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Discriminating organic and conventional foods by analysis of their microbial ecology: An application on fruits

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ABSTRACT

Traceability of foods is mainly done at the administrative level, and the use of analytical tools is rare. Previous studies have demonstrated that microbial ecology analyses at the molecular level (such as PCR-DGGE) could be used to provide food with a unique biological signature that could be linked to the geographical origin of food. The present study aimed at testing this approach to differentiate farming types by analyzing organic and conventional food products. To this end, the microbial ecology of organic and conventional nectarines was analyzed and statistically compared.

Our results show that yeast and bacterial communities were specific of the farming type allowing organic fruits to be discriminated from conventional ones. Several microbial species were identified as potential, biological markers which detection could be used to certify the origin as well as the mode of production of foodstuff. We proposed this analytical tool as a first step to control and authentify organic foods.

1. Introduction

Following various food crises such as the mad cow disease or the recent fraud in the beef meat market, European consumers are more and more perceptible to the quality and the origin of foodstuffs they buy, and food safety became one of their main concerns (Lairon, 2010). As a response to these safety, sociologic and economic problems and within the framework of the globalization, the European regulation relative to the sanitary quality of foodstuffs had to be strengthened. The Food Law (European regulation CE No. 178/2002), applied on the 1st of January 2005, imposes to all food-processing companies of the European Union (EU) to keep consumers informed about the nature of the product and any sanitary problems. Moreover, it imposes the traceability of foodstuffs at all steps of the food production. This regulation applies also to organic food industry.

Organic farming is a method of sustainable production which contributes to the environmental and animal protection by a set of specific agricultural practices. According to the French Agency for Food Safety (Anses, http://www.anses.fr), organic farming is characterized by the use of a positive list of chemicals and Genetically Modified Organisms are prohibited. This mode of production is supervised by a European regulation No. 834/2007 which defines the principles of production, preparation and importation to be respected, the lists of usable products, the practices for every type of breeding and the principles of control, certification, penalty and labelling. The “organic farming” mention is obtained after a period of land conversion of two or three years and a variable period of animal conversion according to species. During this period, organic farmers respect a rigorous specification which favours the respect of the ecosystem (Lairon, 2010).

The strong consumer demand led to the fast increase of the number of farmers committed to this farming type. Academic literature concerning organic farming is very scarce, even if some papers dealing with quality and safety were published these last years (Dangour et al., 2010; Lairon, 2010). Moreover, there are no published results on the agricultural productions from organic farming, in comparison with products from conventional farming. In other words, similar sanitary standards are applied to both agricultures, particularly regarding pesticide residues for example.

The recent crisis of “horse meat” puts in evidence the necessity of better food traceability. In spite of the current European regulation, the administrative documents accompanying foodstuffs (organic or conventional farming) only inform about the country of expedition and the identity of the exporter while the country or region of production or the origins of ingredients remain unknown.

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According to the International Organization for Standardization (ISO 9000-2000), traceability is a risk management tool which allows tracing the progress of foodstuffs ("from the farm to the fork"). Traceability became a constant and compulsory concern for all actors of the food chain: producers, transformers and distributors have to identify and solve critical points, realize self-monitoring, but also, inform consumers about the nature of food products (UE regulation 178/2002). It permits a quicker crisis management and a fast removal of potentially dangerous food from the market. Traceability is one of the main tools that ensure both the effective responsibility of foodstuffs manufacturers, farmers to industry of the food sector and the quality of the end product and also to improve risk estimation and manage effectiveness (Raspor, 2004). However, there is, at the moment, no real analytical tool for food traceability allowing authentication of the product origin or the farming type in a simple, fast and inexpensive way.

Currently, various modern analytical techniques allow determining the origin of food with a certain precision (Peres, Barlet, Loiseau, & Montet, 2007). These methods can be classified in two categories:

i) physico-chemical techniques such as Magnetic Nuclear Resonance (MNR), Near Infra-Red Spectroscopy (NIRS) or Stable Isotope Ratio Analysis (SIRA) and ii) biological techniques (biochemical or molecular biology involving DNA, RNA, proteins or biological molecules analyses).

The skin of fresh foods (vegetables, fruits) is not sterile and carries microorganisms or their fragments. The presence of various microorganisms depends on the external environment of the food matrices (soil ecology, spoilage, insects, diseases), but also microorganisms brought by human activity (Sodeko, Izuagbe, & Ukhun, 1987). Previous works showed that there is a link between the geographical origin of food and the structure of the food microbial flora such as wild and farmed fishes (Doan, Ngoc, Dijoux, Loiseau, & Montet, 2008; Tatsadjieu et al., 2010), fruits (El Sheikha et al., 2009; El Sheikha, Bouvet, & Montet, 2011) and, more recently, on marine salts (Dufossé, Donadio, Valla, Meile, & Montet, 2013). This was performed using a molecular biology method based on the extraction, PCR amplification and DGGE separation of microbial DNA (PCR-DGGE).

The main objective of the present study is to discuss the possibility to apply, for the first time, a molecular microbial ecology approach to discriminate organic from conventional food using rDNA fingerprinting of microorganisms. This work is the first step towards the creation of an analytical tool that will allow the discrimination between foods according their farming type.

2. Materials and methods

2.1. Sampling

Mature samples of nectarines and peaches were collected from orchard and trays stored at 1 °C (for fifteen days for sustainable fruits, one day for organic fruits and nine days for conventional fruits) in the French cooperative "Saveurs des Clos" (Ille sur Tet in the south of France), during august 2011, in an aseptic way with gloves and transported into sterile bags to Cirad laboratories in Montpellier (France). Some fruits were harvested and others were sampled to the cooperative on platters. All these fruits were from the same geographical origin (nearby plots) and had the same variety: Yellow peaches are of the Corundum variety, yellow nectarines belong to the Amber variety, except conventional nectarines from platters that are of the Western Red variety. White nectarines and peaches are of Star Pearl and Amanda varieties, respectively. The fruit sampled were of three different farming types: organic (certified by competent national authority), conventional and sustainable farming (organized by the cooperative as more reasoned than the conventional farming). For each farming type, at least four fruits were sampled and analyzed to ensure the reproducibility of the method.

2.2. Extraction of total DNA

DNA was extracted immediately from fresh fruits skins. Total DNA was extracted by using a method adapted from Masoud, Cesar, Jespersen, and Jakobsen (2004), Ros-Chumillas, Egea-Cortines, Lopez-Gomez, and Weiss (2007) and El Sheikha et al. (2009). About 2 g of peeled skin were separately mixed with 10 mL of sterile peptone water in 50 mL Falcon tubes and incubated on rotating wheel for 30 min at room temperature. Then, 1 mL of the resulting suspension was sampled in Eppendorf tubes containing about 0.3 g of acid washed glass beads (Sigma–Aldrich). The mixture was vortexed vigorously for 15 min in a bead beater instrument (Vortex Genie 2) then centrifuged at 12 000 g for 15 min and the supernatant discarded. The cell pellet was resuspended in 300 μL breaking buffer [2% Triton X-100 (Prolabo), 1% SDS (sodium dodecyl sulfate; Sigma), 100 mM NaCl [(Sigma), 10 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0 (Promega)]. 100 μL of buffer TE (10 mM Tris–HCl pH 8, 1 mM EDTA; Promega), 100 μL of lysozyme solution (25 mg/mL, Eurobio) and 100 μL of proteinase K solution (20 mg/mL, Biosolve) were successively added followed by 20 of incubation at 42 °C. Then 50 μL of 20% SDS were added to each tube, and incubated at 42 °C for 10 min. 400 μL of 2% CTAB (cetyltrimethylammonium bromid, Merck) were added to each tube and incubated at 65 °C for 10 min. The lysates were subjected twice to phenol chloroform extraction by adding 700 μL of phenol/chloroform/isoamyl alcohol mixture (25/24/1, Carlo Erba), manually mixed and then centrifuged at 12 000 g for 15 min. The aqueous layer was transferred to a new Eppendorf tube. The residual phenol was removed by adding 600 μL of chloroform/isoamyl alcohol (25:24:1, Carlo Erba) and centrifuged at 12 000 g for 10 min. The aqueous phase was collected and 0.1 volume of sodium acetate was added (3 M, pH 5), followed by addition of an equal volume of isopropanol and stored at −20 °C for 12 h (overnight). After centrifugation at 12 000 g for 30 min, the supernatant was eliminated, DNA pellets were washed with 500 μL of 70% ethanol, and tubes were centrifuged at 12 000 g for 5 min. The ethanol was then discarded and the pellets were air dried at room temperature for several hours. Finally, the DNA was resuspended in 100 μL of ultra-pure water and stored at −20 °C until analysis. DNA quantities were estimated by electrophoretic migration through a 0.8% agarose gel and by using a UV spectrophotometer (BioSpec-Nano, Shimadzu). Gels were photographed on a UV transilluminator with a CCD camera and Gel Smart 7.3 system (Clara Vision).

2.3. PCR-Denaturing Gradient Gel Electrophoresis (DGGE) analysis

For yeast DNA, a fragment of the D1/D2 region of the 26S rDNA gene was amplified using eukaryotic universal primers: forward, NL1GC (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCC ATA TCA ATA AGC GGA GGA AAA G-3′; Sigma); reverse, LS2 (5′-ATT CCC AAA CAA CTC GAC TC-3′; Sigma), amplifying a 250 bp fragment (Cocolin, Bisson, & Mills, 2000; Kurtzman & Robnett, 1998). For the study of bacterial community, a fragment of the V3 variable region 16S DNA gene was amplified using universal bacterial primers: forward, gc338F (5′ CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG 3′; Sigma); reverse, 518R (5′ ATT ACC GCG GCT GCT GG 3′), amplifying a 190 bp fragment (Ampe, Ben Omar, Moizan, Wacher, & Guyot, 1999; Leesing, 2005; Le Nguyen, Hanh, Dijoux, Loiseau, & Montet,