hours the notes described were and fruity, winey notes. The chemical composition was primarily influenced by fermentation time.

Key words: *Coffea arabica* L., quality, sensory attributes, specialty coffee, *Torulaspora delbrueckii*.

INTRODUCTION

The concept of the quality of products and services has historically sought to meet consumer expectations and satisfaction; coffee is no exception to this trend. Coffee consumers have become increasingly demanding, desiring unique consumption experiences (Lannigan 2020). Thus, according to this quality trend, differentiated coffees are called specialty coffees. For a batch of coffee to be considered specialty, it must be free of defective beans (hard, black and moldy) and impurities (such as stones, sticks and other strange matter). It should be uniform in bean size and color and, in particular, produce a beverage with unique sensory attributes (Lingle 2011).

In the search for specialty coffees, the research and development of new postharvest processes have grown. Coffee fermentation is among the processes that stand out in this area. Fermentation processes with microorganisms are used in the production of foods and are responsible for preserving and bringing new flavors to the product. Studies have revealed the strong presence of microbiota in coffee fruits throughout processing (Chan & Liu 2022, Ferreira

et al. 2023), indicating the feasibility of using fermentative microorganisms naturally present in the fruit as starter cultures for the production of specialty coffees (Bressani et al. 2018, Chan & Liu 2022, Silva et al. 2013). This microbial diversity (bacteria, yeasts and filamentous fungi) in coffee fruits varies (Elhalis et al. 2020b, Gomes et al. 2024) and is influenced by factors such as fruit variety and composition, environment (specific to each geographical region) and processing method (Martins et al. 2020, Vilela et al. 2010).

Yeasts play an essential role in the fermentation process (Elhalis et al. 2020a). The most reported genera are *Pichia*, *Candida*, *Saccharomyces* and *Hanseniaspora* (Junqueira et al. 2019, Zhang et al. 2019a). There are species specific to each region. In Brazil, as for bacteria, there are a wide range of yeasts present in coffee fermentation, with the predominant species being *Debaryomycess hansenii, Pichia ofunaensis*, *P. fermentans, P. anomala, C. railenensis, C. quercitrusa, C. glabrata, Torulaspora delbrueckii, Rhodotorula, Saccharomyces bayanus mucilaginous, Hanseniaspora uvarium*, *Kloeckera* sp., *Meyerozyma caribbica, Wickerhamomyces ciferrii v* and *W. anomalus* (Evangelista et al. 2015, Silva et al. 2008, Vilela et al. 2010).

The main parameters for evaluating the quality of specialty coffees are the sensory characteristics of the beverage. These parameters depend on the chemical composition of the green coffee beans, such as the organic acids, sugars and aromatic compounds present in the cherries at harvest (Cheng et al. 2016), and on the transformations that occur during fermentation, drying and storage. In the case of organic acids the acids citric, malic, and quinic acids as the most prominent in green coffee and in roasted coffee, the increase in overall acidity compared to green is attributed to an increase of formic, acetic, glycolic, and lactic acids that are formed while roasting (Yeager et al. 2023). Notably, during fermentation, complex microbial interactions occur that are capable of changing the composition of coffee beans and, consequently, their sensory attributes (De Bruyn et al. 2017, Haile & Kang 2019, Pereira et al. 2016).

Thus, coffee quality is evaluated mainly by sensory attributes such as acidity, sweetness, body, bitterness and aroma (Sunarharum et al. 2014). Acidity is positively correlated with the presence of some organic acids (Martins et al. 2019). However, there are undesirable acids that impart an unpleasant flavor and aroma to coffee, such as propionic and butyric acids, which can be produced during uncontrolled fermentation processes, leading to deterioration of the beans (Bressani et al. 2021). The aroma is correlated with reducing sugars, amino acids and chlorogenic acids generated by the hydrolysis of macromolecules metabolized by microorganisms (Lee et al. 2015) during fermentation. In addition, acidity and flavor directly affect the sensory quality of the beverage and have been correlated with the controlled fermentation process (Peñuela-Martínez et al. 2018).

Although fermentation processes have been used for thousands of years, studies are rare specific effects of fermentation time and starter culture inoculation on chemical and sensorial composition of coffees, even though they are factors that can strongly influence the the general profile these coffees. Therefore, the objective of this study was to determine the effects of fermentation time and inoculation of *Torulaspora delbrueckii* CCMA 0684 as a starter culture on the chemical composition of fermented coffees. The *Torulaspora delbrueckii* was chosen because it is yeast capable of modifying the nature of the product, providing a differentiated drink with floral and chocolate

sensory notes and increased sweetness (Bressani et al. 2021, Wang et al. 2020).

MATERIALS AND METHODS

Description of the experiment

The coffee was harvested in the city of Presidente Olegário - MG, Fazenda Catuaí. Fruits of *Coffea arabica*, Mundo Novo variety, were collected; the samples included a high percentage of ripe fruits and were later sent to a mechanical washer to separate fruits of different densities. The separated denser fruits were taken to an African bed for manual separation, and only the ripe fruits were selected (Borém 2022).

This selected ripe fruits was divided into two blocks. In the first block of the experiment, natural fermentation of ripe fruits was performed without inoculation (NF), in anaerobic system. In the other block, a starter culture of the yeast *Torulaspora delbrueckii* CCMA 0684 was inoculated into the mature fruits (YF), followed by anaerobic fermentation.

Inoculation and fermentation process

The starter culture *T. delbrueckii* CCMA 0684 was obtained from the Agricultural Microbiology Culture Collection (CCMA, Universidade Federal Lavras, Lavras, Minas Gerais, Brazil). The yeast was previously isolated from *C. arabica* L. coffee fruits during both wet and dry fermentation processes (Vilela et al. 2010). The yeast (stored in a biofreezer at -80 °C) was reactivated in tubes containing 9 mL of liquid solution [sugarcane juice at 16° Brix, yeast extract 10 $g.L^{-1}$ (Merck, Darmstadt, Germany). The culture was incubated at 28 °C for 48 hours, transferred to 90 mL of solution and incubated at 28 °C and 150 rpm for 24 hours. The yeast cells were then transferred to larger volumes of solution until there were enough cells to inoculate the coffee fruits at approximately 1 $x10^{7}$ $cells.g^{-1}$.

The inoculation procedure with starter culture was performed according to the following steps: the coffee fruits, at room temperature, were placed in a sanitized hermetic container, and the cells were inoculated directly onto them and mixed to ensure homogenization. This procedure aimed to put all the coffee fruits in contact with the yeast in solution. Fermentation was performed in a hermetically sealed cylindrical container with a capacity of 100 L. The mixture (coffee fruits + starter culture) remained in this hermetic container. In both blocks, 15 L samples of fermented coffee fruits were collected after 0, 24, 48, 72 and 96 hours of fermentation. These fermentation times were chosen to verify the development of the chemical and sensorial profile that could be generated in periods of 24 hours.

Pulping

After reaching the desired fermentation time, the coffee proceeded to the de-pulping stage using a “Penagos” mechanical pulper (model “DCV-183 Advance Line”). Pulping was performed without adding water to maintain as much mucilage as possible. After the pulping of each sample, the pulper was cleaned to avoid contamination.

Drying

The pulped natural coffees were spread out in a thin layer of approximately 1.5 cm on the African bed to initiate the drying process. During drying, frequent turning over was performed to ensure uniformity in the process. The bean layer thickness increased gradually as drying progressed, reaching a maximum thickness of 5 cm. Drying was completed when the beans reached a water content between 10.8 and 11.5% (wet basis) (Borém & Andrade 2023). Drying took place over a period of 10 to 12 days.

After drying, the samples were stored in a cold chamber. Sensory and chemical analyses

of the coffee were performed after a 30-day rest period.

Sensory evaluation

The coffee was roasted according to the sensory analysis protocol of the Specialty Coffee Association (SCA) Protocols – Cupping Specialty Coffee (Lingle 2011), the color of the roasted and ground beans corresponded to 58.0 units of the Agtron scale. Roasting time was between 8 min and 12 min, and the samples were roasted no more than 24 h before tasting. Five representative cups of each sample were tasted in each analysis, and the concentration used was 8.25 g of coffee in 150 ml of water, adapted from the SCA sensory analysis protocol. The beans were ground according to the protocol, in which 70% to 75% of the particles must pass through a 20-mesh sieve.

Sensory evaluation of the samples was performed by a sensory panel composed of five trained judges with international Q-grader certification. All sample preparation and roasting procedures were performed according to the recommendations of the SCA Protocols – Cupping Specialty Coffee (Lingle 2011). The sensory analysis was approved by the Ethics Committee on Human Research of the Universidade Federal de Lavras – UFLA, under the following certificate number for ethical consideration (CAAE): 40641620.8.0000.5148.

The cupping form consisted of a predefined list of sensory characteristics for aroma, flavor, acidity and body, and the Check All That Apply (CATA) technique was applied. The aroma and flavor attributes were evaluated using a frame containing 51 sensory descriptors, the acidity attribute was evaluated using a frame containing 6 sensory descriptors, and the body attribute was evaluated using a frame containing 5 descriptors. Each judge was required to choose at least one descriptor for each attribute that best represented the perception of the coffee evaluated, and the judge was also allowed to include other descriptors. These sensory descriptors were determined by a panel of judges in a descriptive terminology development session.

The sensory attributes sweetness, acidity, body, bitterness, astringency and aftertaste were evaluated using a linear intensity scale with boundary values of "0", representing absence, and "10", representing the maximum intensity of the attribute (Salvio et al. 2023).

The overall score was assessed on a scale of 0 to 100 using the methodology of the SCA Protocols – Cupping Specialty Coffee (Lingle 2011).

Organic acid profile

Green coffee beans were ground in an 11A basic grinder for about 1 min, with the addition of liquid nitrogen to prevent oxidation.

The determination of organic acids was performed by high-performance liquid chromatography (HPLC) based on the methodology described by Borém et al. (2023b). These compounds were determined by High Performance Liquid Chromatography (HPLC) using 10 mM perchloric acid at a constant flow rate of 0.6 $mL.min^{-1}$ as mobile phase, and the chromatography column used was C610H at 40 °C, monitored by UV spectrophotometry at 210 nm.

Standard solutions of citric, malic, tartaric, succinic, lactic, quinic and acetic acids were used to identify the chromatogram peaks, to compare their retention times and to calculate their concentrations in the samples. The final levels of organic acids were given as the percentage of dry matter (% w/w).

Bioactive compounds

The nonvolatile compounds, such as caffeine, trigonelline and chlorogenic acid, were determined by high-performance liquid chromatography (HPLC) following the methodology from Borém et al. (2023b). The bioactive compounds of the samples of 0.5 g of ground green coffee (in a basic 11A grinder, for about 1 min, with the addition of liquid nitrogen) were extracted in 50 mL of boiling distilled water and placed in a water bath, with boiling water, for 3 min. The extract was filtered through common filter paper and then filtered through a 0.45 µm membrane. The determination of these compounds was performed in a liquid chromatograph (Shimadzu) with a diode array detection system (model SPD_M10A), Discovery C18 chromatographic column (250 x 4.6 mm, 5 µm), and wavelength of 272 nm. The mobile phase consisted of methanol:water:acetic acid (20:80:1) with a flow rate of 1 $mL.min^{-1}$. A calibration curve was prepared for identification and quantitative analysis using standards of caffeine, trigonelline and 5-caffeolquinic acid (5-CQA).

Fatty acid profile

For the determination of fatty acids, the green coffee beans were ground for about 1 min with the addition of liquid nitrogen in a 11A basic grinder and was used for extraction following the methodology described for Borém et al. (2023b). Fatty acids were determined on a Shimadz GC2010 gas chromatograph with a mass spectrometer detector. An SP-2560 column (Supelco) 100 m x 0.25 mm was used with a temperature gradient of 140 °C, 5 min, 4 $°C.min^{-1}$ to 240 °C, remaining at that temperature for 30 min; Injector (1/20 split), at 240 °C and detector at 240 °C; Helium gas was used as carrier gas (2 $mL.min^{-1}$) and 2 µl injection volume. The identification of peaks corresponding to each fatty acid was carried out by comparing them with Supelco37 methylated fatty acid standards. The final levels were given as a percentage of the relative area.

Volatile compounds profile

The roasted coffee beans were ground in a 11A basic grinder (IKA, Brazil) for about 1 minute; liquid nitrogen was added to facilitate maceration and avoid oxidation of the samples. Two grams of roasted and ground coffee from each sample was placed in hermetically sealed vials for analysis of volatile compounds, according to the method of Rabelo et al. (2021). The GC–MS model QP – 2010 SE (Shimadzu) equipped with an NST-100 column (30 m × 0.25 mm × 0.25 µm) that has a polyethylene glycol phase similar to a Carbowax ® column. The vials containing the samples were placed in the equipment and remained in it for 30 min until reaching equilibrium at 70 °C. The volatile phase was injected into the gas chromatograph (GC) with subsequent detection using a mass spectrometer (MS), and the injector temperature was adjusted to 220 °C. Helium gas, used as a carrier gas for the volatile phase, was maintained throughout the analysis at a flow rate of 1 $mL.min^{-1}$. The heating of the oven was programmed as follows: for 6 min, the temperature of the oven was maintained at 25 °C, right after the temperature was heated up to 70 °C at a rate of 10 °C $°C.min^{-1}$, until 95 °C at 5 °C $°C.min^{-1}$, until 115 °C to 10 °C $°C.min^{-1}$, up to 170 °C to 5 °C min^{-1} and, finally, to 215 °C at 40 °C $°C.min^{-1}$. Therefore, the total chromatographic running time was 35 min.

Data were analyzed and compounds were identified using GCMS solution software (version 4.4, Shimadzu Corporation, Japan) and the NIST NIST/EPA/NIH 2014 database. Chemical substances were identified by comparing the MS spectra with the database. The results were expressed as relative percentage area, which corresponds to the peak area for each

identified compound as a proportion of the total chromatogram area of all peaks detected.

Statistical analysis

The experiment was in a completely randomized design, with a 2 x 5 factorial scheme. Using two fermentation microbiota (natural fermentation and fermentation with inoculation of the yeast *T. delbruekii* CCMA 0684) with five fermentation times (0, 24, 48, 72, and 96 hours). Was conducted with three replications, resulting in a total of thirty samples of fermented natural coffee followed by pulping.

The intensity scale data for the attributes sweetness, acidity, bitterness, astringency, aftertaste and overall score were subjected to analysis of variance (ANOVA), and when significant differences were identified in the F test, the Scott-Knott test was applied using SISVAR statistical software (Ferreira 2011).

Multiple factor analysis (MFA) was applied to the descriptors of aroma, flavor, acidity and body. The analyses were performed according to the methodology described by Salvio et al. (2023), using R software (R Development Core Team 2021).

To better understand the relationship between treatment and the profiles of organic acids, fatty acids, bioactive and volatiles compounds, principal component analysis (PCA) was performed using CHEMOFACE statistical software (Nunes et al. 2012). An mxn matrix was constructed relating the contents of the identified chemical compounds and the assigned sensory attributes to the samples evaluated.

RESULTS

Sensory analysis

Analysis of variance of the sensory outcomes (Table I) showed that fermentation time significantly affected the intensity of the acidity, body, and aftertaste attributes, regard less of the addition of yeast. No significant variations in sweetness intensity were observed. The intensity of acidity differed from 24 hours onward and gradually increased up to 72 hours. The intensity of the body differed after 48 hours of fermentation. The aftertaste became longer and more pleasant after 72 hours of fermentation. Variations in the intensity of the different attributes studied resulted in significant variations in the score of the beverage. After 72 and 96 hours of fermentation, the final scores were significantly higher than the final scores of the samples with up to 48 hours of fermentation, regardless of starter culture inoculation.

The results of multiple factor analysis (MFA) of the aroma, flavor, acidity and body attributes of the natural fermented coffees followed by depulping, with and without inoculation with the yeast *T. delbrueckii* CCMA 0684, are presented in Figure 1.

Table I. Final score (0-100 scale) of beverage quality and mean intensity values (0-10 scale) of the sensory attributes sweetness, acidity, body, astringency, bitterness and aftertaste.

Time (h)	Sweetness	Acidity	Body	Astringency	Bitterness	Aftertaste	Score
0	7.41±0.50^{a}	6.67±0.27^{c}	6.94±0.21^{b}	1.09±0.07^{a}	1.15±0.07^{a}	7.31±0.14^{b}	85.58±0.06^{b}
24	7.64±0.19^{a}	7.11±0.25^{b}	7.01±0.35^{b}	1.09±0.38^{a}	1.01±0.16^{a}	7.22±0.25^{b}	86.90±0.21^{b}
48	7.58±0.09^{a}	7.13±0.30^{b}	7.29±0.55^{a}	1.25±0.26^{a}	1.00±0.26^{a}	7.51±0.33^{b}	86.43±0.46^{b}
72	7.77±0.32^{a}	7.74±0.31^{a}	7.57±0.26^{a}	1.29±0.14^{a}	1.15±0.13^{a}	8.00±0.21^{a}	87.82±0.36^{a}
96	7.55±0.15^{a}	7.45±0.46^{a}	7.58±0.48^{a}	1.36±0.23^{a}	1.20±0.19^{a}	8.03±0.13^{a}	88.31±0.33^{a}

*** Different letters in the columns indicate differences according to the Scott-Knott test (P≤ 0.05).**

It is observed in Figure 1aI that the treatments with natural fermentation (NF) and inoculation with *T. delbrueckii* CCMA 0684 (YF) did not differ significantly from each other in the aroma descriptors because the centroids of the two treatments are close.

The graph corresponding to the analysis of fermentation times (Figure 1aII) reveals that the aromatic profile of coffee depends on the interaction between fermentation time and the microbiota present during the process. Samples fermented for 0, 24, 72 and 96 hours were dispersed in different quadrants of the graph, indicating the effect of fermentation time on the aromatic profile of the coffee. The formation of vectors relative to the centroid at 0, 24 and 96 hours (Figure 1aII) represents significant variations in the aromatic characteristics of coffee inoculated with *T. delbrueckii* CCMA 0684 (YF) compared to the aromatic profile of the samples with natural fermentation (NF).

An absence of vectors was observed between 48 and 72 hours of fermentation, and the aromatic profile of coffee inoculated with *T. delbrueckii* CCMA 0684 (YF) did not differ from the aromatic profile of coffee with natural fermentation (NF).

The correlation circle graph (Figure 1aIII) shows that at the initial fermentation times (0, 24 and 48 hours), the coffee presented the main aromatic characteristics of milk chocolate and nut (almond), especially in the coffees inoculated

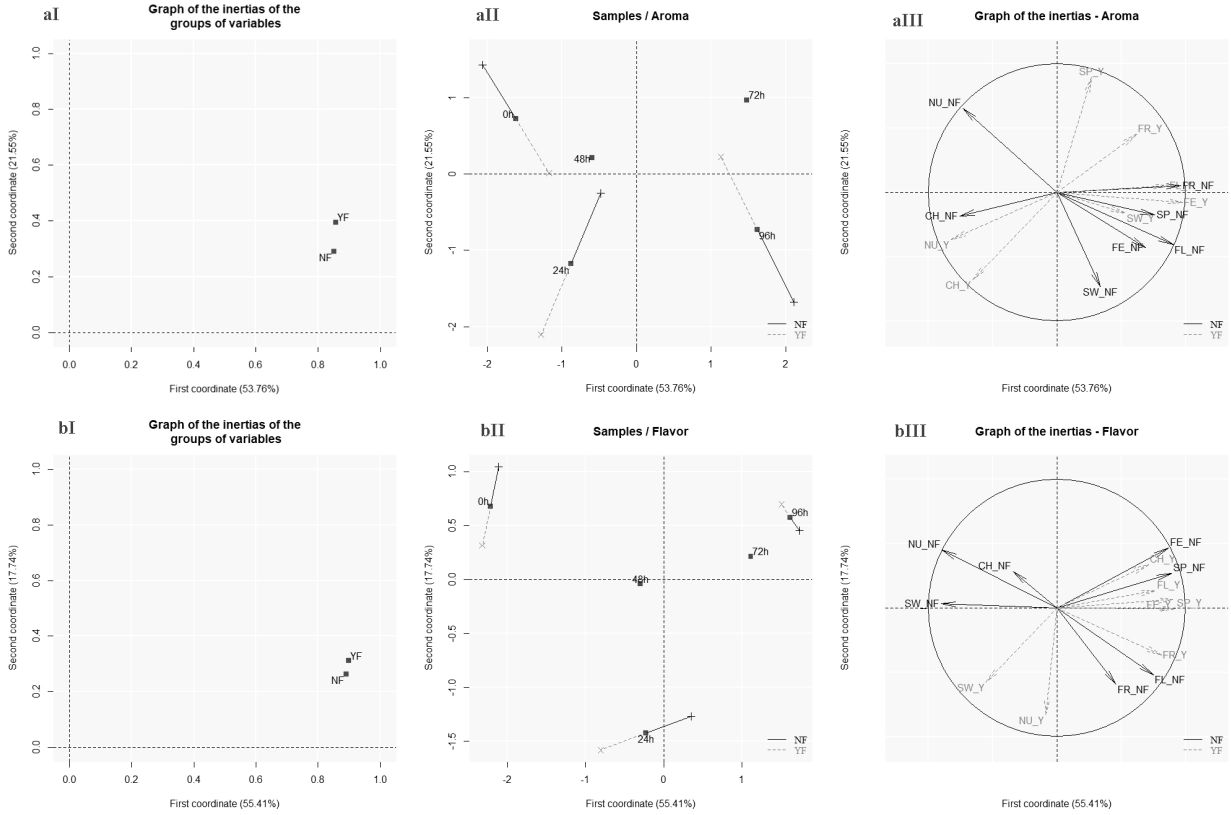

Figure 1. Multiple factor analysis (MFA) of the aroma (a), flavor (b) descriptors in the sensory analysis of fermented natural coffees followed by depulping.
Legend: NF = natural fermentation; YF = fermentation with inoculation with the yeast *T. delbrueckii* CCMA 0684; 0 h = 0 h of fermentation; 24 h = 24 h of fermentation; 48 h = 48 h of fermentation; 72 h = 72 h of fermentation; 96 h = 96 h of fermentation; SW = sweet; NU = nuts; CH= chocolate; SP = spices; EF = fermented; FL = floral; FR = fruity.

with *T. delbrueckii* CCMA 0684. After 72 hours of fermentation, aromatic notes of fermented (vinous, coffee pulp), fruity (tropical fruits), floral and spices predominated, indicating the progress of the fermentation process in the formation of new aromatic compounds.

Fermentation for 96 hours resulted in the greatest aroma differentiation between the inoculated (YF) and natural fermentation (NF) treatments, as indicated by the long and opposite vectors relative to the centroid at this point (Figure 1aII). Analysis of the correlation circle graph for 96 hours of fermentation indicates that natural fermentation (NF) resulted in more intense sweet and fruity aromas than inoculated with yeast (YF). On the other hand, inoculation with yeast intensified the wine notes after 96 hours of fermentation.

When comparing the natural fermentation (NF) and fermentation with *T. delbrueckii* CCMA 0684 (YF), the MFA results for the flavor attribute (Figure 1bI) were similar to the results for aroma, considering the analysis of flavor descriptors.

In the graph corresponding to the analysis of fermentation times (Figure 1bII), the fermentation times are dispersed in different quadrants, as are vectors with significant distance from the centroid, at 0, 24 and 96 hours. This indicates that the coffee flavor profile depends on inoculation and its interaction with fermentation time. In the circle graph of the correlation of flavor characteristics (Figure 1bIII), fermentation times of 0, 24 and 48 hours resulted in flavors with predominantly sweet (caramel and honey) and nutty (almonds) characteristics. At fermentation times of 72 and 96 hours, flavors with notes of fermented (vinous), spices and floral predominated.

Figures 2aI and 1aII show that the treatments with natural fermentation (NF) and inoculation with *T. delbrueckii* CCMA 0684 (YF) did not group together, indicating differences in the acidity attribute as a function of coffee inoculation and fermentation time. The coffees fermented for 0, 24 and 48 hours were characterized by citrus acidity. For the samples fermented for 96 hours, especially in the natural fermentation (NF) treatment (Figure 2aIII), the main descriptor was phosphoric acidity, an acidity characteristic usually considered relatively aggressive and complex.

The inertia graph of the attribute body (Figures 2bI and 2bII) indicates a distinction of the samples in the inoculated fermentation (YF) treatment. Regarding the graph corresponding to the analysis of fermentation times and the treatments with and without inoculation (Figure 2bII), the times of 24, 48 and 72 hours were grouped, indicating their similarity. The point on the graph corresponding to the natural fermentation (NF) sample at 96 hours is located in a different quadrant from the samples fermented for other times, indicating body characteristics different from those of other fermentation times. From the correlation circle graph of body characteristics (Figure 2bIII), it is possible to observe that over the fermentation time, the samples increased in creaminess and viscosity, highlighting the predominance of the viscous body attribute in the samples fermented for 96 hours without inoculation.

Organic acid profile and Bioactive compounds

Citric, malic, quinic, lactic, oxalic, succinic and acetic acids were identified in the beans of fermented natural coffee followed by depulping. The results of principal component analysis (PCA) of the organic acids are shown in Figure 3a.

Two groups were formed as a function of fermentation time: GI comprised the samples with 0 and 24 hours of fermentation; GII comprised the samples with 48, 72 and 96 hours of fermentation. In GI, there was a higher content

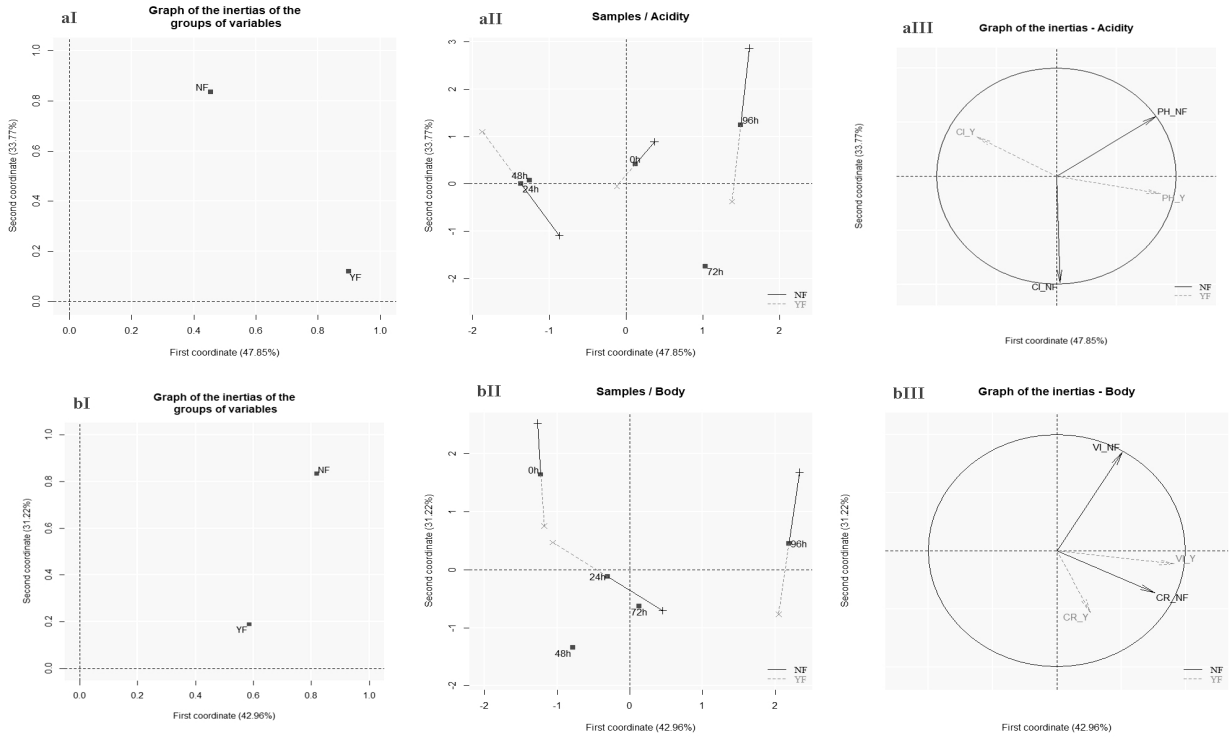

Figure 2. Multiple factor analysis (MFA) acidity (a) and body (b) descriptors in the sensory analysis of fermented natural coffees followed by depulping.
Legend: NF = natural fermentation; YF = fermentation with inoculation with the yeast *T. delbrueckii* CCMA 0684; 0 h = 0 h of fermentation; 24 h = 24 h of fermentation; 48 h = 48 h of fermentation; 72 h = 72 h of fermentation; 96 h = 96 h of fermentation; CI = citric acidity; pH = phosphoric acidity; LI = liqueur; VI = viscous; CR= creamy.

of malic acid. In GII, the samples fermented for longer fermentation times had higher levels of lactic, succinic and acetic acids. The highest levels of acetic acid were associated with the treatments with 96 hours of fermentation. The concentration of citric acid did not contribute to the differentiation of the groups.

It is possible that the increased perceived intensity of the acid taste at longer fermentation times is associated with the increased contents of organic acids, especially lactic, succinic and acetic acids, predominantly under inoculation with yeast.

In addition to the analysis of organic acids, analyses of bioactive compounds, fatty acids and volatile compounds were performed. The analytical results for the bioactive compounds in coffee beans fermented natural coffee followed by depulping are presented in Figure 3b.

In general, there were no significant variations in the contents of trigonelline, chlorogenic acid and caffeine in this study. This means that the use of different fermentation techniques, natural (NF) or with inoculation of yeast *T. delbrueckii* CCMA 0684 (YF), does not affect the levels of the bioactive compounds analyzed.

Fatty acid profile

Nine fatty acids were identified (Figure 4) in all samples analyzed in the different experiments. The fatty acids found were myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0), and behenic (C22:0) saturated fatty acids; the monounsaturated fatty acids were oleic (C18:1), and erucic (C22:1) acids; and the polyunsaturated

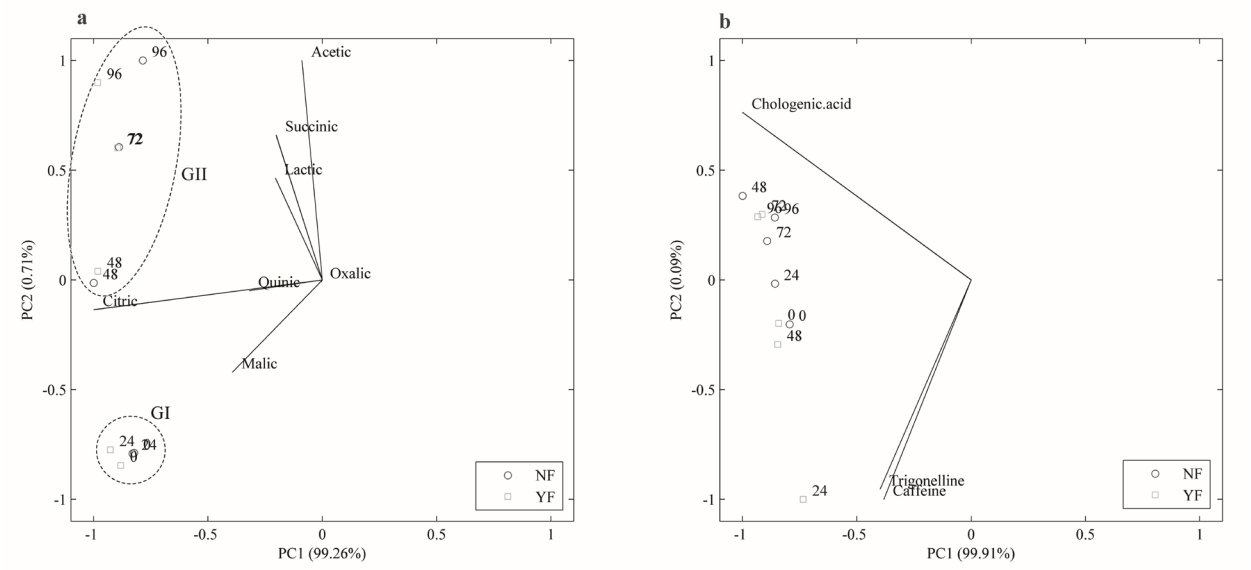

Figure 3. Principal component analysis (PCA) of organic acids and bioactive compound of fermented natural coffees followed by depulping.
Legend: NF = natural fermentation; YF = fermentation with inoculation with the yeast *T. delbrueckii* CCMA 0684; 0 h = 0 h of fermentation; 24 h = 24 h of fermentation; 48 h = 48 h of fermentation; 72 h = 72 h of fermentation; 96 h = 96 h of fermentation. a – PCA- organic acids; b- PCA- bioactive compound.

fatty acids were linoleic (C18:2) and linolenic (C18:3) acids.

The analyzed fatty acids did not yield a clear grouping of samples according to the treatments studied for natural coffee fermentation followed by pulping, considering the levels of fatty acids in the analyzed samples.

Volatile compound profile

Figure 5 shows the results of the analysis of volatile compounds in coffee beans natural fermented followed by depulping.

The main factor that influenced the profile of volatile compounds was the fermentation time, regardless of whether or not there was inoculation. Contrary to expectations, a lower amount of volatile compounds was observed in the longer fermentation processes, possibly due to the degradation and consumption of these compounds by microorganisms during the fermentation of coffee fruits. It is also possible that the fermentation could be influence the generation of non-volatile aroma precursors in coffee beans, which impact could be manifested by intensive roasting in the development of volatile aromatic compounds and sensory perception of fermented and unfermented coffee (Wu et al. 2024)

The main compound responsible for the separation of the samples in time was pentanoic acid, 3-methyl described by The Good Scents Company (2023) as an apple or strawberry odor or as a strong sour odor if found in large concentrations. This compound was found in higher concentrations in the treatments with 96 hours of fermentation. These results are in agreement with the MFA results of sample aroma. According to the MFA, the aromas of coffees fermented after 72 hours were predominantly winey and fruity (yellow and red fruits), and after 96 hours of fermentation, floral and spicy notes predominated.

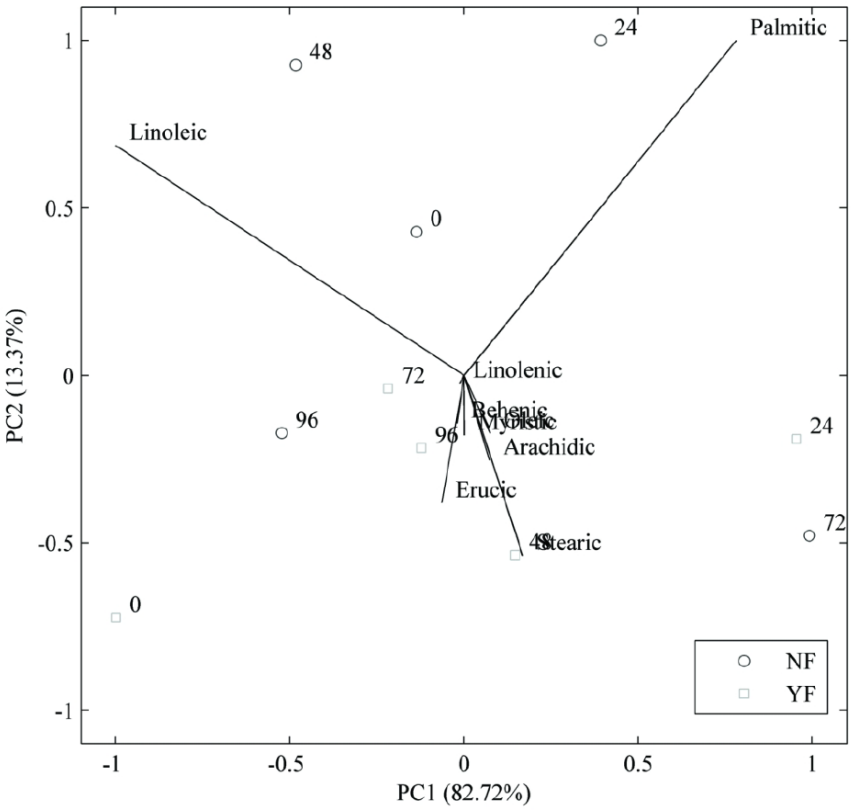

Figure 4. Principal component analysis (PCA) of the fatty acid profile of fermented natural coffees followed by depulping. Legend: NF = natural fermentation; YF = fermentation with inoculation with the yeast *T. delbrueckii* CCMA 0684; 0 h = 0 h of fermentation; 24 h = 24 h of fermentation; 48 h = 48 h of fermentation; 72 h = 72 h of fermentation; 96 h = 96 h of fermentation.

DISCUSSION

From the evaluation of the sensorial and chemical results, it was possible to notice that the samples did not differentiate for natural fermentation and with inoculation of *Torulaspora delbrueckii*. The important differentiation occurs with the fermentation time and it is possible in all aspects analyzed to observe the similarity of the samples fermented at the same time regardless of whether there was yeast inoculation or not.

Predominance of sweetness (brown sugar, caramel and honey), nuts (almonds), chocolate (milk) and herbaceous characteristics were observed at 0, 24 and 48 hours of fermentation. At 72 and 96 hours, other flavors, such as fruity (yellow and red fruits), fermented (winy and alcoholic) and spices, developed.

The organic acids produced by microorganisms during the fermentation process of coffee fruits can influence the sensory characteristics of the beverage in different ways. Butyric, acetic and propionic acids, products of over fermentation, can affect the final quality of the beverage, imparting an oniony and sour taste when present in concentrations higher than 1 $mg.mL^{-1}$ (Farah & Lima 2019, Silva et al. 2013), just as the increase in the overall acidity of roasted coffee is attributed to an increase in formic, acetic, glycolic and lactic acids that are formed during roasting (Yeager et al. 2023). On the other hand, the production of lactic acid can aid in the acidification process of coffee pulp, favoring the development of certain microorganisms without interfering with the final quality of the product (Pereira et al. 2015). Coffee acidity is an important organoleptic parameter that may or may not be desirable, depending on the nature and concentration of the predominant acid in the beverage.

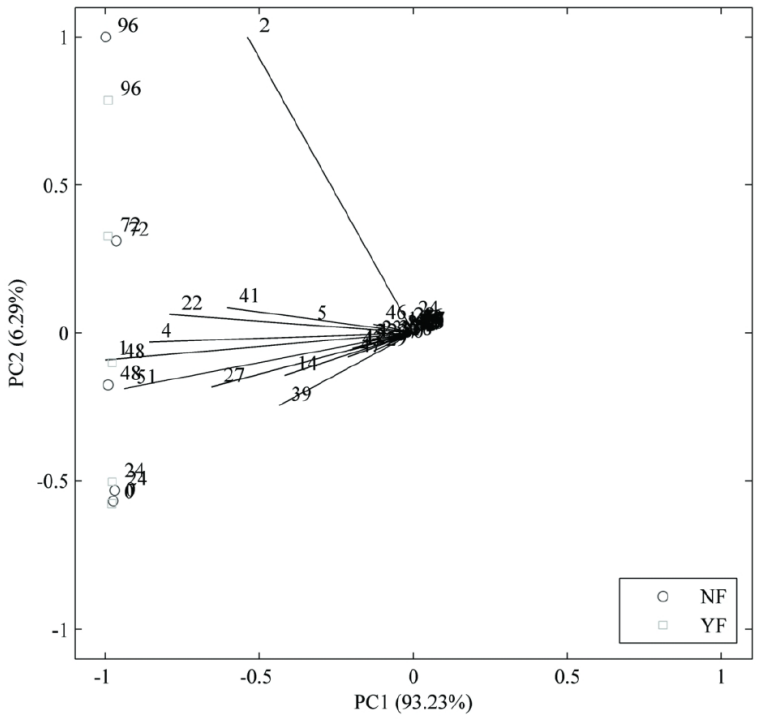

Figure 5. Principal component analysis (PCA) of the volatile fatty compounds of fermented natural coffees followed by depulping.
Legend: NF = natural fermentation; YF = fermentation with inoculation with the yeast *T. delbrueckii* CCMA 0684; 0 h = 0 h of fermentation; 24 h = 24 h of fermentation; 48 h = 48 h of fermentation; 72 h = 72 h of fermentation; 96 h = 96 h of fermentation; 1 =Furan, 2-methyl; 2 = Pentanoic acid, 3-methyl; 3 =Butanal; 4 =Butanal, 2-methyl; 5 =Butanal, 3-methyl; 6 - 2H-Pyran, 3,4-dihydro; 7 = 13-Dioxolane, 2,5-trimethyl; 8 = Furan, 2,5-dimethyl; 9 =Propanal, 2,2-dimethyl; 10 = 2,4-Dimethylfuran; 11 = 3-Heptanone, 5-ethyl, 4-methyl; 12 = 5-Nonylamine; 13 = 3 Buten-2-ol, 2-methyl; 14 = 2,3-Pentanedione; 15 = Vinylfuran; 16 = 2,2-Dimethyl, 3- hydroxypropionaldehyde, 17 = 2-Pentene, 2-methyl; 18 = 1H-Pyrrole, 1-methyl; 19 = 3,4-Hexanedione; 20 = Phenol, 3-methyl; 21 = 1H-Pyrrole, 1-ethyl; 22 = Pyridine; 23 = Pyrazine; 24 = 1-Pentanol; 25 = Furan, 2-methoxymethyl; 26 = 3-Buten-1-ol, 3-methyl; 27 = Pyrazinemethyl; 28 = Imidazole, 4-acetic acid; 29 = N-Nitrosodimethylamine; 30 = Pyrazine, 2,5-dimethyl; 31 = Pyrazine, 2,6-dimethyl; 32 = Pyrazine, ethyl; 33 = Pyrazine, 2,3-dimethyl; 34 = 4-Hydroxy-3-hexanone; 35 = 2-Hydroxy-3-pentanone; 36 = Pyrazine, 2-ethyl-6-methyl; 37 = 1-Hydroxy-2-butanone; 38 = Maleic anhydride; 39 = Furfural; 40 = 4-Ethylamino-N-butylamine; 41 = Acetic acid; 42 = Furfurylformate; 43 = Ethanone, 1,2-furanyl; 44 = 1-Pentyne, 4-methyl; 45 = 2,3-Pentanedione; 46 = 2-Furan-methanolacetate; 47 = 2-Furan-carboxaldehyde, 5-methyl; 48 = Propanoic acid; 49 = 1H-Pyrrole, 2-carboxaldehyde-1-methyl; 50 =Butanoic acid, 4-hydroxy; 51 = 2-Furan methanol; 52 =Butanoic acid, 3-methyl.

A desirable acidity can contribute to the liveliness of the coffee beverage, increasing the perception of other attributes such as sweetness. However, exaggerated acidity can be unpleasant and can be related to unusual tastes in the beverage (Illy & Viani 2005, Lingle 2011). In this study, citric, oxalic, malic, quinic, succinic, lactic and acetic acids were identified in all treatments.

Fermentation time also has a direct effect on the intensity of acidity and is considered the main factor for the increase in and diversification of this attribute in coffee beverages. After 72 hours of fermentation, the acidity of the beverage increased significantly, regardless of the addition of yeast *T. delbrueckii* CCMA 0684. The acidity was characterized predominantly as citrus in samples with up to 48 hours of

fermentation and as malic and phosphoric acid in samples with 72 and 96 hours of fermentation.

The reduction in malic acid concentration may have occurred through the conversion of malic acid into lactic acid by bacteria that occur naturally in coffee (Evangelista et al. 2015). Succinic acid can contribute differently to the final product (Bressani et al. 2020). In the literature, studies are found that report that in the fermentation of grapes for making wine, the starter culture *Torulaspora delbrueckii* is one of the largest producers of succinic acid when compared to other cultures (Ciani & Maccarelli 1998, Puertas et al. 2017). This work does not demonstrate the difference in succinic acid between coffees fermented with the starter culture and natural fermentation, but it justifies the description of vinyl notes achieved for 96-hour coffees fermented with the starter culture (Benito 2018).

Other authors working with the same yeast strain in pulped natural and natural coffees found similar results (Bressani et al. 2021, Chan & Liu 2022, Ferreira et al. 2023, Martinez et al. 2019, Mota et al. 2020, Rocha et al. 2023). Studies have also concluded, as observed in the present study, that the results are more affected by fermentation time than by inoculation with yeast (Mota et al. 2020, Zhang et al. 2019b).

Analysis of the fatty acid profile of the green coffee beans expands the understanding of coffee quality (Rocha et al. 2023). Because, in addition to participating in reactions in the roasting process, during this stage, fatty acids migrate to the outside of the grain, reducing volatilization and loss of aromatic compounds, while contributing to the balance, body and residual flavor of the beverage (Mehari et al. 2019).

The literature has little information relating the fatty acid profile with the sensory quality of the beverage. Figueiredo et al. (2015) found a positive relationship between arachidic, stearic and palmitic saturated fatty acids and the sensory characteristics of coffee beverages and a negative relationship with elaidic unsaturated fatty acids. The authors suggest that these fatty acids may be possible markers of coffee quality.

The oil in coffee beans is composed mainly of triacylglycerols, which have fatty acids in proportions similar to those found in edible oils of vegetable origin (Speer & Kölling-Speer 2006). This oil provides an oily and creamy sensation in the mouth, characteristic of the coffee beverage. This sensation is caused by the ability of oils to cover the surface of the tongue during beverage ingestion (Illy & Viani 2005). In agreement with these studies, the sensory attribute body was positively correlated with palmitic, stearic, arachidic and behenic saturated fatty acids.

The fruity, fermented and floral aromas present in coffee and its beverages are most often derived from the action of microorganisms, i.e., from fermentation processes that use coffee mucilage as a substrate for the production of volatile compounds related to these aromas (Zhang et al. 2019a, b). These characteristics were generally found in the descriptions of the samples in this study.

There are two factors that contribute to the formation of volatile compounds in coffee beans: compounds inherent in the bean itself and microbial metabolites resulting from fermentation processes (Yeretzian et al. 2002). Yeasts produce many low-molecular-weight volatile compounds during the mucilage fermentation process, such as esters, higher alcohols, aldehydes, ketones and terpenoids. Among these compounds, esters (ethyl and acetate esters) are the most abundant group of volatile compounds formed (Pereira et al. 2019). Recent study reported that inoculation with *T. delbrueckii* C0684 in natural coffee produced 2-phenyl-2-butenal, 5-amino-1-naphthol,

3,4-dimethyl-2-pentanone, and 1-hydroxy-2-propanone (Mota et al. 2020). The compound 1-hydroxy-2-butanone is described as having fruity and sweet notes, and was also found in the fermented coffee samples in this research.

The compounds butanal, 2-methyl, associated with malt notes; and pyridine, associated with descriptors such as roast and green notes, were found. The compound 3-methyl pentanoic acid was also found to be associated with apple and strawberry flavors. On the other hand, characteristic compounds of fermented coffees, such as 1,3-dioxolane, 2,4,5-trimethyl, 2-furan methanol acetate and butanoic acid, were found in coffee samples with longer fermentation times and conferred winey and less sweet notes to the beverage.

In general, the main factor that influenced the profile of volatile compounds was the fermentation time. Coffees subjected to long fermentation periods had lower amounts of volatile compounds. Furthermore, these coffees were characterized with significantly better final scores than other fermentation times. This fact corroborates the results found by Borém et al. (2023a) in which, the coffee samples with the highest sensory scores, i.e., those fermented spontaneously for long times, exhibited the least number of volatile compounds. Other researchers also reported that better quality coffees have fewer volatile compounds in unfermented coffees (Toci et al. 2020).

CONCLUSIONS

The main conclusions obtained from the results are as follows:

Fermentation time significantly influenced the sensory description of the coffee beverage, with notes of honey, brown sugar and almond predominating up to 48 hours, for coffees fermented for 72 and 96 hours the notes described were and fruity, winey notes;

The fermentation time increased and changed the acidity of the beverage, with organic acids such as citric, oxalic, malic, quinic, succinic, and lactic contributing to this perception;

The volatile compound profile, a fundamental aspect of chemical composition, was primarily influenced by fermentation time.

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