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ORIGINAL ARTICLE

Microbiological and chemical-sensory characteristics of three coffee varieties processed by wet fermentation

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Abstract

This work evaluated the bacterial diversity during coffee wet fermentation of the three coffee varieties—Mundo Novo (MN), Ouro Amarelo (OA), and Catuaí Vermelho (CV). Isolates were identified by polyphasic techniques: biochemical tests, matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) and DNA sequencing. Chemical compositions were determined by high (HPLC) and gas chromatography-mass spectrometry (GC-MS) and the roasted beans were sensorial evaluated using the cupping test. Thirty-six mesophilic bacteria and six lactic acid bacteria were identified. *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were often found in all varieties. Citric acid was the acid detected in higher concentrations. The volatile profile of the green coffee beans changed during the fermentation in the tank, but more significantly, during the roasting process. These volatiles belonged to the classes of acids, alcohols, aldehydes, and hydrocarbons. Temporal dominance of sensations analysis showed sensorial sensations of acidity (OA and CV), bitterness, chocolate, nuts (MN), and sweetness (CV). The characteristics of each coffee variety were distinct, mainly in relation to total bacteria population, volatile compounds, and sensorial profile. In conclusion polyphasic methodology was efficiently done for bacteria identification; the dominant bacteria might be used for starter cultures and the chemical and sensory analyses helped to understand the changes in coffee fermentation. Our findings are relevant to future select starter bacteria for coffee processing to improve quality and standardization of quality.

Keywords Coffee · Fermentation · Bacteria · Sensorial analysis · Volatile compounds

Introduction

Worldwide coffee sales continue to increase each year, in part due to increased consumption in emerging markets like Turkey and Russia. In traditional coffee markets, such as the European Union, the USA, and Japan, growth is primarily driven by an increased demand for high quality, specialty-grade coffee (ICO 2015). Brazil is the world's largest coffee producer and exporter. Its estimated production for 2018 between 41.74 and 44.55 million of 60-kg bags, with arabica coffee representing 76% of total production (CONAB 2018). Since the market value depends on the coffee's quality, there is a constant search for improvement not only in production but also in quality and safety.

Coffee production consists of several steps, including the fermentation and drying that occurs immediately after harvesting. The fermentation process can be

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performed in three different ways: dry, semi-dry, and wet (Brando and Brando 2015). In the wet method, the beans are first separated from the surrounding cherry by pulping and placed in open tanks with water, where they are allowed to ferment for 6 to 72 h (depending on the environmental temperature), during which the remaining mucilage (a remnant of the fruit exocarp) is degraded and solubilized. The beans are then removed from the tanks and dried in the sun. Epiphytic microorganisms naturally present in coffee cherries play an important role during fermentation and drying, due to consumption of the pulp and mucilage around the fruit and produce compounds that will directly affect the coffee quality (Silva 2015). Some old studies reported the microorganisms present in the coffee wet fermentation (Frank and Cruz 1964; Frank et al. 1965; Agate and Bhat 1966; Avallone et al. 2001), although the methodology used for identification is outdated. More recently, Evangelista et al. (2015) showed that mesophilic and lactic acid bacteria are the major groups of microorganisms involved in the wet fermentation process.

The use of appropriate and polyphasic techniques aids the identification of the microorganisms present throughout the fermentation and will elucidate the diversity and distribution of species involved in this process. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) is a technique that can be used to identify microorganisms. It has been used in studies with different fermented foods, such as meat (Doan et al. 2012), vegetables (Nguyen et al. 2013), silage (Carvalho et al. 2016), cacao (Schwenninger et al. 2016), and kefir vinegar (Viana et al. 2017). In our work, MALDI-TOF MS was used to identify bacteria present during the wet fermentation. In addition, chemical and sensorial analyses were performed to characterize the coffee. The sensorial analysis based on temporal dominance of sensations (TDS) analysis was applied. This analysis has recently been used to describe the sensorial characteristics of coffee, to complement the cup test (Evangelista et al. 2014a, b; Ribeiro et al. 2017b).

The different *Coffea arabica* varieties cultivated in Brazil produce coffee beverages with distinct characteristics. The bacterial profile of these fermentations might be different and needs to be established for each variety, to understand the role of different species or genus in the fermentation process. Ouro Amarelo (OA) and Mundo Novo (MN) varieties have already been studied during semi-dry coffee fermentation and inoculated with yeast. According Ribeiro et al. (2017a), the bean composition (sugars, acids, and volatiles) and the sensory response were different in relation to the inoculated coffee variety. In the present study, our aims were to identify the bacteria present during coffee fermentation of three varieties OA, MN, and Catuaí Vermelho (CV); to chemically characterize coffee beans by liquid and gas chromatography; and to evaluate final quality of the final beverage varieties by cupping taste.

Materials and methods

Wet fermentation coffee

Coffee cherries of the varieties OA, MN, and CV were collected mechanically at the peak of the harvest season (June) on a farm located in Patrocínio in the State of Minas Gerais, Brazil, at 970–1200 m above sea level. Coffee cherries were mechanically pulped in a horizontal machine (Ecoflex, Pinhalense, São Paulo, Brazil). Pulped coffee of each variety was fermented separately in concrete tanks filled with water. The fermentations were performed in duplicate. The content of each tank was homogenized manually at approximately 4-h intervals. The complete degradation of the mucilage layer was determined by hand friction between the coffee beans (Velmourougane 2012). After fermentation, the coffee was transferred to suspended terraces for sun-drying, until it reached 11–12% of moisture, measured using a moisture meter G600i (Geaka, São Paulo, Brazil). The following samples (200 g) were taken during processing: (i) coffee cherries (CC), (ii) pulped coffee at the start of fermentation (PC), (iii) coffee without mucilage (demucilled coffee) at the end of fermentation (DMC), and (iv) dried coffee at the final drying time (DC). It was placed aseptically in sterile plastic bags and transferred to the laboratory in iceboxes for microbiological analyses. For physicochemical analyses, coffee samples were frozen at −20 °C until analysis. The roasted coffee (RC) was used for sensory and volatile compound analysis.

Proximate composition and pectin

The proximate composition was done in coffee cherries of the varieties studied. The moisture determination was performed according to the method 925.09B (AOAC 1995) with modifications. The nitrogen content (method 979.09), protein content (method 920.87, using 6.25 as correction factor), fat content (method 920.97), and ash content (method 923.03) were estimated according AOAC (1995). Carbohydrate content was estimated as nitrogen-free extract.

The pectin was performed according to the methodology described by McCready and McComb (1952), using the spectrophotometric determination at 520 nm and total soluble solids were performed according to Bitter and Muir (1962). The pH was determined by potentiometric analysis. The analyses were performed in triplicate.

Microbiological analysis

Quantification, isolation, and phenotypic characterization

Aliquots (10 g) of the sample CC, pulped coffee at the start of fermentation (PC), coffee without mucilage at end of fermentation, and dried coffee at final drying time were added to flasks with 90 mL of sterile peptone water (in g L^{-1}: 1 bacteriological peptone [Himedia, Mumbai, India]), homogenized in an orbital shaker (280 rpm—20 min) and used for decimal serial dilution. Mesophilic bacteria were enumerated by spread plating on Nutrient Agar (Himedia, Mumbai, India) and lactic acid bacteria on MRS agar (Merck, Darmstadt, Germany) with added nystatin (Merck) to inhibit growth of yeasts and filamentous fungi. The plates were incubated at 30 °C and 35 °C for 72 h, for mesophilic and acid lactic bacteria, respectively. The morphological characteristics of the colonies (cell size, cell shape, edge, color, and brightness) were recorded and the square root of the number of colonies counted for each morphotype was purified by streaking on new agar plates (Senguna et al. 2009). The phenotypic characterization of the bacterial colonies was performed using Gram staining, catalase and oxidase activities, and motility tests (Holt et al. 1994). The pure cultures were stored in an ultra-freezer at − 80 °C in the same broth culture media used for plating, containing 20% glycerol (*w*/*w*).

MALDI-TOF sample preparation, measurement, and data analysis

The isolates obtained (321 colonies) from plating were grown on plates using specific culture medium for each taxonomic group, as described above. The purified cultures were streaked on new agar plates and incubated at 30 °C and 35 °C for 24 and 48 h for mesophilic and acid lactic bacteria respectively, and then 20 mg of each colony was aseptically transferred to microtubes. The methodology of protein extraction, equipment calibration, and method of data analysis were done as described by Carvalho et al. (2016). The equipment used was a MALDI-TOF microflex LT spectrometer (Bruker Daltonics, Bremen, Germany). Each isolate was analyzed in triplicate.

Molecular identification

Representative strains from each cluster performed by MALDI-TOF and isolates that were not possible be identified using this technique (score < 1.7) were submitted for molecular analyses. A total of 152 isolates were amplified for 16S rRNA analysis, using the primers 27F and 1512R (Devereux and Willis 1995). The amplified PCR products were sent for sequencing to the Macrogen USA – Humanizing Genomics (MD, USA). The sequences were aligned using the CLC Main Workbench 7.7.1 (Quiagen®) sequence alignment editor and were compared to the EzTaxon database server (Kim et al. 2012; EzTaxon 2017) based on 16S rRNA sequence data for the identification of isolates.

Acids and sugars analysis

Organic acids (malic, lactic, acetic, butyric, propionic, citric, oxalic, succinic, and tartaric acids) and sugars (fructose, glucose, and sucrose) were analyzed.

The following samples were analyzed: pulped coffee at the start of fermentation (PC), coffee without mucilage (demucilled coffee) at end of fermentation (DMC), and dried coffee (DC) at the final drying time. Samples were extracted as described by Ribeiro et al. (2017a).

The extracts were analyzed using a HPLC system (Shimadzu, Japan). A Shimpack SCR-101H (7.9 mm × 30 cm) column was used with a 100 mM solution of perchloric acid, with a flow rate of 0.6 mL per min as the mobile phase. The oven temperature was kept at 50 °C for the analysis of acids, detected with a 210-nm UV detector, and at 30 °C for the analysis of sugars, detected with a refractive index detector. The quantification of compounds was performed using calibration curves constructed with different concentrations of standard compounds [malic, propionic, and citric acids (Merck, Germany); lactic, oxalic, and tartaric acids (Sigma Chemical, USA); acetic and succinic acids (Sigma Aldrich, Germany); butyric acid (Riedel-deHaen, Germany)] and analyzed using the same conditions as for the samples. Analyses were performed in duplicate.

Volatile compounds

The samples analyzed by GC-MS were pulped coffee at the start of fermentation, coffee without mucilage at end of fermentation, dried coffee at final drying time, and roasted coffee (RC). Volatile compounds were extracted using manual headspace solid-phase microextraction (HS-SPME).

GC/MS analysis was carried out with a GC-MS-QP2010 from Shimadzu on an Agilent HP-FFAP column (30 m × 0.25 mm × 0.25 μm). The extraction and parameters of analysis were done according to Ribeiro et al. (2017a). Chromatographic and mass spectral data were analyzed with GC-MS solution software from Shimadzu. Compounds were identified by comparison of their mass spectra to the NIST mass spectral library and linear retention index, relative to a series of n-alkanes.

Sensory characteristics analysis

Cupping test

Sensory analysis for coffee roasting and brewing was performed by three certified specialty coffee judges (Q graders), using the methodology proposed by the Specialty Coffee Association (SCA) (Lingle 2011). Each sample was analyzed five times. The evaluated sensory attributes were fragrance/aroma, flavor, acidity, body, balance, aftertaste, overall impression, uniformity, sweetness, and clean cup. Besides these attributes, tasters were asked to describe the characteristic flavors of each coffee (Ribeiro et al. 2017a).

Temporal dominance of sensations

The temporal dominance of sensations (TDS) analysis was performed according to Pineau et al. (2009) and under the same conditions as described in Ribeiro et al. (2017a). Eleven panelists were selected, five women and 11 men, in the range of 20–30 years and were enrolled in training sessions to familiarize themselves with the tasting attributes used in this analysis. The tasters were trained to use the software (Sensomaker, version 1.8) and procedures for data acquisition.

The tests were performed in closed cabins with white illumination at the Sensory Analysis Laboratory, Food Science Department, Federal University of Lavras (Lavras, MG). The panelists evaluated each sample twice.

Statistical analysis

The data of proximate composition, pectin, temperature, pH value, and cup quality were evaluated by analysis of variance (ANOVA) and the Scott-Knott test was used for comparison between means, considering each treatment in isolation. A 3 × 3 factorial arrangement of treatment was used to analyze the results of carbohydrates and organic acids: three varieties (Ouro Amarelo, Mundo Novo, and Catuaí Vermelho) and three collected samples (pulped coffee, demucilled coffee, and dried coffee). Data were analyzed under the following model:

$$X = \mu + \mathrm{Var}j + \mathrm{Samp}k + \mathrm{Var} \times \mathrm{Samp}jk + \mathrm{e}jk$$

where μ = global mean; Var*j* = varieties effect (*j* = OA, MN, CV); Samp*k* = sample collection time effect (*k* = PC, DMC, and DC); Var × Samp*jk* = effect of interaction between varieties and sample collection time; and e*jk* = experimental error.

The tests were performed using Sisvar 5.6 software (Ferreira 2014). Significance level was defined at $p < 0.05$ level.

The analysis of the TDS data was made using the Sensomaker Software (Nunes and Pinheiro 2012) and plotting as TDS curves showing the percentage of subjects which selected the attribute as dominant at a specific time (Pineau et al. 2009).

Results

The water temperature in the fermentation tank was 21 to 16 °C, while the environmental temperature ranged from 28 to 15 °C and the pH in final of fermentation reached around 4 (begging at 5.5) (Fig. 1). Fermentation was finished when the complete degradation of the mucilage layer was tested by friction between the coffee beans. Each variety presented a different fermentation time to complete the process, with 18, 19, and 23 h for OA, MN, and CV varieties, respectively.

Proximate composition and pectin

Proximate composition and pectin content were evaluated in coffee cherries of varieties OA, MN, and CV (Table 1). There was significant difference in carbohydrate and soluble pectin contents, among the coffee varieties. The OA and MN varieties presented high carbohydrate content (16.47 and 16.89%), while the OA variety showed high content of soluble pectin (0.33%). The other chemical compounds analyzed were very similar among the varieties. The pulp and mucilage composition were reflected in the normal fermentation time of each variety and explained why the fermentation time for OA and MN was closer than that for CV.

Microbiological analysis

The bacterial population present in coffee cherry, pulped coffee at start of fermentation, coffee without mucilage at end of fermentation, and dried coffee at final drying time was quantified by plating (Table 2). The total mesophilic bacteria started with 4.84, 4.69, and 4.10 log CFU per g for OA, MN, and CV, but decreased throughout the process until it reached values of 2.48, 2.78, and 2.70 log CFU per g for OA, MN, and CV. The largest population of mesophilic and lactic acid bacteria was different in each sample (for each processing step) and coffee variety. The expressive mesophilic bacteria were detected in CC and DMC samples. Similar behavior in the population counting was observed with lactic acid bacteria (6.15, 5.49, and 3.64 log CFU per g for OA, MN, and CV). Most of the population values declined until dried coffee (3.11, 3.75, and 3.96 log CFU per g for OA, MN, and CV).

Three hundred twenty-one bacteria isolates were identified by a combination of MALDI-TOF and sequencing of 16S rRNA. All strains that were identified by

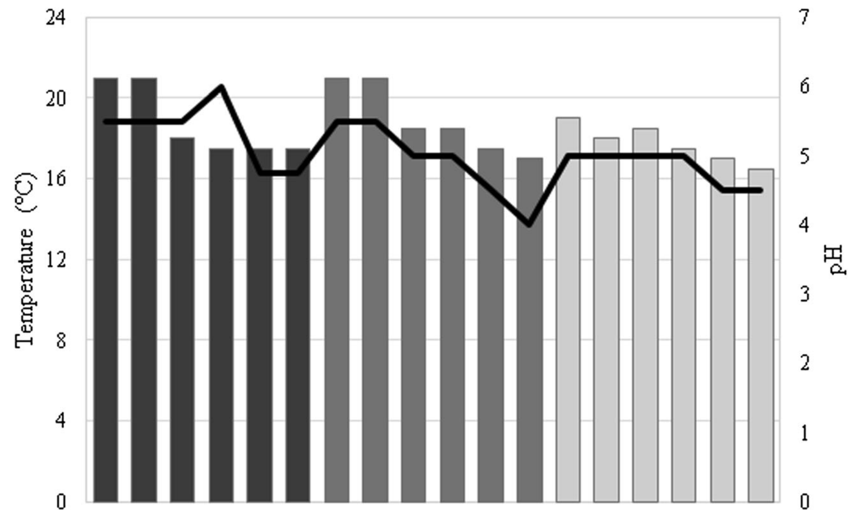


Fig. 1 Temperature (°C) and pH of tank water of coffee processed by wet fermentation of varieties Ouro Amarelo (■), Novo Mundo (■), and Catuaí Vermelho (■). Data are presented as mean. Standard deviation of mean ranged from 1.75 to 0.23. There was no statistical difference at $p < 0.05$ by Scott-Knott test

sequencing were deposited in NCBI (National Center for Biotechnology Information) database and are represented in Table 2. Thirty-five species of mesophilic bacteria belonging to 14 genera were identified, comprised of 17 species identified in OA and MN and 15 species in CV variety. The abundance of each microorganism was calculated in relation to the total population in each stage of the processing. The lactic acid bacteria population was less diversified in terms of species. Six LAB species were identified, belonging to three genera. Four LAB distinct species were identified in OA, five were identified in MN, and three in the CV variety (Table 2).

All the strains of bacteria identified in the study were deposited in the Culture Collection of Agricultural Microbiology (http://www.ccma.dbi.ufla.br) and were coded as CCMA (as listed below). The species found in the all three varieties were *Bacillus cereus* group CCMA 1219–1227, 1243, and 1275–1276; *Cellulosimicrobium cellulans* CCMA 1165–1191; *Enterobacter cloacae* CCMA 1159–1164; *Lactobacillus plantarum* group CCMA 1058–1080; *Leuconostoc mesenteroides* CCMA 1081–1127, and *Enterococcus hirae* CCMA 1130 and 1132–1140.

Table 1 Proximate composition of cherry coffee fruit freshly harvested from the coffee plant varieties Ouro Amarelo, Mundo Novo, and Catuaí Vermelho in wet weight

Composition (%)	Varieties		
	Ouro Amarelo	Mundo Novo	Catuaí Vermelho
Moisture	63.27a	63.68a	62.64a
Fatty content	1.28a	1.25a	1.40a
Protein content	4.34a	4.72a	3.99a
Ash content	1.71a	1.77a	1.58a
Carbohydrate	16.47a	16.89a	12.39b
Total pectin	1.10a	2.30a	1.02a
Soluble pectin	0.33a	0.29b	0.25b
pH	5.03a	4.94a	4.90a

Data are presented as mean. Values with different letters are significant at $p < 0.05$ by Scott-Knott test. Standard deviation of mean ranged from 2.32 to < 0.01

Some species were found only in one of the Ouro Amarelo varieties: *Acetobacter indonesiensis* CCMA 1129; *Arthrobacter luteolus* CCMA 1158; *Bacillus asahii* CCMA 1247; *B. clausii* CCMA 1229–1231 and 1283; *B. licheniformis* CCMA 1233; *B. safensis* CCMA 1232, *Paenibacillus cookii* CCMA 1267; *P.konsidensis* CCMA 1253; *Staphylococcus warneri* CCMA 1146; and *Enterococcus casseliflavus* CCMA 1141.

In Mundo Novo variety, *Bacillus humi* CCMA 1217; *B. simplex* CCMA 1216; *Brevibacillus parabrevis* CCMA 1218; *Gluconobacter oxydans* CCMA 1145; *Lysinibacillus macroides* CCMA 1280–1281; *Microbacterium testaceum* CCMA 1151; *Paenibacillus lactis* CCMA 1268; *Pantoea dispersa* CCMA 1278,1201–1207; *Enterococcus faecium* CCMA 1131; and *Enterococcus faecalis* CCMA 1128 were present.

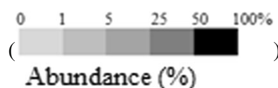
In Catuaí Vermelho variety, *Arthrobacter gandavensis* CCMA 1156; *A. koreensis* CCMA 1157; *Microbacterium paraoxydans* CCMA 1152; *Rhizobium pusense* CCMA 1200; *Rhodococcus pyridinivorans* CCMA 1213–1214; and *R. rhodochrous* CCMA 1282 were the identified ones.

Some species prevailed over the others, such as *Bacillus megaterium* CCMA 1245 in OA variety; *B. subtilis* group CCMA 1234, *Pantoea dispersa* CCMA 1201–1207 and 1278 and *P. vagans* CCMA 1208–1210 in MN variety; and *B. subtilis* group CCMA 1235–1242 and 1244 in CV variety. *Lactobacillus plantarum* group, *L. mesenteroides*, and *Enterobacter hirae* were present in all varieties and at all collection points (Table 2).

Table 2 Bacteria population present in the coffee cherry and during wet processing, identified using MALDI-TOF and 16S rRNA region sequencing

Species identified	Log CFU per g	Accession number	Ouro Amarelo				Mundo Novo				Catuaí Vermelho			
			CC	PC	DMC	DC	CC	PC	DMC	DC	CC	PC	DMC	DC
Mesophilic bacteria														
Total Population (log CFU per g)			4.10	3.95	4.60	2.48	4.69	4.38	2.60	2.78	4.84	3.36	4.84	2.70
Acetobacter indonesiensis	2.00	BAMW01000001												
Arthrobacter gandavensis	3.46	n.s.												
Arthrobacter koreensis	2.60	AY116496												
Arthrobacter luteolus	2.30	BCQM01000025												
Bacillus asahii	2.95	AB109209												
Bacillus cereus group	4.19	AE016877												
Bacillus clausii	3.26	X76440												
Bacillus hornickiae	2.30	FR749913												
Bacillus humi	3.18	AJ627210												
Bacillus licheniformis	2.00	AE017333												
Bacillus megaterium	2.70	JJMH01000057												
Bacillus pumilus group	4.26	ABRX01000007												
Bacillus safensis	2.90	ASJD01000027												
Bacillus simplex	4.00	BCVO01000086												
Bacillus subtilis group	4.87	ABQL01000001												
Brevibacillus parabrevis	2.00	D78463												
Cellulosimicrobium cellulans	4.61	CAOI01000359												
Enterobacter asburiae	3.67	BBED01000197												
Enterobacter cloacae	3.38	CP009854.1												
Gluconobacter oxydans	2.00	X73820												
Lysinibacillus fusiformis	3.28	AB271743												
Lysinibacillus macroides	2.30	LGCI01000008												
Microbacterium paraoxydans	2.48	BCRH01000180												
Microbacterium testaceum	2.00	X77445												
Paenibacillus cookii	2.00	AJ250317												
Paenibacillus konsidensis	4.00	EU081509												
Paenibacillus lactis	2.00	AY257868												
Pantoea bagglomerans	3.26	AJ233423												
Pantoea dispersa	4.46	DQ504305												
Pantoea vagans	4.43	EF688012												
Rhizobium pusense	3.80	jgi.1102370												
Rhizobium radiobacter	4.01	AJ389904												
Rhodococcus pyridinivorans	3.30	LRRI01000001												
Rhodococcus rhodochrous	3.30	n.s.												
Staphyococcus warneri	2.30	L37603												
Species identified	**Log CFU per g**	**Accession number**	**Ouro Amarelo**				**Mundo Novo**				**Catuaí Vermelho**			
			CC	**PC**	**DMC**	**DC**	**CC**	**PC**	**DMC**	**DC**	**CC**	**PC**	**DMC**	**DC**
Acid lactic bacteria														
Total Population (log CFU per g)			6.15	5.54	4.07	3.11	5.49	4.66	3.84	3.75	3.63	3.96	4.98	3.96
Lactobacillus plantarum grupo	5.31	ACGZ01000098												
Leuconostoc mesenteroides	6.20	CP012009												
Enterococcus hirae	3.37	CP003504												
Enterococcus casseliflavus	2.00	n.s.												
Enterococcus faecium	2.00	AJKH01000109												
Enterococcus faecalis	3.00	ASDA01000001												

Database accession number of EzTaxon (http://www.ezbiocloud.net/); *n.s.*, not sequenced, identified by MALDI-TOF with a score above 2.000; *CC*, coffee cherry; *PC*, pulped coffee; *DMC*, demucilled coffee; *DC*, dried coffee

()

Acid and sugar analyses

In general, sugar did not show significant differences in the collected samples, except for fructose in the CV variety (ranging from 7.55 mg g^{-1} in pulped coffee to 12.09 mg g^{-1} in demucilled coffee) (Table 3). Regarding coffee varieties, CV showed differences from the other varieties. At the beginning of fermentation, CV showed lower fructose concentration (7.55 mg g^{-1}). In dried coffee, CV showed high glucose (8.60 mg g^{-1}) and fructose (13.57 mg g^{-1}) concentrations and lower sucrose concentration (33.53 mg g^{-1}). Sucrose predominated throughout the process, being the main sugar present in dried coffee, showing concentrations of 40.06, 46.53, and 33.53 mg g^{-1}, in OA, MN, and CV, respectively, followed by fructose 8.67, 8.52, and 13.67 mg g^{-1}.

The main acids involved during coffee fermentation (citric, malic, succinic, and acetic acids) were detected and quantified (Table 3) and lactic, butyric, propionic, oxalic, and tartaric acids were not detected in any of the samples. Citric acid occurred at the highest concentration, increasing from the start of fermentation to dried coffee. The maximum concentration of citric acid was 6.86, 8.94, and 5.69 mg g^{-1} in OA, MN, and

Table 3 Carbohydrates and organic acids during the wet processing of Ouro Amarelo, Mundo Novo e Catuaí Vermelho varieties, effects of different samples and *p* value

Compounds (mg g^{-1}) and varieties	Samples			*p* value			SEM*
	Fermentation			Variety (V)	Samples (S)	V × S	
	Pulped coffee (PC)	Demucilled coffee (DMC)	Dried coffee (DC)				
Glucose							
Ouro Amarelo	7.69aA	5.28aB	4.31bB	< 0.01	< 0.01	< 0.01	0.27
Mundo Novo	7.90aA	5.53aB	3.63bC				
Catuaí Vermelho	8.83aA	4.30aB	8.60aA				
Fructose							
Ouro Amarelo	9.48aB	11.69aA	8.66bB	0.06	< 0.01	< 0.01	0.31
Mundo Novo	9.48aB	12.19aA	8.52bB				
Catuaí Vermelho	7.55bB	12.09aA	13.57aA				
Sucrose							
Ouro Amarelo	11.63aB	10.35aB	40.06aA	0.40	< 0.01	0.01	1.44
Mundo Novo	11.76aB	14.74aB	46.54aA				
Catuaí Vermelho	17.37aB	21.03aB	33.53bA				
Citric acid							
Ouro Amarelo	3.85aB	2.94aB	6.86bA	0.07	< 0.01	< 0.01	0.25
Mundo Novo	3.66aB	3.89aB	8.94bA				
Catuaí Vermelho	4.64aA	4.54aA	5.69aA				
Malic acid							
Ouro Amarelo	0.55aB	0.44aB	1.04bA	0.95	< 0.01	< 0.01	0.02
Mundo Novo	0.48aB	0.35aB	1.24aA				
Catuaí Vermelho	0.63aB	0.52aB	0.91bA				
Succinic acid							
Ouro Amarelo	2.61bA	0.74bB	2.14aA	< 0.01	< 0.01	< 0.01	0.18
Mundo Novo	3.76aA	3.85aA	1.78aB				
Catuaí Vermelho	4.42aA	3.37aB	1.66aC				
Acetic acid							
Ouro Amarelo	1.55bA	1.04cA	0.83aA	< 0.01	< 0.01	< 0.01	0.11
Mundo Novo	2.23aB	7.00aA	0.83aC				
Catuaí Vermelho	2.65aA	2.10bA	0.62aB				

Data are presented as mean. For each column, mean values with different lowercase letters are significant at $p < 0.05$ by Scott-Knott test. For each row, mean values with different capital letters are significant at $p < 0.05$ by Scott-Knott test. *Standard error of the means. *PC*, pulped coffee; *DMC*, demucilled coffee; *DC*, dried coffee

CV respectively, in dried coffee (DC). The same behavior was observed for malic acid, reaching the final concentration of 1.04, 1.24, and 0.91 mg g^{-1} in OA, MN, and CV varieties, respectively. Succinic acid, unlike citric acid, showed a decrease from fermentation to drying. Similar behavior was observed for acetic acid, which showed a final concentration of 0.83, 0.83, and 0.62 mg g^{-1} in OA, MN, and CV varieties, respectively.

Volatile compounds

Gas chromatography, coupled with mass spectrometry (GC-MS), in combination with headspace solid-phase microextraction (HS-SPME) detected 100 volatile compounds. Among these, 53 were detected in green coffee (Supplementary material) and 55 in roasted coffee (Supplementary material).

The 53 compounds identified in green coffee were aldehydes (16), alcohols (13), acids (5), hydrocarbons (5), ketones (4), esters (4) lactones (2), and phenols, furans, pyrazines, and terpenes (1 each). A few novel volatile metabolites (1 in OA and 5 in MN) were detected after fermentation in the tank, compared to the unfermented beans. The subsequent drying step has a more significant impact on the volatile profile, yielding 12 (MN, CV) and 14 (OA) novel metabolites belonging to several different chemical classes. The compounds heptanoic acid and 2-ethyl-1-hexanol were detected only in beans of the variety OA. No qualitative differences were found in the volatile profiles of MN and CV in the dried beans.

Among the 55 compounds detected in roasted coffee were pyrazines (14); ketones (9); pyrroles (7); furans (4); furaldehydes (3); lactones (3); aldehydes, esters, hydrocarbons, and phenols (2 each); and acids, alcohols, pyrans, pyridines, terpenes, triazoles, and thiophenes (1 each).

Sensory analysis

Two different sensory analyses were carried out to describe the flavor characteristics of the coffee beverages. The cupping test (Fig. 2) and temporal dominance of sensations (TDS) were used to characterize some attributes of each coffee variety (Fig. 3).

The final score of the cupping test was 83.04, 81.50, and 82.33 for the OA, MN, and CV varieties, respectively. The variety OA showed higher scores for all attributes except for the balance, which was higher for the variety CV (Fig. 2). Panelists characterized the coffee from OA beans by the descriptors chocolate, vanilla, creamy body, clean, and pleasant finish. Mundo Novo was described as milk chocolate, almonds, and pleasant finish. Catuaí Vermelho was defined as caramel flavor, citric acidity, creamy body, and long finish. Statistical analysis was performed; however, there was no statistical difference at $p < 0.05$ by Scott-Knott test. Although there is no difference between the notes of the cupping test, the varieties present distinct characteristics that were highlighted in the TDS analysis.

The TDS curves (Fig. 3) showed the descriptions of the sensations as sweet, bitter chocolate, nuts, astringent, acidic, fruity, and bitter, in each variety. Acidity was the dominant attribute in the OA variety (5 to 15 s). The dominant sensation in MN coffee was bitter at first (5 to 20 s), followed by bitter chocolate (20 to 25 s) and ending with nuts (29 s). The variety CV presented acidity as the dominant sensation at the beginning of the analysis (between 5 and 10 s) followed by astringency (between 10 and 15 s) and ending with a dominant sweet sensation (15 to 25 s). This sweetness may correspond with the caramel aroma observed by the tasters in the cupping test. However, a little astringency was observed in this variety, which was not desirable.

Discussion

During coffee fermentation, several factors influence the microbiota diversity, metabolites formation, and consequently the final coffee quality. Among these factors are as follows: the coffee varieties, fermentation method (solid or submerged fermentation in water, open or closed systems, continuous or sporadic, static or agitated), epiphytic microorganisms, temperature, pH, and acidity. One of the major issues involving the wet fermentation is the process control and determination of the ending point of fermentation. In the present study, the fermentation time was between 18 and 23 h, which is generally recommended for wet processing. A decisive factor in achieving coffee quality in wet process is fermentation time; therefore, fermentations lasting more than 40 h are not desirable (Quintero and Molina 2015). The ending point of fermentation can be determined based on either observations or empiric measurements. The measurement of pH value was established as a parameter to determine the fermentation ending. The lowering of pH value was due to microbial metabolism (Silva et al. 2008). Bacteria and yeasts present in coffee produced a considerable quantity of acids, specialty citric acid, as shown in Tables 2 and 3.

Bacteria are the main microbiota group present during wet processing of coffee (Avallone et al. 2001). According to Avallone et al. (2002) and Hamdouche et al. (2016), lactic acid bacteria had a significant influence on the final coffee quality; besides, these bacteria might be used as microbial markers of wet fermentation. Here, LAB presented a larger population than mesophilic bacteria. LAB may perform

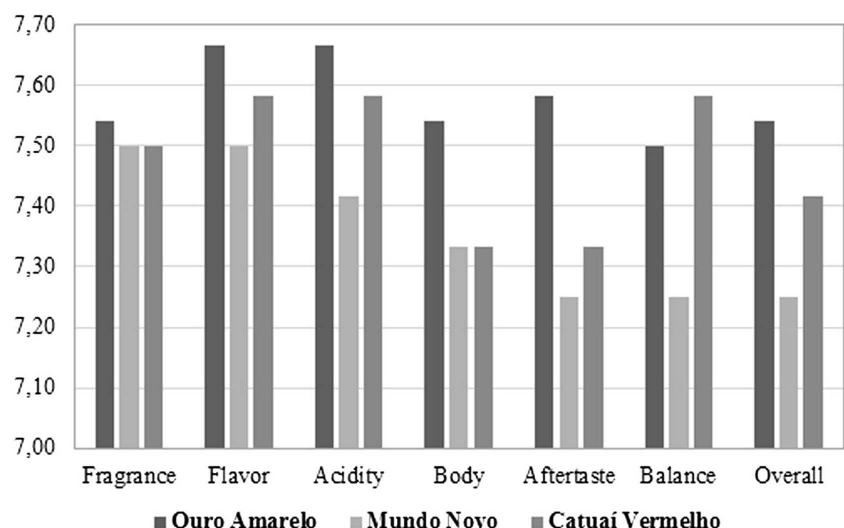


Fig. 2 Scores of cup test (SCAA) of coffee processed by wet fermentation of varieties Ouro Amarelo, Novo Mundo, and Catuaí Vermelho. Data are presented as mean. Standard deviation of mean ranged from 0.38 to < 0.01. There was no statistical difference at $p < 0.05$ by Scott-Knott test

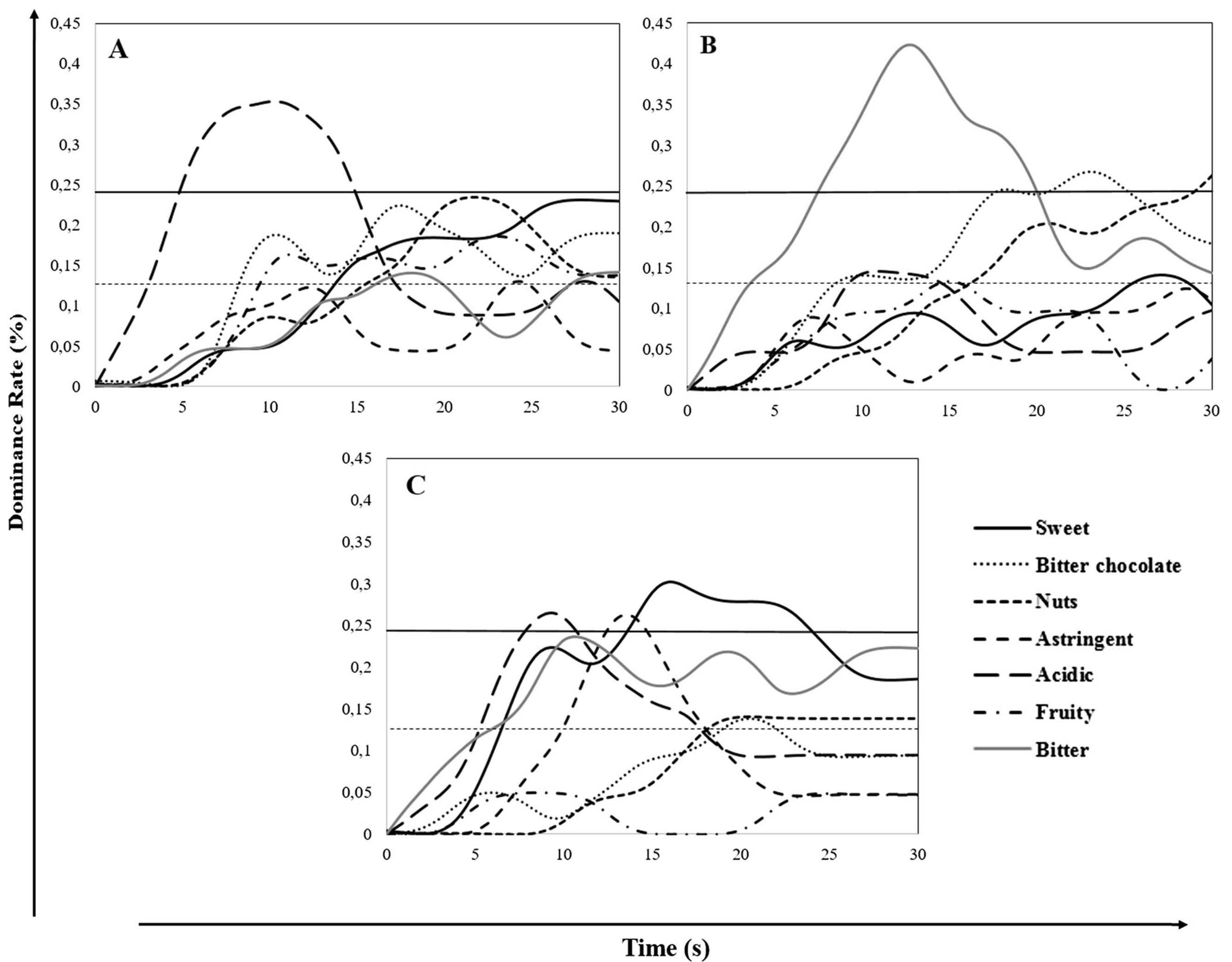


Fig. 3 Temporal dominance of sensation (TDS) curves of the coffee processed by wet fermentation of Ouro Amarelo (A), Mundo Novo (B), and Catuaí Vermelho (C) varieties

alternative pathways of use of pyruvate under specific growth conditions. Compounds such as diacetyl, acetoin, and consequently 2,3-butanediol can be formed from the degradation of pyruvate; these produce fruity, creamy, and buttery aromas (Von Wright and Axelsson 2012). In this work, 2,3-butanediol was detected in all varieties in the dried coffee, and possibly, these compounds influenced the sensory results of our final product. Among the coffee varieties studied, the variety MN showed the highest diversity of LAB. *Leuconostoc mesenteroides* was the main species found in wet process, reaching values of 6 log CFU per g. This species was also reported by Evangelista et al. (2015) and Vaughan et al. (2015) in coffee samples.

The highest diversity of the species identified for MN and CV varieties was observed in the coffee fruit, while in the OA variety, it was at the beginning of the fermentation (Table 2). The MN variety showed the highest concentration of carbohydrates (Table 1), which might justify the great variety of species found. The species present during the wet fermentation of coffee might vary according to the region where the fermentation is carried out (Evangelista et al. 2015) and, as noted here and for the first time reported according to coffee variety.

The main genera of mesophilic bacteria found were *Enterobacter*, *Cellulosimicrobium*, *Pantoea*, and *Bacillus*. These genera are usually found in wet fermentation and among mesophilic bacteria (Evangelista et al. 2015; Lee et al. 2015; Vaughan et al. 2015). However, the present study isolated bacteria from the genus *Arthrobacter*, *Microbacterium*, and *Paenibacillus*, which have already been described in the literature as commonly found in natural and semi-dry process (Sakiyama et al. 2001; Evangelista et al. 2014a, b; Silva 2015).

MALDI-TOF MS was used for clustering of bacterial isolates. Some isolates could not be identified at the species level and representatives of each group and isolates with score > 1.70 (accurate identification to the genus level) were selected for ribosomal region sequencing to

confirm the species. MALDI-TOF MS is an effective method for the identification and detection of different microbial groups and has been applied in various areas (Pavlovic et al. 2012; Santos et al. 2015).

Fructose and glucose concentrations decreased at the end of the fermentation process (Table 3), while sucrose increased throughout the process. This increase in the sucrose concentration might have resulted from the action of enzymes, such as sucrose phosphate synthase, present in the coffee beans. This enzyme is involved in the synthesis of sucrose, which contributes to control the import and mobilization of this carbohydrate (Wendler et al. 1990). The presence of microorganisms can have influenced the action of this enzyme (due to pH changes) and the sucrose formation process in the grain because the production of acids altered the fermentative environment (Ribeiro et al. 2017a). Recently, Cheng et al. (2016) described the importance of these sugars in coffee processing, since these carbohydrates are essential precursors for volatile and non-volatile compounds, such as furans, pyrazine, aliphatic acids, and hydroxymethyl furfural which were detected in the present study. It is still good to emphasize that glucose is an important precursor of citric acid (Papagianni et al. 1999) which is the main acid detected in the present study.

Acidity is often much desired in coffee and the highest score for acidity was detected in the OA variety, described as citric acid-like. This could be due to the presence of *Bacillus*, especially *B. licheniformis*, which are known producers of citric acid (Soccol et al. 2006). The nuts sensation perceived might correspond to the almond aroma that was recognized in the same beverage by the tasters in the cupping test.

The temperature of the fermentation tank increased following the ambient temperature. The qualitative analysis of the volatiles after fermentation and dried coffee indicated that metabolic activity of microorganisms occurred during both steps of the process. Changes in the production of compounds could be explained by different factors such as specific metabolites (it cannot be inferred whether the compound is a microbial metabolite or plant metabolism). The immersed coffee beans in tank fermentation adsorb the resulting compounds from the microbial metabolism from mucilage degradation; further, the intensities of the aromas and chemical compounds present in the coffee might be affected due to environmental factors (Quintero and Molina 2015).

It seems that coffee varieties might be a greater influence than the environmental conditions, for bacteria diversity and volatile compounds. The composition of the volatiles in the roasted bean may be improved using starter cultures (Evangelista et al. 2014a, b; Lee et al. 2015). We suggest that isolates identified in the study could be tested in later studies as starter cultures from these varieties.

Secondary metabolites produced during fermentation and drying could also directly or indirectly influence coffee aroma (Lee et al. 2015). All treatments were considered as specialty coffees, as the SCA scores were above 80, which is consistent with the compounds identified by GC-MS analysis (Supplementary material), where compounds were found to impart flavor to the final product. Consumers are constantly searching for good-quality coffees. Thus, understanding of the coffee microbiota contributes to improving the sensory quality of coffee beverages. The present study demonstrated the sensory differences that exist between coffee varieties. The variety of coffee had a large influence on the response the yeast presented, exercising a predominant impact on the coffee quality (Ribeiro et al. 2017a, b). The TDS analysis showed that despite having the same scores, samples from different varieties caused different sensorial sensations, which may be the influence of microbiota present and metabolites produced and may be influenced by varietal characteristics (Table 3).

Conclusion

The dominant and common bacteria found in wet fermentation were *Enterobacter cloacae*, *Leuconostoc mesenteroides*, and *Lactobacillus plantarum*. *Leuconostoc mesenteroides* showed high dominance over the other species and probably have an important influence on wet coffee fermentation. The bacteria diversity during the fermentation of the three coffee varieties (Ouro Amarelo, Mundo Novo, and Catuaí Vermelho) provided useful information about the possible starter cultures that can be used in wet processing of coffee. Tests with these isolates should be made to better understand of their capacity for inoculation and use as starter culture.

The knowledge of the chemical composition of green and roasted coffee also helped the selection of a more suitable coffee variety for wet fermentation. The sensorial analysis of the coffee using two techniques (cup test and TDS) allowed a better description of the sensorial characteristics of the varieties. These differences may be linked to the microbiological quality of each variety.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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