Food Microbiology 46 (2015) 368–382

Contents lists available at ScienceDirect

Food Microbiology

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

Physico-chemical characterization of natural fermentation process of Conservolea and Kalamàta table olives and developement of a protocol for the pre-selection of fermentation starters

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ARTICLE INFO

Article history:
Received 13 May 2014
Received in revised form
21 July 2014
Accepted 26 August 2014
Available online 6 September 2014

Keywords:
Table olives
Fermentation
Microorganism selection
GC–MS analysis

ABSTRACT

Table olives are one of the most important traditional fermented vegetables in Europe and their world consumption is constantly increasing. Conservolea and Kalamàta are the most important table olives Greek varieties. In the Greek system, the final product is obtained by spontaneous fermentations, without any chemical debittering treatment. This natural fermentation process is not predictable and strongly influenced by the physical-chemical conditions and by the presence of microorganisms contaminating the olives. Natural fermentations of Conservolea and Kalamàta cultivars black olives were studied in order to determine microbiological, biochemical and chemical evolution during the process. Following the process conditions generally used by producers, in both cultivars, yeasts were detected throughout the fermentation, whereas lactic acid bacteria (LAB) appeared in the last staged of the process. A new optimized specific protocol was developed to select autochthonous yeast and LAB isolates that can be good candidates as starters. These microorganisms were pre-selected for their ability to adapt to model brines, to have beta-glucosidase activity, not to produce biogenic amines. Chemical compounds deriving by microbiological activities and associated to the three different phases (30, 90 and 180 days) of the fermentation process were identified and were proposed as chemical descriptors to follow the fermentation progress.

1. Introduction

Table olives are one of the most important and popular traditional fermented vegetables in Western world and in particular in Southern European countries. This product, together with olive oil, represents an important food in the Mediterranean diet. World production of table olives is estimated (2013–2014 season) in 2,574,500 tons and the 27% of this production (698,000 tons) is located in the European Union (EU) (IOOC, 2013). Spain has a leading position in table olive production with 513,100 tons, followed by Greece with 94,000 tons, by Italy with 74,000 tons and Portugal with 11,900 tons. Table olives consumption is constantly increasing throughout both EU and the world and producer countries are also the most important consumers. According to their relevance on the international markets, Kalamàta and Conservolea are the most important table olive varieties together with Manzanilla, Sevillana and Hojiblanca and to a lesser extent Bella di Cerignola and Ascolana Tenera (Anon., 2003). Conservolea represents the most economically important cultivar in Greece, corresponding to at least 80–85% of Greek olive production, whereas Kalamàta is the second most important cultivar used in the production of Greek table olives for domestic and foreign market (Garrido-Fernandez et al., 1997). The most important industrial preparations of table olives are the green olives by Spanish style, the black oxidized olives by Californian method and the naturally black olives by Greek style (Garrido-Fernandez et al., 1997).

In the Greek system, the final product is obtained placing directly olives into brine, without any debittering pre-treatment and it is characterized by a fruity aroma and a slightly bitter taste. Today, this debittering process is carried out by spontaneous

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http://dx.doi.org/10.1016/j.fm.2014.08.021
0740-0020/

fermentations, not predictable and strongly influenced by the physical-chemical conditions (salt content, pH, aerobic/anaerobic conditions and temperature), the availability of fermentable substrates and by the presence of microorganisms contaminating the drupes (De Castro et al., 2002; Tassou et al., 2002; Alvarez et al., 2003; Abriouel et al., 2011). To standardize the fermentation process and consequently improve the quality of the final products, the identification of chemical descriptors for monitoring the fermentation is highly requested. The primary purpose of table olive fermentation is to achieve a preservation effect and enhance the organoleptic attributes of the processed product. Natural fermentation is mainly promoted by yeasts and lactic acid bacteria (LAB), naturally associated with drupes (Brenes et al., 2004; Romero et al., 2004).

Homo and hetero-fermentative LAB, which can produce lactic acid and other organic acids, are the most important group of bacteria in olives. Homo fermentative LAB such as *Lactobacillus*, *Streptococcus* and *Pediococcus* and hetero fermentative LAB such as *Leuconostoc* and some members of *Lactobacillus* are commonly detected in fermented olive preparations (Abriouel et al., 2012; Randazzo et al., 2012).

LAB are able to improve the aroma and flavor characteristics of the product (Panagou et al., 2008), to enhance the olive preservation due to a progressive acidification of the fermenting brine and the production of antimicrobial compounds and bacteriocins (Marsilio et al., 2005). *Lactobacillus plantarum* and *Lactobacillus pentosus* were used in different studies as starters to control fermentation processes, demonstrating that these microorganisms have the potential to allow the microbiological control of the process, increase the lactic acid yield and improve the quality of the final product (Lamzira et al., 2005; Marsilio et al., 2005; Sabatini et al., 2008; Panagou et al., 2008; Servili et al., 2006).

Yeasts can play both a positive and a negative role in table olive processing (Arroyo-López et al., 2008). In fact, yeasts are able to produce desirable volatile compounds and metabolites that improve the organoleptic properties (Garrido-Fernández et al., 1995), to enhance the growth of LAB (Tsapatsaris and Kotzekidou, 2004; Segovia Bravo et al., 2007) and to biodegrade phenolic compounds (Ettayebi et al., 2003). On the other hand, yeasts may cause gas pocket formation because of CO_2 production at the early stage of fermentation (Lamzira et al., 2005) and softening of the olive tissue (Hernández et al., 2007).

The aim of the present work was (i) to study the microbiological, biochemical and chemical profiles associated with natural fermentation of Conservolea and Kalamàta cultivars black olives, (ii) to identify chemical descriptors associated with the fermentation progress and (iii) to pre-select, by optimized specific protocols, autochthonous yeast and LAB isolates that can be good candidates as starters. For the first time in this study yeast and LAB strains suitable to be used as candidate mixed autochthonous starter cultures were isolated and chemical compounds associated to the three different phases of the fermentation process were identified in Conservolea and Kalamàta black olives.

2. Materials and methods

2.1. Olive samples and fermentation method

The pilot-scale fermentations were performed in triplicate on olive samples of Conservolea and Kalamàta cultivars in an industrial plant (Agricola Nuova Generazione, Martano, Lecce, Italy). Healthy black olives (90 kg) were collected at the black stage of ripening and washed with tap water to eliminate plant materials and superficial contaminants. The olives were then selected (caliber above 10–12 mm), washed and placed in plastic vessels of 30 kg capacity filled with 20 L of 8% NaCl (wt/vol). The olives were allowed to ferment at ambient temperature (8–30 °C).

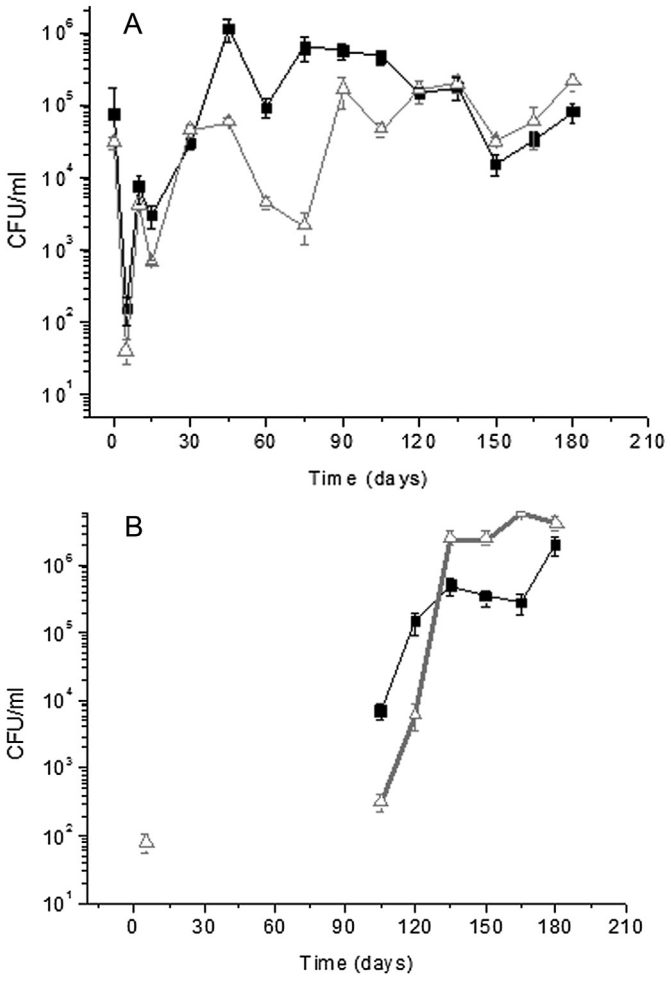

Fig. 1. Yeast and LAB evolution during Conservolea —■— and Kalamàta —△— fermentation. Yeast (A) and LAB (B) total counts (Log CFU/ml) of Conservolea and Kalamàta naturally fermented table olives.

2.2. Isolation of microbial population

To isolate epiphytic yeasts, *Enterobacteriaceae* and Lactic Acid Bacteria (LAB) from the olives, 10 drupes per sample were washed in 50 ml of sterile water on a rotary shaker at 200 rpm for 30 min. The sediment was recovered after centrifugation at 5000 × *g* for 10 min at room temperature and suspended in 0.5 ml of 0.1% (wt/vol) peptone water. The suspension was added with one volume of sterile glycerol and stored at −80 °C until microbiological analysis.

Salinity, pH and temperature were evaluated during fermentation at the following time points: 0, 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180 days (Fig. 2). At each different fermentation time, 7.5 ml aliquots of brines were collected diluted with one volume of sterile 100% glycerol and stored at −80 °C for further analysis.

Microbiological analyses on epiphytic microorganisms from olives and brines were performed by diluting samples serially with 0.1% (wt/vol) peptone water and applying them to agar slants containing the following media: Man, Rogosa and Sharpe Agar (MRS, LABM, UK) added with 0.05 g/l of nystatin for LAB identification and incubated at 30 °C under anaerobic conditions for