Chapter 6

Yeast Ecology of Fermented Table Olives: A Tool for Biotechnological Applications

Dimitrios Anagnostopoulos, Despina Bozoudi and Dimitrios Tsaltas

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.70760

Abstract

The table olive is considered to be a traditional fermented vegetable in the Mediterranean countries and its production and consumption is recently spreading all around the world. The presence of yeasts is very important during olive fermentation due to their double role. On one hand, yeasts maintain desirable biochemical activities (lipase, esterase, β-glucosidase, catalase, production of killer factors, etc.) with essential technological applications in this fermented vegetable. On the other hand, spoilage activity may be shown. However, recent studies have reported that yeasts coming from table olives would be a new source of potential probiotics. Indeed, many yeast species found in table olive processing, have been reported to demonstrate such properties. Thus, starter cultures technology will play significant role, not only in olive fermentation by controlling the safety and the quality of the final product, but also in consumer's health.

Keywords: yeast, ecology, table olives, starter cultures, probiotic, spoilage, flavor, aroma

1. Introduction

Table olives are one of the most important and well known fermented vegetables, with an estimated worldwide production currently reaching 2.5 million tons per year [1]. Not only the production, but also the consumption of this food is closely related to the culture and diet of some Mediterranean countries (Greece, Spain, Italy, France, Portugal, Egypt, and Turkey), which are the main producers [2]. Other countries such as USA, Peru, Argentina and Australia, are also rising as competitive producers [3].

Yeasts play a vital role during table olives fermentation process principally on the safety, the quality and the flavor of the final product [4]. On one hand, yeasts are responsible for properly driving the fermentation, due to their constant presence throughout the fermentative process. It is generally accepted, that they can produce compounds with important organoleptic attributes determining the quality and flavor of the final product [3]. More specifically, they are able to produce desirable biochemical activities such as lipase, esterase, β-glucosidase, catalase, production of killer factors, which have several technological applications in the fermented table olives. On the other hand, yeasts may also cause spoilage of olives, during fermentation, packing and storage, due to the production of bad odors and flavors, the accumulation of CO_2 leading to swollen containers and gas pocket effects, the clouding of brines, the softening of fruits and the degradation of lactic acid. The technological implications and functional properties of yeasts have been extensively reviewed [5–9]. It has been reported that they could exert a fundamental role for the fermentation of green-treated olives and black-naturally fermented ones [6]. Moreover, many yeast species, which were found in table olive processing, such as *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae, Pichia membranifaciens and Kluyveromyces lactis*, have been reported to exhibit some interesting properties, such as enhancement of the organoleptic characteristics of the fruits, degradation of polyphenols, enhancement of the growth of lactic acid bacteria (LAB) and antioxidant action [5, 9]. Therefore, the selection of the most appropriate yeast strains to be used as starters, in combination with LAB or not, is a promising future target which may improve the added value of the final product [5].

2. Biology and physiology of yeasts from fermented table olives

Yeasts are single-celled microbes, larger than bacteria, belonging to the kingdom of Fungi. Nowadays 1500 species are identified which corresponds approximately to 1% of all described fungal species [10]. Both their survival and growth are ensured by consuming starches and sugars. Plenty of yeasts are commercially available and can provide an inexpensive source for biochemical and biotechnological applications (classic and molecular) [11].

Generally, in the heterotrophic organisms, such as yeasts, the energy and carbon metabolism have an interconnection between them, i.e., anabolism is connected with catabolism. ATP is provided by the oxidation of organic molecules which also play an important role in the biosynthesis, because they can be used firstly as carbon sources and eventually as energetic currency for all kinds of cellular work. Although, for instance, table olive yeasts species contain a wide set of carbon sources (polyols, alcohols, organic acids and amino acids) that can help in their growth, they prefer to metabolize sugars. There are a lot of data related to the metabolism of different carbon sources, with the most widely studied sugars being hexoses (glucose, fructose, galactose or mannose) and disaccharides (maltose or sucrose), as well as compounds with two carbons (ethanol or acetate) [5, 12].

The metabolic pathway of the central carbon metabolism is mainly identical between different yeast species, suggesting that these microorganisms might constitute a metabolic homogenous

group. Nevertheless, the mechanisms for nutrient uptake, the number of different isoenzymes and the regulation of fermentation and respiration differ substantially [13], making yeasts a highly heterogeneous and complex metabolic group.

Moreover, the presence of volatile compounds, such as ethanol, glycerol, higher alcohols, and, to a lesser extent, acetaldehyde in brines can be directly attributed to the metabolic activity of yeasts together with heterofermentative bacteria [12].

On the contrary, methanol, detected in brine [14], is not related with the yeast metabolism, because its activity is due to pectinolytic enzymes. The presence of these enzymes in the drupes is favored by an improper handling of raw material and by poor fermentation conditions [15].

2.1. Enzymatic activities

Yeasts ensure their survival in tissues, and are connected with the digestion of host's proteins and other organic molecules due to a large array of physiological traits and enzymatic activities [16, 17]. It is characteristic that the kind of medium used for their isolation and growth is an important adaptive factor which determines the technological activities of the strains selected [18, 19]. Although many surveys have underlined the potential of unconventional and extreme environments as a source of natural biodiversity for the isolation and selection of useful microorganisms [20], it is the secretion of extracellular prote ases which has been studied extensively in yeasts isolated from these environments [21, 22]. Esterases from yeasts are also gaining industrial interest with applications in laundry detergents and in dairy industries [23, 24], while little attention has been paid to lipases from yeasts [7, 25, 26]. Although peptic enzymes for industrial uses have been so far produced by molds and bacteria [27], the pectinolytic activity of yeasts has, however, been studied with ambiguous results [28, 29].

Yeasts can also produce plenty of other interesting enzymes like invertase, zymase, hexose phosphatase, maltase, reductase, carboxylase, melibiase, and endo-tryptase. However, all kind of yeasts do not comprise the same range of enzymes. Hence, different yeasts are functioning differently toward the degradation of sugars. It is generally accepted that one yeast can ferment one sugar [30]. Since today, it is known that three of the aldo-hexose sugars, called dextrose, d-mannose, and d-galactose, are exclusively fermentable by yeasts. It has been reported that yeasts which ferment dextrose, can also use mannose and laevulose [30]. Due to the enzyme invertase, yeasts can firstly reverse and secondly ferment cane-sugar. However, they are unable to ferment milk-sugar, because of the lack of the enzyme lactase.

2.2. Functional properties

Despite the fact that research has been focused almost exclusively on LAB, as being extremely useful in table olives microbiota [31], recent studies have been negotiating the significant contribution of yeasts to table olives fermentation [3, 5, 9], during the whole process and adding value to the final product. The selection of yeasts to be used as starter cultures is an unclear

and complicated procedure, which includes three main steps. The first one is the selection of promising species, which should have some interesting properties. The second step is the validation of those microorganisms on laboratory scale and finally the third one, and the most important, is the demonstration at large scale [32].

Starter cultures must be selected for their functional traits, and for their ability to dominate at the fermentation process, as well as leading to a stable final product. Thus, the screening of those yeast strains is very important. Esterase and lipase enzymatic activities are desirable in yeasts because of their ability to improve the organoleptic characteristics of olives through the configuration of volatile compounds that can be produced by the catabolism of free fatty acids [9, 32–34].

Yeasts with β-glucosidase activity are also good candidates, because they can be used for the hydrolysis of oleuropein, removing the natural bitterness present in brined olives and thus, avoiding the placement of olives into large amounts of water [3, 35]. However, limited data are available in the literature about the contribution of these species on table olive fermentation process [7, 17, 34]. Furthermore, very few strains which produce nontoxic compounds, like biogenic amines (BA) (spermine, spermidine, agmatine) was proposed as a new criterion for the selection of yeasts as starter cultures for many fermented products [36, 37]. Many yeast species which have the ability to produce BA can be found in olives. As a coincidence, this trait should be investigated as another critical point for the selection of proper starter cultures in the near future.

It is noteworthy to mention the probiotic profile of some table olives yeasts. Generally, probiotics are microorganisms which could have beneficial impact on the human organism [38]. This positive impact would exist only if those microorganisms are safe for the host, metabolically active within the gastrointestinal tract and are being consumed, either as food components or as non-food preparations. For instance, *Saccharomyces boulardii*, a member of *S. cerevisiae* species, is the only yeast with clinical role and proven probiotic efficiency in double blind clinical studies [39]. Thus, finding other yeast strains with probiotic characteristics and especially those isolated from table olives is of great interest.

The probiotic potential of yeasts isolated from fermented table olives has been documented to some extent [8, 35]. In particular, Psani & Kotzekidou, [35] found *Torulaspora delbrueckii* and *Debaryomyces hansenii* strains to be quite tolerant in high bile salt concentrations and low pH values. Moreover, the fact that the inoculation of these strains were able to inhibit food borne pathogens such as *Listeria monocytogenes, Bacillus cereus* and *Salmonella typhimurium* is remarkable. Furthermore, data from Ref. [8] showed that *P. membranifaciens* and *Candida oleophila* strains have similar properties but with a different spectrum of inhibition zones (for *Escherichia coli, Salmonella enteritidis* and *Staphylococcus aureus*). Another significant number of yeast species, such as *K. lactis, D. hansenii, T. delbrueckii* and *S. cerevisiae*, have shown tolerance to traverse the gastrointestinal tract, inhibit the enteropathogens, adhere the intestinal $CaCO_2$ cell line and present an immunostimulatory activity [40–42]. In particular, *S. cerevisiae* has shown its ability to prevent the survival of *E. coli* O157:H7 under simulated gastrointestinal conditions by the production of ethanol [43]. Although research of olive yeast strains with the properties mentioned above is a promising task, it is important for olive yeasts to adhere on to the olive skin and survive during storage and/or packaging, in order to be ingested by consumers at elevated numbers.

Except from their probiotic properties, yeasts could positively affect human health in other ways, as well. For instance, diverse strains of *K. lactis*, *S. cerevisiae*, and *Issatchenkia orientalis* show great ability to reduce cholesterol serum levels [40]. Secondly, phytate has a strong chelating ability to form insoluble complexes with divalent minerals of nutritional importance such as zinc, calcium, magnesium and iron. Due to a lack of some required enzymes, humans cannot degrade phytate complexes in the gastrointestinal tract. It is known that dephosphorylation of phytate is catalyzed by phytases, which are widespread in yeast species such as *I. orientalis*, *W. anomalus*, *S. cerevisiae*, *T. delbrueckii*, and *K. lactis* [41, 44]. Thus, yeasts could be included in humans' diet in order to help them to do so.

Folates (vitamin B9) are considered essential co-factors in the biosynthesis of nucleotides and play an important role in cellular replication and growth. It is common that mammals cannot synthesize folates and a potential solution is for yeasts to help them to do so because they contain a folate biosynthesis pathway and can produce natural folates. Some of those species which have a high folate biosynthesis pathway are the *S. cerevisiae* and *Candida glabrata* [41].

Other species of yeasts, such as the diverse yeast strains isolated from table olives, belonging to the *P. membranifaciens* and *P. farinosa* species have the ability to produce B-complex vitamins [8, 45]. This means that yeasts can synthesize a number of bioactive compounds which can serve as natural antioxidants.

Moreover, researchers are interested in screening of yeasts for free-radical-scavenging activity. For instance, *W. anomalus* produced the highest activity in a laboratory medium [46]. The production of bioactive antioxidants may retard the oxidative degeneration of fatty substances and improve human health. In any case, probiotic yeasts could be able to adhere onto the olive epidermis, and thus, be ingested by consumers.

Once researchers are very interested in the organoleptic features of olives, yeasts have some very essential and strain-specific metabolic properties, such as esterase and lipolytic activities. The former has frequently been detected, while the isolation of strains with lipoytic activities has been reported to a lesser extent [7]. Enterase positive yeasts are covetable because the fact that they are able to meliorate the flavor of olives from the formation of esters coming from free fatty acids. Strong lipase activity, as well as, weak activity have been detected both in vitro in some yeast species. The former has been reported in *Candida boidinii*, *D. hansenii* and *T. delbrueckii* while the latter only in *P. membranifaciens* [34, 35]. Those authors have emphasized the change of the free fatty acids composition of olives in the presence of yeast populations in contrast with sterile conditions, indicating that lipases produced by these microorganisms modify the characteristics of fruit lipids and therefore its organoleptic characteristics.

3. Yeast ecology of table olives

According to Ref. [4] it has been reported, that yeasts are responsible for the fermentation process of natural black olives. However, to green olives, the fermentation is driven by LAB. Black olive fermentations have been studied in different countries, mainly in the Mediterranean (Italy, Spain, Portugal, Greece, and Morocco) and showed some yeast species biodiversity.

Despite this fact, a few species were dominant, such as *P. membranifaciens*, *Saccharomyces oleaginosus*, *Pichia anomala*, *C. boidinii* and *T. delbrueckii* [47–50]. However, since LAB are partially inhibited in directly brined green and natural black olives due to the presence of phenolic compounds, yeasts became highly important for the fermentation process [3].

Nowadays, several studies have focused on yeasts microbiota situated on the surface of olive fruits. Some of those studies are summarized in **Table 1**. 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 [10, 61]. However, molecular methods have started to be used recently, for the identification of yeasts coming from table olives fermentation. One of the most applicable methods is the Denaturating Gradient Gel Electrophoresis (DGGE-PCR), which is more precise than any classical method and is based on (i) restriction fragment length polymorphism (RFLP) analysis obtained after cutting the amplified 5.8S rRNA gene and the associated intergenic spacers ITS with endonucleases [62], and (ii) the direct sequencing of the D1/D2 domains of the 26S rRNA gene amplified with primers NL1 and NL4 [63] or the 5.8S-ITS region amplified with primers ITS1 and ITS4 [62].

The information following below is a short description of microbial ecology studies at different cultivars and processing steps within the major table olives production countries of Mediterranean in an attempt to draw conclusions on their yeast colonization (**Figure 1**).

3.1. Spain

According to Ref. [12], *S. cerevisiae*, *Issatchenkia occidentalis* and *Geotrichum candidum* were identified from Spanish naturally green seasoned table olives (cv. Alorena). In the same study the researchers identified *C. boidinii* and *Hanseniaspora guilliermondii* from the preservation stage of ripe black olives. During the fermentation of Arbequina naturally green table olives in Spain [52, 53], *C. boidinii, C. sorsoba, Candida diddensiae, K. lactis, P. membranifaciens, W. anomalus, P. kluyveri*, and *Rhodotorula glutinis* were found. In a study looking at the Spanish yeast biodiversity of oleic ecosystems, the yeast biodiversity in the fresh table olive, crushed olives and olive pomace from Arbequina and Cornicabra varieties, has been found to contain *Pichia caribbica, Lachancea fermentati and Nakazawaea holstii,* as the most important isolated species [54]. According to Ref. [7], researchers 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 Alorena olives, but for Gordal and Manzanilla cultivars, *Candida tropicalis*, *P. galeiformis* and *W. anomalus* were found. Recently, other scientists [57] used a culture-independent approach based on the PCR-DGGE analysis for the identification of yeasts associated with Alorena de Malaga olive fermentation, and found that in cold fermented olives the most essential yeasts were *S. cerevisiae* and *Candida apicola*. Finally, it has been shown that the most important species throughout the storage period of table olives were *S. cerevisiae* and *Pichia galeiformis*, although *C. boidinii* was present at the last stages of the process and *P. membranifaciens* was detected at an earlier stage of the Hojiblanca cultivar storage [34].

To summarize, the hierarchically most prominent yeast on Spanish table olives are *P. membranifaciens, C. boidinii, C. diddensiae, S. cerevisiae* and *P. anomala*.

Yeasts/stage	Country/variety	Method	Ref.
Saccharomyces cerevisiae, *Issatchenkia occidentalis, Geotrichum candidum* (during process)	Spanish green seasoned table olives	Sequencing of 26S rRNA, D1/D2 region	[12]
Candida boidinii, *Hanseniaspora guilliermondii* (final product)	Ripe black olives	Sequencing of 26S rRNA, D1/D2 region	[12]
P. anomala, *C. boidinii*, *Debaryomyces etchelsii* (during process)	French black olives	RFLP, Sequencing of D1/D2 region	[51]
C. boidinii, *Candida sorsoba, Candida diddensiae, P. membranifaciens, Kluyveromyces lactis, P. anomala, P. kluyveri*, *Rhodotorula glutinis* (during process)	Spain, Arbequina table olives	RFLP	[52, 53]
*Pichia caribbica, Lachancea fermentati, Nakazawaea holstii (*during process)	Spain, Arbequina and Cornicabra	Sequencing of 5.8 rRNA, ITS1-ITS2 region	[54]
Metschnikowia pulcherrima, Debaryomyces hansenii, Aureobasidium pullulans (during process)	Greece, Conservolea black olives	Sequencing of 5.8 rRNA, ITS1-ITS2 region	[55]
P. membranifaciens, P. anomala (final product)	Greek black olives	Sequencing of 5.8 rRNA, ITS1-ITS2 region	[55]
Candida parapsilosis, P. guilliermondii, P. kluyveri (during process)	Sicilian green table olives.	RFLP, Sequencing of 26 s rRNA D1/D2 region	[56]
S. cerevisiae, *Pichia galeiformis*, *C. boidinii* (final product), and *P. membranifaciens* (during possess)	Hojiblanca	RFLP, Sequencing of 5.8 s rRNA, ITS1-ITS2 region	[34]
C. diddensiae, *S. cerevisiae* and *P. membranifaciens* (during process)	Spain/ Alorena	RFLP, Sequencing of 26 s rRNA D1/D2 region	[7]
Candida tropicalis, *P. galeiformis* and *P. anomala* (during process)	Spain/Gordal and Manzanilla	RFLP, Sequencing of 26 s rRNA D1/D2 region	[7]
S. cerevisiae and *Candida apicola* (during process)	Spain/ Alorena de Malaga	PCR-DGGE of 26SrRNA	[57]
S. cerevisiae, P. anomala, C. diddensiae, Issatchenkia orientalis (during process)	Sicilian green table olives	PCR-DGGE of 26 s rRNA	[58]
P. membranifaciens, Pichia fermentans, S. cerevisiae, *Candida oleophila*. (during process)	Portuguese brined olive	RFLP, Sequencing of 26 s rRNA D1/D2 region	[8]
Citeromyces matritensis, Zygotorulaspora mrakii, S. cerevisiae (during process)	Portugal, Manzanilla	Sequencing of 26 s rRNA D1/D2 region	[59]
P. membranifaciens (during process)	Greece, Conservolea	Sequencing of 5.8 s rRNA, ITS1-ITS2 region	[60]

Table 1. Scientific reports of microbial ecology studies reporting the isolation of yeasts from different cultivars of table olives and different fermentation processes and different Mediterranean countries.

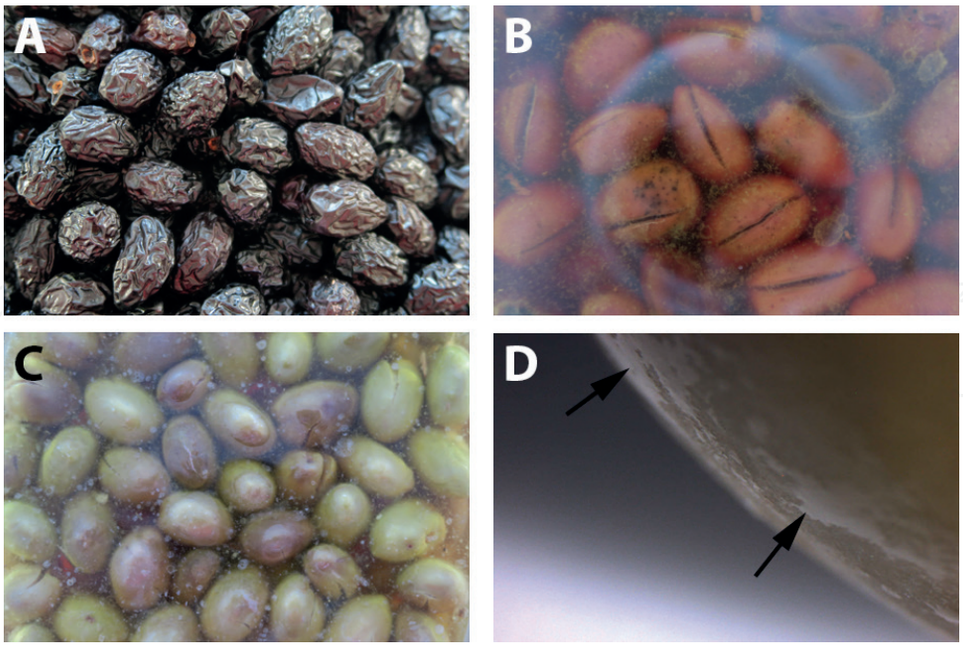

Figure 1. (A) Cyprus naturally black olives with salt, (B) Kalamata naturally black olives in brine, (C) Cyprus green cracked olives in brine, (D) Colonized surface of Cyprus green cracked olives (arrows show the formation of microbial colonies viewed under stereomicroscope).

3.2. Greece

In relevant scientific work of Greek scientists it has been discovered that *Metschnikowia pulcherrima, D. hansenii* and *Aureobasidium pullulans* were the dominant yeast species at the beginning of the fermentation process of Greek Conservolea black olives [55]. These researchers found a new yeast species associated with this type of fermentation, named *Candida olivae*. Species heterogeneity changed during fermentation and both *P. membranifaciens* and *P. anomala* became the only dominant yeasts at the end of the fermentation. A similar work focusing on microbial heterogeneity during aerobic and modified atmosphere packaging storage of Conservolea natural black olives found that *P. membranifaciens* was dominated in all pouches regardless of gas composition with a frequency more than 80% during storage [60]. Summarizing *P. membranifaciens* is the dominant yeast on Conservolea black olives followed by *P. anomala.*

3.3. Italy

In Ref. [56], it has been revealed that in Sicilian environment in four Italian olive cultivars (Bradofina, Castriciana, Nocellara del Belice and Passalunara) and one Spanish (Manzanilla) the presence of *P. kluyveri followed by Candida parapsilosis and P. guilliermondii* during the entire fermentation period is clear. In a similar work [58] also in Sicily with green table olives of cultivar Nocellara dell' Etna, researchers found *S. cerevisiae, P. anomala, C. diddensiae,* and *I. orientalis* during the process. Finally, when researchers [6] studied the technological and spoiling characteristics of yeast microflora isolated from cultivar Bella Di Cerignola table olives, most prominent yeasts isolated were *Candida famata* and *C. guilliermondii*. Summarizing the Italian yeast biodiversity in green olives is seems that *P. guilliermondii* and *P. kluyveri* are likely to play a crucial role in their fermentation.

3.4. France

In Ref. [51] it has been identified that *P. anomala*, *C. boidinii* and *Debaryomyces etchelsii* were dominant species in French black olives of Nyons area (South France).

3.5. Portugal

According to Ref. [8], researchers studied the yeast population associated with Portuguese brined green olives of cultivars Galega and Cordovil, fermentation was mainly driven by *P. membranifaciens, P. fermentans, S. cerevisiae* and *C. oleophila*. In a similar work [59], it has been found that during the initial phases of cracked green Manzanilla olive fermentation a great diversity of yeasts was observed; however, as the process was evolving, the biodiversity decreased with the fermentative yeasts *Citeromyces matritensis, Zygotorulaspora mrakii* and *S. cerevisiae* becoming the dominant species. These species though are reported as high risk spoilage microorganisms contrary to *P. membranifaciens* reported by [8].

Concluding this brief overview of yeast ecological studies in fermented table olives around the Mediterranean it becomes apparent that among Candida species, *C. boidinii, C. diddensiae, C. famata* (formerly *D. hansenii*), *C. guilliermondii* (formerly *H. guilliermondii*), and *C. oleophila* are the most prominent. Among Pichia species, *P. membranifaciens, Pichia anomala* (*W. anomalus*) and *P. fermentans* are the most prominent. From other species we tend to isolate more *A. pullulans, Debaryomyces etchellsii, G. candidum, I. occidentalis, K. lactis, Rhodotorula spp., S. cerevisiae* and *Z. mrakii.* These yeasts are plentiful for detailed studies on their role during table olive fermentations and could offer great opportunities for biotechnological tools as starter cultures.

4. Biotechnological applications of table olives yeast strains

Yeast microbiota in olives is very heterogeneous and can be altered depending on the olive cultivar, region, type of fermentation process, salt concentration, pH, nutrients, oxygen and interactions with other microorganisms [5, 57, 64–69]. In table olive fermentations, yeasts are an important group of microorganisms that act as both desirable and spoilage microorganisms and it is important to evaluate their biodiversity in table olive fermentations [3, 4]. As a positive effect, some yeast species isolated from table olives, such as *Debaryomyces*, *Pichia* and *Candida* are known to include a considerable number of strains with a killer character and it has been found, that *W. anomalus* protects olives from unsaturated fatty acid oxidation and peroxide formation [3, 70, 71], relating also to the antioxidant activity of yeasts [7, 8]. Moreover, it has been reported that *W. anomalus* and *S. cerevisiae* isolated from diverse table olive fermentations, have phytase enzymes that are required for the degradation of phytate complexes [3, 41, 44]. On the other hand, *W. anomalus* is important yeast for olive fermentation, but it may also have a role in the deterioration of olives at the end of fermentation [72]. It has been reported that some olive-related yeast strains, such as *W. anomalus*, can produce enzymes that could cause softening of the olives as an unfavorable property [33].

According to Ref. [73], the combination of yeasts with LAB, has resulted to an improvement in growth of the LAB. As a result, the production of lactic acid was improved, thanks to the

greater availability of the necessary nutrients by the yeasts activity and lysed cells. Indeed, recent studies have shown that the growth of LAB could be stimulated by the use of yeasts, as for example *L. plantarum*, improved its growth when *D. hansenii* was inoculated in olive brine [74]. Moreover, *L. pentosus's* performance was rapidly improved, with the use of *S. cerevisiae* as a starter in green table olive solutions [73]. In the same study, it has been proved that the production of lactic acid was increased, as well. Yeasts seem to be active microorganisms during the fermentation of table olives, synthesizing substances such as vitamins, amino acids and purines, or breakdown complex carbohydrates, which are essential for the growth of *Lactobacillus* species [75].

Therefore, selection of the most appropriate yeasts for starters should be firstly based on strains possessing the best enzymatic activities as mentioned earlier and secondly to their ability to predominate during fermentation. Moreover, it is needed to have a high resistance to salt and low pH values. Predictive microbiology is seemed to be a valuable tool for the discrimination and selection of the most promising strains, determining the influence of environmental variables on yeast growth [5]. A problem usually occurring in such applications is to find the appropriate methodology to manage such a large amount of data, which is necessary when researchers have to analyze several biochemical activities or growth data from a considerable number of strains.

Multivariate analysis techniques offer a viable approach in solving this setback. For instance, multivariate analysis approach to study growth and qualitative activity data of a number of yeasts isolated from Bella di Cerignola table olives [6] has been used. Principal Component Analysis (PCA) clearly differentiated and assisted the selection and discrimination of several *W. anomalus* and *C. boidinii* isolates with high global desirable activity levels [76].

However, in some cases, dominant yeasts could create products with milder taste and less self-life preservation [4, 15]. This problem was reported in [65] during fermentation of natural black olives at different NaCl levels and temperatures. Moreover, an excessive growth of fermented yeast (7 log10 CFU mL^{-1}) could produce a vigorous production of CO_2 resulting in penetrating olives and damaging the fruits [64]. The use of high levels of NaCl during fermentation (8% in the equilibrium) could privilege the growth of yeasts against LAB [4, 65]. In accordance with Ref. [33], it has been found that some strains of *Rhodotorula minuta* and *D. hansenii* in green table olive fermentations are having this ability. Finally, strains of *R. glutinis, R. minuta* and *R. rubra* could grow, form pellicles in olive brines and produce polygalacturonases causing a softening of olives kept in storage [77].

Moreover, yeasts present in packed olives can produce an excess gas (CO_2) leading to swollen containers, clouding of the brines, or produce off-flavors and off-odors [4]. Furthermore, it has been reported that yeasts identified currently as *S. cerevisiae* and *P. anomala* spoiled the olives through a combination of gas-pocket formation and softening [78]. Fortunately, yeasts from table olives are almost entirely nonpathogenic. The inhibitory effect of sorbic acid, benzoic acid and their salts on yeasts growth to stabilize table olive packing, were reported previously [5, 79, 80].

5. Future perspectives

At this moment, the scientific community has gathered significant amount of knowledge about the ecology of fermented table olives, physiology, biochemistry and genetics of yeasts isolated from table olives fermentation and more data are soon going to be added with the use of high throughput sequencing techniques. Despite the large amount of beneficial properties that these microorganisms could offer to the final product, recent findings showed that yeasts are not only in the cover brines, but also on the fruit epidermis, which indicates a promising source of probiotic strains [43, 81]. The production of an innovative and functional food having the advantages of probiotic yeasts, can contribute to a final product with added value, even higher than it already has. From the research so far, it is clear that yeasts from table olives show an interesting technological and probiotic profile. However, further research is needed as far as their physiology, ecology, biochemistry is concerned. Also the study of yeasts are needed in molecular level throughout the fermentation, in order to be explained their prevalence or not in the final product, especially when it has to do with probiotic microorganisms. Undoubtedly, the full potential of table olive related yeasts have not been fully determined and many challenges are awaiting research, dissemination and industrial exploitation.

The microbiota of olives varies somewhat from region to region, from cultivar to cultivar and from type to type of processing [5, 12, 33, 34, 76]. For this reason and due to the importance of yeasts to the final product, it is a challenge to investigate and combine the diversity of yeasts in table olives around regions as a geographical indication. This could lead to the introduction of new table olives as PDO or PGI products, especially for EU countries. Finally, starter cultures can play an essential role in olive fermentation by controlling the safety and the quality of the final product. For extensive information on selection of yeasts as starter cultures for table olives, Refs. [32, 82] are thorough reviews with step by step procedures to follow.

Next Generation Sequencing (NGS) approaches have recently started to appear in the subject with most recent the work enhancing the knowledge on fungal communities in directly brined Alorena de Malaga green olives fermentations using metabarcoding analysis [83]. Along these lines more publications are expected in the forthcoming years.

However, NGS technologies are providing massive amount of data, many times difficult to interpret at logical, applied level. Recently, a very important effort on databasing, visualizing and exploring the food bacterial communities based on network analysis has announced preliminary results [84]. FoodMicrobionet, the platform prepared by the inclusion of 17 bacterial studies on dairy products, dairy starter cultures, raw and fermented meat, doughs and sourdoughs and fermented vegetables is attempting to analyze nodes and network properties while building an interactive web-based visualization. By this the researcher can explore the relationships between Operational Taxonomic Units (OTUs) and samples in order to identify core and sample specific bacterial communities. A similar approach will be very much in use for yeast/fungal studies of various matrices. The above if combined with a high throughput screening technique for aroma formation like the one used in [85] may provide exciting results with a plethora of new interrelationships. Similarly, a recent work with Sicilian table

olives (cv. Nocellara Etnea) [86] investigates the bacterial community and its dynamics during the fermentation of the olives and its effect on metabolome formation.

In the following years we expect more detailed studies in the field of table olives microbial ecology and biotechnology, as well as gaining valuable knowledge for the related microbial functions that can be applied far beyond olive fermentations.

Author details

Dimitrios Anagnostopoulos, Despina Bozoudi and Dimitrios Tsaltas*

*Address all correspondence to: dimitris.tsaltas@cut.ac.cy

University of Technology, Limassol, Cyprus

References

[1] IOC (International Olive Oil Council), 2013. Statistical of Table Olive Production. Available from: http://www.internationaloliveoil.org/estaticos/view/132-world-table-olive-figures [Accessed: Jan 24, 2013].

[2] Bonatsou S, Benítez A, Rodríguez-Gómez F, Panagou EZ, Arroyo-López FN. Selection of yeasts with multifunctional features for application as starters in natural black table olive processing. Food Microbiology. 2015;**46**:66-73

[3] Arroyo-López FN, Romero-Gil V, Bautista-Gallego J, Rodríguez-Gómez F, JiménezDíaz R, García-García P, Querol A, Garrido-Fernández A. Yeasts in table olive processing: Desirable or spoilage microorganisms? International Journal of Food Microbiology. 2012;**160**:42-49

[4] Garrido-Fernández A, Fernandez Diez MJ, Adams RM. Table Olives: Production and Processing. London: Chapman and Hall; 1997

[5] Arroyo-López FN, Querol A, Bautista-Gallego J, Garrido-Fernández A. Role of yeasts in table olive production. International Journal of Food Microbiology. 2008;**128**:189-196

[6] Bevilacqua A, Perricone M, Cannarsi M, et al. Technological and spoiling characteristics of the yeast microflora isolated from Bella di Cerignola table olives. International Journal of Food Science & Technology. 2009;**44**:2198-2207

[7] Bautista Gallego J, Rodriguez-Gomez F, Barrio E, Querol A, Garrido-Fernandez A, Arroyo-Lopez FN. Exploring the yeast biodiversity of green table olive industrial fermentations for technological applications. International Journal of Food Microbiology. 2011;**147**:89-96

[8] Silva T, Reto M, Sol M, et al. Characterization of yeasts from Portuguese brined olives, with a focus on their potentially probiotic behaviour. LWT – Food Science and Technology. 2011;**44**:1349-1354

[9] Rodriguez-Gomez F, Bautista-Gallego J, Romero-Gil V, et al. Influence of yeasts on the oil quality indexes of table olives. Journal of Food Science. 2013;**78**:1208-1217

[10] Kurtzman CP, Fell JW, Boekhout T. The Yeasts, A Taxonomic Study. 5th ed. Amsterdam: Elsevier; 2011

[11] Kurtzman CP, Fell JW. The Yeast, A Taxonomic Study. 4th ed. Amsterdam: Elsevier; 1998

[12] Arroyo-López FN, Durán-Quintana MC, Ruiz-Barba JL, Querol A, Garrido-Fernández A. Use of molecular methods for the identification of yeast associated with table olives. Food Microbiology. 2006;**23**:791-796

[13] Flores CL, Rodriguez C, Petit T, Gancedo C. Carbohydrate and energy-yielding metabolism in non-conventional yeasts. FEMS Microbiology Reviews. 2000;**24**:507-529

[14] Sanchez I, Palop L, Ballesteros C. Biochemical characterization of lactic acid bacteria isolated from spontaneous fermentation of 'Alamgro' eggplants. International Journal of Food Microbiology. 2000;**59**:9-17

[15] Panagou EZ, Tassou CC. Changes in volatile compounds and related biochemical profile during controlled fermentation of cv. Conservolea green olives. Food Microbiology. 2006;**23**:738-746

[16] Krajewska-Kulak E. Hydrolytic activity of *Candida albicans* and their susceptibility to antimycotics. Medical Science Monitor. 1998;**4**:1643-3750

[17] Krajewska-Kulak E. Enzymatic biotypes of the yeast-like fungi strains and their susceptibilities to antimycotics isolated from ontocenosis of the urogenital system. Mikologia/Medical Mycology. 2002;**9**:2083-5744

[18] Brasch J. Enzyme patterns of dermatophytes. Mycoses. 1994;**37**:1439-0507

[19] Plomer-Niezgoda E, Baran E. Evaluation of extracellular activity of hydrolytic enzymes of selected mold fungi. Mikologia Lekarska. 1997;**4**:141-145

[20] Bull AT, Goodfellow M, Slater JH. Biodiversity as a source of innovation in biotechnology. Annual Review of Microbiology. 1992;**46**:219-252

[21] Abranches J, Morrais PB, Rosa CA, Mendonca-Hagler LC, Hagler HN. The incidence of killer activity and extracecullar proteases in tropical yeast communities. Canadian Journal of Microbiology. 1997;**43**:328-336

[22] Bossi A, Bonizzato L, Zapparoli G. Acidic extracellular proteases from microrganisms of fairly acidic niche. Protein and Peptide Letters. 2006;**13**:737-741

[23] Burden DW, Eveleigh DE. Yeasts-diverse substrates and production. In:Yeast Technology, Spencer JF, Spencer DM, editors. Berlin: Springer-Verlag; 1990. p. 199-227

[24] Bordes F, Barbe S, Escalier P, Mourey L, André I, Marty A, Tranier S. Exploring the conformational states and rearrangements of Yarrowia lipolytica lipase. Biophysical Journal. 2010;**99**(7):2225-2234

[25] Basaran P, Hang YD. Purification and characterization of acetyl esterase from *Candida guilliermondii*. Letters Applied Microbiology. 2000;**30**:167-171

[26] Athenstaedt K, Daum G. Tgl4p and Tgl5p, two triacylglycerol lipases of the yeast *Saccharomyces cerevisiae* are localized to lipid particles. J BiolChem. 2005;**280**(45):37301-37309

[27] Sakai N, Mano S, Nozaki K, Takaya H. Journal of the American Chemical Society. 1993; **115**:7033

[28] Charoenchai C, Fleet GH, Henschke PA, Todd BEN. Screen of non-saccharomyces wine yeasts for the presence of extracellular hydrolytic enzymes. Australian journal of grape wine Research. 1997;**3**:2-8

[29] Strauss RS, Pollack HA. Epidemic increase in childhood overweight 1986-1998. Journal of the American Medical Association. 2001;**286**:2845-2848

[30] Kiehn E, Wong B, Edwards F, Armstrong D. Comparative recovery of bacteria and yeasts from Lysis centrifugation and a conventional blood culture system. Journal of Clinical Microbiology. 1983;**18**:300-304

[31] Hurtado A, Reguant C, Bordons A, Rozes N. Lactic acid bacteria from fermented table olives. Food Microbiology. 2012;**31**:1-8

[32] Bevilacqua A, Corbo MR, Sinigaglia M. Selection of yeasts as starter cultures for table olives: A step-by-step procedure. Frontiers in Microbiology. 2012;**3**:194

[33] Hernández A, Martín A, Aranda E, Pérez-Nevado F, Córdoba MG. Identification and characterization of yeast isolated from the elaboration of seasoned green table olives. Food Microbiology. 2007;**24**:346-351

[34] Rodriguez-Gomez F, Arroyo-Lopez FN, Lopez-Lopez A, Bautista-Gallego J, Garrido Fernandez A. Lipolytic activity of the yeast species associated with the fermentation/ storage phase of ripe olive processing. Food Microbiology. 2010;**27**:604-612

[35] Psani M, Kotzekidou P. Technological characteristics of yeast strains and their potential as starter adjuncts in Greek-style black olive fermentation. Springer Science+Business Media B.V. 2006;**22**:1329-1336

[36] Caruso M, Fiore C, Contursi M, Salzano G, Paparella A, Romano P. Formation of biogenic amines as criteria for the selection of wine yeasts. World Journal of Microbiology and Biotechnology. 2002;**18**:159-163

[37] Landete JM, Ferrer S, Pardo I. Biogenic amine production by lactic acid bacteria, acetic bacteria and yeast isolated from wine. Food Control. 2007;**18**:1569-1574

[38] FAO/WHO. Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. Córdoba, Argentina: Joint Food and Agricultural Organization of the United Nations and World Health Organization Expert Consultation Report; 2001

[39] Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhoea: A meta-analysis of masked, randomised, placebo-controlled trials. The Lancet Infectious Diseases. 2006;**6**:374-382

[40] Kourelis A, Kotzamanidis C, Litopoulou-Tzanetaki E, Scouras ZG, Tzanetakis N, Yiangou M. Preliminary probiotic selection of dairy and human yeast strains. Journal of Biological Research of Thessaloniki. 2010;**13**:93-104

[41] Moslehi-Jenabian S, Lindegaard Pedersen L, Jespersen L. Beneficial effects of probiotic and food borne yeasts on humanhealth. Nutrients. 2010;**2**:449-473

[42] Pennacchia C, Blaiotta G, Pepe O, Villani F. Isolation of *Saccharomyces cerevisiae* strains from different food matrices and their preliminary selection for a potential use as probiotics. Journal of Applied Microbiology. 2008;**105**:1919-1928

[43] Etienne-Mesmin L, Livrelli V, Privat M, Denis S, Cardot JM, Alric M, BlanquetDiot S. Effect of a new probiotic *Saccharomyces cerevisiae* strain on survival of *Escherichia coli* O157:H7 in a dynamic gastrointestinal model. Applied and Environmental Microbiology. 2011;**77**:1127-1131

[44] Olstorpe M, Schnüren J, Passoth V. Screening of yeast strains for phytase activity. FEMS Yeast Research. 2009;**9**:478-488

[45] Ruiz Barba JL, Jiménez Diaz R. Availability of essential B-group vitamins to *Lactobacillus plantarum* in green olive fermentation brines. Applied and Environmental Microbiology. 1995;**61**:1294-1297

[46] Gazi MR, Hoshikuma A, Kanda K, Murata A, Kato F. Detection of free radical scavenging activity in yeast culture. Bulletin of the Faculty of Agriculture of Saga University. 2001;**86**:67-74

[47] Marquina D, Peres C, Caldas FV, Marques JF, Peinado JM, Spencer Martin J. Characterization of the yeast population in olives brines. Letters in Applied Microbiology. 1992;**14**:279-283

[48] Marquina D, Toufani S, Llorente P, Santos A, Peinado JM. Killer activity in yeast isolated from olive brines. Advances in. Food Science. 1997;**19**:41-46

[49] Fernández González MJ, Gárcia P, Garrido-Fernández A, Durán Quintana MC. Microflora of the aerobic preservation of directly brined green olives from Hojiblanca cultivar. Journal of Applied Bacteriology. 1993;**75**:226-233

[50] Kotzekidou P. Identification of yeast from black olives in rapid system microtitre plates. Food Microbiology. 1997;**14**:609-616

[51] Coton E, Coton M, Levert D, Casaregola S, Sohier D. Yeast ecology in French cider and black olive natural fermentations. International Journal of Food Microbiology. 2006;**108**:130-135

[52] Hurtado A, Reguant C, Esteve-Zarzoso B, Bordons A, Rozès N. Microbial population dynamics during the processing of Aberquina table olives. Food Research. 2008; **41**:738-744

[53] Hurtado A, Reguant C, Bordons A, Rozès N. Influence of fruit ripeness and salt concentration on the microbial processing of Arbequina table olives. Food Microbiology. 2009;**26**:827-833

[54] Romo-Sánchez S, Alves-Baffi M, Arévalo-Villena M, Úbeda-Iranzo J, Briones-Pérez A. Yeast biodiversity from oleic ecosystems: Study of their biotechnological properties. Food Microbiology. 2010;**27**:487-492

[55] Nisiotou AA, Chorianopoulos N, Nychas GJE, Panagou EZ. Yeast heterogeneity during spontaneous fermentation of black Conservolea olives in different brine solutions. Journal of Applied Microbiology. 2010;**108**:396-405

[56] Aponte M, Ventorino V, Blaiotta G, Volpe G, Farina V, Avellone G, Lanza CM, Moschetti G. Study of green Sicilian table olive fermentations through microbiological, chemical and sensory analyses. Food Microbiology. 2010;**27**:162-170

[57] Abriouel H, Benomar N, Lucas R, Gálvez A. Culture-independent study of the diversity of microbial populations in brines during fermentation of naturally fermented Alorena green table olives. International Journal of Food Microbiology. 2011;**44**:487-496

[58] Muccilli S, Caggia C, Randazzo CL, Restuccia C. Yeast dy-namics during the fermentation of brined green olives treated inthe field with kaolin and Bordeaux mixture to control the olive fruit fly. International Journal of Food Microbiology. 2011;**148**:15-22

[59] Alves M, Gonçalves T, Quintas C. Microbial quality and yeast population dynamics in cracked green table olives' fermentations. Food Control. 2012;**23**:363-368

[60] Doulgeraki A, Hondrodimou O, Iliopoulus U, Panagou EZ. Lactic acid bacteria and yeast heterogeneity during aerobic and modified atmosphere packaging storage of natural black Conservolea olives in polyethylene pouches. Food Control. 2012;**26**:49-57

[61] Barnett JA, Payne RW, Yarrow D. Yeasts: Characteristics and Identification. 2nd ed. Cambridge: University Press; 1990

[62] Esteve-Zarzoso B, Belloch C, Uruburu F, Querol A. Identification of yeasts by RFLP analysis of the 5.8 rRNA gene and the two ribosomal internal transcribed spacers. International Journal of Systematic Bacteriology. 1999;**49**:329-337

[63] Kurtzman CP, Robnett C. Identification and phylogeny of ascomycetous yeast from analysis of nuclear large subunit (26S) ribosomial DNA partial sequences. Antonie Van Leeuwenhoek. 1998;**73**:331-371

[64] Duran-Quintana MC, Garcia-Garcia P, Garrido-Fernández A. Establishment of conditions for green table olive fermentation at low temperature. International Journal of Food Microbiology. 1999;**51**:133-143

[65] Tassou CC, Panagou EZ, Katsaboxakis KZ. Microbiological and physicochemical changes of naturally black olives fermented at different temperatures and NaCl levels in the brines. Food Microbiology. 2002;**19**:605-615

[66] Alvarez DME, Sánchez A, Lamarque AL. Naturally black olives: Comparison of three processes for fermenting cv. 'Farga' olives. Olivae. 2003;**97**:47-51

[67] Chorianopoulos NG, Boziaris IS, Stamatiou A, Nychas GJE. Microbial association and acidity development of unheated and pasteurised green table olives fermented using glucose or sucrose supplements at various levels. Food Microbiology. 2005;**22**:117-124

[68] Corsetti A, Perpetuini G, Schirone M, Tofalo R, Suzzi G. Application of starter cultures to table olive fermentation: An overview on the experimental studies. Frontiers in Microbiology. 2012;**3**:1-6

[69] Tofalo R, Schirone M, Perpetuini G, Angelozzi G, Suzzi G, Corsetti A. Microbiological and chemical profiles of naturally fermented table olives and brines from different Italian cultivars. Antonie Van Leeuwenhoek. 2012;**102**:121-131

[70] Llorente P, Marquina D, Santos A, Peinado JM, Spencer-Martins I. Effect of salt on the killer phenotype of yeasts from olive brines. Applied and Environmental Microbiology. 1997;**63**:1165-1167

[71] Hernández A, Martín A, Córdoba MG, Benito MJ, Aranda E, Pérez-Nevado F. Determination of killer activity in yeasts isolated from the elaboration of seasoned green table olives. International Journal of Food Microbiology. 2008;**121**:178-188

[72] Pitt JI, Hocking AD. Yeast. Fungi and Food Spoilage. New York: Springer; 2009

[73] Segovia Bravo K, Arroyo-López FN, Garcia Garcia P, Duran Quintana MC, GarridoFernández A. Treatment of green table olive solutions with ozone. Effect on the polyphenol content on *Lactobacillus pentosus* and *Saccharomyces cerevisiae* growth. International Journal of Food Microbiology. 2007;**114**:60-68

[74] Tsapatsaris S, Kotzekidou P. Application of a central composite design and response surface methodology to the fermentation of olive juice by *Lactobacillus plantarum* and *Debaryomyces hansenii*. International Journal of Food Microbiology. 2004;**95**:157-168

[75] Viljoen BC. Yeast ecological interactions. Yeast–yeast, yeast bacteria, yeast–fungi interactions and yeasts as biocontrol agents. In: Querol A, Fleet H, editors. Yeasts in Food and Beverages. Berlin: Springer–Verlag; 2006. p. 83-110

[76] Rodríguez-Gómez F, Romero-Gil V, Bautista-Gallego J, Garrido-Fernández A, Arroyo-López FN. Multivariate analysis to discriminate yeast strains with technological applications in table olive processing. World Journal of Microbiology and Biotechnology. 2012;**28**:1761-1770

[77] Vaughn RH, Jakubczyk T, MacMillan JD, Higgins TE, Dave BA, Crampton VM. Some pink yeast associated with softening of olives. Applied Microbiology. 1969;**18**:771-775

[78] Vaughn RH, Stevenson KE, Dave BA, Park HC. Fermenting yeast associated with softening and gas-pocket formation in olives. Applied Microbiology. 1972;**23**:316-320

[79] Rodriguez de la Borbolla Alcalá JM, FernándezDíez MJ, González Cancho F. El empleo de ácidosórbico y sus sales en las aceitunasaderezadas. Grasas y Aceites. 1961;**12**:10-15

[80] Marsilio V, Cichelli A. Influencia del sorbatopotásico y delbenzoatosódicosobre la estabilidad de las aceitunas de mesa ensalmuera. Grasas y Aceites. 1992;**43**:66-74

[81] Domínguez-Manzano J, Olmo-Ruiz C, Bautista-Gallego J, Arroyo-López FN, Garrido-Fernández A, Jiménez-Díaz R. Biofilm formation on abiotic and biotic surfaces during Spanish style green table olive fermentation. International Journal of Food Microbiology. 2012;**157**:230-238

[82] Bonatsu S, Tassou CC, Panagou EZ, Nychas G-JE. Table olive fermentation using starter cultures with multifunctional potential. Microorganisms. 2017;**5**:30. DOI: 10.3390/microorganisms5020030

[83] Arroyo-López FN, Medina E, Ruiz-Bellido MA, Romero-Gil V, Montes-Borrego M, Landa BB. Enhancement of the knowledge on fungal communities in directly brined Alorena de Malaga green olive fermentations by metabarcoding analysis. PloS One. 2016;**11**(9). DOI: 10.1371/journal.pone.0163135

[84] Parente E, Cocolin L, De Filippis F, Zotta T, Ferrocino I, O'Sullivan O, Neviani E, De Angelis M, Cotter PD, Ercolini D. FoodMicrobionet: A database for the visualisation and exploration of food bacterial communities based on network analysis. International Journal of Food Microbiology. 2016;**219**:28-37

[85] Gamero A, Quintilla R, Groenewald M, Alkema W, Boekhout T, Hazelwood L. High-throughput screening of a large collection of non-conventional yeasts reveals their potential for aroma formation in food fermentation. Food Microbiology. 2016;**60**:147-159

[86] Randazzo CL, Todaro A, Pino A, Pitino I, Corona O, Caggia C. Microbiota and metabolome during controlled and spontaneous fermentation of Nocellara Etnea table olives. Food Microbiology. 2017;**65**:136-148