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Improvement of coffee beverage quality by using selected yeasts strains during the fermentation in dry process

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ABSTRACT

Coffee is an important commercial product to Brazil with its consumption distributed globally. The aim of this work was to evaluate the potential of yeast strains as starter cultures for dry fermentation of washed and non-washed coffee beans. Four yeast strains (*Saccharomyces cerevisiae* UFLA YCN727, *S. cerevisiae* UFLA YCN724, *Candida parapsilosis* UFLA YCN448 and *Pichia guilliermondii* UFLA YCN731) were inoculated separately in washed and non washed coffee cherries and in the control was not added any of the starter cultures. The fruits inoculated were spread on trays and placed on a terrace until the coffee beans reached 11% of moisture. Samples were collected for evaluation of the persistence of the inoculum by PCR-DGGE, and for chemical composition by HPLC and GC-FID. Sensory analysis was performed using the Temporal Dominance of Sensations (TDS) methodology. In all tests the yeasts persisted until the end of fermentation. There was no propionic and butyric acid production in concentrations that could compromise the final quality of the beverage. Forty-eight volatile compounds were identified, some were similar for green and roasted coffee. The most abundant class of compounds was alcohols (11–27%) followed by furan in roasted grains (~27%), and aldehydes (~13%) in green grains. The coffee inoculated with yeast showed sensations of flavors higher than the control coffee indicating increased sensory quality. The treatment with *C. parapsilosis* UFLA YCN448 showed dominance rate higher (near 1) for the sensation of caramel. In non-washed coffee those sensations were not pleasant in relation to the washed coffee, except when *P. guilliermondii* UFLA YCN731 was inoculated, suggesting that washing the fruit before the fermentation process positively influenced the final product quality. A coffee with special aroma of caramel, herbs and fruits could be produced using the starter cultures *C. parapsilosis* UFLA YCN448 and *S. cerevisiae* UFLA YCN727 in coffee processed by the dry method.

1. Introduction

Coffee is an important commercial product to Brazil with its consumption distributed globally. Nowadays, consumers can choose the most preferred type of beverage from the most full-bodied to the lightest flavors, organic or conventional, pale to dark, or with fruited flavors or with essences.

After harvesting, coffee fruits are processed to allow a spontaneous or indigenous fermentation to occur. The fermentation can be either a dry or a wet process, and sometimes a combination of both, which is called semi-dry process (Esquivel & Jiménez, 2012; Vilela, Pereira, Silva, Batista, & Schwan, 2010). After harvesting the coffee fruits might be washed or not prior to being spread out in thin layers of 5–8 cm thick on cement patios, where they remain exposed to the sun until they reach 11–12% moisture content. Fermentation of the pulp and mucilage within the fruit occurs during this period which may last up to 20 days which also corresponds to the fruit drying period.

Dry processing is characterized by being fully aerobic, which retains a larger concentration of glucose and fructose in the fruits, as they are less consumed in the seed metabolism (Knopp, Bytof, & Selmar, 2005). These sugars and pectins present in the mucilage will allow microorganisms' growth, especially bacteria and yeasts. The access of the epiphytic microorganisms to the pulp can be given by the action of pectinolytic and cellulolytic enzymes, opening the micropores on the skin-peel-bark due to the loss of water by the opening of the peduncle after harvest.

Coffee quality is a highly complex trait, and depends on physical and sensory qualities as moisture content, defects, bean size, some chemical compounds and preparation of a sample to perform cup tasting (Leroy et al., 2006). The presence of microorganisms may interfere with some of these features. During coffee fermentation some pectinolytic microorganisms are associated with the degradation of the pulp and mucilage (rich in polysaccharides) producing alcohols and acids and other metabolic compounds that interfere in the final beverage quality. Silva et al. (2013) studied these characteristics to select microorganisms to be used as culture starters on coffee fermentation.

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There are different kinds of coffee beverages characterized by different nuances in terms of body, aroma, acidity and astringency. There are many factors that influence the final beverage, and the action of microorganisms is one of them (Esquivel & Jiménez, 2012). The microbial metabolites produced in this period can diffuse into the grains and influence the beverage final quality. The microbial diversity in this process is high and several species of bacteria, yeasts, and filamentous fungi have been identified (Silva, Batista, Abreu, Dias, & Schwan, 2008).

The fermentative activity of the microbiota naturally present in coffee fruits has been discussed by some authors (Masoud, Cesar, Jespersen, & Jakobsen, 2004; Masoud, Poll, & Jakobsen, 2005; Silva, Schwan, Sousa Dias, & Wheals, 2000; Silva et al., 2013). However, there is still a need to understand the influence of specific groups of strains in coffee quality processed by dry, semi-dry and wet methods. The aim of this work was to use selected yeasts to inoculate natural coffee fermented by dry process in order to improve the final beverage quality.

2. Material and methods

2.1. Reagents

Malic, propionic and citric acids were purchased from Merck (Germany), lactic acid was purchased from Sigma-Chemical (EUA), acetic and succinic acids were purchased from Sigma-Aldrich (Germany), and butyric acid was purchased from Riedel-deHaen (Germany).

2.2. Sampling

Coffee cherries were manually harvested at the mature stage (red cherries) from a farm 750–800 m above sea level situated in Lavras in the state of Minas Gerais, Brazil. Half of the harvested coffee was immersed in clean water and immediately transferred to a tray (this fraction was named for washed coffees). Washed and non-washed fruits

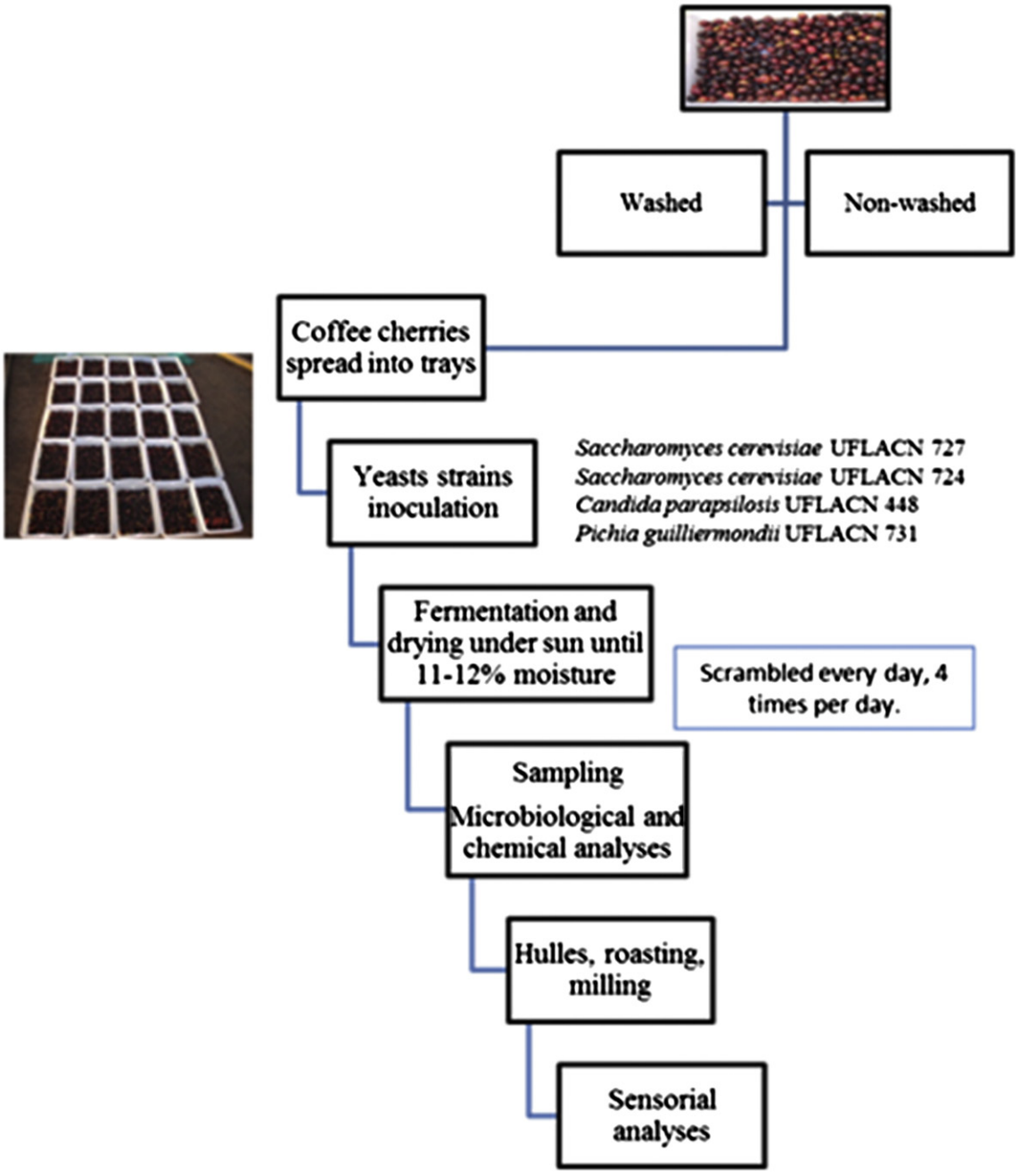

Fig. 1. Flow chart of the methodology used.

were spread (600 g) on a tray in layers of 5 cm and processed using the dry method (coffee cherries were fermented and sun dried until they reached 11–12% of moisture). The ambient temperature ranged from 14.6 to 28.2 °C, and relative humidity from 39 to 72%. Everyday, the fruits were scrambled, as usually done, in dry process at farms. Samples were collected aseptically each 4 days, placed in sterile plastic bags, and transferred to the Microbiology Laboratory of the Federal University of Lavras (UFLA) for microbiological and physicochemical analyses.

2.3. Microorganisms used as cultured starters

The yeast isolates used as culture starters were *Saccharomyces cerevisiae* UFLA YCN727, *S. cerevisiae* UFLA YCN724, *Candida parapsilosis* UFLA YCN448 and *Pichia guilliermondii* UFLA YCN731 belonging to the Culture Collection of the Microbial Physiology Laboratory at the Biology Department, UFLA, Lavras, MG, Brazil. These yeasts had been previously isolated from coffee fruit (*Coffea arabica* L. var. Acaia) during dry and semi-dry processes and their potential for use as culture starters in coffee fermentation was evaluated in previous work (Silva et al., 2013).

Each inoculum stored in the freezer − 80 °C was reactivated in YEPG medium (in g L^{-1}: glucose 20.0, yeast extract 10.0, peptone soy 10.0 and agar 20.0) incubated at 28 °C/150 rpm, in increasing volumes, until they reached approximately 10^8 CFU/mL (*C. parapsilopsis* UFLA YCN448; *S. cerevisiae* UFLA YCN724 and UFLA YCN727 and, respectively; *P. guilliermondii* UFLA YCN731). The biomass was harvested after centrifugation and resuspended with 10 mL of distilled water. The control fermentation was carried out without inoculated microorganisms but 10 mL of water was sprayed in order to have the same humidity as the other treatments. All fermentations were carried out in triplicate as a natural process method. Fig. 1 shows the methodology flowchart.

2.4. Enumeration of microorganisms

Each sample (two grams) was added to a bottle containing 18 mL of saline–peptone water (1 g L^{-1} bacteriological peptone, Himedia). After mixing for 20 min at 150 rpm in an orbital shaker, ten-fold dilutions were prepared. Microorganisms were counted using two different culture media: PCA (in g L^{-1} tryptone 5; yeast extract 2.5; glucose 1; agar 15) was used as a general medium for the viable bacteria population and YEPG (in g L^{-1}: glucose 20.0, yeast extract 10.0, peptone soy 10.0 and agar 20.0) was used for yeasts. Following inoculation, plates were incubated at 28 °C for 48 h. After that, the morphological characterisation was done and the population was estimated.

2.5. Microbial community analysis through PCR-DGGE

2.5.1. Total DNA extraction and PCR analysis

Coffee samples from the initial and final fermentation periods were collected to DGGE analysis. Three grams of sample was mixed with 5 mL of Milli-Q water for 10 min, the fluids were centrifuged 100 ×*g* for 10 min at 4 °C. The pellet was used for DNA extraction. Total DNA was extracted from samples at three periods of fermentation (initial, intermediate and end) using the DNA Purification from Tissues protocol [QIAamp DNA Mini Kit (Qiagen, Hilden, Germany)] in accordance with the instructions of the manufacturer. After purification, the samples were PCR-amplified and analyzed in 1% agarose gel and the final samples were stored at − 20 °C until further use.

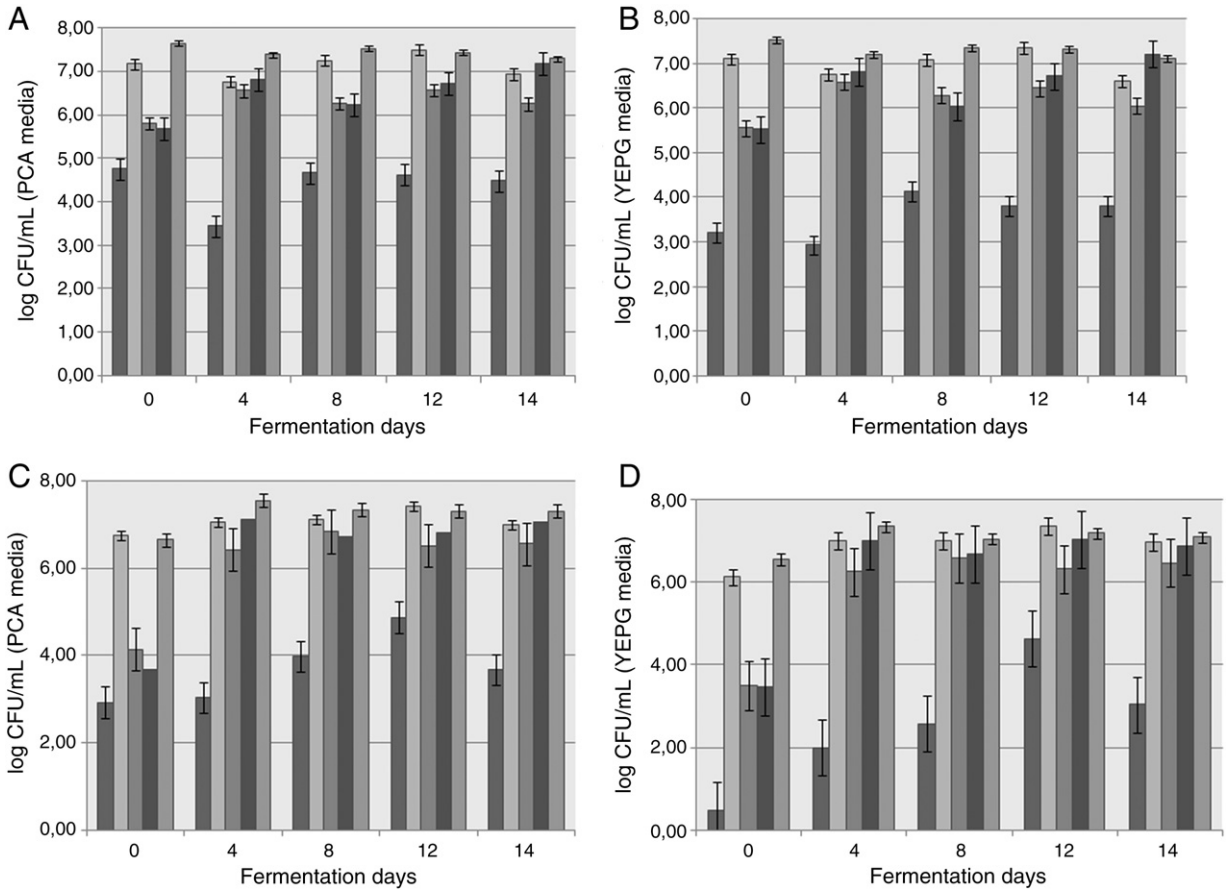

Fig. 2. Total population (PCA media-A and C) and yeast population (YEPG media-B and D) from the inoculation of 4 yeast strains selected during the fermentation of dry-processed, non-washed coffee (A and B) and washed coffee (C and D). Control (■), *C. parapsilosis* UFLA YCN448 (■), *S. cerevisiae* UFLA YCN724 (■), *S. cerevisiae* UFLA YCN727 (■), *P. guilliermondii* UFLA YCN731 (■).

The DNA from the bacterial community was amplified with the 338fgc primers (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG-3′) (the GC clamp is underlined) and 518r (5′-ATT ACC GCG GCT GCT GG-3′), which span the V3 region of the 16S rRNA gene (Ovreas, Forney, & Daae, 1997). A fragment of the D1-region of the 26S rRNA gene was amplified with the eukaryotic universal primers NL1GC (5′ CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA TAT CAA TAA GCG GAG GAA AAG-3′) (the GC clamp is underlined) and LS2 (5′-ATT CCC AAA CAA CTC GAC TC-3′), which amplified a fragment of approximately 250 bp (Cocolin, Bisson, & Mills, 2000). PCR was carried out in a final reaction volume of 25 μL containing 0.625 U Taq DNA polymerase (Promega, Milan, Italy), 2.5 μL 10 × buffer, 0.1 mM dNTP, 0.2 mM of each primer, 1.5 mM $MgCl_2$ and 1 μL of extracted DNA. The amplification was performed according to Ramos et al. (2010). Aliquots (3 μL) of the amplification products were analyzed by electrophoresis on 1% agarose gels before they were used for DGGE.

2.5.2. PCR-DGGE analysis

The PCR products were separated in polyacrylamide gels [8% (w/v) acrylamide:bisacrylamide (37.5:1)] in 1 × TAE buffer with a DCode system apparatus (BioRad Universal Dcode Mutation Detection System, Richmond, CA, USA). Optimal separation was achieved with a 15–55% urea-formamide denaturing gradient for bacteria community and 20–60% for the yeast community (100% correspondent to 7 M urea and 40% (v/v) formamide).

Electrophoresis was conducted at a constant voltage of 130 V for 6 h (bacteria and yeasts) and at a constant temperature of 60 °C. Following electrophoresis, the gels were stained with SYBR-Green I (Molecular Probes) (1:10.000 v/v) for 30 min. The images were visualized and photographed using a Transilluminator (LPix®).

2.5.3. DGGE bands sequencing

Selected bands from the PCR-DGGE gels were excised with a sterile blade and placed in 50 μL of sterile Milli-Q water at 4 °C overnight to allow the DNA to diffuse out of the polyacrylamide matrix and re-

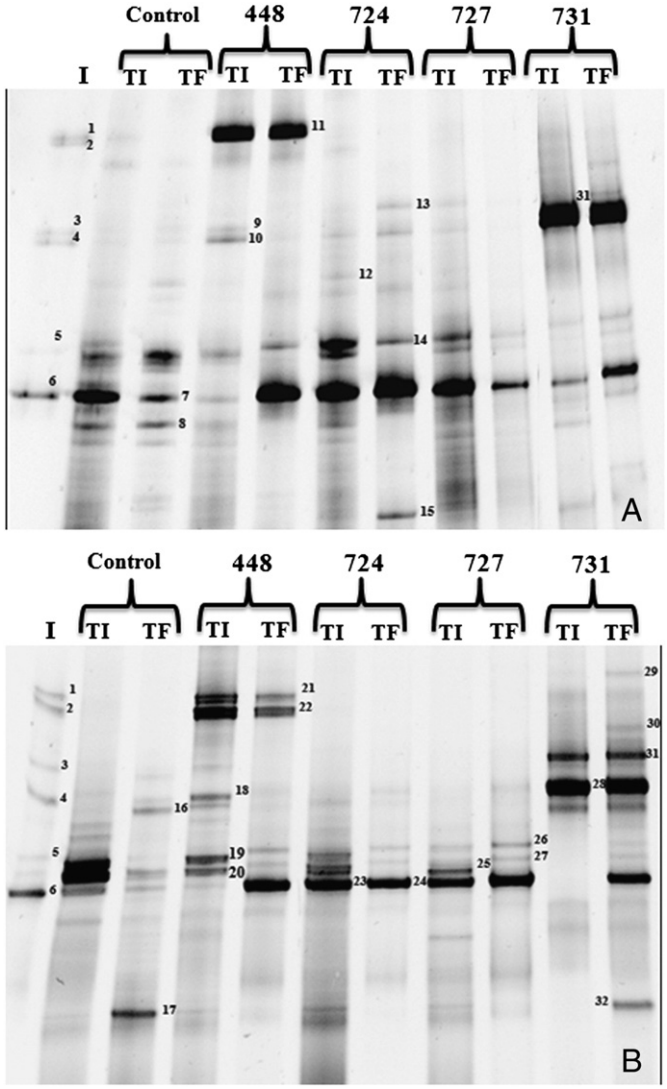

Fig. 3. DGGE analyses of yeast communities found in the beginning (TI) and at the end (TF) of the fermentation process of washed (A) and non-washed coffee (B). I = mixture of the strain inoculates in fruit. The numbers over the spots represent the yeast strains: *C. parapsilosis* UFLA YCN448, *S. cerevisiae* UFLA YCN724, *S. cerevisiae* UFLA YCN727, *P. guilliermondii* UFLA YCN731. The identities of the bands and access number are: 1, 2, 11, 21,22 = *Candida parapsilosis* (FJ009291); 3, 4, 9, 10, 18, 28, 31 = *Pichia guilliermondii* (EF197951); 5, 6, 7, 14, 23, 24 = *Saccharomyces cerevisiae* (EU649672); 8,12, 26, 27 = *Mitchella repens* (JQ417238); 13, 16 = *Pichia kluyveri* (JQ219339); 15, 17, 29, 32 = *Torulaspora delbrueckii* (EU879961); 19 = *Hanseniaspora opuntiae* (DQ872866); 20, 25 = *Debaryomyces hansenii* (FJ475230); 30 = unidentified. Similarity above 99%.

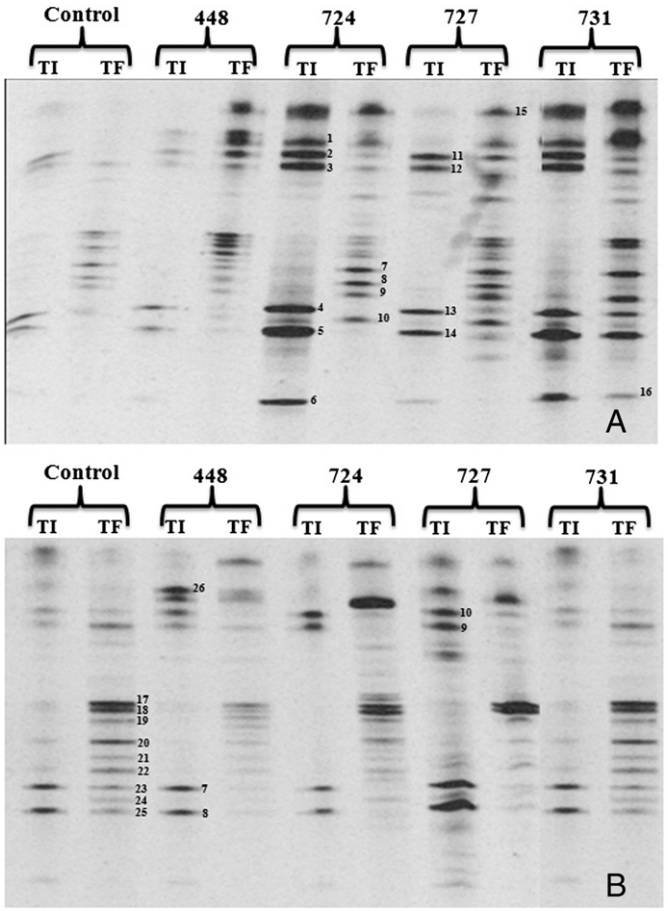

Fig. 4. DGGE analyses of bacteria communities found in the beginning (TI) and at the end (TF) of the fermentation process of washed (A) and non-washed coffee (B). The identities of the bands and access number are: 1 = Uncultured bacterium (GU301232), 6, 16 = Uncultured bacterium (GU301189); 2, 10 = *Erwinia billingiae* (JQ353778); 3, 9 = *Leuconostoc mesenteroides* (GU458344); 4,7,23 = *Halospirulina* sp. (JX912466); 5,8,25 = unidentified; 11, 20 = *Pantoea agglomerans* (JN645943); 12, 21 = *Pantoea dispersa* (JQ659939); 13, 22 = *Pantoea* sp. (FR692005); 14, 24 = *Pectobacterium* sp. (FJ784694); 15 = *Cryptosporidium environmental* (JQ178286); 17 = *Pantoea* sp. (EU741017); 18 = *Pantoea brenneri* (HF585161); 19 = *Pantoea eucrina* (JX865456). Similarity above 98%.

amplified with the 338fgc and 518r primers for bacteria and NL1 and LS2 for yeast.

The DNA fragments were purified with a QIAEX II gel extraction kit (Qiagen, Chatsworth, CA, USA) and the PCR products were sequenced by UNESP University (Jaboticabal, São Paulo, Brazil). The sequences were compared with those available in the GenBank database with the BLAST algorithm (National Center for Biotechnology Information, Maryland, USA).

2.6. Organic acids analysis

Organic acids (malic, lactic, acetic, butyric, propionic, citric, oxalic, succinic and tartaric acid) were analyzed using a high-performance liquid chromatography system Shimadzu model (Shimadzu Corp., Japan) with a UV detector at 210 nm. Three grams of sample was mixed with 5 mL of Milli-Q water for 10 min and the fluids were centrifuged 10,000 ×*g* for 10 min at 4 °C two times. Samples were microfiltered through a 0.2 μm cellulose acetate filter and directly injected (20 μL) onto the chromatographic column. A Shimpack SCR-101H (7.9 mm × 30 cm) column operating at 50 °C was used to achieve chromatographic separation of water-soluble acids that were eluted with 100 mM of perchloric acid at a flow rate of 0.6 mL/min. The acids were identified by comparison with retention times of authentic standards. The quantification of compounds was performed using calibration curves constructed with injections (at the same conditions of samples) of different concentrations of standard compounds.

2.7. Volatile compounds analysis

2.7.1. Extraction of volatile by headspace-SPME

Coffee samples were macerated with nitrogen for headspace analysis. A carboxen/poly (dimethylsiloxane) (DVB/CAR/PDMS) type 75 μm SPME fiber (Supelco Co., Bellefonte, PA, USA) was used to extract volatile constituents from the coffee headspace. Two grams of coffee was placed in a 15 mL hermetically sealed flask, and heated for 10 min at 60 °C, to reach sample headspace equilibrium. Then, volatile compounds were extracted by placing the SPME fiber in the headspace for 30 min at 60 °C. For compound desorption, the fiber was placed in the GC injection port heated at 230 °C for 5 min.

2.7.2. HS-SPME/GC analysis

The analysis of volatile compounds of green and roasted beans was performed using a gas chromatograph (GC), Shimadzu model 17A equipped with an FID (flame ionization detector) and a capillary DB wax column (30 m × 0.25 mm i.d. × 0.25 μm) (J&W Scientific, Folsom, Calif., U.S.A.). The oven temperature was maintained at 50 °C for 5 min, raised to 190 °C by increments of 3 °C/min, and then maintained at 190 °C for 10 min. Injector and detector temperatures were kept at 230 and 240 °C, respectively. The carrier gas (N_2) was maintained at a flow rate of 1.2 mL/min. Volatile compounds were identified by comparing the retention times of the compounds with those of standard compounds injected under the same conditions (Duarte et al., 2010). The relative percentages of individual compounds were calculated from the total area of volatiles on the chromatograms (Petisca, Pérez-Palacios, Farah, Pinho, & Ferreira, 2013).

2.8. Sensory analysis

The samples were prepared according to the Specialty Coffee Association of America (SCAA, 2012). The coffee grains were roasted in a Probat roaster, "Probatino" model, having a capacity of 150 g and ground in a Pinhalense ML-1 electric mill. A panel of trained coffee tasters, with Q-Grader Coffee Certificate was employed for this study. Two methodologies were applied to evaluate the sensory

Table 1
Organic acid production by yeasts in the natural washed and natural non-washed coffee in the beginning and in the end of fermentation process. The organic acids were evaluated in pulp + seed and seed.

Fermentation samples	Fermentation Time (days)	Organic acids (g/kg)											
		PULP + SEED						SEED					
		Citric	Malic	Succinic	Lactic	Acetic	Propionic	Citric	Malic	Succinic	Lactic	Acetic	Propionic
No-washed coffee													
Control[a]	0	0.41	2.78	6.46	0.04	nd[f]	nd	0.02	1.04	1.59	0.02	nd	nd
Control	14	1.87	3.14	17.70	nd	0.08	nd	0.36	0.70	3.06	nd	nd	nd
UFLA YCN448[b]	0	0.06	4.48	5.35	nd	nd	nd	0.07	2.03	1.82	nd	nd	nd
UFLA YCN448	14	0.77	2.00	4.87	1.37	nd	nd	0.07	0.55	1.24	0.20	nd	nd
UFLA YCN724[c]	0	0.67	4.39	7.82	0.01	nd	nd	0.06	1.12	2.00	nd	nd	nd
UFLA YCN724	14	1.34	1.81	4.61	nd	nd	nd	0.59	0.90	2.21	nd	nd	nd
UFLA YCN727[d]	0	0.11	3.83	3.41	nd	7.34	0.85	0.05	1.48	1.73	nd	nd	nd
UFLA YCN727	14	2.07	2.43	6.54	nd	4.86	2.09	0.33	0.49	1.20	0.02	nd	nd
UFLA YCN731[e]	0	0.32	3.92	6.11	0.05	10.52	0.40	0.09	0.41	0.49	0.01	2.07	0.11
UFLA YCN731	14	2.03	1.41	5.36	nd	2.99	1.55	0.39	0.49	1.48	nd	1.46	0.43
Washed coffee													
Control	0	0.25	4.76	5.52	nd	nd	nd	0.02	0.97	0.89	0.02	nd	nd
Control	14	0.82	2.80	4.76	nd	nd	nd	0.22	1.01	2.05	nd	nd	nd
UFLA YCN448	0	0.43	4.22	3.20	0.01	nd	nd	0.15	0.34	0.11	nd	nd	nd
UFLA YCN448	14	1.43	3.40	4.42	0.06	nd	nd	0.29	1.34	1.93	0.11	nd	nd
UFLA YCN724	0	nd	2.63	1.55	nd	nd	nd	0.05	1.34	0.75	nd	nd	nd
UFLA YCN724	14	1.07	4.03	9.32	0.14	nd	nd	0.25	0.57	0.90	0.03	nd	nd
UFLA YCN727	0	0.14	1.31	1.07	nd	2.10	0.14	0.10	0.79	0.91	nd	nd	nd
UFLA YCN727	14	1.38	1.87	4.81	0.04	4.95	1.77	0.26	0.72	2.52	0.01	nd	nd
UFLA YCN731	0	0.55	2.60	2.47	nd	7.68	0.28	0.16	0.43	0.55	nd	1.63	0.07
UFLA YCN731	14	2.08	2.11	7.75	nd	7.70	1.46	0.41	0.51	1.44	nd	2.27	0.36

The samples show a standart deviation around 0.02.
[a] Control = without inoculums.
[b] UFLA YCN448 = *Candida parapsilosis* UFLA YCN448.
[c] UFLA YCN724 = *Saccharomyces cerevisiae* UFLA YCN724.
[d] UFLA YCN727 = *Saccharomyces cerevisiae* UFLA YCN727.
[e] UFLA YCN731 = *Pichia guilliermondii* UFLA YCN731.
[f] nd = not detectable.

Table 2
Relative percentage of volatile compounds extracted from green and roasted non-washed coffee.

	%									
	Green bean	Roasted bean	Green bean	Roasted bean	Green bean	Roasted bean	Green bean	Roasted bean	Green bean	Roasted bean
Compounds/Yeast	Control[a]	Control[a]	448[b]	448[b]	724[c]	724[c]	727[d]	727[d]	731[e]	731[e]
Acetal										
1,1-dietoxyethane	0.14	0.47	0.06	0.75	0.03	0.72	0.03	0.63	0.05	0.73
Ketones										
2,3-butanedione	0.58	0.09	0.29	0.08	0.25	0.11	0.35	nd	0.33	nd
2-nonanone	nd[f]	1.56	nd	2.20	nd	1.93	nd	1.59	nd	2.14
Verbenone	nd	1.18	nd	1.52	nd	1.35	nd	1.01	nd	1.42
Total ketones	0.58	2.83	0.29	3.80	0.25	3.39	0.35	2.60	0.33	3.56
Alcohols										
Methanol	0.46	1.90	0.33	2.28	0.22	2.38	0.23	1.78	0.23	2.07
1-Propanol	0.51	0.07	0.37	0.08	0.63	0.07	0.38	0.07	0.46	0.06
2-Methyl-1-propanol	nd	nd	nd	nd	nd	nd	nd	nd	0.08	nd
1-Butanol	1.05	0.03	1.73	0.04	0.40	0.02	0.65	0.05	0.27	nd
3-Methyl-1-butanol	nd	nd	0.37	nd	nd	nd	0.23	nd	0.19	nd
1-Pentanol	nd	5.01	nd	6.22	nd	5.77	nd	5.66	nd	6.17
2-Heptanol	1.12	2.52	0.38	3.25	0.70	2.81	0.30	2.55	0.33	2.90
3-Mehtyl-1-pentanol	0.23	nd	0.73	nd	0.68	nd	0.46	nd	0.63	nd
1-Hexanol	nd	nd	nd	nd	nd	nd	nd	0.02	nd	nd
Linalool	4.98	0.29	5.35	0.54	4.60	0.72	6.97	0.90	8.12	0.67
1,2-Propanediol	nd	0.27	nd	0.26	nd	0.34	nd	0.28	nd	0.32
a-Terpeniol	nd	0.05	nd	nd	nd	0.04	nd	nd	nd	nd
b-Citronellol	nd	0.23	nd	0.20	nd	0.17	nd	0.17	nd	0.20
Geraniol	nd	0.05	nd	0.03	nd	0.04	nd	nd	nd	nd
2-Phenylethanol	0.45	0.11	1.67	0.17	1.02	0.29	1.85	0.25	1.84	0.24
Menthol	nd	0.11	0.21	nd	nd	nd	nd	0.10	nd	nd
Total alcohols	8.81	10.64	11.14	13.07	8.25	12.65	11.07	11.83	12.15	12.63
Aldehydes										
Acetaldehyde	1.76	0.59	1.61	0.52	1.12	0.47	1.09	0.42	1.28	0.54
Butyraldehyde	4.73	0.05	9.98	0.27	18.70	0.44	14.82	0.80	10.75	0.34
Hexanal	0.28	nd	0.15	nd	0.16	nd	0.09	nd	0.23	nd
Octanal	nd	0.40	nd	0.26	nd	0.39	nd	0.51	nd	0.42
Decyl aldehyde	nd	nd	nd	0.38	nd	0.35	nd	0.26	nd	0.35
Total aldehydes	6.77	1.04	11.74	1.43	19.98	1.65	16	1.99	12.26	1.65
Acids										
Propionic acid	nd	nd	nd	nd	nd	0.33	nd	nd	nd	nd
Isobutyric acid	nd	10.32	nd	9.25	nd	8.72	nd	8.97	nd	9.33
Butyric acid	nd	0.10	nd	0.11	nd	0.11	nd	0.10	nd	0.10
Hexanoic acid	nd	0.06	nd	0.09	0.44	0.09	0.50	0.09	0.18	0.08
Decanoic acid	nd	0.14	nd	0.19	nd	0.16	nd	nd	nd	0.08
Total acids	nd	10.62	nd	9.64	0.44	9.41	0.5	9.16	0.18	9.59
Esters										
Ethyl acetate	0.06	0.02	nd	0.01	nd	0.01	nd	0.02	nd	0.01
Propyl acetate	2.49	0.83	1.09	0.66	0.90	0.71	0.54	0.59	0.92	0.67
Isobutyl acetate	0.44	nd	0.33	nd	0.28	nd	0.22	nd	0.20	nd
Ethyl butyrate	1.03	0.07	1.73	0.08	1.89	0.08	1.30	0.07	1.51	0.06
Isoamyl acetate	nd	nd	nd	nd	1.03	nd	1.43	nd	0.80	nd
Ethyl lactate	nd	nd	nd	nd	nd	nd	nd	nd	0.18	nd
Ethyl octanoate	nd	0.01	nd	0.02	nd	0.02	nd	0.02	nd	nd
Diethyl malonate	nd	0.36	nd	0.37	nd	0.39	nd	0.33	nd	nd
Phenyl acetate	nd	0.32	nd	0.34	nd	0.31	nd	0.34	nd	0.35
Propyl butyrate	nd	0.07	nd	nd	nd	0.02	nd	nd	nd	0.02
Diethylsuccinate	nd	0.02	nd	nd	nd	nd	nd	nd	nd	nd
Total esters	4.02	1.70	3.15	1.48	4.1	1.54	3.49	1.37	3.61	1.11
Phenols										
Guaiacol	nd	0.39	nd	0.23	nd	0.39	nd	0.20	nd	0.20
Furans										
Furfuryl alcohol	nd	12.40	nd	10.76	nd	9.81	nd	12.27	nd	11.22
Furfuryl acetate	nd	3.67	nd	3.08	nd	3.72	nd	4.33	nd	3.58
Furfuryl propionate	nd	0.31	nd	0.21	nd	0.26	nd	0.29	nd	0.33
Furfural	nd	14.39	nd	13.80	nd	13.01	nd	10.81	nd	11.93
5-methylfurfural	1.19	0.01	0.76	nd	0.74	0.01	0.52	nd	0.51	nd
Total furans	1.19	30.77	0.76	27.85	0.74	26.81	0.52	27.7	0.51	27.06
Total GC area	59,223	521,920	61,355	431,442	108,068	487,511	76,741	537,204	103,307	482,993

[a] Control = without inoculums.
[b] UFLA YCN448 = *Candida parapsilosis* UFLA YCN448.
[c] UFLA YCN724 = *Saccharomyces cerevisiae* UFLA YCN724.
[d] UFLA YCN727 = *Saccharomyces cerevisiae* UFLA YCN727.
[e] UFLA YCN731 = *Pichia guilliermondii* UFLA YCN731.
[f] nd = not detectable.

Table 3
Relative percentage of volatile compounds extracted from green and roast whased coffee.

Compounds/yeast	%									
	Green bean	Roasted bean	Green bean	Roasted bean	Green bean	Roasted bean	Green bean	Roasted bean	Green bean	Roasted bean
	Control[a]	Control[a]	448[b]	448[b]	724[c]	724[c]	727[d]	727[d]	731[e]	731[e]
Acetal										
1,1-Dietoxyethane	0.17	0.50	0.02	0.39	0.02	0.54	0.01	0.43	0.02	0.77
Ketones										
2,3-Butanedione	0.29	0.09	0.12	0.06	0.14	0.07	0.08	0.09	0.17	nd
2-Nonanone	nd[f]	1.48	nd	1.46	nd	1.58	nd	1.35	nd	1.41
Verbenone	nd	0.98	nd	0.90	nd	0.98	nd	0.85	nd	0.86
Total ketones	0.29	2.55	0.12	2.42	0.14	2.63	0.08	2.29	0.17	2.27
Alcohols										
Methanol	0.45	1.72	0.12	1.60	0.13	1.68	0.18	1.60	0.07	1.88
1-Propanol	1.35	0.05	0.80	0.05	0.83	0.06	0.83	0.07	0.64	0.06
1-Butanol	0.57	0.03	nd	nd	nd	0.02	nd	nd	0.12	nd
1-Pentanol	nd	5.94	nd	5.22	nd	6.69	nd	5.27	nd	5.85
2-Heptanol	0.79	2.77	0.39	2.54	nd	2.94	0.28	2.71	nd	2.61
3-Mehtyl-1-pentanol	0.43	nd	0.30	nd	0.53	nd	0.46	nd	0.42	nd
1-Hexanol	nd	nd	nd	0.03	nd	nd	nd	0.01	nd	nd
Trans-3-hexen-1-ol	nd	0.27	nd	0.23	nd	0.22	nd	0.24	nd	0.21
Linalool	6.30	0.37	3.92	0.91	3.87	0.76	4.96	1.18	4.60	0.89
1,2-Propanediol	nd	0.24	nd	0.30	nd	0.34	nd	0.28	nd	0.36
b-Citronellol	nd	0.24	nd	0.19	nd	0.19	nd	0.18	nd	0.19
2-Phenylethanol	0.77	0.12	1.05	0.21	1.05	0.18	1.38	0.23	1.20	0.17
Menthol	nd	0.10	nd	0.07	nd	0.07	nd	nd	0.14	0.08
Total alcohols	10.66	11.85	6.58	11.35	6.41	13.15	8.09	11.77	7.19	12.3
Aldehydes										
Acetaldehyde	2.17	0.51	1.33	0.38	0.85	0.33	0.76	0.30	1.12	0.47
Butyraldehyde	3.23	0.10	12.31	0.75	10.60	0.56	17.05	0.89	14.28	0.36
Hexanal	nd	nd	nd	nd	0.06	nd	nd	nd	nd	nd
Octanal	nd	0.49	nd	0.58	nd	0.33	nd	0.34	nd	0.58
Decyl aldehyde	nd	0.25	nd	0.24	nd	0.25	nd	0.20	nd	0.24
Total aldehydes	5.4	1.35	13.64	1.95	11.51	1.47	17.81	1.73	15.4	1.65
Acids										
Isobutyric acid	nd	9.55	nd	8.22	nd	8.12	nd	8.03	nd	9.48
Butyric acid	nd	0.10	nd	0.09	nd	0.10	nd	0.08	nd	0.10
Hexanoic acid	nd	nd	nd	0.09	nd	0.08	nd	0.08	nd	0.08
Decanoic acid	nd	0.05	nd	nd	nd	nd	nd	nd	nd	nd
Total acids	nd	9.7	nd	8.4	nd	8.3	nd	8.19	nd	9.66
Esters										
Ethyl acetate	0.07	0.02	nd	0.00	nd	0.01	nd	0.01	nd	0.01
Propyl acetate	2.44	0.79	0.57	0.67	0.56	0.65	0.26	0.59	0.51	0.71
Isobutyl acetate	0.34	nd	0.17	nd	0.16	nd	0.12	nd	0.18	nd
Ethyl butyrate	1.09	0.05	3.11	0.04	3.77	0.08	2.63	0.05	3.17	0.07
Isoamyl acetate	nd	nd	nd	nd	nd	nd	1.47	nd	nd	nd
Ethyl octanoate	nd	0.02	nd	0.02	nd	0.02	nd	0.02	nd	0.04
Diethyl malonate	nd	0.21	nd	0.32	nd	0.18	nd	0.26	nd	0.25
Phenyl acetate	nd	0.38	nd	0.36	nd	0.40	nd	0.41	nd	0.42
Propyl butyrate	nd	0.03	nd	0.03	nd	0.04	nd	0.04	nd	0.04
Diethylsuccinate	nd	0.03	nd	nd	nd	0.04	nd	nd	nd	0.04
Total esters	3.94	1.53	3.85	1.44	4.49	1.42	4.48	1.38	3.86	1.58
Phenols										
Guaiacol	nd	0.21	nd	0.21	nd	0.19	nd	0.19	nd	0.19
Furans										
Furfuryl alcohol	nd	15.49	nd	13.56	nd	14.02	nd	14.14	nd	13.89
Furfuryl acetate	nd	3.97	nd	3.94	nd	5.02	nd	4.56	nd	4.96
Furfuryl propionate	nd	0.24	nd	0.18	nd	0.30	nd	0.25	nd	0.27
Furfural	nd	9.99	nd	9.96	nd	8.02	nd	7.61	nd	9.15
5-Methylfurfural	0.89	0.01	0.42	nd	0.39	nd	0.31	nd	0.48	nd
Total furans	0.89	29.7	0.42	27.64	0.39	27.36	0.31	26.56	0.48	28.27
Total GC area	48,789	642,420	85,915	550,900	114,407	623,276	118,141	620,499	107,172	597,224

[a] Control = without inoculums.
[b] UFLA YCN448 = *Candida parapsilosis* UFLA YCN448.
[c] UFLA YCN724 = *Saccharomyces cerevisiae* UFLA YCN724.
[d] UFLA YCN727 = *Saccharomyces cerevisiae* UFLA YCN727.
[e] UFLA YCN731 = *Pichia guilliermondii* UFLA YCN731.
[f] nd = not detectable.

characteristics of coffee. The first sensory evaluation was conducted according to SCAA (SCAA, 2012) standards, assessing ten attributes: Fragrance, Flavor, Aftertaste, Acidity, Body, Uniformity, Balance, Sweetness, Cleanliness and Score. After that, the temporal dominance of sensations (TDS) analysis was used (Pineau et al., 2009). The most relevant attributes for describing the temporal evolution

of sensations induced by each sample were selected from the main lists by those trained coffee experts. The attributes selected were: chocolate, acidic, bitter, fermented, fruity, herbaceous, caramel, and spicy. The total duration of the proof taste was 20 s. The panelists imbibed the coffee, and moved it around in their mouths for 3 s before swallowing it. The evaluation continued until no sensation was perceived at a maximum of 20 s. The act of swallowing the sample facilitated the temporal sensory perception of the product, according to reports of the panelists. Samples coded with three digits were submitted in balanced order (Wakeling & Macfie, 1995). The panelists evaluated each sample in triplicate. Data were recorded using the SENSOMAKER Software (Nunes & Pinheiro, 2012) and plotted as TDS curve showing, for each sample, the percentage of subjects which selected the attribute as dominant at a specific time, i.e. the dominance rate (Pineau et al., 2009).

2.9. Statistical analysis

Data of volatile compounds and sensory analysis were statistically analyzed by principal component analysis (PCA) using SENSOMAKER Software (Nunes & Pinheiro, 2012). An $m \times n$ matrix was built with the relative areas of the n identified chromatographic peaks for the m samples, for volatile compounds. An $m \times n$ matrix was built with the values of the n attributes for the m samples, for sensory analysis. The data were auto scaled.

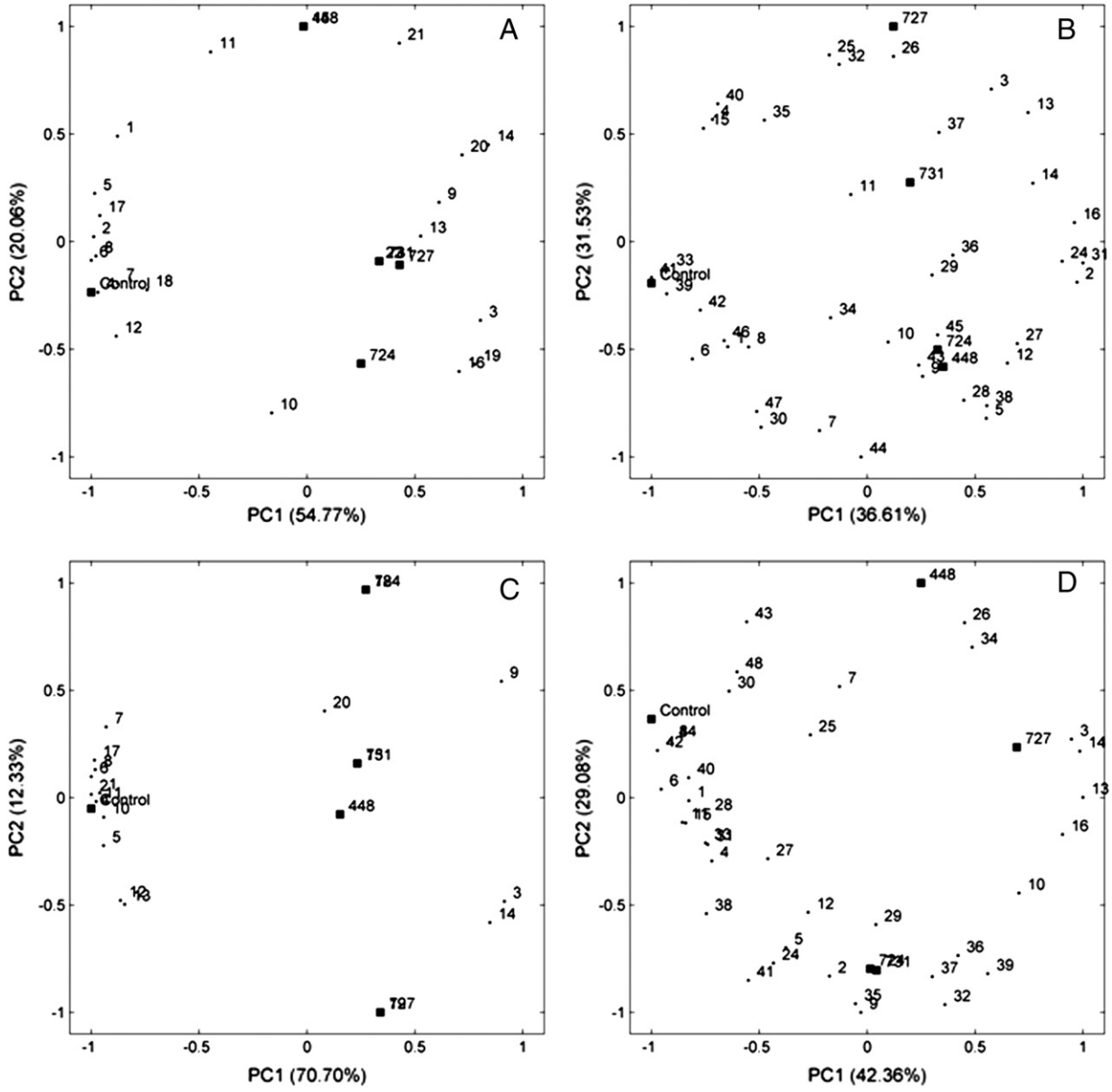

Fig. 5. Principal component analysis loading plot of the following volatile compounds during the fermentation coffee by dry process in non-washed (A — green bean and B — roasted bean) and washed (C — green bean and D — roasted bean) coffee fruits inoculated with the UFLA YCN448, UFLA YCN724, and UFLA YCN727 and UFLA YCN731 yeast strains and control (no yeast inoculated): 1 — Acetaldehyde, 2 — 1,1-Dietoxyethane, 3 — Butyraldehyde, 4 — Ethyl acetate, 5 — Methanol, 6 — Propyl acetate, 7 — 2,3-Butanedione, 8 — 5-Methylfurfural, 9 — Ethyl butyrate, 10 — 1-Propanol, 11 — 1-Butanol, 12 — 2-Heptanol, 13 — Linalool, 14 — 2-Phenylethanol, 15 — Menthol, 16 — Hexanoic acid 17 — Isobutyl acetate, 18 — Hexanal, 19 — Isoamyl acetate, 20 — 3-Mehtyl-1-pentanol, 21 — 3-Methyl-1-butanol, 22 — 2-Methyl-1-propanol, 23 — Ethyl lactate, 24 — 1-Pentanol, 25 — Octanal, 26 — 1-Hexanol, 27 — 2-Nonanone, 28 — Verbenone, 29 — Ethyl octanoate, 30 — Furfural, 31 — Decyl aldehyde, 32 — Furfuryl acetate, 33 — Isobutyric acid, 34 — Diethyl malonate, 35 — Furfuryl propionate, 36 — 1,2-Propanediol, 37 — Phenyl acetate, 38 — Butyric acid, 39 — Propyl butyrate, 40 — Furfuryl alcohol, 41 — Diethylsuccinate, 42 — b-Citronellol, 43 — Guaiacol, 44 — Decanoic acid, 45 — Propionic acid, 46 — a-Terpeniol, 47 — Geraniol, 48 — Trans-3-hexen-1-ol.

3. Results and discussion

3.1. Yeast population during the coffee fermentation

Total microbial and yeast counts showed no significant differences during the fermentation processes with different strains inoculated in washed and non-washed coffee cherries (Fig. 2). However, it was possible to observe that the microbial population was higher in non-washed coffees, which was already expected due to the physical removal of microorganisms during washing.

The bacteria population was 1.57 times higher than yeasts in the treatments where coffee cherries were washed before fermentation (Fig. 2). The identification of bacteria and yeasts population in dry processed coffee has been reported by Silva et al. (2000, 2008). Usually, bacteria and yeasts predominate in the early stages of fermentation due to the high content of water present in the fruits (Silva et al., 2008). The yeast population was higher in the treatments where selected strains were inoculated than in the control. These results showed that the inoculated strains were able to compete with the epiphytic microorganisms already present in coffee fruits, and to use the coffee fruit pulp as substrate. Another result was that they could persist throughout the fermentation process.

Analyzing each inoculated strain, it was observed that the population of *C. parapsilosis* UFLA YCN448 in non-washed coffee was constant during the fermentation. However, this strain showed growth of 1 log cycle in treatments with washed coffee. *S. cerevisiae* UFLA YCN724 and UFLA YCN727 strains showed no difference in population between them, although it was below that of *C. parapsilosis* UFLA YCN448. The

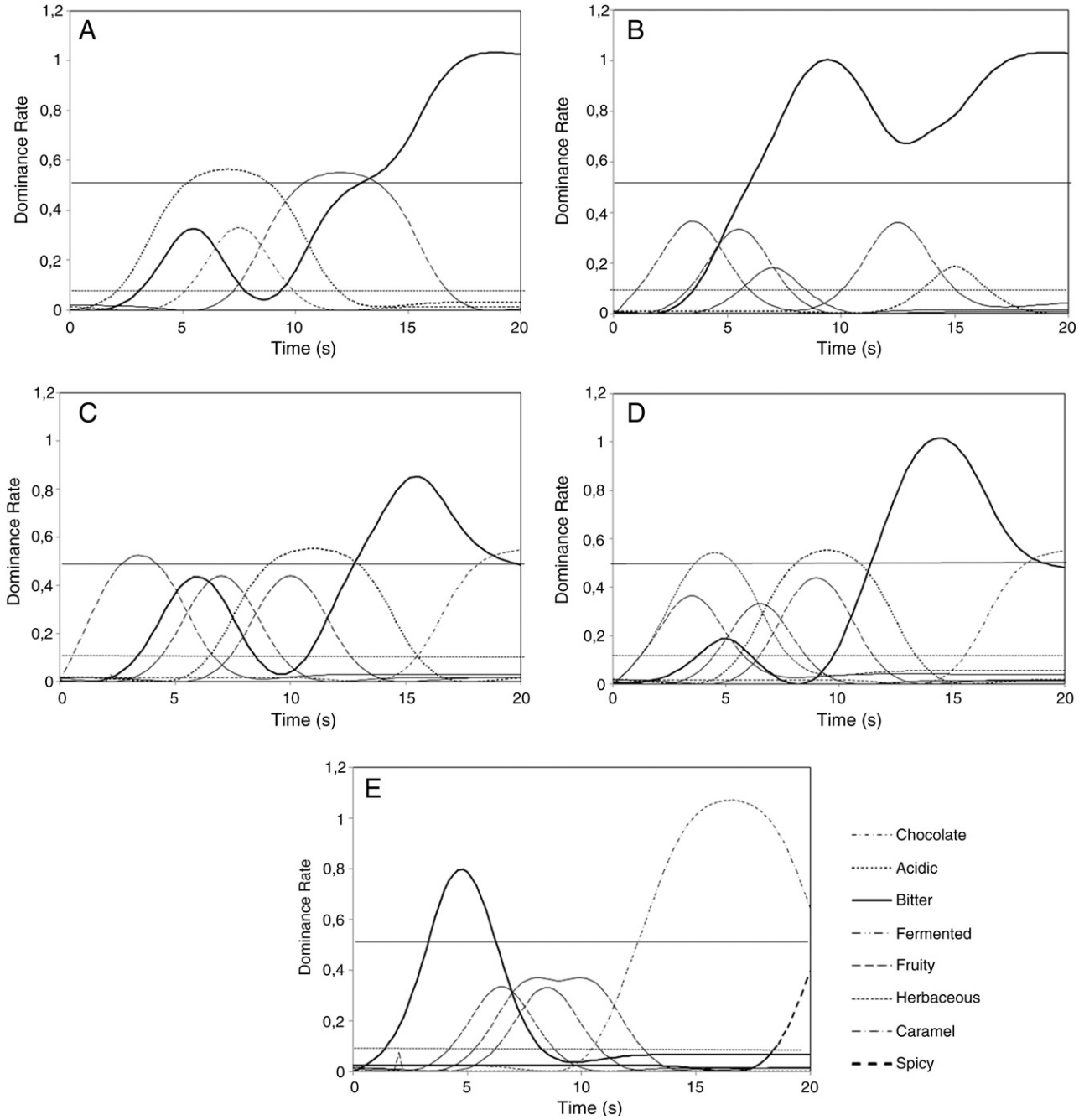

Fig. 6. TDS graphical representation for the washed coffee samples after the roasting from beans inoculated with yeasts strains (Control (A); *C. parapsilosis* UFLA YCN448 (B), *S. cerevisiae* UFLA YCN724 (C), *S. cerevisiae* UFLA YCN727 (D), *P. guilliermondii* UFLA YCN731 (E)).

P. guilliermondii UFLA YCN731 strain showed better growth in the first 4 days of fermentation in washed coffee. The four yeast strains could be indicated as culture starters for coffee dry processing considering aspects of persistence and dominance (Fig. 2).

3.2. DGGE analysis

The starter cultures were detected throughout the fermentation process (Fig. 3) in DGGE gels. *C. parapsilosisis* (1 isolate) and *P. guilliermondii* (1 isolate) and *S. cerevisiae* (two isolates) showed a different band pattern identified as bands 1 and 2 (*C. parapsilosis*), 3 and 4 (*P. guillliermondii*), and 5 and 6 (*S. cerevisiae*). The multiple banding patterns of the strains may be due to sequence heterogeneity generating multiple copies of the 18S rRNA (Nübel, Garcia-Pichel, Kuhl, & Muyzer, 1999).

S. cerevisiae was detected from samples where they were inoculated and also in the control samples, suggesting that this strain is epiphytic on coffee fruits and present in the environmental conditions during the fermentation process (Fig. 3A). All four strains tested here were able to persist until the end of the fermentation period. *S. cerevisiae* UFLA YCN724 strain did not show a reduction of its population observing the intensity of the band in PCR-DGGE (Fig. 3A). This observation was because DGGE is a semi-quantitative method and the initial concentration of the population of each inoculum was known. *Toruslapora delbrueckii* (band 15), *Pichia kluyveri* (band 13) and *Candida* sp. (band 31) were found at the beginning of fermentation (lower population compared to inoculum) but had their populations reduced at the final period of fermentation.

Yeasts were also found in samples from the control of non-washed coffee. The presence of *Hanseniaspora opuntiae* (band 19), *Debaryomyces hansenii* (bands 20 and 25), *T. delbrueckii* (bands 17 and 32) and *P. kluyveri* (band 16) (Fig. 3B) was detected. Bands 5 and 6, identified as *S. cerevisiae*, were detected in all fermentations. *S. cerevisiae* has been reported as one of the naturally found yeasts in coffee fermentation (Silva et al., 2008).

All inoculated strains were detected in all fermentation periods, which indicated that they were able to compete with the great diversity of naturally present microbe species in coffee fruits. The coffee fruits presented drastic changes in the physicochemical composition of the fruit such as the decrease of nutrients and the reduction of water activity (0.6) at the end of the fermentation period.

During fermentation with UFLA YCN724 and UFLA YCN727 strains (Fig. 3B) it was possible to observe the presence of *T. delbrueckii* in some samples, but it did not persist until the end of the fermentation, probably due to competition for nutrients with the inoculated strains. At the end of the fermentation inoculated with UFLA YCN731 strain it was also possible to detect *T. delbrueckii* (band 32) and *S. cerevisiae* (band 24).

The bacteria diversity present was generally greater than the yeast diversity (Fig. 4). This finding has also been reported by Vilela et al. (2010). *Erwinia billingiae* (bands 2 and 10), *Leuconostoc mesenteroides* (bands 3 and 9) *Halospirulina* sp. (bands 4 and 7), *Pantoea agglomerans* (bands 11), *Pantoea dispersa* (band 12), and uncultured *Pantoea* (band 13) were the bacteria found during coffee fermentation inoculated with yeasts. The presence of several species of bacteria was easily noted in the DGGE gel (Fig. 4B) of samples from coffee with or without inoculum. The profile found in microbial fermentations with UFLA YCN448 and UFLA YCN724 strains was very similar to the profile of the control sample (Fig. 4B), suggesting that these inoculum were not able to inhibit bacteria. For all samples, regardless of the inoculated strain, it was possible to observe the presence of the bacteria *Pantoea* sp. (band 17) and *Pantoea brenneri* (band 18) at the end of the fermentation process.

One of the DGGE bands was identified as *Mitchella repens* (bands 8, 12, 26, and 27), which is a plant belonging to the *Rubiaceae* family. The identification of a plant was possible due to the use of universal primer for the eukaryota; consequently the DNA of the plant was also amplified. This species was also identified by Masoud et al. (2004) during coffee fermentation in Tanzania. These authors reported that *M. repenses* is a plant that belongs to the botanical genus *Mitchella* of the family Rubiaceae, to which the genus *Coffea* belongs.

3.3. HPLC analysis

Coffee seeds have all the precursors needed to generate typical flavor and aroma during the roasting operation (Joët et al., 2010). However, the growth of microorganisms during the processing stages may confer additional flavor notes due to metabolites produced by fermentation, and their subsequent potential to migrate into the seed.

Tartaric and butyric acids were not detected in none of the analyzed samples. The absence of butyric acid indicates that the coffee fruits did not undergo fermentation. The acetic (maximum concentration 10.52 g/kg) and propionic (2.09 g/kg) acids (Table 1) are important microbial metabolites in the final quality of the coffee beverage (Bertrand et al., 2012; Lopez, Bautista, Moreno, & Dentan, 1989). These acids were found when *S. cerevisiae* and *P. guilliermondii* strains were inoculated, or when *S. cerevisiae* was naturally present (Fig. 3). Acetic, malic and citric acids contribute to the acidity of the final beverage (Buffo & Cardelli-freire, 2004). Usually, the acetic acid is easily accumulated in warm environmental temperatures ($T > 22$ °C) (Bertrand et al., 2012). The temperature during the days of fermentation was highly variable, with values between 14.6 and 28.2 °C (data not shown), which probably did not show a positive correlation with the accumulation of acetic acid.

It is important to observe that the simple presence of the specific organic acids is not responsible for the interference in the final quality but their concentrations might affect the coffee quality. In this case, the higher concentration of acetic acid observed was 10.52 g/kg in the pulp + seed sample in non-washed coffee inoculated with *P. guilliermondii* UFLA YCN731 but the concentration was lower inside the seed (2.07 g/kg). All tested samples showed low concentrations of propionic acid (0.07–2.09 g/kg). These acids should not be present in concentrations higher than 1 mg/mL for a good coffee beverage (Lopez et al., 1989).

3.4. GC analysis

GC was done to evaluate the chemical composition of the volatiles from the fermentation and roasting. Forty-eight compounds (Tables 2 and 3) including hydrocarbons (1 compost), ketones (3), alcohols (17), aldehydes (5) and acids (5) linked from post-harvest processing, esters (11), phenols (1) and furans (5) from thermal origin were detected. Alcohols were the most abundant compounds (11–27%) followed by furan in roasted grains (~27%) and aldehydes (~13%) in green grains (Tables 2 and 3). Esters are important compounds from a sensory point of view present in green coffee or which are produced during post-harvest processing (Cantergiani et al., 2001). However, higher concentrations of esters might confer over-fermented flavor. In our work, only 3.89% of the total compounds were esters in green coffee.

More than 800 volatile compounds were detected in roasted and green coffees (Farah, Monteiro, Calado, Franca, & Trugo, 2006), but not all of them are responsible for coffee flavor (Bertrand et al., 2012). The majority of the compounds detected in green coffee persist during roasting, and some compounds are precursors of flavor compounds during roasting as sucrose and amino acids (Mailard reaction), carbohydrates, chlorogenic acids, nitrogen-containing substances, and lipids (Yeretzian, Jordan, Badoud, & Lindinger, 2002). Two factors contribute to the formation of volatile compounds in coffee: microbial metabolites formed during fermentation, and the compounds of the inherent grains (Yeretzian et al., 2002). These factors can be very variable in different regions of coffee production. The composition and concentration of volatiles may be influenced by the environment, the variety of the plant, the chemistry of soil, altitude and even storage conditions (Yeretzian et al., 2002). Bertrand et al. (2012) observed that climatic conditions, such as temperature, interfere with the formation of volatile compounds in coffee. In places with high environmental temperatures

(20–25 °C), there is predominance of earthy and green aromas, and in regions showing mild temperatures (14–16 °C), the dominance of fruity aroma and overall quality is generally observed (Bertrand et al., 2012).

Some compounds were detected only in green beans and in grains after roasting, while others were detected in both (Tables 2 and 3). Of the compounds tested, 2,3-butanedione, 3-methyl-1-butanol, linalool, 1,3-butanediol, 2-phenylethanol, hexanal, nonanal, hexanoic acid and guaiacol were correlated with lower quality of the beverage (Bertrand et al., 2012; Guyot, Cros, & Vincent, 1982; Toci & Farah, 2008).

Acetoin, acetaldehyde, isoamylacetate and furans are compounds that confer caramel, floral and fruity flavors to the final beverage (Czerny & Grosch, 2000; Sanz, Maeztu, Zapelena, Bello, & Cid, 2002). Other compounds had antioxidant action such as furans and acetic acid (Fuster, Mitchell, Ochi, & Shibamoto, 2000; Yanagimoto, Lee, Ochi, & Shibamoto, 2002, 2004) and antifungal activity as isobutylacetate (Masoud et al., 2005).

Comparing the results of washed and non-washed coffee, a difference in the concentration of 18 compounds (Tables 2 and 3) was observed, and that in general, the-non washed coffee presented these compounds at higher concentrations. Hexanal is a compound that characterizes the typical odor of green beans before the roasting process (Czerny & Grosch, 2000) and it was detected only in non-washed coffee. Some of these compounds characterize the loss of sensory quality and might identify which defect is present, for example, 2,3-butanedione, 3-methyl-butanol, linalool and hexanal characterize the insect damage; high concentration of propionic acid is related to overfermentation; hexanoic acid is found only in roasted coffee (Toci & Farah, 2008). The

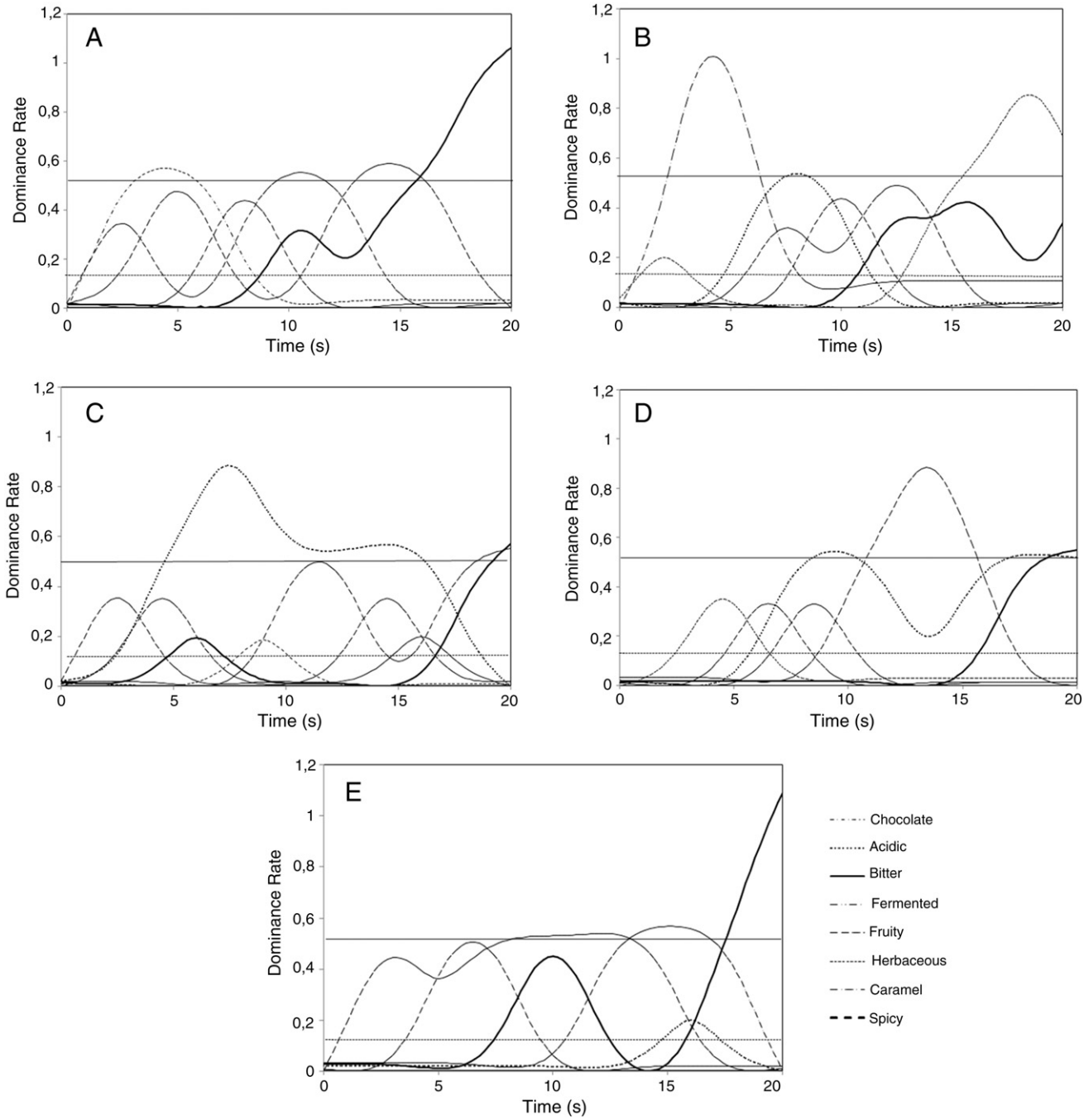

Fig. 7. TDS graphical representation for the non-washed coffee samples after the roasting from beans inoculated with yeasts strains (Control (A); *C. parapsilosis* UFLA YCN448 (B), *S. cerevisiae* UFLA YCN724 (C), *S. cerevisiae* UFLA YCN727 (D), *P. guilliermondii* UFLA YCN731 (E)).

two compounds that impart pleasant fruit flavor to the final beverage were decanoic acid and acetaldehyde, which were found in higher concentrations in non-washed coffee.

The PCA analysis allowed the correlation between some volatile compounds and the four yeasts in green beans and after roasting. Each axis of the graph shows the percentage of total explained variation (Fig. 5A and B). Fig. 5A shows that two principal components explained 54.77 and 31.54% of total variance. The Control was characterized by the presence of 2-heptanol, 2,3-butanedione, hexanal, propyl acetate, 5-methylfurfural, 1,1-dietoxyethane, isobutyl acetate, and methanol. Among the other treatments, UFLA YCN727 and UFLA YCN731 were grouped in the positive part of the PC1 and characterized by linalool, ethyl butyrate. Compared to the roasted coffee (Fig. 5C), it was observed that Control treatment kept the profile of volatile compounds, while the other treatments presented a different behavior concerning to the volatile profile. A similar behavior (as described above) was observed for Control treatment in Fig. 5B (PC1 36.61% of total variance; PC2 31.53% of total variance) and D (PC1 42.36% of total variance; PC2 29.08% of total variance).

Depending on the processing (washed and non-washed), the inoculated yeasts behaved differently in both green and roasted coffee; e.g. the UFLA YCN724 and UFLA YCN448 yeasts for non-washed roasted beans (Fig. 5B) were grouped together and characterized by ethyl butyrate, 1-propanol, guaiacol, and propionic acid. The same yeasts were plotted separately (Fig. 5D) for washed roasted beans and correlated with different volatile compounds (except for the UFLA YCN724 correlated by ethyl butyrate). Comparing washed and non-washed coffees, it was possible to observe that the volatile profile was quite different; showing that washing the coffee changed the final characteristics of the product.

3.5. Sensory analysis

The temporal dominance of sensations consists in assessing iteratively at each specific time until the sensations end, which sensation is dominant and in scoring its intensity (Labbe, Schlich, Pineau, Gilbert, & Martin, 2009). Figs. 6 and 7 show the TDS curves for the washed and non-washed coffee fermented via dry or natural process with yeasts inoculated as culture starters. The samples from the control treatment of washed coffees (Fig. 6A) showed an early perception as caramel sensation and after 5 s the aroma was dominated by chocolate and fruit, and finally after 20 s it was possible to feel a strong bitter flavor. The samples from the treatments with starter cultures showed sensory differences when compared with the control treatment, except for the ones inoculated with UFLA YCN731 (Fig. 6B–E).

Each inoculated sample showed a strongly dominant sensation and different between them, for example, when samples of treatment with UFLA YCN448 showed a strong feeling of caramel 5 s, UFLA YCN724 showed a strong acidic sensation between 5 and 10 s, UFLA YCN727 showed a strong fruity feeling 15 s after ingestion. All grains tested and inoculated with *C. parapsilosis* UFLA YCN448 (Fig. 6B) had the highest rate of dominance (near 1) for the sensation of caramel and, at the end, (about 20s) the feeling was predominantly herbaceous. Thus, it was possible to observe that in all washed coffee treatments with selected yeasts inoculum showed greater sensations and differences compared to control, suggesting an increase in flavor and therefore greater acceptance of tasters.

The control samples of non-washed coffee (Fig. 7A) showed bitterness as the first perceived aroma/flavor, followed by the acidity and chocolate (between 5 and 10 s) and finally after 20 s a strong bitterness flavor. Bitterness feeling in late 20 s was also prevalent in the washed coffee without yeast inoculation. The grains inoculated with UFLA YCN448 (Fig. 7B) showed initial sensation of caramel (however weak) when there was a predominant and strong feeling of bitterness after 10 s. Grains inoculated with UFLA YCN724 (Fig. 7C) showed that the initial sensation was perceived as fermented with predominance after 20 s of the bitterness feeling. Grains inoculated with UFLA YCN727 (Fig. 7D) showed an initial sensation of caramel (weak) with final predominance (20 s) of a bitterness feeling. Coffee beans inoculated with UFLA YCN731 (Fig. 7E) showed an initial bitterness sensation but with an intense chocolate sensation after 20 s. Thus, washed coffees inoculated with yeasts as starter culture may be used in blends to achieve different flavor.

Non-washed coffee grains inoculated with yeast presented more pleasant sensations than the experiments where the coffee was washed before inoculation, except for inoculation with the UFLA YCN731 strain. These results suggested that washing the coffee fruits before the fermentation process influenced the final quality of the product. The non-washed coffee also had the lowest scores (73.25 on average) in the cup proof compared with washed coffee samples (77.18 average rate).

Thus, it was possible to conclude that it is possible to use selected yeasts for the fermentation of natural or dry coffee processing. The inoculated yeasts persisted during the entire fermentation and resulted in a beverage with a distinctive flavor (caramel and fruity) and good sensory quality. Further research should focus on choosing selected strains for inoculation in washed coffee fermented by dry method in order to increase the pleasant sensations obtained for non-washed coffee. The use of starter cultures in coffee fermentation is an economically viable alternative to obtain a differentiated coffee, adding value to the product and standardizing the dry process.

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