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Influence of anaerobic fermentation and yeast inoculation on the viability, chemical composition, and quality of coffee

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ABSTRACT

Microbial metabolites produced during fermentation migrate into the coffee and can influence the decrease in seed viability and coffee quality. This study evaluated the effects of physiological changes in seed viability on the sensory quality of the beverage using starter cultures through self-induced anaerobic fermentation (SIAF) in *Coffea arabica* L. for natural and pulped coffee. The yeasts were monitored by real-time polymerase chain reaction (qPCR). High-performance liquid chromatography (HPLC) detected citric, malic, and succinic acids in all fermented coffees. Furthermore, lactic acid was mainly identified in those coffees processed by the SIAF method. Volatile compounds (40) were detected by gas chromatography-mass spectrometry (GC-MS). Alterations in physiological quality were identified, with decreased embryonic viability and cell membrane damage by tetrazolium and electrical conductivity tests. All fermented coffees obtained scores above 80 points. The *Torulospora delbrueckii* yeast got the best score (86.50) in pulped coffee, and the *Candida parapsilosis* yeast received the highest score (85.90) in the natural coffee using the SIAF method. The coffees were characterized by aromas and flavors of citrus, caramel, honey, chocolate, and chestnut. The coffee fermentation process with yeast inoculation affected the coffee bean viability but not the beverage's sensory quality, indicating that the use of yeast starters by SIAF favors the production of specialty coffees with differentiated sensory characteristics.

1. Introduction

Specialty coffees have changed international trade in recent years, from selling regular coffee to a special product (Sittipod et al., 2019), causing an increase in their popularity in global markets and driving the search for new postharvest technologies that help the production of specialty coffees (Córdoba et al., 2021). Traditionally, postharvest processing of coffee is carried out using natural, pulped, and wet methods, which are considered responsible for some differences observed in the sensory quality of coffee (Nadaleti et al., 2022; Selmar et al., 2014). During postharvest processing, fermentation occurs spontaneously by epiphytic microorganisms (bacteria, yeasts, and fungi), which process the coffee mucilage (Silva et al., 2013). The production of microbial metabolites can reach the interior of the seed, thus leading to beneficial (organic acids of interest, esters, alcohols, sugars) or harmful effects (undesirable organic acids and toxins) on the quality of coffee beans (Elhalis et al., 2021; Hadj Salem et al., 2020; Wang et al., 2019, 2022). In addition, during the postharvest period, physiological processes associated with germination begin inside the seed (respiration and cell division). As a result, they can use energy reserves, putting the embryo's viability at risk and causing the risk of a negative impact on the sensory quality of the coffee beverage (da Silva et al., 2019; Kitzberger et al., 2020; Selmar et al., 2006, 2014).

When the coffee fruit reaches maturity, the seed is ready to begin germinating. However, this metabolic process is inhibited by a dormancy process caused by natural inhibitors (abscisic acid), the shell (a protective barrier that creates an impenetrable environment), and the lack of environmental conditions (moisture, oxygen, light, and

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temperature) (Taiz et al., 2017). During the postharvest period, the start of the metabolic processes of germination may be linked to the appropriate conditions and the elimination of natural inhibitors present in coffee (Selmar et al., 2006; Taiz et al., 2017).

The use of starter cultures (mainly yeast strains) has emerged as a promising option to improve the quality and modify the sensory profile of coffee (Cassimiro et al., 2022; Elhalis et al., 2021; Mahingsapun et al., 2022). These microbial starters may be used in closed bioreactors, thus creating an anaerobic environment that allows for self-induced anaerobic fermentation (SIAF) (da Mota et al., 2022; Pereira et al., 2022). The SIAF method consists of gradual CO_2 production driven by microbial metabolism, improving the fermentative performance of lactic acid bacteria (LAB) and yeasts and increasing the production of metabolites (organic acids, alcohols, and volatile composts) that intensify the aromas and flavors desired in coffee (Cassimiro et al., 2022; Martinez et al., 2019).

Consequently, the present study aimed to evaluate the effects of decreased seed viability on the sensory quality of the coffee beverage for natural and pulped postharvest coffee (*Coffea arabica* L. variety Topázio Amarelo) processed using starters (*Saccharomyces cerevisiae* CCMA0543, *Candida parapsilosis* CCMA0544, and *Torulospora delbrueckii* CCMA0684) by SIAF compared with that of coffee processed by a SIAF control and a conventional process (without bioreactors) through physiological, real-time polymerase chain reaction (qPCR), chemical and sensory analysis.

2. Materials and methods

2.1. Coffee

Coffee cherries (*Coffea arabica* L.) of the 'Topazio Amarelo' variety were harvested at an altitude of 850 m above sea level at the farm Cafés Monte Alegre (S 1°59′56″, W 54°4′58″) in Alfenas, Minas Gerais (M.G.), Brazil.

2.2. Yeast

The *Saccharomyces cerevisiae* (CCMA0543), *Candida parapsilosis* (CCMA0544), and *Torulaspora delbrueckii* (CCMA0684) yeasts belonging to the Agricultural Microbiology Culture Collection (CCMA) of the Department of Biology at UFLA, Lavras, M.G., Brazil, were selected as starter cultures for their ability to survive throughout the fermentation process and for generating chemical and sensory changes that improve coffee quality (Silva et al., 2013). The yeasts stored at −80 °C were reactivated and grown according to Martins et al. (2022). Cells were recovered by centrifugation (3200×*g*; 10 min) and resuspended in sterile water (500 mL) until reaching a concentration of 10^7 and 10^8 cells/mL in coffee.

2.3. Coffee fermentation processing

The natural and pulped coffee (40 L) was processed without and with yeast inoculation in high-density polyethylene cylindrical bioreactors with a 50 L capacity using the method of SIAF (da Mota et al., 2022; Pereira et al., 2022). Five treatments were performed: conventional processing (the traditional process where the coffee is harvested and then dried in the sun on cement platforms, with the addition of pulping for pulped coffee), SIAF control (without inoculation), *S. cerevisiae* (CCMA0543), *T. delbrueckii* (CCMA0684), and *C. parapsilosis* (CCMA0544). All the treatments were carried out in triplicate. During fermentation, the temperature and humidity of the environment and the temperature and concentration of coffee solids (% TSS) were monitored until stabilized (Table 1) (da Mota et al., 2022; Pereira et al., 2022). The fermentation time for both natural and pulped coffee was 180 h, and the end of fermentation occurred by decreasing the coffee temperature (Table 1). Samples were taken at the beginning of fermentation (0 h) and at the end of drying (480 h). The coffee was dried immediately in the sun on suspended terraces until reaching 11% moisture.

Table 1
Monitoring room temperature, moisture, coffee temperature and total soluble solids (% TSS) at 0, 96 and 180 h of fermentation.

Coffee processing	Treatments	Time (h)	Room temperature (°C)	Moisture (%)	Coffee temperature (°C)	% TSS
Natural coffee	Control (SIAF)	0	20	45	16	25
	S. cerevisiae (SIAF)	0	20	45	16	25
	T. delbrueckii (SIAF)	0	20	45	16	25
	C. parapsilosis (SIAF)	0	20	45	16	25
	Conventional process	0	19	47	15	25
	Control (SIAF)	96	19	44	19	20
	S. cerevisiae (SIAF)	96	18	45	19	18
	T. delbrueckii (SIAF)	96	18	45	19	20
	C. parapsilosis (SIAF)	96	18	46	19	18
	Conventional process	96	17	51	15	23
	Control (SIAF)	180	20	45	18	nd
	S. cerevisiae (SIAF)	180	20	45	18	nd
	T. delbrueckii (SIAF)	180	20	45	18	nd
	C. parapsilosis (SIAF)	180	20	45	18	nd
	Conventional process	180	20	45	16	nd
Pulped coffee	Control (SIAF)	0	19	47	16	26
	S. cerevisiae (SIAF)	0	19	48	16	26
	T. delbrueckii (SIAF)	0	17	48	16	26
	C. parapsilosis (SIAF)	0	17	48.5	16	26
	Conventional process	0	17	49	15	26
	Control (SIAF)	96	21	40	19	19
	S. cerevisiae (SIAF)	96	20	40	18	15
	T. delbrueckii (SIAF)	96	20	43.5	18	17
	C. parapsilosis (SIAF)	96	17	49	19	18
	Conventional process	96	17	49	16	nd
	Control (SIAF)	180	20	45	18	nd
	S. cerevisiae (SIAF)	180	20	45	18	nd
	T. delbrueckii (SIAF)	180	20	45	18	nd
	C. parapsilosis (SIAF)	180	20	45	18	nd
	Conventional process	180	18	47	17	nd

SIAF: self-induced anaerobic fermentation.

2.4. Monitoring of inoculated yeast populations

The inoculated yeast population was monitored by qPCR using a QIAamp DNA Mini Kit (Qiagen, Hilden Germany) to extract DNA from sample strains at the end of processing (480 h). All yeast species were cultured separately on YEPG agar at 28 °C for 24 h. They were serially diluted (1:10) from 10^8 to 10^3 cells/mL, measuring each point of the standard curve in triplicate for use in qPCR (Batista et al., 2015). For this analysis, it was necessary to use specific primers (Supplementary Table S1) for each yeast species.

2.5. Analysis of chemical compounds

2.5.1. Determination of organic acids by high-performance liquid chromatography (HPLC)

Organic acids (acetic, citric, lactic, malic, oxalic, succinic, and tartaric) were evaluated by HPLC (Shimadzu Corp., Japan) with a UV detector at 210 nm. The natural and pulped coffee was evaluated at the beginning and end of drying (0 and 480 h). The operating conditions were described by Evangelista et al. (2014). For each sample, 10 g of coffee was mixed twice with 10 mL of Milli-Q water for 5 min, and the solutions (20 mL) were centrifuged at 12,745×*g* for 10 min at 4 °C. The pH value of the samples was adjusted to 2.11 with perchloric acid and centrifuged again. Then, the supernatant was filtered through 0.22 μm cellulose acetate membranes. The samples obtained were stored at −18 °C until analysis.

2.5.2. Analysis of volatile compounds by headspace/solid-phase (HS-SPME) microextraction/gas chromatography/mass spectrometry (GC/MS)

Volatile compounds were extracted from roasted coffee and green coffee at the end of postharvest processing using a manual headspace solid-phase microextraction (HS-SPME) procedure and a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane SPME fiber (Supelco Co., Bellefonte, PA., USA) (Evangelista et al., 2014). Two grams of coffee from each treatment were ground with liquid nitrogen and placed in a 15 mL hermetically sealed flask. After equilibration at 60 °C for 15 min, the volatile compounds were extracted at 60 °C for 30 min. Injections were performed by fiber exposition for 2 min. A GCMS-QP2010 (Shimadzu) equipped with mass spectrometry (MS) and a silica capillary Carbo-Wax 20 M (30 m × 0.25 mm x 0.25 mm) column were used for GC/MS analysis. The oven temperature was held at 60 °C for 5 min, followed by a gradient from 60 °C to 230 °C at 10 °C/min, and held at 230 °C for 15 min. The carrier gas (He) was used at a flow rate of 1.95 mL/min. The volatile compounds were identified by comparing the mass spectra to the NIST11 library. In addition, an alkane series (C10–C40) was used to calculate each compound's retention index (RI) and compare them to the scholarly literature's RI values.

2.6. Tetrazolium test

Fifty seeds from each fermentation protocol with four replicates were soaked in water (36 h). The embryos were then extracted and kept in a polyvinylpyrrolidone (PVP) antioxidant solution until submerged in a 0.5% 2, 3, 5 triphenyl chloride tetrazolium solution in dark flasks at 30 °C for 2 h. Finally, a longitudinal cut was made in the middle of the embryos to perform the viability analysis of the embryo with a 10x stereoscopic magnifying glass to visualize its internal and external structures, better classifying them as viable and nonviable according to the location and extension of the stained areas (Clemente et al., 2012).

2.7. Electrical conductivity (E.C.)

Four replicates of 25 seeds of each fermentation treatment were first weighed and then soaked in containers with 37.5 mL of deionized water, keeping them in BOD at a constant temperature of 25 °C for 24 h. After that period, the electrical conductivity of the solution containing the seeds was read in $\mu S.cm^{-1}. g^{-1}$ (Malta et al., 2005).

2.8. Sensory evaluation

Five trained coffee tasters with Q-Grader certificates evaluated the coffee samples, following the sample preparation protocol of the Specialty Coffee Association. The following attributes were evaluated: fragrance, aroma, flavor, aftertaste, acidity, body, uniformity, balance, sweetness, cleanliness, defects, and general score according to the SCA Cupping Protocol (SCA, 2018, p. 14).

2.9. Statistics

The treatments were applied through a 5x2 mixed factorial design (yeast x processing method) to evaluate sensory analysis, organic acids, electrical conductivity, and tetrazolium test. The tests for organic acids, electrical conductivity, and tetrazolium were also compared before (0 h) and after the fermentation process (480 h) using Student's *t*-test. A 3x2 mixed factorial design (yeast x processing method) was used for qPCR analysis at the end of processing (480 h) to monitor the inoculated yeast population. An analysis of variance (ANOVA) was performed using the Scott Knott test with a 5% significance level ($p \leq 0.05$) and with a significance level of $p \leq 0.088$ for the tetrazolium test, using the Sisvar software version 5.6 (Ferreira, 2014). In addition, a principal component analysis (PCA) was performed for the volatile compounds using the SensoMaker program v. 1.92 (UFLA, Lavras, Brazil) (Pinheiro et al., 2013).

3. Results

3.1. Population of yeasts by qPCR

The population of *S. cerevisiae, C. parapsilosis,* and *T. delbrueckii* yeasts at the end of processing was evaluated by qPCR (Fig. 1). The inoculated yeasts grew and maintained viability during fermentation. The growth of the *C. parapsilosis* population was not stimulated by using the SIAF method, and it was minimally present in the conventional process. In inoculated fermentation using SIAF, a higher population of *T. delbrueckii* (8.10 $\log_{10}$ cells/g) was observed in the natural coffee, while *S. cerevisiae* (8.21 $\log_{10}$ cells/g) showed the highest population in pulped coffee. The inoculated fermentation with *T. delbrueckii* reached 8.10 $\log_{10}$ cells/g, followed by *S. cerevisiae* with 7.71 $\log_{10}$ cells/g for the natural coffee method. In the pulped method, the treatment inoculated with *S. cerevisiae* had the highest population with 8.21 $\log_{10}$ cells/g, followed by *T. delbrueckii* with 7.31 $\log_{10}$ cells/g.

In addition, the monitoring of the yeasts in the SIAF control treatment showed that the yeast *S. cerevisiae* obtained the highest presence in the coffees without inoculation, with 6.98 $\log_{10}$ cells/g in the natural coffee and 7.89 l $\log_{10}$ cells/g in the pulped coffee. Followed by the *T. delbrueckii* yeast with 7.21 $\log_{10}$ cells/g in natural coffee and 6.93 $\log_{10}$ cells/g in pulped coffee, and the yeast with the lowest population present was *C. parapsilosis* with a population of 3.32 $\log_{10}$ cells/g in natural coffee and 2.98 $\log_{10}$ cells/g in pulped coffee.

The conventional processing was the treatment with the lowest yeast population in both methods. For example, the yeast *S. cerevisiae* with 2.44 $\log_{10}$ cells/g obtained the highest population in the natural coffee, and the yeast *C. parapsilosis* with 2.92 $\log_{10}$ cells/g had the highest population in the pulped coffee.

3.2. Evaluation of organic acids

The acids (acetic, citric, lactic, malic, oxalic, succinic, and tartaric) are present in the coffee at the beginning (freshly harvested coffee before fermentation for 0 h) and at the end (end of drying for 480 h) of processing were evaluated in natural and pulped coffees. At the beginning of the processing (freshly harvested coffee), citric, malic, and

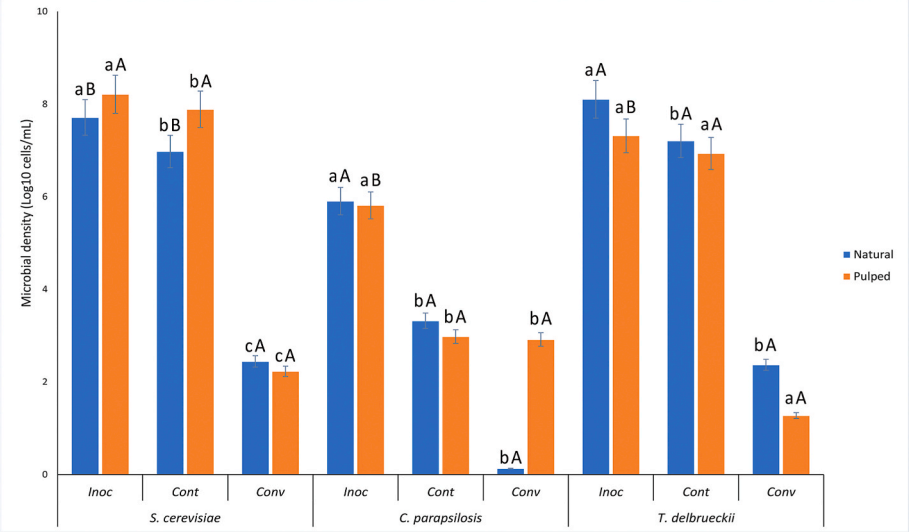

Fig. 1. *S. cerevisiae* (CCMA0543), *T. delbrueckii* (CCMA0684), and *C. parapsilosis* (CCMA0544) population by qPCR at the end of coffee drying (480 h). Means for microbial density at the end of coffee processing followed by the same lowercase (treatments) and uppercase (processing method) letter did not differ from each other by the Scott–Knott test ($p \leq 0.05$). Inoc = Inoculated, Cont = Control, Conv = Conventional process.

succinic acids were identified (Table 2); oxalic, tartaric, lactic, and acetic acids were not identified.

The acids concentration showed significant differences ($p \leq 0.05$) between the natural and pulped coffee processing; however, no difference was obtained among fermentations. For example, citric acid was detected in high concentrations in natural (4.85 g/kg) and pulped (3.01 g/kg) coffee at the beginning of the process (0 h).

By the end of processing, citric, malic, succinic, and lactic acids were detected, and high lactic acid production was observed in coffees fermented by SIAF (Table 2). In addition, natural coffee had a higher production of acids (citric, malic, succinic, and lactic acids) than pulped coffee in the treatments processed by SIAF. On the other hand, conventional processing showed higher production of acids (citric, malic, succinic, and lactic acids) in pulped coffee than in natural coffee. Significant differences ($p \leq 0.05$) were found in acid production between the treatments. *T. delbrueckii* had the highest citric acid production, with 7.29 g/kg in natural coffee and the conventional process with 4.04 g/kg for pulped coffee. For succinic acid, the SIAF control presented the highest concentration with 2.38 g/kg in natural coffee and in pulped coffee conventional processing with 1.60 g/kg. For lactic acid, *S. cerevisiae* was 12.15 g/kg in natural coffee, and *C. parapsilosis* was 4.36 g/kg in pulped coffee. Moreover, conventional processing obtained the highest yields for malic acid, with 2.02 g/kg in natural coffee and 2.75 g/kg in pulped coffee.

3.3. Volatile compounds

A total of 40 volatile compounds were detected between green (14) and roasted (39) coffees (Supplementary Table S2). These compounds were classified into 14 groups: pyrroles (6), furans (6), esters (5), acids (4), alcohols (3), ketones (3), pyrazines (3), pyridines (2), lactones (2), aldehydes (2), phenols (1), pyrans (1), thiophenes (1) and others (1) (Flamet, 2001). Ethyl oleate, octadecanoic acid, and ethyl ester were detected only in coffee processed by natural coffee in green and roasted coffee. Principal component analysis (PCA) analyzed the GCMS results and correlated the volatile compounds detected with each treatment and processing method. Fig. 2 shows the PCA results, with numbers (1) for green coffee and (2) for roasted coffee and letters (A) for natural and (B) for pulped coffee. In green coffee, PC1 and PC2 explained 79.41% and 77.49% of the total variance for natural and pulped coffee, respectively.

In natural green coffee, there is a relationship between *C. parapsilosis* yeasts and the compounds linoleic acid ethyl ester, hexanoic acid, 2-methylbutyl ester, hexadecanal, and n-nonadecanol, and between SIAF control 2-pentadecanone, the conventional process with compound pentadecanal and *S. cerevisiae* and *T. delbrueckii* do not show a direct correlation with any specific compound.

In roasted coffee, PC1 and PC2 explained 77.77% and 77.79% of the total variance for natural and pulped coffee, respectively. In natural coffee, the SIAF control was related to Compounds 2-thiophenemethanol, ethanone, 1-(1H-pyrrol-2-yl), and 1H-pyrrole, 1-(2-furanylmethyl). The conventional process with compounds 3- pyridinol

Table 2
Organic acids detected (g/kg) in coffee before processing (freshly harvested 0 h) and at the end (480 h) of postharvest processing for the natural and pulping coffee.

Time (h)	Treatments	Organic acids (g/kg)							
		Citric Acid		Malic Acid		Succinic Acid		Lactic Acid	
		Natural	Pulped	Natural	Pulped	Natural	Pulped	Natural	Pulped
0	Freshly harvested	4.85*	3.01*	1.13*	1.74*	0.76* B	1.17*	0.00*	0.00*
480	*S. cerevisiae* (SIAF)	3.23 dA*	2.52 bB*	0.14 bA	0.00 bA	1.49 bA	0.48 dB*	12.15 bA	4.89 aB
	C. parapsilosis (SIAF)	4.15 cA*	2.21 bB*	0.07 cA	0.00 bA	1.65 bA	0.59 dB*	8.80 dA	4.36 aB
	T. delbrueckii (SIAF)	7.29 aA	3.00 bB*	0.29 bA	0.00 bA	1.74 bA	1.21 cB	10.50 cA	1.20 cB
	Control (SIAF)	5.46 bA*	3.95 aB*	0.33 bA	0.09 bB	2.38 aA	1.60 bB	13.20 aA	3.50 bB
	Conventional process	3.13 dB*	4.40 aA*	2.02 aB	2.75 aA	1.26 bB*	2.22 aA*	0.00 eA*	0.29 dA

Means followed by the same lowercase letter (columns) and uppercase (rows) do not differ from each other by the Scott–Knott test ($p \leq 0.05$). Means followed by (*) do not differ from freshly harvested coffee using Student's *t*-test.
SIAF: self-induced anaerobic fermentation.

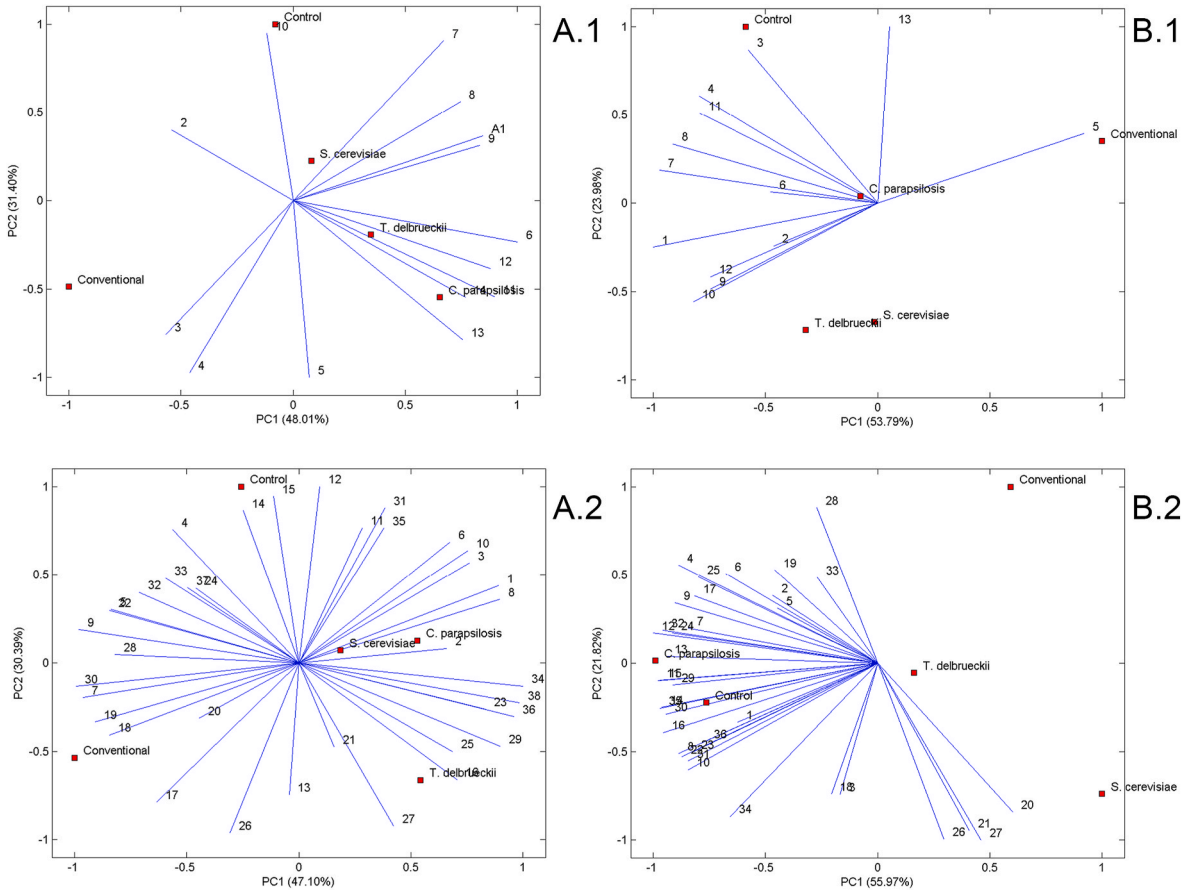

Fig. 2. Principal component analysis (PCA) of volatile compounds detected in natural (A) and pulped (B) processes in green (1) and roasted coffees (2). Volatile compounds: (1) 1H-Pyrrole, 1-(2-furanylmethyl), (2) 1H-Pyrrole-2-carboxaldehyde, (3) 1H-Pyrrole-2-carboxaldehyde, 1-methyl, (4) 2(5H)-Furanone, (5) 2-Furancarboxaldehyde, 5-methyl, (6) 2-Furanmethanol, (7) 2-Furanmethanol, acetate, (8) 2-Heptadecanone, (9) 2-Pentadecanone, (10) 2-Pentadecanone, 6,10,14-trimethyl, (11) 2-Pyrrolidinone, 1-butyl, (12) 2-Thiophenemethanol, (13) 3-Methyl-2-pyrazinylmethanol, (14) 3-Pyridinol, (15) 3-Pyridinol, 2-methyl, (16) 4-Hydroxy-2-methylacetophenone, (17) 4-Methyl-5H-furan-2-one, (18) 5-Hydroxymethylfurfural, (19) Benzyl alcohol, (20) Butanoic acid, 4-hydroxy, (21) Caffeine, (22) Decanoic acid, (23) Ethanone, 1-(1H-pyrrol-2-yl), (24) Ethanone, 1-(2-furanyl), (25) Ethyl Oleate, (26) Furfural, (27) Hexadecanal, (28) Hexadecanoic acid, methyl ester, (29) Hexanoic acid, 2-methylbutyl ester (30) Indole, (31) 9,12-Octadecadienoic acid, ethyl ester, (32) Morpholine, 4-octadecyl, (33) n-Nonadecanol, (34) Octadecanoic acid, ethyl ester, (35) Octanoic acid, (36) n-hexadecanoic acid, (37) Pentadecanal, (38) Phenylethyl Alcohol, (39) Pyrazine, 2,6-dimethyl, (40) Pyrazine, 3-ethyl-2,5-dimethyl. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and 5-hydroxymethylfurfural, *C. parapsilosis* with compound phenylethyl alcohol, *T. delbrueckii* with 1H-pyrrole-2-carboxaldehyde, 2- pentadecanone, and decanoic acid, and *S. cerevisiae* did not show a direct correlation with any specific compound.

In pulped green coffee, SIAF control is related to hexanoic acid, 2-methylbutyl ester, and the conventional process is related to caffeine, *T. delbrueckii*, *C. parapsilosis* and *S. cerevisiae* did not present a correlation. A correlation was observed between *C. parapsilosis* yeast and the SIAF control for pulped roasted coffee. The SIAF control showed a correlation with the compounds 2(5H)-furanone, 4-methyl-5H-furan-2-one, 2-thiophenemethanol, 1H-pyrrole-2-carboxaldehyde, and benzyl alcohol, *C. parapsilosis* with 3-methyl-2-pyrazinylmethanol, 2-furanmethanol, ethanone, 1-(1H-pyrrol-2-yl), 2-heptadecanone, 1H-pyrrole, 1-(2-furanylmethyl), 1H-pyrrole-2-carboxaldehyde, 1-methyl, indole, and ethanone, 1-(2-furanyl), *S. cerevisiae* with n-hexadecanoic acid, and the conventional process, and *T. delbrueckii* did not show any correlation.

3.4. Tetrazolium test

Table 3 shows the results of the tetrazolium test, with significant differences ($p \leq 0.088$) between treatments and processing methods and between the times of freshly harvested coffee before fermentation (0 h) and coffee at the end of drying (480 h).

Table 3
Seed viability according to a tetrazolium test at sampling times 0 and 480 h of postharvest processing for the natural and pulping coffee.

Time (h)	Treatments	Viability (%)	
		Natural	Pulped
0	Freshly harvested	87.5*	85*
480	*S. cerevisiae* (SIAF)	52.5 bA	60.0 aA
	C. parapsilosis (SIAF)	67.5 bA	60.0 aA*
	T. delbrueckii (SIAF)	67.5 bA	70.0 aA*
	Control (SIAF)	65.0 bA	70.0 aA*
	Conventional	90.0 aA*	60.0 aB*

Means followed by the same lowercase letter (columns) and uppercase (rows) do not differ from each other by the Scott–Knott test ($p \leq 0.088$). Means followed by (*) do not differ from freshly harvested coffee using Student's *t*-test.
SIAF: self-induced anaerobic fermentation.

In general, we can say that the pulped coffee processing method has caused less embryonic damage than natural coffee processing. However, only the treatment of natural coffee conventionally obtained the same results as coffee before treatments (0 h).

3.5. Electrical conductivity (E.C.)

Before processing, at time 0, a statistically significant difference ($p \leq 0.05$) was found between the processing methods (Table 4). At the end of processing (480 h), the results showed statistically significant differences ($p \leq 0.05$) between processing methods and between treatments. The conventional processing obtained the lowest electrical conductivity values in the natural (259.53 μS cm $^{-1}$. g $^{-1}$) and pulped (209.00 μS cm $^{-1}$. g $^{-1}$) methods, indicating the better physiological quality of coffee by this treatment.

3.6. Sensorial analysis

Sensory analysis showed that all coffees scored above 80 and were classified as specialty coffees. When inoculated with *T. delbrueckii* (86.50), pulped coffee obtained the best scores; natural coffee showed better results with *C. parapsilosis* (85.90). The coffees processed by the conventional method obtained the lowest scores (83.75 for natural coffee and 84.25 for pulped coffee). Fig. 3 shows the intensity of sensory attributes (sweetness, acidity, body, astringency, bitterness, and aftertaste) that contributed to the final score for each treatment. Likewise, these coffees were characterized by aromas and flavors of citrus fruits, caramel, honey, chocolate, and chestnuts for natural coffee and flavors and aromas of citrus fruits, nuts, chocolate, caramel, and chestnuts for pulped coffee. In addition, the Q-graders detected aromas and fermented flavors (of wine) in the natural coffees processed by SIAF.

4. Discussion

SIAF is a new technology applied to the fermentative processing of coffee in closed bioreactors, creating a modification in the fermentation environment by limiting the availability of oxygen and allowing the development of facultative aerobic microorganisms (Cassimiro et al., 2022; da Mota et al., 2022). The anaerobiosis caused by SIAF generates a decrease in cellular respiration in the seed, forced to carry out anaerobic respiration, which translates into greater consumption of nutrients to produce the energy necessary for maintenance and development functions, affecting the viability of the seed (Taiz et al., 2017). Kleinwächter and Selmar (2010) demonstrated that the anaerobic process intensified the consumption of sugars by the seed. Therefore, microbiological interactions are created that favor the development of yeasts through different metabolic pathways (Feldmann, 2012; Takagi et al., 2015). However, the growth of microorganisms during fermentation generates physicochemical changes in the beans, loss of water, and changes in organic compounds (acids, sugars, volatile) that provide additional flavors and aromas through the metabolites produced in this process, which can migrate to the bean, producing different flavors and aromas that favor the quality of a beverage (Evangelista et al., 2014; Silva et al., 2013).

Table 4
Electrical conductivity values (μS.cm $^{-1}$. g $^{-1}$) for coffee beans before fermentation (0 h) and at the end of drying (480 h) of natural and pulped coffee.

Time (h)	Treatments	Electrical conductivity (μS.cm $^{-1}$. g $^{-1}$)	
		Natural coffee	Pulped coffee
0	Freshly harvested	256.03*	206.50*
480	*S. cerevisiae* (SIAF)	398.35 bA	563.35 cB
	C. parapsilosis (SIAF)	381.85 bA	417.70 bA
	T. delbrueckii (SIAF)	376.80 bA	462.10 bB
	Control (SIAF)	309.68 aA	417.10 bB
	Conventional process	259.53 Ab*	209.00 Aa*

Means followed by the same lowercase letter (columns) and uppercase (rows) do not differ from each other by the Scott–Knott test ($p \leq 0.05$). Means followed by (*) do not differ from freshly harvested coffee using Student's *t*-test.
SIAF: self-induced anaerobic fermentation.

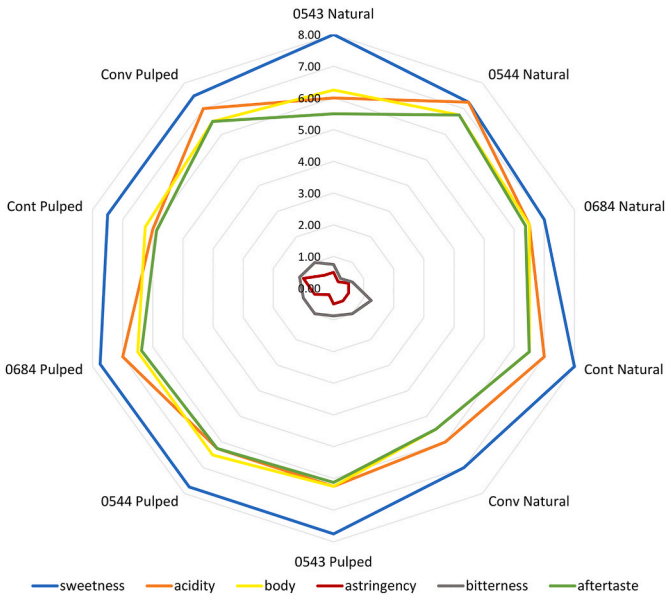

Fig. 3. Sensory attribute intensity. Treatments: 0543 Nat = *S. cerevisiae* Natural, 0544 Nat = *C. parapsilosis* Natural, 0684 Nat = *T. delbrueckii* Natural, Cont Nat = Control Natural, Conv Nat = Conventional processing Natural, 0543 Pulp = *S. cerevisiae* Pulped, 0544 Pulp = *C. parapsilosis* Pulped, 0684 Pulp = *T. delbrueckii* Pulped, Cont Pulp = Control Pulped, Conv Pulp = Conventional processing Pulped.

The use of yeast starter (*C. parapsilosis, T. delbrueckii,* and *S. cerevisiae*) during the coffee fermentation process has been used in different investigations (da Mota et al., 2022; Martins et al., 2022) showing the yeast's ability to carry out fermentation and remain viable until the end of the drying process. In addition to guaranteeing the sensory quality of the beverage through the production of the desired sensory attributes.

Epiphytic microorganisms participate in coffee fermentation, competing for available nutrients (pectin, sugars, amino acids, polysaccharides) among themselves and with starter cultures (Silva et al., 2013). The microbial metabolites produced can migrate to the interior of the bean, altering the internal chemical composition of the bean and providing organic compounds (amino acids, sugars, volatile compounds) that modify the sensory profile of the coffee (Hadj Salem et al., 2020; Pereira et al., 2022; Wang et al., 2022).

At the end of coffee processing, the inoculated yeasts (*S. cerevisiae, C. parapsilosis,* and *T. delbrueckii*) maintained populations at least 1 log above the populations found in the SIAF control and conventional processing. These yeasts were identified both in the SIAF control and in the conventional processing, which was expected, as these yeasts are naturally in coffee in populations between 10^2 and 10^6 cells/mL (Silva et al., 2013; Vilela et al., 2010). Through the generation of suitable environments for the growth of these microorganisms (presence and absence of oxygen), environmental conditions were different from the conditions outside the bioreactors (Cassimiro et al., 2022; da Mota et al., 2022), observing differences between the treatments in bioreactors and the conventional processing that was directly the terraces. *T. delbrueckii* and *S. cerevisiae* exceeded six $\log_{10}$ cells/mL in the SIAF control and four $\log_{10}$ cells/mL above conventional processing, thus demonstrating that SIAF favors the growth of these microorganisms.

Coffee fruits naturally contain organic acids in their composition, such as citric, malic, and succinic acids, which influence the perceived acidity of the beverage (Bressani et al., 2021). However, acid degradation occurs during coffee fermentation, as well as an increase in their concentration, due to the metabolism of microorganisms (Ribeiro et al., 2017). In complex fermentative processes, these acids are used as a carbon source by the microbiota present, such as in the fermentation of

citric acid by lactic acid bacteria (LAB), increasing or decreasing its concentration during coffee processing as a product generated by the metabolism of microorganisms (Martinez et al., 2019). These compounds will influence the perceived acidity in coffee, an essential attribute of coffee quality combined with sweetness, bitterness, and aroma, which improves the beverage's sweetness (Sunarharum et al., 2014). Therefore, producing organic acids during coffee fermentation plays a promising role in creating specialty coffees with desired sensory characteristics (Cassimiro et al., 2022; Wang et al., 2019).

Lactic acid, absent at the beginning of processing, becomes the main acid during fermentation, with the highest quantification compared to the other acids detected. Similar results were reported by da Mota et al. (2022). This lactic acid in the coffee is related to the decarboxylation of the malic acid by LAB, with a decrease in malic acid observed compared to that detected at the beginning of processing (Cassimiro et al., 2022; Leeuwenhoek, 1999). Furthermore, citric, malic, succinic, and lactic acid in green beans affect the formation of volatile compounds during roasting, such as pyrazines, furans, and esters (Elhalis et al., 2021).

The volatile compounds identified in green coffees, alcohols, esters, ketones, aldehydes, and caffeine, are responsible for aromas characteristic of coffee with aromas of citric fruits, nuts, and florals (Flamet, 2001; Sunarharum et al., 2014), indicating that the coffees were free of defects (rotted, over-fermented, brocaded, moldy coffees) (Elhalis et al., 2021). Roasted coffees had an increased number of volatile compounds due to the several reactions produced during roasting (Maillard reaction, Strecker degradation) (Flamet, 2001; Prakash et al., 2022). Thirty-nine compounds detected in 14 classes were dominated by seven classes: Pyrroles (7, provide sweet, smoky, herbaceous, mushroom, woody aroma), Furans (6, provide the characteristic aroma and flavor of coffee, sweet, fruity, nutty, caramel), Esters (4, fruity flavors, original notes and flavors produced by fermentative metabolites), Acids (4, contribute to the characteristic aroma of the coffee, contribute to the quality of coffee, characteristic fruity flavors), Alcohols (3, characteristic flavors of coffee, enhance the aroma and flavor of coffee), Ketones (3, characteristic aromas of coffee), Pyrazines (3, aromas and flavors characteristic of roasted coffee, chocolate, roasted walnuts, roasted peanuts, hazelnuts, popcorn, herbal) (Flamet, 2001; Prakash et al., 2022).

When evaluating coffee's seed vigor and cell membrane integrity before and at the end of processing through tetrazolium and electrical conductivity tests, a decrease in seed viability was observed, except for conventional processing in natural coffee, which maintained the same viability as freshly harvested coffee. Bytof et al. (2007) and Selmar et al. (2006) describe the function of the husk as a protective barrier that avoids the beginning of metabolic processes within the seed, preventing the consumption of energetic reserves. All the coffees processed by SIAF decreased their physiological quality, with statistically significant differences ($p \leq 0.088$) between the treatments and the processing. The decrease in the viability of the embryos observed by the tetrazolium test is influenced by the metabolic reactions of microorganisms and the beginning of the metabolic processes of the seed during fermentation (Selmar et al., 2014). These metabolic processes (cellular respiration, cell division, and cell maintenance processes, among others) can use the nutritional reserves inside the bean, transforming them into other compounds. It is also possible that the decrease in available oxygen caused by SIAF forces the seed to carry out cell breathing by anaerobic processes, which results in greater nutrient consumption (reserves) for energy production (Pereira et al., 2022; Selmar et al., 2014; Taiz et al., 2017). For the conventional processing of pulped coffee, the behavior was similar to that of the treatments processed by SIAF, possibly due to the loss of protection from the husk to the coffee bean (Selmar et al., 2008, 2014).

Similar electrical conductivity results were observed with statistically significant differences ($p \leq 0.05$) between treatments and processing methods. This test can show the rupture of the cell membrane and the loss of control of its permeability, by indirect evaluation of the degree of structuring of the cell membrane, by determining the number of ions leached in the soaking solution, such as sugars, amino acids, organic acids, proteins and phenolic substances, and inorganic ions, such as K^{+}, Ca^{+2}, Mg^{+2}, $Na^{+,}$ and Mn^{+2} (Marcos-Filho, 2015). These tetrazolium and electrical conductivity tests showed that the physiological quality (theoretical capacity of the seeds to express their vital functions in favorable and unfavorable environmental conditions) of the fermented coffees began to decrease (Marcos Filho, 2015; Pazmiño-Arteaga et al., 2022).

The aroma and flavor were different for each treatment, showing different behavior for each yeast, as observed in other studies (Bressani et al., 2021; Evangelista et al., 2014; Martinez et al., 2019; Martins et al., 2019). Notes of citrus fruits, caramel, honey, chocolate, and chestnuts were perceived in all the treatments that adopted the natural method and notes of fermentation (wine) for those processed by SIAF. In addition, the SIAF control produced a coffee beverage with a wood flavor, which is considered a defect (Flamet, 2001). The pulped coffee was characterized by flavors and aromas of citrus and nuts, chocolate, caramel, and chestnuts. These sensory characteristics are influenced by volatile composition, such as benzyl alcohol related to fruit flavors, Phenylethyl alcohol with floral aromas (roses), 1h-pyrrole, 1- (2-Furanylmethyl) coffee and fruited, Pyrazine, 3-Ethyl-2,5 -Dimethyl, relating cocoa, nuts, and roasted potatoes, Butanoic acid, 4-hydroxy with sweet caramel and creamy taste (Bressani et al., 2018; Martinez et al., 2019). In addition, other compounds related to the caramelization of sugars generate flavors and aromas characteristic of coffee (bitterness, acidity, caramel, and smoked, among others) (Flamet, 2001; Prakash et al., 2022).

The coffee processed by SIAF obtained the best scores, and the lowest scores were observed for coffees processed by the conventional method. These results showed that processing and starter cultures affect viability, which helps to control the fermentation process and potentiate the quality of coffee. Conventional coffee processed by the natural method was the only treatment that did not show a decrease in seed viability. However, this treatment did not obtain the best sensory scores, demonstrating that the seed's viability does not influence coffee's sensory quality.

5. Conclusions

The fermentation process negatively affects the viability of the coffee seed without negatively affecting the sensory quality of the beverage. The SIAF favors the production of specialty coffees since all fermented coffees were considered specialty coffees with scores above 80. The use of yeasts as starter cultures in the processing of coffee stimulates the production of differentiated specialty coffees, producing a variation in the composition of volatile compounds and generating differentiation in the sensory characteristics of the beverage. The use of closed bioreactors by the SIAF method favors the production of lactic acid in coffee. The *T. delbrueckii* yeast obtained the best score of 86.50 for pulped coffee, and the *C. parapsilosis* yeast obtained the highest score of 85.90 for natural coffee. Natural coffee produced higher amounts of organic acids and a better distribution of volatile compounds than pulped coffee.

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Authors statement

Emerson Josue Martinez Jimenez and Pâmela Mynsen Machado Martins - Conducting a research and investigation process, specifically performing the experiments in the Microbiology lab. Wrote the first draft of the manuscript. Ana Luiza de Oliveira Vilela and Sttela Dellyzete

Veiga Franco da Rosa – a critical review, commentary, or revision – of the manuscript. Nadia Nara Batista - Formulation and plan the project, critical review, commentary, or revision – of the manuscript. Disney Ribeiro Dias - Conceptualization, Methodology, critical review, commentary, or revision – of the manuscript. Rosane Freitas Schwan - Ideas; formulation or evolution of overarching research goals and aims. Financial resources and final critical review of the manuscript pre and pos submission.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2022.102218.

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