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Conducting starter culture-controlled fermentations of coffee beans during on-farm wet processing: Growth, metabolic analyses and sensorial effects

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ABSTRACT

In this study, the potential use of *Pichia fermentans* YC5.2 as a starter culture to conduct controlled coffee bean fermentations during on-farm wet processing was investigated. Inoculated fermentations were conducted with or without the addition of 2% (w/v) sucrose, and the resultant microbial growth and metabolism, bean chemistry and beverage quality were compared with spontaneous (control) fermentation. In both inoculated treatments, *P. fermentans* prevailed over indigenous microbiota and a restricted microbial composition was observed at the end of fermentation process. The inoculation also increased the production of specific volatile aroma compounds (e.g., ethanol, acetaldehyde, ethyl acetate and isoamyl acetate) and decreased the production of lactic acid during the fermentation process. Sucrose supplementation did not significantly interfere with the growth and frequency of *P. fermentans* YC5.2 inoculum but maintained high levels of wild bacteria population and lactic acid production similar to the spontaneous process. In roasted beans, the content of sugars and organic acids were statistically ($p < 0.05$) similar for all the treatments. However, the inoculated fermentations were shown to influence the volatile fraction of roasted coffee beans by increasing the concentration of yeast-derived metabolites compared to control. Sensory analysis of coffee beverages demonstrated that the use of the YC5.2 strain was favorable for the production of high-quality coffees with distinctive characteristics, e.g., intense perception of 'vanilla' taste and 'floral' aromas. In conclusion, the use of *P. fermentans* YC5.2 in coffee processing was shown to be a viable alternative to control the fermentation step and to ensure consistent quality of finished products.

1. Introduction

The quality of a coffee beverage is strongly related to the chemical changes occurring during the roasting process but is also dependent on the postharvest processing (Correa, 2014; Mussatto, Machado, Martins, & Teixeira, 2011). Three different methods are employed in producing countries to process coffee fruit, referred to as dry, wet and semi-dry (Pandey et al., 2000). Wet processing is used mainly for arabica coffee: the ripe fruits are de-pulped and then submitted to 24–48 h of underwater tank fermentation and dried until a final water content of 10–12% is achieved (Avallone, Guyot, Brillouet, Olguin, & Guiraud, 2001; Murthy & Madhava Naidu, 2012). In dry processing, in contrast, entire coffee fruits are dried (in the sun) on platforms and/or on the floor without prior removal of the pulp (Silva, Batista, Abreu, Dias, & Schwan, 2008). Semi-dry processing is a combination of both methods, in which coffee fruits are de-pulped, but the fermentation process occurs directly under the sun on a platform (Vilela, Pereira, Silva, Batista, & Schwan, 2010).

During on-farm wet processing, the fermentation step is carried out to eliminate any mucilage still stuck to the beans and helps improve beverage flavor by producing microbial metabolites, which are precursors of volatile compounds formed during roasting (Mussatto et al., 2011; Pereira et al., 2014). The microorganisms responsible for the fermentation are indigenous species that originate as natural contaminants of the process, including yeasts, bacteria and filamentous fungi. Surveys have shown that the most frequently occurring yeast species during coffee fermentation are *Pichia kluyveri*, *Pichia anomala*, *Hanseniaspora uvarum*, *Saccharomyces cerevisiae*, *Debaryomyces hansenii* and *Torulaspora delbrueckii* (Silva et al., 2008; Vilela et al., 2010; Silva, 2014). In addition, bacteria with pectinolytic activity belonging to the genera *Erwinia*, *Klebsiella*, *Aerobacter*, *Escherichia* and *Bacillus*, and a variety of filamentous fungi are often isolated as well (Avallone, Brillouet, Guyot, Olguin, & Guiraud, 2002; Silva et al., 2008). These fermenting organisms utilize the bean pulp as a carbon and nitrogen source and

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produce significant amounts of ethanol, acetic and lactic acids and other microbial metabolites, resulting in lowered pH (from 5.5–6.0 to 3.5–4.0) (Avallone et al., 2001; Pereira et al., 2014).

Over the years many studies have demonstrated that the coffee bean fermentation process needs to be well controlled to ensure the development of microorganisms that give a high-quality beverage with good coffee aroma (Agate & Bhat, 1966; Avallone et al., 2002; Evangelista et al., 2014; Silva et al., 2013). Failure in fermentation can result in the development of microorganisms that adversely affect coffee character and flavor (Frank, Lum, & Delacruz, 1965; Pereira et al., 2014). Presently, coffee fermentation is conducted as a simple, traditional process and still relies on the naturally occurring microbiota of the fresh raw materials. A controlled coffee fermentation by the use of starter cultures may guarantee a standardized quality and reduce the economic loss for the producer, as has occurred with many other fermented foods and beverages, such as cheese, yogurt, bread, beer and wine (Schwan, Pereira, & Fleet, 2014; Steinkraus, 2004).

Good candidate functional starter cultures are mainly wild-type strains that originate from the natural ecosystem, as these usually exert more elaborate metabolic activities in comparison with industrial bulk starters, often from diverse sources (Leroy, Verluyten, & De Vuyst, 2006; Pereira, Miguel, Ramos, & Schwan, 2012; Wouters, Grosu-Tudor, Zamfir, & De Vuyst, 2013). In this direction, the frequent appearance of *Pichia* yeasts as prevailing in coffee fermentations (Masoud, Poll, & Jakobsen, 2005; Pereira et al., 2014; Silva et al., 2008) makes this common genus a promising candidate for starter culture trials. Furthermore, several *Pichia* strains possess functional properties, such as the capacity to degrade the coffee bean mucilage (pectinolytic activity), to resist stress conditions prevailing in coffee fermentation matrix, to produce flavoring compounds and to inhibit ochratoxigenic filamentous fungi growth during coffee fermentation (Masoud, Poll, & Jakobsen, 2005; Masoud & Jespersen, 2006; Silva et al., 2013; Evangelista et al., 2014; Pereira et al., 2014). All these features make their application as starter cultures even more interesting.

This study was aimed at the implementation of *P. fermentans* YC5.2 as a starter culture to conduct controlled coffee bean fermentations during wet processing. It is a competitive and aromatic yeast strain isolated from coffee processing that has successfully been used as a starter culture in coffee fermentation trials on a laboratory scale (Pereira et al., 2014).

2. Materials and methods

2.1. Microorganism and lyophilized inoculum preparation

The yeast strain used in this study, *P. fermentans* YC5.2 (LPPII-UFPR Culture Collection, Paraná, Brazil), was originally isolated from a spontaneous coffee fermentation process and selected as detailed by Pereira et al. (2014). For biomass production, pre-culture was prepared by inoculating 100 mL of YEPG broth (1% yeast extract [Merck], 2% peptone [Himedia], 2% glucose [Merck] at pH 5.6) with 1 mL of the thawed stock culture and incubating for 48 h at 30 °C and 120 rpm. This preculture was transferred to 3-L Erlenmeyer flasks containing 1 L YEPG broth and grown for 24 h at 30 °C and 120 rpm. Subsequently, 400 mL of the resulting yeast culture was transferred to a 6-L Erlenmeyer flask containing 4 L YEPG broth and grown for 24 h at 30 °C and 150 rpm. The culture was centrifuged for 15 min at 4500 *g* and 4 °C, washed in distilled water and centrifuged again. Lyophilized culture was prepared by resuspending the pellet in skimmed UHT milk, rapidly freezing in an ethanol–dry ice mixture and freeze-drying in a Modulyod Freeze Dryer 230 (Thermo Electron Corporation, Waltham, USA) under negative pressure of 50 mBar at −45 °C. The lyophilized culture was analyzed for total viable cell count by standard dilution method on YEPG agar and conditioned in packs of 10 g.

2.2. Fermentation experiments

The fermentation experiments were conducted at the Fazenda Apucarana localized in the Cerrado Mineiro region at Minas Gerais state, Brazil. The Apucarana farm is situated at 1270 m above sea level atop the mineral-rich dome of an unformed volcano and is known for consistently producing high-quality coffees. Freshly harvested coffee cherries (*Coffee arabica* var. Catuí) were depulped using a BDSV-04 Pinhalense depulper (Pinhalense, Sao Paulo, Brazil) to obtain beans with mucilage. Fermentations were conducted in cement tanks with inner dimensions 2.42 × 1.94 × 0.96 m containing 20 kg of depulped beans and approximately 500 L of fresh water in accordance with the local wet processing method. The lyophilized starter culture was rehydrated by adding water at 37–40 °C (10 g/L) and stirred gently over a period of 5 min. This solution was spread into the fermentation tank to reach a concentration of 10^7 cells/mL. During the fermentation trials, three different batches were performed: (i) spontaneous (noninoculated control); (ii) inoculated; and (ii) inoculated, supplemented with 2% (w/v) sucrose. Sucrose supplementation was performed to determine the effect of this additional carbon source on the growth of *Pichia fermentans* inoculum. The fermentations were conducted simultaneously and repeated three times. The depulped coffee beans were fermented for 24 h and then sun-dried until 11–12% moisture was reached. The environmental temperature was 24–32 °C (day-time temperature) and 12–15 °C (night-time temperature).

2.3. Sampling and pH

Samples (liquid fraction plus beans) were withdrawn in triplicate (0 and 24 h) at random to perform microbial counts and metabolite target analysis. At every sampling point, the pH of the fermenting mass was measured with a portable pH meter, model AK90 (AKSO, São Leopoldo, Brazil).

2.4. Microbiological analyses

2.4.1. Enumeration of microorganisms

Ten-milliliter samples were homogenized in 90 mL saline–peptone water (0.1% [vol/vol] bacteriological peptone [Himedia], 0.8% [vol/vol] NaCl [Merck, Whitehouse Station, NJ]) in a Stomacher at normal speed for 5 min (10^{-1} dilution) and diluted serially. Yeasts were enumerated by surface inoculation on YEPG agar containing 100 mg/L chloramphenicol (Sigma) to inhibit bacterial growth. Nutrient agar containing 0.1% cycloheximide was used as a general medium for count of viable bacteria population. The plates were incubated at 30 °C for 48 h. Following incubation, the number of colony-forming units (CFU) was recorded.

2.4.2. Verification of inoculum dominance

To verify inoculum dominance, colonies of *P. fermentans* YC5.2 were distinguished from the indigenous yeasts through a DNA approach. All yeast colonies from the YEPG plates at an appropriate dilution (see "Enumeration of microorganisms") were transferred into single wells of a 96-well plate containing 40 μL sterile distilled water and heated to 95 °C for 10 min. One μL of each yeast extract was used directly in a *P. fermentans*-specific PCR primer protocol (Pereira et al., 2014) that contained 12.5 μL of Mix GoTaq® Green Master 1X (Promega, São Paulo, Brazil) and 0.3 μM of each *P. fermentans*-specific primer (PFF2 — 5′GAAGGAAACGACGCTCAGAC3′ and PFR2 — 5′ ATCTCTTGGTTCTCGCATCG3′). Amplification products were separated by electrophoresis on a 0.7% (w/v) agarose gel, detected by ethidium bromide staining and visualized by UV transillumination. A ladder marker (GeneRuler 100 bp DNA Ladder Plus, Fermentans) was used as a size reference. A 136-bp amplification product pointed to the identification of *P. fermentans*, and the estimated average levels (log cfu/mL) were obtained at the sampling time. To confirm the identification of *P. fermentans*, the 5.8S ITS rRNA gene region of 36 representative yeast isolates was