Food Control 65 (2016) 112–120

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Discrimination of post-harvest coffee processing methods by microbial ecology analyses

Yasmine Hamdouche [a, *], Jean Christophe Meile [a], D. Nadège Nganou [b], Noël Durand [a], Corinne Teyssier [c], Didier Montet [a]

[a] CIRAD-UMR Qualisud, TA B-95/16, 73, rue Jean-François Breton, 34398 Montpellier Cedex 5, France
[b] Department of Food Science and Nutrition, Food Microbiology Laboratory, National School of Agro-Industrial Sciences, University Sciences, University of Ngaoundere, P. O. Box. 455 Ngaoundere, Cameroon
[c] University of Montpellier, CIRAD-UMR Qualisud, TA B-95/16, 73, Jean-François Breton, 34398 Montpellier Cedex 5, France

ARTICLE INFO

Article history:
Received 19 November 2015
Received in revised form
13 January 2016
Accepted 14 January 2016
Available online 18 January 2016

Keywords:
Coffea arabica
Coffea canephora
Microbial
Bacterial
Fungal communities
PCR-DGGE
Post-harvest processes

ABSTRACT

Two main types of processing are applied to coffee: the dry and wet processes. The aim of this study was to measure the impact of post-harvest processing on coffee microbial ecology and search for microbial markers of process discrimination. Coffee samples were collected in three farms in two locations of West Cameroon (Bafoussam and Dschang). Samples of *Coffea arabica* were collected along wet and dry methods and *Coffea canephora* samples were collected only during treatment using the dry method. Bacterial and fungal communities present on coffee beans were determined by using a molecular method PCR-DGGE (Denaturing Gradient Gel Electrophoresis) generating 16S rDNA and 26S rDNA profiles for bacteria and fungi. Results showed that microbial ecology structures were different depending on three main parameters: i) the method applied, ii) the coffee species and iii) their geographical origin. Statistical analysis applied allowed to measure the impact of all parameters and showed that the method was the major parameter influencing microbial communities' structure. Microbial DNA markers that appear specific of coffee origin, species or treatment were identified, such as lactic acid bacteria (LAB) which were detected only during wet process. They are specific of the fermentation step. DNA from *Wallemia* sp. was only detected in Robusta coffee beans (*Coffea canephora*). The study of microbial communities at the molecular level appears as a promising approach to discriminate between coffee processing types.

1. Introduction

The world total coffee production was estimated at 8.4 billion tons in 2014. Africa and South America represented respectively 11.5% and 29.2%, when Asia and Oceania represent 46.6% of the total production (ICO Annual Review, 2014). Coffee production in Cameroon encountered a drop over the past thirty years. Despite the decline of production, the Cameroonian coffee is still exported to major destinations, for example, Robusta to Italy (more than 40% of total Cameroon Robusta exports in 2007–2008) and Arabica to Germany (more than 70% of total Cameroon Arabica exports in 2007–2008) (ONCC-NCCB, 2009).

Coffee trees belong to the genus *Coffea* of the *Rubiaceae* family which constitutes about 80 species and several hundred varieties. The most common species of coffee and the most cultivated in the world are *Coffea arabica* and *Coffea canephora* known as Arabica and Robusta coffee respectively. Coffee beverages are mainly made from these species.

Several studies on the discrimination of coffee varieties based on differences in their chemical composition have been published. Most of them were based on the analysis of their aroma compounds (Blank, Sen, & Grosch, 1992, Sanz, Maeztu, Zapelena, Bello, & Cid, 2002), by authentication and quantification of coffee products using Fourier transform infrared spectroscopy (Briandet, Kemsley, & Wilson, 1996; Rubayiza & Meurens, 2005), by quantitative determination of their polysaccharide content (Fischer, Reimann, Trovato, & Redgwell, 2001), by analysis of their chemical composition (Martin et al., 1998) or by the composition of amino acid enantiomers (Casal, Alves, Mendes, Oliveira, & Ferreira, 2003). Molecular methods were also applied to discriminate coffee varieties as PCR-RFLP (Spaniolas, May, Bennett, & Tucker, 2006).

Coffee processing must begin immediately after the fruit

* Corresponding author.
E-mail address: yasminehamdouche@gmail.com (Y. Hamdouche).

http://dx.doi.org/10.1016/j.foodcont.2016.01.022
0956-7135/

harvesting to prevent the pulp from fermentation and deterioration. Different post-harvest practices play an important role in preserving and enhancing the intrinsic quality of coffee. The two main types of processes applied to coffee are: i) the natural process (dry method) which is usually applied to *Robusta coffee*, and washed process (wet method) (Teixeira et al., 1995) which is mostly applied to Arabica. Dry method is the oldest, simplest, and cheapest method. It produces 'natural' coffees (Clarke & Macrae, 1985; 1987). In the dry method, the harvested cherries are first sorted and then sun-dried. Drying operation is the most important stage of this process, since it affects the final quality of green coffee obtained after husking.

The wet method produces a fully washed parchment coffee (parchment devoid of mucilage) that is fermented in water. This process helps to preserve the intrinsic qualities of the bean, producing a homogeneous green coffee with very few damaged beans. Coffee treated by this process is regarded as a high quality coffee and is sold as higher price when compared to natural coffee (Rothfos, 1980). Coffee cherry pulp and mucilage consist primarily of water (76%), proteins (10% DM), fibers (21% DM) and minerals (8% DM) (Silva, Schwan, Dias, & Wheals, 2000). Coffee pulp and mucilage are natural substrates for the growth of microorganisms. Bacteria, yeast, and fungi have been shown to be implicated in coffee processing, notably during coffee fermentation. Microbial activities degrade the components of pulp and mucilage and induce the biochemical transformations necessary for natural fermentation (Silva et al., 2000; 2008). Many microbial species have been isolated from coffee cherries during natural or dry fermentation comprising 44 genera and 64 different species, as reported by Silva, Batista, Abreu, Dias, and Schwan (2008).

UE regulation 178/2002 established the general requirements of food law, established the European Food Safety Authority and required the determination of traceability in processes.

Previous works have implemented a traceability tool for the determination of geographical origin of fruits (El Sheikha et al., 2009) and coffee (Nganou et al., 2012) by microbial global analysis using Denaturing Gradient Electrophoresis (PCR-DGGE). This molecular approach was previously applied to study the dynamics of OTA producing fungi during coffee processing (Durand, 2012) and to assess the microbial diversity of semi-dry processed coffee (Vilela, De M.Pereira, Silva, Batista, & Schwan, 2010). Recent studies showed the use of PCR-DGGE in order to evaluate the potential of yeasts as starter cultures for dry method of coffee fermentation and to evaluate the microbiota present during coffee fermentation in wet method (Evangelista et al., 2014; 2015).

Others tools were used to discriminate coffee processes such as metabolomics approaches. For example, Ongo et al. (2012) and Jumhawan, Putri, Marwani, Bamba, and Fukusaki (2013) applied such methods to discriminate coffee treated by original process using animal "Asian Palm Civet" from others coffees. In this study, we are interested in tracing coffee by analyzing the microbial communities, because this product is developed with the help of microorganisms.

This rapid fingerprinting method could be employed as an analytical tool to trace the history of the exported products and to monitor the dynamics of microbiota during processing steps. To this end, we used PCR-DGGE to generate 16S rDNA and 26S rDNA profiles and determine the bacterial and fungal communities associated with *Coffea arabica* and *Coffea canephora* from two major producing locations in Cameroon (Bafoussam and Dschang) along their processing. We showed that this approach could be used to accurately measure differences in microbial ecology structures according to the type of coffee processing used. Thus, this approach could be used for traceability to discriminate between coffee from different origin and production mode.

2. Materials and methods

2.1. Sampling

Coffee samples were collected in two locations situated in western Cameroon, Bafoussam and Dschang. Meteorological data were noted in May 2014 (month of harvest). The mean temperature was 22.1 °C in Bafoussam and Dschang, mean humidity was 0.0165 and 0.0162 kg/kg. The geographical coordinates of the sampling area were 5°28′0, 72″N − 10°25′0, 48″E and 5°27″N − 10°04′00″E respectively. For each location, the samples were collected from three different sites (farms), the distance between site B1 and B2 was 12 km, between site B2 and B3 was 10 km and between site B1 and B3 was 22 Km in Bafoussam. In Dschang, there was 7 km between site D1 and D2, 5 km between D2 and D3, and 12 Km between D1 and D3.

Five hundred grams of coffee samples were collected from bags of 50 kg (100 g at 5 different parts of the coffee bag). *Coffea Arabica* samples were collected after brewing at two steps during wet method (hull coffee and green coffee) and dry method (husk coffee and green coffee) but *Coffea canephora* samples were collected only during dry process (Fig.1). All samples were stored at room temperature (25 °C) until analysis.

2.2. Total DNA extraction

From 10 g of coffee beans, total DNA (including microbial DNA) was extracted according to the method described previously by Hamdouche et al. (2015). The quantity and purity of extracted DNA were verified by a UV spectrophotometer (Biospec-Nano), and by electrophoresis through a 0.8% agarose gel in 1X TAE buffer (containing 40 mM Tris−HCl, pH 7.4, 20 mM sodium acetate, 1.0 mM Na_2-EDTA; Eppendorf) with a 1 Kb molecular weight ladder (Promega). After running at 100 V for 30 min, the gels were stained for 15 min in Gel Red solution (50 μg mL^{-1}; Biotium), then observed and photographed on a UV transilluminator, using a black and white CCD camera (Scion Company) and Gel Smart 7.3 system software (Clara Vision).

2.3. PCR amplification

For bacterial communities analyses: A 160 bp fragment of the V3 variable region of 16S rDNA was amplified using gc-338f (5′-GCG CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCA TCC TAC GGG AGG CAG CAG-3′, Sigma) and 518r (5′-ATT ACC GCG GCT GCT GG-3′, Sigma) DNA primers (Ampe, Omar, Moizan, Wacher, & Guyot, 1999; Leesing, 2005; Øvreas, Forney, Dae, & Torsvik, 1997). (A 40-pb GC-clamp (Sigma) was added to the forward primer in order to ensure that the fragment of DNA will remain partially double stranded and that the region screened is in the lowest melting domain (Sheffield, Beck, Stone, & Myers, 1989)). Each mixture (final volume 50 μL) contained about 100 ng of template DNA, DNA primers at 0.2 μM, deoxyribonucleotide triphosphate (dNTPs) at 200 μM, 1.5 mM $MgCl_2$, 5 μL of 10X of reaction Taq buffer $MgCl_2$ free and 1.25 U of a-Taq polymerase (Promega). In order to increase the specificity of amplification and to reduce the formation of spurious by-products, DNA was amplified in a "touchdown" PCR as follow: first cycle at 94 °C for 3 min, followed by 10 touchdown cycles of denaturation at 94 °C for 1 min annealing at 65 °C (1st cycle) to 55 °C (10th cycle) for 1 min and extension at 72 °C for 1 min, then 25 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min. The extension step was increased to 10 min in the last cycle.

For fungal communities analyses: a 250 bp fragment of D1/D2 region of the 26S rDNA was amplified using eukaryotic universal