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Different inoculation methods for semi-dry processed coffee using yeasts as starter cultures

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ABSTRACT

This study evaluates the behavior of *Saccharomyces* (*S.*) *cerevisiae* (CCMA 0543), *Candida* (*C.*) *parapsilosis* (CCMA 0544), and *Torulaspora* (*T.*) *delbrueckii* (CCMA 0684) as starter cultures for semi-dry processed coffee using two inoculation methods: (1) direct inoculation and (2) bucket inoculation. The microbial population was evaluated by plating and real-time polymerase chain reaction (qPCR). The microbial metabolic changes of both bucket and direct inoculation methods during fermentation were evaluated using high performance liquid chromatography (HPLC), and gas chromatography–mass spectrometry (GC–MS). A sensorial test was also carried out. Citric and succinic acids were detected throughout the fermentation period. Chlorogenic acid concentration levels were higher for the bucket method after roasting. Roasted coffee beans also had a higher caffeine concentration, with the exception of the *T. delbrueckii* (CCMA0684) assay. Acids, pyrazines and pyridines were the main volatile compounds in both green and roasted coffee beans. Coffee cupping results proved that both inoculation methods scored well in terms of coffee quality. The bucket method favored the permanence of the microorganisms during coffee processing, especially the treatment inoculated with *S. cerevisiae*.

1. Introduction

Coffee is a dark-brown, slightly bitter beverage made from ground and roasted coffee beans, which is widely consumed and considered popular due to its stimulant properties and composition (Ballesteros, Teixeira, & Mussatto, 2014; Contreras-Calderón et al., 2016). South and Central America, the Caribbean, Africa and Asia are the main coffee-producing areas (Restuccia, Spizzirri, Parisi, Cirillo, & Picci, 2015). Brazil is the major global coffee producer and exporter, with 55,000 thousand bags produced in 2016 (ICO, 2017). Within Brazil, Minas Gerais, Espírito Santo, and São Paulo are the main coffee producing states (CONAB, 2017).

Coffee fermentation occurs naturally, regardless of the process, in order to remove the mucilage from seeds and reduce water content, with beans being dried simultaneously until moisture content is reduced to percentages between 11 and 12% (Silva et al., 2013). Most microorganisms responsible for fermentation are autochthonous (bacteria, yeast, and filamentous fungi). The population of each microbial group may vary depending on the processing method and the water loss extent (Silva, Schwan, Dias, & Wheals, 2000). During certain processing stages, the contribution of acetic acid, lactic acid, caffeine, chlorogenic acids, and other compounds improves coffee flavor and is beneficial to health. Coffee beans have antioxidant and antidiabetic properties that can reduce cholesterol levels (Belguidoum, Amira-Guebailia, Boulmokh, & Houache, 2014). Measuring these compounds before and after processing is important due to their influence by coffee variety, geographical origin, and microbiota during fermentation and roasting.

The role of yeasts is essential for coffee fermentation, preventing oxigenic filamentous fungi growth and boosting the production of pectinolytic enzymes, which help the degradation of coffee mucilage and pulp (Ramos, Silva, Batista, & Schwan, 2010; Silva et al., 2013). Using yeasts as starter cultures during coffee fermentation can improve the quality of the end product. Evangelista, Silva, et al. (2014) have reported promising results after the direct inoculation of *Saccharomyces*, *Pichia* and *Candida* yeast strains over coffee beans.

The use of starter cultures is an increasingly common practice and has shown positive results for several types of fermented foods and beverages - wine (Sun, Gong, Jiang, & Zhao, 2014); cheese (Galli, Martin, da Silva, Porto, & Spoto, 2016); cocoa (Visintin et al., 2017), and in industrial processes, such as lignocellulosic ethanol production (Favaro, Basaglia, & Casella, 2014), production of second-generation biofuels (Steensels et al., 2014), compounds production (human insulin,

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hepatitis vaccines, and human papillomavirus vaccines) (Hou, Tyo, Liu, Petranovic, & Nielsen, 2012). Molecular and biochemical techniques to evaluate starter cultures have been applied to different foods in previous research (Batista, Ramos, Ribeiro, Pinheiro, & Schwan, 2015; Bressanello et al., 2017; Evangelista, Silva, et al., 2014; Menezes et al., 2016). Starter culture populations have also been monitored by qPCR (Gil-Serna, González-Salgado, González-Jaén, Vázqueza, & Patiño, 2009; Michel et al., 2016; Postollec, Falentin, Pavan, Combrisson, & Sohier, 2011). In combination, results from GC–MS methods become relevant because the processes separates volatile compounds according to the stated conditions and generates data about unknown compounds from fermentation (Batista et al., 2015; Bressanello et al., 2017; Ziółkowska, Wąsowicz, & Jeleń, 2016).

Despite the use of starter cultures, the methodology of inoculation and selection might change and/or improve the quality of the final product, making some special coffees. There is still significant room for improvement for example, the best way to inoculate these strains; the effective activity of these microorganisms in coffee fermentative processes. The reason for this is that these coffee fermentative processes rarely use the most suited or best-performing strain. The aim of this study was to evaluate two different methods of inoculation (direct and bucket inoculation methods), to find out which would have a better effect on the persistence of yeast starter. *S. cerevisiae* (CCMA 0543), *C. parapsilosis* (CCMA 0544), and *T. delbrueckii* (CCMA 0684) were used as starter cultures and their persistence during coffee processing were evaluated by use of qPCR. The effect of inoculation on the chemical composition of the bean (sugars, acids, and volatiles) was analyzed using liquid and gas chromatography. Sensorial analysis (coffee cupping) of the resulting beverage was carried out to confirm whether inoculation contributed to coffee quality.

2. Material and methods

2.1. Coffee cherries

Cherries from *C. arabica* variant Catuaí Amarelo were obtained from a producing farm near Lavras, Minas Gerais, Brazil. Cherries were processed using a semi-dry method, which consisted of depulping and washing, leaving only mucilage and parchment (Brando & Brando, 2015).

2.2. Starter cultures, fermentation and drying

Three yeast strains—*S. cerevisiae* (CCMA 0543), *C. parapsilosis* (CCMA 0544) and *T. delbrueckii* (CCMA 0684)—from the Culture Collection of Agricultural Microbiology (CCMA) in Lavras were used as starter cultures (Evangelista, Silva, et al., 2014). Each yeast was grown in 1 L of YEPG medium (20 g/L glucose, 10 g/L yeast extract, 10 g/L peptone soy, and pH 3.5), at 28 °C for 24 h until they reached a concentration of 10^9 cells/mL for inoculation. After, cells were washed and diluted in 500 mL of water for inoculation.

Depulped cherries were inoculated using two methods: (1) direct inoculation by spraying the yeast solution on depulped beans placed in wooden frames (1 m × 1 m) with plastic nets right after coffee processing (Evangelista, Silva, et al., 2014), and (2) inoculation of yeast solution on depulped cherries placed in polystyrene buckets for 16 h. Subsequently, fermented beans were transferred to wooden frames for drying. A total of six treatments and two controls (not inoculated) containing 10 kg of depulped cherries were carried out: four treatments used direct inoculation (Treatment 1 – control, coffee not inoculated; Treatment 2 – coffee inoculated with *S. cerevisiae* (CCMA 0543); Treatment 3 – coffee inoculated with *C. parapsilosis* (CCMA 0544) and Treatment 4 – coffee inoculated with *T. delbrueckii* (CCMA 0684); four treatments used the bucket method (Treatment 5 – control, coffee not inoculated; Treatment 6 – inoculated with *S. cerevisiae* (CCMA 0543); Treatment 7 – inoculated with *C. parapsilosis* (CCMA 0544) and Treatment 8 – inoculated with *T. delbrueckii* (CCMA 0684)). All treatments were performed empirically, without any type of control under environmental conditions, the ambient temperature ranged from 14.6 to 28.2 °C. During the fermentation and drying process, samples of approximately 100 g were collected in sterile plastic bags (at times 0, 16, 64,112, 256, and 352 h). All treatments were fermented and sun dried until a moisture content of 10 to 11% was reached (measured using a water activity meter, provided by Paw kit) (Evangelista, Silva, et al., 2014).

2.3. Microbial counts

2.3.1. Cultivation methods

Initial (0 h) and final (352 h) samples of fermented beans (10 g) were homogenized in 90 mL saline-peptone water (0.1% (v/v) bacteriological peptone (Himedia) and 0.8% (v/v) NaCl (Merck, Whitehouse Station, NJ)) in a stomacher at normal speed for 5 min, serially diluted, and then plated in triplicate. YEPG (in g/L: glucose20 (Merck), yeast extract 10 (Merck), peptone soy 10 (Himedia), and agar 20 (Merck), with pH 3.5), MRS agar containing 0.1% (w/v) nystatin, and Nutrient agar containing 0.1% (w/v) nystatin were used to count the total yeast, lactic acid bacteria (LAB), and mesophilic bacteria. Plates were incubated at 30 °C for 48 h (Silva et al., 2000).

2.3.2. Quantitative polymerase chain reaction (qPCR)

Total DNA was extracted from samples at fermentation times 0, 16, 64, 112, 256, and 352 h, using the “DNA Purification from Tissues” protocol (QIAamp DNA Mini Kit (Qiagen, Hilden, Germany)) in accordance with the manufacturer's instructions.

Specific primers for *S. cerevisiae* (Díaz, Molina, Nahring, & Fischer, 2013), *C. parapsilosis* (Hays, Duhamel, Cattoir, & Bonhomme, 2011) and *T. delbrueckii* (Zott et al., 2010) were used (Supplemental material 1). The specificity of each primer pair was confirmed by searching in GenBank using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). A qPCR analysis was used to quantify *S. cerevisiae*, *C. parapsilosis*, and *T. delbrueckii*, as described by Batista et al. (2015). Three independent qPCR assays were performed for each treatment. For standard curves, all yeast species were cultivated in YEPG broth at 28 °C for 24 h. The yeast populations were then counted using a Neubauer chamber. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and serially diluted (1:10) from 10^{10} to 10^3 cell/mL. Each point of the calibration curve was measured in triplicate.

2.4. Chemical analysis

2.4.1. Carbohydrates, organic acids, glycerol, and ethanol by HPLC

The samples were evaluated after 0, 16, 112, 256, and 352 h of fermentation. For extraction, 3 g of fermented beans were homogenized with 20 mL of Milli-Q water by vortexing for 10 min at room temperature. Then, fluids were centrifuged twice at 100 × *g* for 10 min at 4 °C, and were filtered through a 0.2 mm cellulose acetate filter. Same was done for bean interior. In addition, expect parchment of beans was removed. Carbohydrates, organic acids, glycerol, and ethanol analyses were performed with a Shimadzu liquid chromatography system (Shimadzu Corp., Japan) equipped with a dual detection system consisting of a UV–vis detector (SPD 10Ai) and a refractive index detector (RID-10Ai). A Shimadzu ion exclusion column Shim-pack SCR-101H (7.9 mm × 30 cm) was set at an operating temperature of 30 °C for ethanol and glycerol and 50 °C for acids. Perchloric acid (100 mM) was used as the eluent at flow rate of 0.6 mL/min. Calibration curves were constructed with different concentrations of standard compounds. Analysis was done in triplicate.

2.4.2. Caffeine, chlorogenic acid and trigonelline

Measurement of the non-volatile compounds (caffeine, chlorogenic acid [5-CGA], and trigonelline) in times 0, 112, 352 h of fermentation