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Review

Yeasts in table olive processing: Desirable or spoilage microorganisms?

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ABSTRACT

Yeasts are unicellular eukaryotic microorganisms isolated from many foods, and are commonly found in table olive processing where they can play a double role. On one hand, these microorganisms can produce spoilage of fruits due to the production of bad odours and flavours, the accumulation of CO_2 leading to swollen containers, the clouding of brines, the softening of fruits and the degradation of lactic acid, which is especially harmful during table olive storage and packaging. But on the other hand, fortunately, yeasts also possess desirable biochemical activities (lipase, esterase, β-glucosidase, catalase, production of killer factors, etc.) with important technological applications in this fermented vegetable. Recently, the probiotic potential of olive yeasts has begun to be evaluated because many species are able to resist the passage through the gastrointestinal tract and show beneficial effects on the host. In this way, yeasts may improve consumers' health by decreasing cholesterol levels, inhibiting pathogens, degrading non assimilated compounds, producing antioxidants and vitamins, adhering to intestinal cells or by maintaining epithelial barrier integrity. Many yeast species, usually also found in table olive processing, such as *Wicherhamomyces anomalus*, *Saccharomyces cerevisiae*, *Pichia membranifaciens* and *Kluyveromyces lactis*, have been reported to exhibit some of these properties. Thus, the selection of the most appropriate strains to be used as starters, alone or in combination with lactic acid bacteria, is a promising research line to develop in a near future which might improve the added value of the commercialized product.

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1. Introduction

The olive, the fruit of the *Olea europaea* tree, is a fleshy drupe with a high fat content, a bitter component (oleuropein) and a low sugar concentration. Such characteristics prevent the direct consumption of olives and have promoted a series of processes, which can differ considerably from region to region, in order to make them edible. The Trade Standard Applying to Table Olives (IOOC (International Olive Oil Council), 2004) defined this food as: 'the product obtained from suitable olive cultivars, processed to remove their natural bitterness, and preserved (by natural fermentation, heat treatment or preservatives) with or without brine until consumption'.

Nowadays, the table olive is one of the major fermented vegetables of the food industry. The International Olive Oil Council (IOOC (International Olive Oil Council), 2012) estimates that table olives' world production reached around 2,440,000 t in the 2010/2011 season.

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Most table olives are produced in the Mediterranean countries with Spain, Turkey, Egypt, Greece and Italy as the main contributors. There are also important productions in the USA, Argentina, Peru and Australia. Thus, table olive processing is widespread around the world and represents an important economic source for producing countries.

The most important industrial elaborations of table olives are: i) alkali treated green olives (the so-called Spanish style), which represent about 60% of production, ii) ripe olives by alkaline oxidation (the so-called Californian style), and iii) untreated or directly brined olives (green, turning colour or naturally black). There are also many other traditional/industrial ways of processing table olives. A complete description of the different types of olive elaborations can be found in Fernández Díez et al. (1985) and Garrido Fernández et al. (1997).

Microorganisms play an important role in the production of table olives. Diverse groups are involved throughout olive fermentation determining the safety, quality and flavour of the final product, but *Enterobacteriaceae*, *Propionibacteriaceae*, lactic acid bacteria (LAB) and yeasts are the most relevant microorganisms (Fernández Díez et al., 1985; Garrido Fernández et al., 1997). The growth, for long period of time, of the two former groups of microorganisms is habitually considered negative because they can produce product spoilage and their presence can also compromises the safety of the final product. On the contrary, LAB have been widely studied due to the prodeuction of lactic acid and antimicrobial compounds (bacteriocins), which originate from the rapid and safe acidification of brines. Also, these microorganisms can degradate oleuropein, producing the biological debittering of the fruits. Finally, yeasts can play a double role during table olive processing, acting as desirable or spoilage microorganisms. However, it must be emphasised that there is little information available about the real effects of yeasts on the organoleptic properties of the fruits and their interactions with the other microorganisms present during olive fermentation or packaging.

The present review deals with the favourable and negative aspects of the use of yeasts in table olive processing. The final goal is to assess their potential application as starter cultures in this fermented vegetable, especially in those elaborations where their presence is more abundant such as in directly brined green and natural black olives. As olives are not lye treated in these preparations, LAB are partially inhibited due to the presence of phenolic compounds, and the role played by yeasts is even more relevant (Garrido Fernández et al., 1997; Ruiz Barba et al., 1993).

2. Yeast species isolated from table olive processing

Yeasts are unicellular eukaryotic microorganisms with an enormous importance in food and beverage industries (Querol and Fleet, 2006). They are classified in the kingdom fungi, with approximately 1500 species described (Kurtzman and Fell, 2006). In the past, the characterization of yeasts associated with table olives was mainly made by morphological and biochemical methods comparing the obtained results with diverse taxonomic keys (Barnett et al., 1990; Kurtzman et al., 2011). The process was complex and laborious, although the methodology permitted the isolation and characterization of a great variety of genera and species around the world for diverse types of table olive elaborations (Balatsouras, 1967; Borcakli et al., 1993; Durán Quintana et al., 1986; González Cancho, 1965, 1966a, 1966b; González Cancho et al., 1975; Hernández et al., 2007; Kotzekidou, 1997; Marquina et al., 1992, 1997; Mrak et al., 1956; Pelagatti, 1978).

However, molecular methods have recently started to be routinely used for the identification of yeasts associated with table olive processing. These techniques confer a higher accuracy degree in the final identification than classical biochemical methods. They are mainly based on: i) the restriction fragment length polymorphism (RFLP) analysis obtained after cutting the amplified 5.8S rRNA gene and the associated intergenetic spacers ITS with a battery of endonucleases (Esteve-Zarzoso et al., 1999), and ii) the direct sequencing of the D1/D2 domains of the 26S rRNA gene amplified with primers NL_1 and NL_4 (Kurtzman and Robnett, 1998) or the 5.8S-ITS region amplified with primers ITS_1 and ITS_4 (Esteve-Zarzoso et al., 1999). The generated data are then compared with those existing in diverse international data bases such as yeast-id.com (University of Valencia and CSIC) for RFLP analysis, or GenBank from NCBI for sequencing data. Molecular techniques are rapid, easy to perform and more precise, eliminating part of the subjectivity that usually accompanies the output of the biochemical test. Tofalo et al. (2012) have also developed a real-time quantitative PCR assay to directly detect and quantify the total number of yeasts besides the three species (*Wickerhamomyces anomalus*, *Pichia guillermondii* and *Pichia kluyveri*) associated with table olives. The targeted region were the internal transcribed spacers ITS (rDNA) and the conserved sequences of the variable D1/D2 domains of the 26S rRNA gene. The assay was able to unequivocally distinguish the three mentioned species from other yeasts and LAB generally associated with this type of processes.

Using molecular methods, Arroyo López et al. (2006) identified the species *Saccharomyces cerevisiae*, *Issatchenkia occidentalis* and *Geotrichum candidum* from Spanish green seasoned table olives (without lye treatment), and *Candida boidinii* and *Hanseniaspora guilliermondii* from the preservation stage of ripe black olives. Coton et al. (2006) identified *W. anomalus*, *C. boidinii* and *Debaryomyces etchelsii* as the predominant species in French black olives. Hurtado et al. (2008, 2009) found the species *C. boidinii*, *Candida sorsoba*, *Candida diddensiae*, *Candida membranifaciens*, *Kluyveromyces lactis*, *Pichia membranifaciens*, *W. anomalus*, *P. kluyveri*, and *Rhodotorula glutinis* during the processing of *Arbequina* table olives in Spain. Romo-Sánchez et al. (2010) studied the yeast biodiversity from Spanish fresh olive fruits, crushed olives and olive pomace from Arbequina and Cornicabra varieties. *Pichia caribbica*, *Lachancea fermentati* and *Nakazawaea holstii* were the most commonly isolated species, followed by *Pichia mississippiensis*, *Kluyveromyces thermotolerans* and *Saccharomyces rosinii*.

Nisiotou et al. (2010) reported that *Metschnikowia pulcherrima* was the dominant yeast species at the onset of the spontaneous fermentation of Greek black olives, followed by *Debaryomyces hansenii* and *Aureobasidium pullulans*. They also found a new yeast species associated with this type of fermentation, named as *Candida olivae*. Species heterogeneity changed as fermentation proceeded and *P. membranifaciens* along with *W. anomalus* evolved as the main yeasts of this olive elaboration prevailing at the end of the process. Aponte et al. (2010) revealed the presence of *Candida parapsilosis*, *P. guilliermondii* and *P. kluyveri* during the entire fermentation period of Sicilian green table olives. Rodríguez-Gómez et al. (2010) studied the yeast microflora associated with the olive storage of Manzanilla and Hojiblanca cultivars in acidified brines. The two most important species identified in both cultivars were *S. cerevisiae* and *Pichia galeiformis*, which were present throughout the storage period, whilst *C. boidinii* was detected during the later stages of the process. The species *P. membranifaciens* was detected only in the early stages of the Hojiblanca cultivar storage.

Bautista-Gallego et al. (2011) carried out the molecular identification by means of a RFLP analysis and sequencing of a total of 199 yeast isolates obtained from Spanish industrial green table olive fermentation. *C. diddensiae*, *S. cerevisiae* and *P. membranifaciens* were the most abundant yeast species isolated from directly brined Aloreña olives, whilst for Gordal and Manzanilla cultivars they were *Candida tropicalis*, *P. galeiformis* and *W. anomalus*. In the case of Gordal and Manzanilla green olives processed according to the Spanish style, the predominant yeasts were *D. etchellsii*, *C. tropicalis*, *P. galeiformis* and *K. lactis*. Recently, Abriouel et al. (2011) used a culture-independent approach based on the PCR-DGGE analysis for the identification of yeasts associated with Aloreña de Málaga olive fermentation, and found that in cold fermented olives the most relevant yeasts were