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Behavior of yeast inoculated during semi-dry coffee fermentation and the effect on chemical and sensorial properties of the final beverage

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ABSTRACT

Pulped Mundo Novo and Ouro Amarelo coffee beans were inoculated with *Saccharomyces cerevisiae* (CCMA 0200 and CCMA 0543) during semi-dry coffee fermentation and compared with a non-inoculated control. Samples were collected throughout the fermentation process (12 days) to evaluate the persistence of the inoculum by Real-Time quantitative PCR (qPCR). Also, the chemical composition of the beans was determined by HPLC and GC–MS and the roasted beans were sensorial evaluated using the cupping test. *S. cerevisiae* CCMA 0543 had an average population of 5.6 log cell/g (Ouro Amarelo cultivar) and 5.5 log cell/g (Mundo Novo cultivar). Citric, malic, succinic and acetic acid were found in all samples, along with sucrose, fructose, and glucose. There were 104 volatile compounds detected: 49 and 55 in green and roasted coffee, respectively. All coffee samples scored over 80 points in the cupping test, indicating they were specialty-grade. Inoculation with the CCMA 0543 strain performed better than the CCMA 0200 strain. This is the first time that qPCR has been used to assess the persistence of the inoculated strains populations during coffee processing. Strain CCMA 0543 was the most suitable as an inoculant due to its enhanced persistence during the process and number of volatile compounds produced.

1. Introduction

Coffee is one of the most widely distributed beverages in the world and ranks among the five most traded agricultural commodities (FAO, 2009). Coffee beans grow inside the fruits of the *Coffea arabica* tree. Upon harvesting, several methods are available to transform the fresh, wet beans to the dried green beans that are traded on the international markets. The choice of this method (wet, dry or semi-dry) influences the chemical composition of the bean as well as the sensory characteristics of the coffee beverage (Silva, 2014).

In the semi-dry method, coffee beans are separated from the fruit by pulping. This step removes the exocarp and most of the mesocarp. The beans are then collected to dry in open air, typically for 10–15 days (depending on weather circumstances). Drying is considered complete when the beans reach 10–12% moisture content. Simultaneous with this drying step, a fermentation process occurs during which, microorganisms degrade remnants of the mesocarp (also called mucilage) that still adhere to the beans (Batista, Chalfoun, Batista, & Schwan, 2016).

In a study of the microbiota associated with the semi-dry process, Vilela, Pereira, Silva, Batista, and Schwan (2010) observed a microbial succession with bacterial species prevailing in the early stages of fermentation, reaching log 7 cfu/g during the first 24 h of fermentation. Yeasts dominated the later stages (as the moisture content decreased) reaching log 6.9 cfu/g after 5 days. According to Velmourougane, Bhat, Gopinandhan, and Panneerselvam (2011), delays during the semi-dry fermentation and drying process can trigger outgrowth of filamentous fungi, potentially leading to food safety risks and decreased beverage quality. In our lab, Evangelista, Miguel et al. (2014) and Evangelista, Silva et al. (2014) inoculated several yeast starter cultures during semi-dry processing of Acaiá cultivar and found that some produced coffee with distinct flavor characteristics in comparison with non-inoculated control samples. However, these data need to be validated through repeat experiments at various farms and harvest seasons, with other varieties and on a commercially relevant scale.

In the present work, two coffee bean varieties (Ouro Amarelo and Mundo Novo) were, respectively, inoculated with two strains of *Saccharomyces cerevisiae*-CCMA 0200 and CCMA 0543 (formerly UFLA YCN727) during semi-dry coffee fermentation. The persistence of the inoculated strains populations was confirmed using Real-Time quantitative PCR (qPCR). The effect of the inoculations on the chemical

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composition of the bean (sugars, acids, and volatiles) was analyzed using gas and liquid chromatography. Finally, a cupping test was performed to evaluate the sensory characteristics of the coffee beverage obtained by these treatments.

2. Materials and methods

2.1. Microorganisms used as starter cultures

Saccharomyces cerevisiae CCMA 0200 (previously isolated from sugar cane, formed UFLA CA11), and *S. cerevisiae* CCMA 0543 (formed UFLA YCN 727), were isolated during semi-dry fermentation of coffee beans (*Coffea arabica* L. var. Acaiá) (Vilela et al., 2010) were used as the starter cultures. These strains belong to the Culture Collection of Agricultural Microbiology (CCMA, Federal University of Lavras, Lavras, Minas Gerais, Brazil). *S. cerevisiae* CCMA 0543 already is used as starter culture for another coffee variety (Evangelista, Miguel et al., 2014; Evangelista, Silva et al., 2014) and CCMA 0200 is used for another fermentative process (Ribeiro, Duarte, Dias, & Schwan, 2015).

A lyophilized culture of *S. cerevisiae* CCMA 0200 (produced by LNF, Bento Gonçalves, Brazil) was weighed in sterile water at the concentration necessary to inoculate the coffee beans with 5 log cells/g. A culture of *S. cerevisiae* CCMA 0543 stored at −80 °C was reactivated in YEPG tubes containing 9 ml of liquid medium [glucose 20 g/l (Merck, USA), yeast extract 10 g/l (Merck, USA), and bacteriological peptone 10 g/l (Himedia, India), pH 3.5]. The cultures were incubated at 28 °C for 48 h, and then transferred to YEPG (90 ml) and incubated at 28 °C, 150 rpm for 24 h. The yeast cells were transferred to incremental volumes of YEPG until a sufficient number of cells was produced to inoculate the coffee beans with approximately 5 log cells/g. The cells were recovered by centrifugation (7000 rpm, 10 min) and re-suspended in sterile water.

2.2. Harvest and coffee processing

Ouro Amarelo (OA) and Mundo Novo (MN) coffee cultivars were collected mechanically on a farm located in Patrocínio in the state of Minas Gerais, Brazil, at 970–1200 m above sea level, during the harvest of June 2014. Only ripe cherries were used to perform the experiment, since coffee processed with ripe cherries is naturally sweet and have floral and fruit notes, while unripe cherries may taste grassy, green, or astringent (Batista et al., 2016). The exocarp and mesocarp of the coffee fruit were separated from the beans in a horizontal pulper (Ecoflex, Pinhalense, São Paulo, Brazil). The pulped coffee beans (60 kg) were spread on suspended terraces. Each treatment was inoculated separately with *S. cerevisiae* CCMA 0200 and CCMA 0543 and the control samples were not inoculated. The yeasts cells suspended in sterile water were sprinkled over the coffee cherries and mixed manually and aseptically. The fermentations were performed in duplicate. The pulped coffee beans remained on the suspended terraces, where they were regularly turned to ensure uniform drying. The fermentation and drying were considered complete when the beans reached a moisture content of 11–12%. Samples were collected at 0, 24, 48 and 284 h (the final drying time). For each sample, 300 g of beans were aseptically collected in sterile plastic bags and immediately transferred to the Microbial Fermentation Laboratory of the Federal University of Lavras, in iceboxes. Samples were stored at −18 °C until microbiological and physicochemical analyses were performed, and at −8 °C for sensory analyses.

2.3. DNA extraction from pulped coffee and real-time PCR

The total DNA from pulped coffee was extracted using 3 g of sample. Each treatment was mixed with 5 ml of ultrapure water for 10 min, then centrifuged at 9000 rpm, 4 °C for 10 min. The pellet was used for DNA extraction. Total DNA was extracted from samples during the fermentation [at 0, 24, 48 and 284 h (end of drying)] using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions.

Specific primers for the *S. cerevisiae* were amplified with the primers SC-5fw 5′-AGGAGTGCGGTTCTTTCTAAAG-3′ and SC-3bw 5′-TGAAATGCGAGATTCCCCCA-3′, which span the 26S rDNA region and amplify products 150–200 bp in length (Díaz, Molina, Nähring, & Fischer, 2013). The specificity of primer pair was confirmed by searching in GenBank using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).

Real-time PCR was carried out using the Rotor-Gene Q System (Qiagen, Hombrechtikon, ZH, Switzerland). Each reaction comprised 12.5 μl 2× Rotor-Gene SYBR Green PCR Master Mix (Qiagen, Stockach, Konstanz, Germany), 0.8 μM of each primer (Invitrogen, São Paulo, SP, Brazil) and 1 μl template DNA extracted from coffee beans, for a total volume of 25 μl. The mixture was heated to 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s, and annealing/extension at 60 °C for 15 s. The cycling temperature was then increased by 1 °C every 5 s from50°C to 99 °C to obtain the melting curve. All analyses were performed in triplicate. The DNA concentration in the samples was limited to 50 ng per analysis, except for the standard curves, which were prepared from samples containing a known number of yeast cells. For the standard curves, all yeast species were cultivated in YPD agar at 28 °C for 24 h. The cells were counted using a Neubauer chamber. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and serially diluted (1:10) from 8 to 3 log cell/ml. Each point on the calibration curve and DNA of samples was measured in triplicate.

2.4. Analysis of acids and sugars by high-performance liquid chromatography (HPLC)

Pulped coffees were ground to a fine powder using an IKA A11 analytical mill. In a 50 ml Erlenmeyer flask, 3 g of powder was mixed with 20 ml of ultrapure water and extracted for 10 min, at room temperature, while stirred with a magnetic bar. The extracts were decanted and centrifuged at 10,000 rpm, 4C for 10 min. To remove sample components that may precipitate on the chromatographic column, the supernatant was adjusted to pH 2.11 using 200 mM perchloric acid solution before centrifuging a second time under the same conditions. The second supernatant was filtered using a 0.22 μm cellulose acetate filter. The filtered extract was stored at −18 °C until analysis.

The extracts were analyzed using an HPLC system (Shimadzu). A Shim-pack SCR-101H (7.9 mm × 30 cm) column was used with a 100 mM solution of perchloric acid and a flow rate of 0.6 ml/min as the mobile phase. The oven temperature was maintained at 50 °C for analysis of the acids, detected with a UV detector at 210 nm, and at 30 °C for analysis of the sugars, detected with a refractive index detector. Analysis were done in triplicate.

2.5. Analysis of volatile compounds by gas chromatography/mass spectrometry (GC/MS)

Volatile compounds were extracted using a manual headspace-solid phase microextraction procedure (HS–SPME) with a divinylbenzene/carboxen/polydimethylsiloxane 50/30 μm SPME fiber (Supelco Co., Bellefonte, PA, USA). Pulped coffee (2 g) was macerated with liquid nitrogen and placed in a 15 ml hermetically sealed vial. After equilibration at 60 °C for 10 min, the volatile compounds were extracted at 60 °C 30 min. Desorption time on the column was 5 min.

A GCMS-QP2010 (Shimadzu), equipped with an Agilent HP-FFAP column (30 m × 0.25 mm × 0.25 μm) was used for GC/MS analysis. The oven temperature was maintained at 50 °C for 5 min, then raised to 190 °C at 3 °C/min and maintained at 190 °C for 10 min. The injector and detector were maintained at 230 and 240 °C, respectively. The He carrier gas was maintained at a flow rate of 1.2 ml/min. Analysis were done in duplicate.