Chapter 13

Biotechnology can Improve a Traditional Product as Table Olives

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Additional information is available at the end of the chapter

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Abstract

Table olives are fermented vegetables very popular in the world and especially in the Mediterranean countries. Five main styles (Spanish or Sevillian, Castelvetrano, Siciliano, Californian, and Greek) are diffused to produce commercial products, beside several traditional styles. Although the main preparation methods of table olives are known for a long time, they are not yet optimized systems, and each of them is characterized by advantages and disadvantages. The use of NaOH for green olive debittering is responsible for the elimination of many aroma compounds and nutritionally important molecules. High volumes of heavily contaminated wastewaters are produced during olive processing. Spontaneous fermentation processes used to ferment black or green olives are difficult either to monitor or control. Microbial starters, selected for specific bio/technological and safety traits, can be useful to (i) improve the table olives organoleptic characteristics, (ii) control the fermentation process and significantly reduce the time to obtain a final product, (iii) monitor the correct evolution of the process, (iv) ensure the maintenance and/or improvement of nutritional and healthy features of the product, (v) protect table olives from undesired spoilage and pathogenic microorganisms, (vi) produce table olives as a carrier of microorganisms with probiotics characters, and (vii) enhance product stability and shelf life.

Keywords: table olives, starters, organoleptic traits, nutritional characteristics, probiotics

1. Introduction

Table olives are one of the most important and popular fermented vegetables in Western world and in particular in Southern European countries. Table olives world production was estimated to be 2,742,500 tons in 2015–2016 season (**Figure 1**).

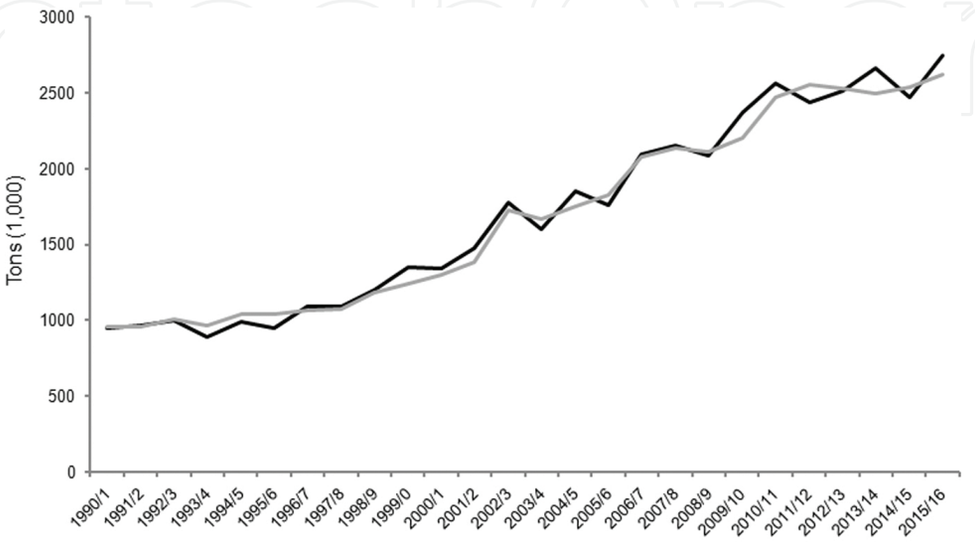

Figure 1. Table olives world production (▬) and consumption (▬). Adapted from data reported in [1].

The 29% of this production (796,000 tons) is located in the European Union (EU). Spain has a leading position in table olive production with 514,000 tons, followed by Greece (210,000 tons), Italy (50,000 tons), and Portugal (17,500 tons) [1]. Among the countries of the Mediterranean Basin, Egypt, Turkey, Algeria, and Morocco are the main producers and consumers (**Figure 2**).

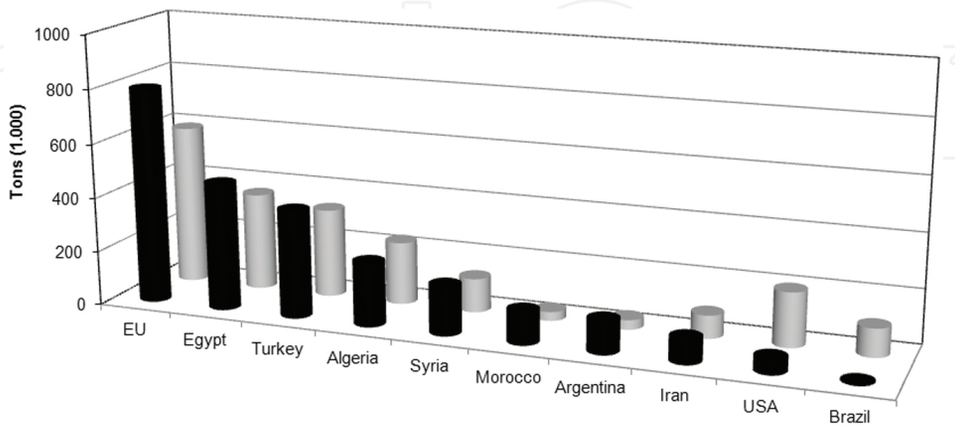

Figure 2. Table olives production ( ) and imports ( ) in main producing and importer countries. Adapted from data reported in [1].

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According to International Olive Council Standard, the term "table olive" means the product prepared from the sound fruits of varieties of the cultivated olive trees that are chosen for their production of olives whose volume, shape, flesh-to-stone ratio, fine flesh, taste, firmness, and ease of detachment from the stone make them particularly suitable for processing; treated to remove its bitterness and preserved by natural fermentation, or by heat treatment with or without the addition of preservatives; packed with or without covering liquid.

Table olives are classified according to the degree of ripeness of the drupes (green olives, olives turning color, and black olives), trade preparations (treated olives, natural olives, dehydrated and/or shriveled olives, olives darkened by oxidation, and specialties), and styles (whole, pitted, stuffed, salad, and other). They are produced by processing raw olives with the objective of eliminating their natural bitterness, which is mainly due to oleuropein and other phenolics [2]. The main commercial types of table olives are processed according to five styles: Spanish (or Sevillian), Castelvetrano, Siciliano, Californian, and Greek [3], although several other traditional styles also exist for the preparation of treated and natural table olives [4].

The two main commercial table olives preparations, lye-treated olives (Spanish and Castelvetrano styles) and brine-soaked olives (Greek style) are industrially produced by spontaneous fermentation, but, currently, these processes are difficult to be monitored and controlled [5]. The spontaneous process cannot ensure either the correct evolution of the process or the good quality and safety standards of the final product. Controls of the presence of biogenic amines and toxins in table olives commercial preparations need to be increased [6].

In green olives productions, the NaOH is used as chemical debittering system. This treatment is economic, characterized by a simple implementation, and an easy standardization. However, simultaneously to the debittering effect, it causes the elimination of many aroma compounds together with nutritional and health important molecules. The process produces also high volumes of heavily contaminated wastewaters. Besides, the use of NaOH for debittering organic table olives is prohibited in many countries [7].

The employment of starter cultures of *Lactobacillus plantarum* and *L. pentosus* can be used as an alternative to NaOH for debittering. This strategy has the advantage to control the fermentation process and to improve the quality of the final product [8]. Lactic acid fermentation is considered the key step in spontaneous fermentation processes. It promotes (i) debittering of the olives through oleuropein hydrolysis, (ii) lowering of brine pH, which prevents the growth of spoilage and pathogenic microorganisms, and (iii) the enhancement of a correct flavor and texture profile in the final product [9, 10].

It has also been demonstrated that yeasts, producing desirable metabolites and volatile compounds, are able to improve the organoleptic properties. Yeasts can also enhance the growth of lactic acid bacteria (LAB) and degrade phenolic compounds. A role of yeasts as starters has been recently proposed for production of table olive [11–15].

There is an increasing interest in lowering NaCl concentration (now 8–10%) and in shortening the fermentation time (8–12 months) in order to obtain a healthier product suitable to reach the market very soon.

Recently, Bleve et al. described a novel method based on the sequential use of autochthonous yeast and bacterial strains to shorten the time of fermentation, to standardize the process, and to improve organoleptic and nutritional properties of olives [16].

The future challenges will be to investigate some strains for their probiotic characteristics, in order to produce functional olives. Indeed, several studies demonstrated that the use of LAB as starter for table olive production can produce beneficial effects on human health [17, 18]. Also yeast strains have been evaluated for their probiotic properties [13, 15]. The nutritional and health-related compounds associated to fermented table olives (or derivatives) could be assessed by *in vitro* and *in vivo* analyses. The results of these assays can produce precise information on the importance of these compounds for the prevention and/or treatment of several human and animal diseases (i.e., gastrointestinal, cardiovascular, neurodegenerative diseases, and tumors).

Table olives are considered by many food scientists as the "food of the future" owing to the healthy bioactive compounds they contain. In fact, table olives, together with olive oil, represent an important food of the Mediterranean diet and are perceived to have positive nutritional and therapeutic effects. Monounsaturated fatty acids, as found in olives are known to be healthier than polyunsaturated and saturated fats. In addition, epidemiological studies indicate that olive biophenols have a role in lowering incidence of several chronic and heart diseases [19, 20].

2. Production methods

Table olives, directly harvested from trees, need to be processed in order to reduce or eliminate their bitter taste and to obtain a product ready to be consumed. Different commercial preparations of table olives are produced using procedures inherited and opportunely modified from traditional methods. As previously extensively described by Boskou et al. [21], the main methods including fermentation steps to obtain the final product are water-cured olives produced by soaking olives in water over a week or more and then placing them in brine where a fermentation process can occur; Greek-style or "natural" olives and Sicilian-style green olives spontaneously fermented in brines; lye-treated olives (Spanish or Sevillian style, Castelvetrano method) produced by a first treatment with alkali (NaOH) and, after washing olives with water to remove NaOH, by a second step in brine to obtain a partial or complete fermentation of the drupes.

Other methods for black olives not involving fermentation are known as Californian and Spanish styles. The drupes are debittered by lye and soaking in brine. They are also aerated insufflating air to oxidize the pigments and immersed in ferrous gluconate or ferrous lactate solution in order to stabilize a uniform black color. Table olives can also be produced by traditional methods diffused in Mediterranean Basin using lime (CaO) and olive wood ash, or they can be dried and debittered without chemicals by using salt or heat treatment. Some cultivars of olives resulted naturally debittered also by parasite fungi directly on the tree without necessity of further treatment. They can also undergo a natural sweetening during

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ripening on the tree, although genetic and biochemical mechanisms involved in this last phenomenon are until now unknown.

3. Biotechnological approaches to produce table olives

The fermentation process, generally performed by indigenous microorganisms, is one of the best and oldest procedures of treating food products to transform and preserve them. However, as already demonstrated in several food products (wine, beer, bread, yogurt, cheese, sake, chocolate, etc.), spontaneous fermentations are uncontrolled and not predictable. These spontaneous processes are inefficient since they do not ensure the expected quality and safety characteristics of the final product, the sensorial and structure features, the limitation, or absence of growth of harmful or undesired spoilage organisms [22].

In order to obtain a more controlled process and to improve the quality and safety levels of table olives, the selection and use of starter cultures is diffusing. In fact, several studies demonstrated the usefulness and the benefits of starters in table olives production [5, 9, 15, 23].

3.1. Starter selection

The microbiota associated to olives can be different among the different cultivars. Microorganisms detected in table olives and brines belong to members of bacteria (lactic acid bacteria, Enterobacteriaceae, *Pseudomonas, Staphylococcus, Clostridium*, etc.), yeasts, and moulds. Enterobacteriaceae, *Clostridium*, and *Pseudomonas* are generally associated to raw olives and to the beginning of fermentation. They are completely eliminated at the end of the process, especially due to the low pH [24–26] (**Figure 3**).

The presence of hazardous pathogens such as *C. botulinum* has to be adduced to incorrect processing, heat treatment, packaging, and transportation [27].

The most studied group of bacteria is lactic acid bacteria (homo and hetero fermentative), since they are responsible for the sugar conversion to organic acids and in particular to lactic acid. The different table olives production methods can influence microbial population present in raw olives and their evolution during fermentation (**Figure 3**). *Lactobacillus coryniformis, L. plantarum, L. pentosus*, and *Leuconostoc mesenteroides* have been detected and isolated in Spanish style green olives across the process. Also *Enterococcus* spp., *Pseudomonas* spp., and *Staphylococcus* spp. are associated to olives produced by this method. Bacterial biodiversity associated to natural green and natural cracked green olives is richer than that present in Spanish style olives, treated with NaOH [28] (**Figure 3**). In black olives, bacteria belonging to Enterobacteriaceae, *Kocuria, Swaminathania, Acetobacter*, and *Pseudomonas* were detected only at the initial stage of fermentation, except for *Swaminathania* that has been found, in some cases, also at the end of fermentation. Bleve et al. [29, 30] reported the presence of LAB (*Lactobacillus* sp., *L. plantarum, L. pentosus, Leuconostoc mesenteroides*) associated to the final stage of fermentation (120–180 days) in Leccino and Kalamàta cultivars (**Figure 3**).

Yeasts found in olives belong to the genera *Candida, Debaryomyces, Hanseniaspora, Issatchenkia, Kluyveromyces, Pichia, Rhodotorula, Saccharomyces, Torulaspora, Wickerhamomyces, Zygosaccharomyces, Zygotorulaspora*, with some differences between green and black olives (**Figure 4**). Yeasts are detectable throughout the fermentation process in all table olive cultivars.

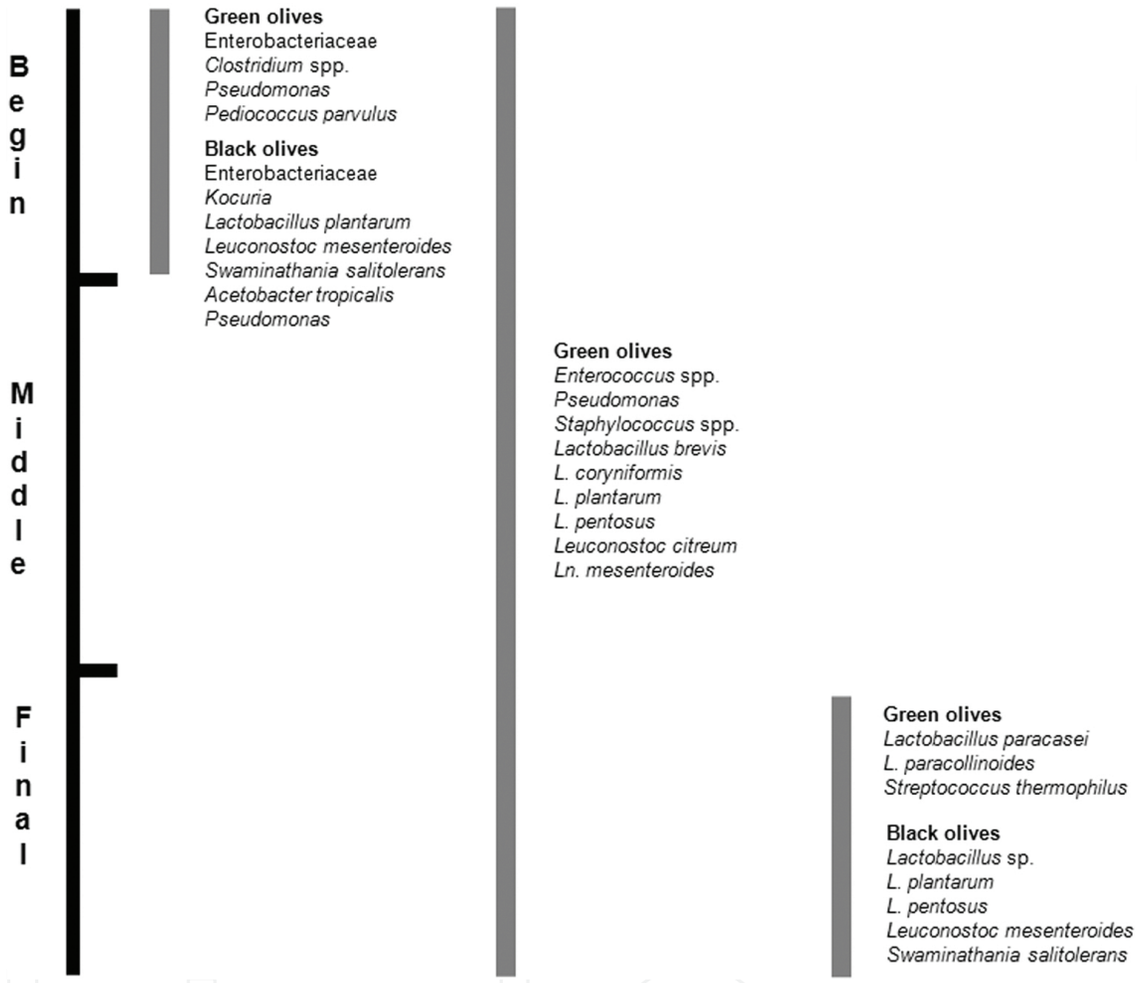

Figure 3. Main genera and species of bacteria associated to different production stages of green and black table olives. Adapted from Heperkan et al. [32]; Bleve et al. [29], [30].

Also the mould genera *Aureobasidium* and *Geotrichum* have been isolated from green and black naturally fermented olives, whereas isolates belonging to the genus *Penicillium* and *Aspergillus* were isolated by naturally fermented black olives [21, 31, 32].

The introduction of LAB and yeasts starter cultures in table olives production can also be motivated by the difficulty to monitor and control spontaneous fermentation in the industrially production of black as well as several cultivars of green olives [33–35]. Starter cultures are preparations of microorganisms, live, or resting, generally present in high cell number, which can be added to enhance, accelerate, and improve a fermentation process by their metabolic activities.

LAB have been considered very important since they are able to debitter olives, low brine pH, limit the spoilage and the presence of pathogens, and develop a correct flavor and texture in the final product. Several studies proposed the use of *Lactobacillus plantarum* and/or of *L. pentosus* as starter cultures among the possible available technological approaches [9, 10, 15, 33, 36–38].

The use of yeasts as starters cultures has been recently proposed for production of table olive [11, 13, 14, 35, 39], since they can improve the organoleptic properties [11, 40], enhance the growth of LAB [12, 41], and biodegrade phenolic compounds [42].

Moreover, the possibility to use simultaneous or sequential inocula of yeasts and LAB in green and black olives has been proposed [12, 15, 37, 41, 43]. The presence of yeasts together with LAB can produce a significant improvement of the sensorial quality of olives. They can also favor LAB growth rate, help in Enterobacteriaceae reduction, sensitively shorten the time needed to obtain the final product.

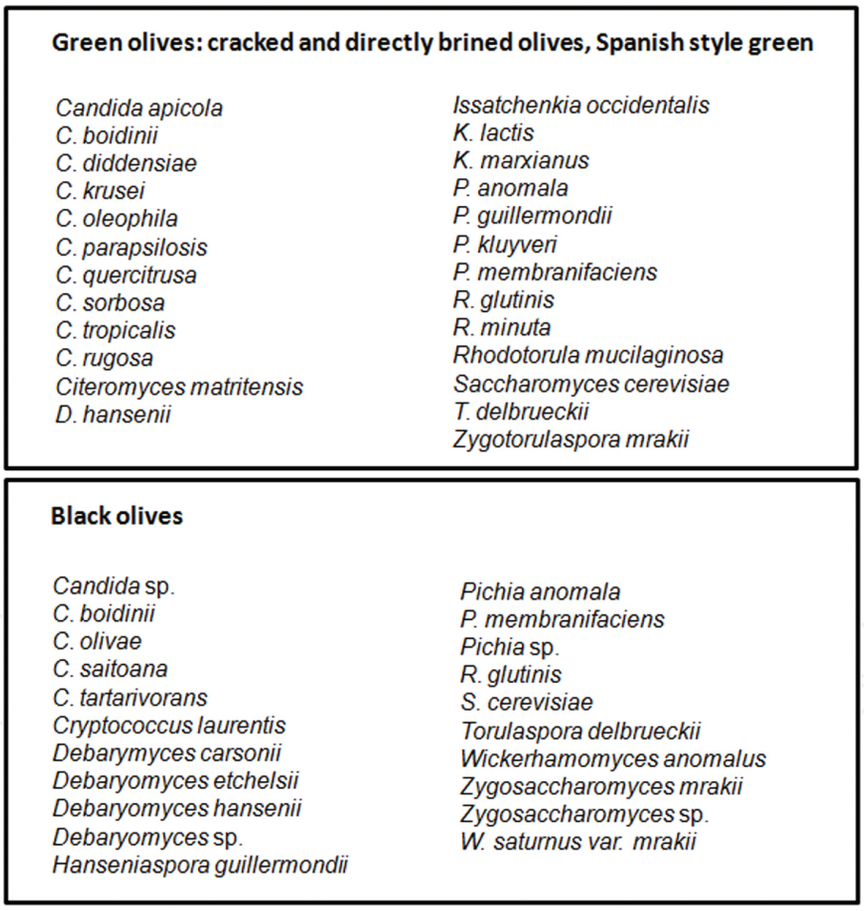

Figure 4. Main species of yeasts associated to green and black table olives. Adapted from Heperkan et al. [32]; Bleve et al. [29], [30].

Moulds can be responsible of undesirable effects on table olives quality. They can alter olive taste and appearance and can be responsible of mycotoxins production [32]. These microor-

ganisms need to be deeply studied in order to evaluate their possible positive role in table olive processing.

LAB and yeast strains to be used as starter cultures can be selected among microorganisms associated to a spontaneous fermentation. In a first step, they can be selected on the basis of their characteristics and abilities: (i) to lower pH (by homo or hetero-fermentative metabolism for LAB, by fermentative metabolism for yeasts); (ii) to survive and to grow in the presence of different constraints (poor nutrient substrate like olives in brine, low pH, high salt level, presence of phenols, wide range of temperatures), (iii) to produce lactic acid and other organic acids; (iv) to metabolize phenols and, in particular, to degrade oleuropein, which is the main compound responsible for the bitter taste in olives; (v) to develop desired flavors (volatile compounds); (vi) to produce no biogenic amines, which represent an emerging concern in table olives, wine and other fermented products [44–46]; (vii) to possess esterase and lipase activities that have a role in improving the aromatic profile of fermented olives (by increasing their free fatty acid content); (viii) to have no proteolytic and pectolytic activities, which could have a negative impact on olive quality since they are related to olive softening; (ix) to have functional (probiotics and health-promoting) properties. Several laboratory tests have been developed to select yeasts and LAB for all of these features. In a laboratory-scale, the most promising isolates can be tested for their ability to dominate the indigenous microbiota by predominant growth or by production of antagonistic substances during table olives fermentation.

The selected LAB and yeast isolates can be then tested in a pilot-scale fermentation (200 kg) in order to mimic the industrial conditions of fermentation. The best performing isolates can be proposed for industrial-scale fermentation in tanks of 3–8 tons.

3.2. Influence of starter cultures on table olives chemical and aromatic profile

The distribution and structure of the chemical constituents of olive fruit is complex and depend on variety, cultivation practices, geographical origin, and the level of maturation. Olive fruit's average composition is water (50%), protein (1.6%), fat (22%), carbohydrate (19.1%), cellulose (5.8%), inorganic substances (1.5%) and phenolic compounds (1–3%).

Both in green and black olive fermentations, lactic, citric, tartaric, and acetic acids were found to be the major metabolic products in drupes and in the brines [9, 47, 48], responsible for a decrease in pH value (about 4.0), satisfactory for naturally black olive fermentation [29, 30].

Although in the literature there are many data about aroma compounds in olive oil, very little is known about the quali-quantitative composition of volatile compounds in table olives. Among table olives, more attention has been placed on the characterization of the volatile fraction of the fermented black olives. Little is known about volatile fraction of green olives, probably because their volatile profiles are less rich, due to the NaOH treatment; the latter affects many precursors of the volatile compounds.

The formation of flavor compounds in table olives is a dynamic process mainly occurring during fermentation carried out by LAB and yeasts, along with a variety of contaminating microorganisms, which produce a variety of volatile compounds [49]. Volatile and semivolatile organic compounds are responsible for the olive complex flavor that in turn can influence the

consumer's preference. The "green odor" of unripe olives was associated to the presence of C5 and C6 volatile compounds (alcohols and aldehydes) originating from the activity of lypoxygenase metabolic pathway [50]. Hexanol and 2-hexenal are the major contributors to the characteristic green odor of olives and of many fruit and vegetable fermented foods. In spontaneous fermentation of black olives, the main product is ethanol that derives from the metabolic activity of different yeasts and hetero-fermentative LAB and is very important for the organoleptic properties of the final product [51].

C6 alcohols such as 1-hexanol and *cis*-3-hexen-1-ol, characterized by a "vegetal" and "herbaceous" aromas, seem to be linked to the different yeast strain used [52]. As already observed in wines, the relevant presence of the ethyl-acetate ester at the end of fermentation adds complexity to the aroma of the final product [53]. The high level of isoamyl alcohols indicates the role of yeasts in driving the process. In particular, 2 + 3 methyl-1-butanol (isoamyl alcohol, fruity-winey notes), hexanol (fruity-green notes) and *cis*-3-hexen-1-ol (green notes) are very important both in olives and brines. Other higher alcohols (1-propanol and 2-methyl-1 propanol) derive from the reduction process of aldehydes, but can also be linked to the microbial deamination process of amino acids [54]. Hexanal, (*Z*)-hex-3-enol, hexanol, (*Z*)-hex-3-enol acetate and hexyl acetate, detectable at various concentrations were reported to be related to the lipoxygenase activity [29, 30, 49].

Fatty acids, formed enzymatically during fermentations constitute an important group of aroma compounds that can contribute to the aroma complexity of table olives [29, 30]. Terpenes production is closely linked to cultivars, geographical area, climatic conditions and proliferation of specific pests and microorganisms characteristic of a given production area [55]. The presence of styrene can increase during fermentation [15]. This compound could be linked to an environmental contaminants and/or produced by L-phenylalanine deamination and decarboxylation of *trans*-cinammic acid [60] or by the dehydration of 2-phenylethanol.

The use of selected starter cultures has been proposed for Spanish- and Greek-type, green and black, olives to improve fermentation performance. Starters can accelerate and control the process, reduce undesired off-flavors and enhance quality of the final product by the development of typical and peculiar sensorial and taste characteristics.

In the evolution of volatile compounds during spontaneous fermentation of different black olive cultivars (Leccino, Cellina di Nardò, Conservolea and Kalamàta), Tufariello et al. [15] identified three main temporary steps characterized by the presence of chemical descriptors: aldehydes at the first stage (30 days), higher alcohols and styrene in the middle (90 days), and ethyl esters and fatty acids at the end of fermentation (third fermentation stage, 180 days). These descriptors could help in monitoring the fermentation process of other black olive cultivars as well as of naturally fermented green olives.

In starter-driven fermentations carried out by sequential inoculum of yeast LAB strain, three main stages have been described. The first stage (30 days) is characterized by high aldehydes content, compounds responsible of herbaceous flavors in fruits and vegetables. The second stage (60 days) is characterized by the presence of higher alcohols, styrene [56] and terpenes, compounds correlated with the metabolic activities of inoculated yeast starter strains. The third

final fermentation stage (90 days), mainly characterized by the presence of acetate esters (isoamyl acetate, ethyl acetate), esters (ethyl hexanoate and ethyl octanoate), and acids, probably due to the different pathways undertaken by LAB enzymes.

The use of starter microorganisms significantly reduced the time of fermentation process from 180 to 90 days. The first stage of fermentation shifted from 90 (spontaneous fermentation) to 60 days (starter-driven fermentation) and the second step shifted from 180 (spontaneous fermentation) to 90 days (starter-driven fermentation). The use of sequential inoculation strategy of selected yeast and LAB starters produced a volatile profile richer in compounds that can be associated to attributes such as fruity, winey-sweet and herbaceous. A significant reduction of volatile phenols and hydrocarbons was observed.

For Moresca and Kalamàta table olives inoculated with selected starter cultures of *L. plantarum*, a shift from herbal notes to fruity, sweet, and floral profile has been reported. In inoculated samples, a significant increase of higher alcohols (isoamylalcohols, 1-propanol, 2-methyl-1-propanol, phenylethylalcohol), esters (ethyl butanoate), and acetate esters (isoamyl acetate, ethyl acetate) was also observed [57].

Grounta et al. [58] demonstrated that the use of *L. pentosus* B281 produced table olives with good physical and chemical features and sensory properties highly appreciated by expert panelists. The coinoculation of *L. pentosus* B281 and *P. membranifaciens* M3A in brines of Conservolea olives developed a proper fermentation process, producing a final product with good sensory attributes and a milder acid gustatory sensation. This product could be suitable for consumers who do not appreciate the acid taste of natural black fermented olives and prefer milder tastes. No off-odors associated to abnormal fermentation (i.e., butyric, putrid fermentation, or zapateria spoilage) were detected by the panelists [58].

In green olives, most studies have been carried out for the selection of starter cultures able to control fermentation in brines after lye treatment. This process generally needs 3–7 months to be completed. It is mainly driven by LAB belonging to lactobacilli, *Leuconostoc* and *Pediococcus* spp. There are few studies on the volatile fraction and its evolution during the fermentation process. Panagou and Tassou [59] studied the evolution of the volatiles in green table olives (Conservolea cv.) treated with NaOH and then inoculated with *L. plantarum* or *L. pentosus*. The use of starters produced an acceleration of fermentation process. The final products contained increased concentrations of lactic and acetic acid as well as volatile molecules such as ethanol, methanol, acetate esters and isobutyric, isovaleric, and propionic acids. The sensorial characteristics ascribable to typical lactic fermentation were obtained in the final product also inoculating the strains *L. pentosus* B281 and *L. plantarum* B282, as single or combined cultures to ferment Spanish-style produced Halkidiki green olives [8].

In order to improve the fermentation of directly brined green olives, the application of the "pied de cuve" technology has been proposed [38]. Partially fermented brines deriving from a previous spontaneous fermentation were used to produce green olives with improved aroma and taste complexity. In comparison with brines deriving from previous fermentations performed with the starter *L. pentosus* OM13, undesired off-odors and off-flavors were not detected and a good control of microorganism spoilage was obtained. The use of selected

strains of lactobacilli and yeasts accelerated the fermentation process of directly brined Bella di Cerignola green olives [37], a cultivar traditionally debittered in the Spanish style. In inoculated samples, major compounds that significantly increased were ethanol, acetic acid and ethyl acetate. There was also an increase in the level of esters (fruity nuances), alcohols (fruity, floral and sweet notes), and acids except for propanoic acid. A decrease of aldehydes content was also observed [37].

Volatile compounds confer peculiar sensorial characteristics and contribute to the aroma "fingerprint" of single table olives variety. Then, it could be important to promote the use of new descriptors other than those linked to taste (crispness, sourness, bitterness, and astringency), appearance (brightness, intense green color, etc.) [2]. New descriptors should be able to describe floral, fruity, green, winey as well other similar notes (**Figure 5**).

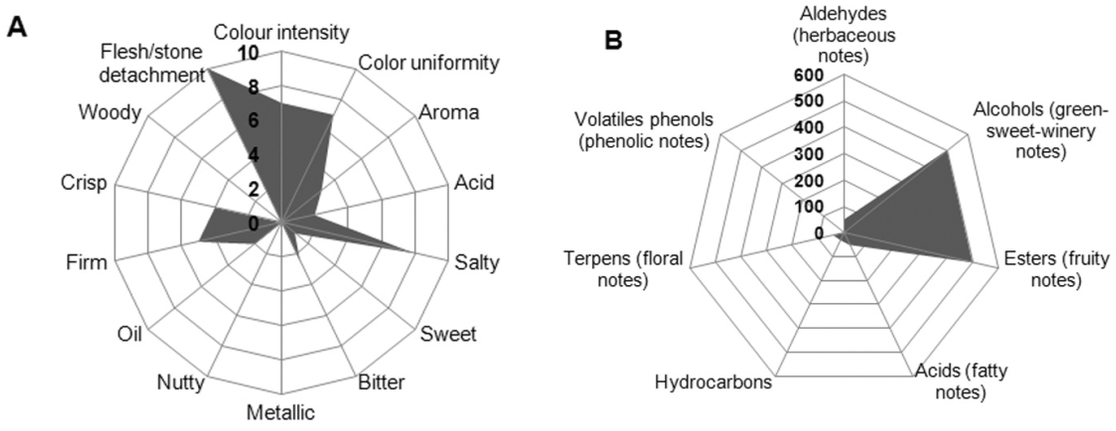

Figure 5. Spider plot showing the main organoleptic attribute intensities identified by trained panel (A) and (B) radar plot of all volatiles classes associated to black olives fermented by yeast and LAB starters.

To link chemical data to sensory data, it is necessary to evaluate the perception thresholds of the volatiles, defined as the lowest concentration capable of producing a sensation. The contribution of each volatile compound to odor profile can be quantified by its odor activity value (OAV). OAV is the ratio of the compound concentration to its odor threshold. In the table olives sector, these thresholds are not available, so it is not still possible to establish the role of each volatile compound as odorant in the multiplicity of olives aroma components.

3.3. Influence of microbial fermentation on table olives nutritional profile

In the past decades, olive oil and table olives have been attracting interest, mostly due to their beneficial effects on health. Table olives contain several nutritional components that largely depend on the olive variety, the cultivation conditions, the maturation stage of the olive fruit, and the processing method. The consumption of table olives thus allows the dietary introduction of bioactive components, such as triterpenic acids, α-tocopherol, biophenols, and fatty acids. These compounds are known to be responsible for a variety of health benefits. More specifically, olive fruits are remarkably rich in maslinic and oleanolic acids [60]. These triterpenic acids are located in the epicarp of the olive fruit and they constitute the main substances

of the surface waxes [61, 62]. Some studies indicate that maslinic and oleanolic acids possess health beneficial activities such as anti-inflammatory, antioxidant [63–65], antimicrobial [66], antiviral [67], cardioprotective [68], antihypertensive [69], antihyperlipidemic [70, 71], antidiabetic [72, 73], and even antitumor [73–77]. It is worthwhile noting that the content of these bioactive compounds in table olives is significantly higher than in olive oil [78].

NaOH treated green and black olives contain low levels of these compounds in comparison with naturally fermented olives [78]. Indeed, the NaOH treatment leads to the solubilization of maslinic and oleanolic acids into the alkaline and washing solutions. The resulting final product contained significantly reduced levels of these compounds.

In Greek-style preparations, the fermentation of black table olives driven by selected starter cultures can preserve the triterpenic acid content. The amount of these molecules was around 1000–2000 mg/kg olive flesh, much higher than the values observed in extra virgin olive oils [Bleve G., unpublished]. These observations confirm that table olives can be considered a dietary natural source of triterpenic acids.

The health benefits of olive oil and table olives are also attributed to their high content in monounsaturated fatty acid (MUFA). Commonly recognized as a high-fat food (about 80–85% of the calories in olives come from fat), olives provide a high content of oleic acid. Linoleic acid and α-linolenic acid are present in small amounts. Owing to the content of MUFA, the consumption of table olives can prevent and reduce the risk of cardiovascular diseases, regulate cholesterol levels, stimulate transcription of LDL-cholesterol receptor mRNA, and reduce breast cancer risks [79–81]. In Spanish-, Californian-, and Greek-style processes, triglylglycerols composition remains unaffected, although fatty acid composition of both green and black olives, shows differences depending on the ripeness degree. The concentration of oleic acid, the most abundant fatty acid in green and black olives, showed differences depending on the stage of maturity the producing methods [82]. When considering the PUFA/SFA ratio, green and directly brined table olives showed a value >0.4. This is a value recommended by the nutritional guidelines [83]. In particular, a significantly high PUFA/SFA ratio was found in directly brined olives [84, 85]. The use of selected starter cultures for black olives fermentation ensured a PUFA/SFA ratio >0.4 in the final product.

The olive fruit is also highly valuable for the presence of α-tocopherol (TC), β-carotene (BC) [80], and biophenols. TC acts as the major radical scavenging antioxidant and efficiently interrupts the propagation of lipid oxidation chain [86]. Several works described a protective action of TC on human health against different pathologies. It contributes to reduce the effects of inflammations and it defends the body against the negative effects of free radicals [87]. When cultivars of black table olives were fermented using selected starter cultures, Vitamin E and carotenes levels were found more constant.

Among biophenols, tyrosol, hydroxytyrosol, luteolin, and oleuropein are the main species found in olives [88–93]. The latter compound is mainly responsible for the bitter taste of unprocessed olives. Other phenolic compounds are verbascoside, 3,4-dihydroxyphenylglycol [94], anthocyanins, flavonoids, and phenolic acids [88]. Mechanisms postulated for chemical

and microbial oleuropein degradation and the effects on valuable phenols caused by different table olives processing methods are described in details by Boskou et al. [21].

In Greek-style fermented olives (driven by natural microbiota or by starter cultures), a higher content of total phenols was detected than that observed in lye treated olives [15, 29, 30, 37, 95, 96]. During the process, a complete hydrolysis of oleuropein and its aglycone takes place in olive flesh by yeasts and LAB β-glycosidase and esterase activities. In fact, high levels of hydroxytyrosol and tyrosol, together with verbascoside, caffeic acid, vanillic acid, and hydrocaffeic acid were detected in the final products. Table olives are a very good source of hydroxytyrosol which is known to possess a high antioxidant and free radical scavenging activity [95, 97]. Table olives together with virgin olive oil are the only edible source of hydroxytyrosol; in olive oil, however, the bound forms of this compound prevail.

Changes in the profiles of bioactive compounds caused by metabolic activities of microbial starters can produce variations in the bioaccessibility and/or bioavailability of these metabolites. The role of microorganisms and the effects of their activities on these bioactive compounds need to be further elucidated. Table olives can be used to ensure a "positive" or "optimal" dietary intake of these compounds. They represent a source of phytochemicals useful for the prevention of several diseases and the promotion of human health (**Figure 6**).

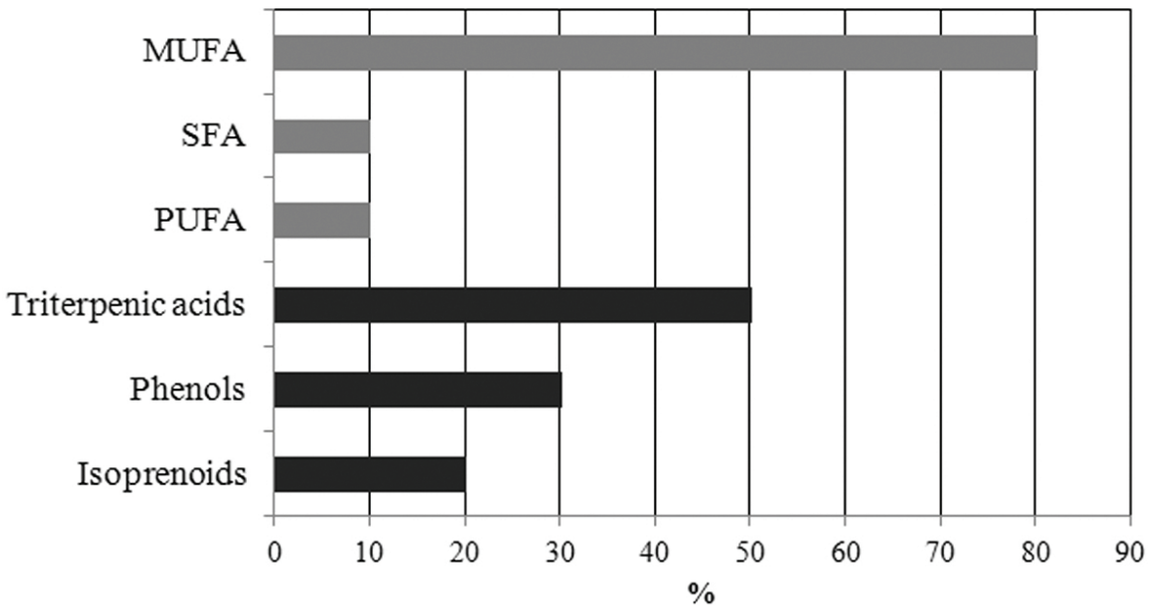

Figure 6. Profile of the main nutritional traits (% on drupe fresh weight) associated to black table olives fermented by yeast and LAB starters.

3.4. Use of starter cultures as probiotics

Another important character for the selection of potential starter cultures is referred to probiotic traits that beneficially influence intestinal microflora and health [98]. LAB, mainly the strains belonging to genera *Lactobacillus, Bifidobacterium*, and *Streptococcus*, are microor-

ganisms generally considered for probiotics preparations. The term "probiotic" is defined by a United Nations and World Health Organization Expert Panel as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [99]. Probiotics are live microorganisms that have a beneficial effect on the host by influencing the composition and or metabolic activity of the flora of the gastrointestinal (GI) tract. Selected LAB probiotics strains can have beneficial properties. They can enhance the immune system responses, improve resistance to infection, protect against certain types of cancer, lower serum cholesterol levels, and reduce the incidence of coronary heart disease. They are also involved in the prevention or treatment of peptic ulcer disease, treatment of intractable diarrhea during antibiotic therapy, reduction of allergic inflammation, production of antimicrobial substances, reduction of symptoms of lactose intolerance, and the enhancement of the nutrients bioavailability [100–102].

Probiotic characteristics are strongly required for microorganisms to be used for food production. These attributes can be conferred to a food by microbial preparations different or by the same microorganisms used as starter cultures. In the first case, the interaction between probiotics strains and traditional starter cultures must be considered, since some probiotics strains may have effects on organoleptic properties of the food product or can influence negatively the starter culture bacteria [103]. In the latter case, strains to be proposed as starters need to be selected also for probiotics traits.

Recent studies have focused on the use of table olives as a carrier of LAB probiotic strains as well as on the evaluation of the fermentation performances of these probiotics [39, 101, 104, 105]. *L. paracasei* strain (IMPC2.1) was able to successfully colonize both the olive surface [102] and human gut [104], also driving the fermentation [106]. Moreover, LAB strains directly isolated from fermented olives have been proposed as probiotics starters instead of bacteria from human and animal sources. By this approach, some strain of *L. pentosus, L. plantarum*, and *L. paracasei* ssp. *paracasei* isolated from fermented olives showed desirable *in vitro* probiotic properties as well as good aptitude to be employed as starter cultures [8, 18].

During the past few years, some researchers have identified yeast species with potential probiotic properties, such as *C. boidinii, C. oleophila, D. hansenii*, and *P. membranifaciens* [35, 107, 108].

As proposed by different authors [14, 23, 109], for these microorganisms, probiotics traits are: (i) the resistance and/or survival to gastric pH conditions and to bile salts, (ii) the capacity to adhere to intestinal mucosa, and (iii) the antimicrobial activities against intestinal and food-borne pathogens. In addition, several other health promoting factors can be considered in order to promote yeast starters as probiotics. They are the production of B vitamins and the reduction of the intestinal proinflammatory response as an antagonistic effect of yeasts or probiotic bacteria toward pathogen; the ability to reduce cholesterol serum levels [110]; the ability to biodegrade phytate complexes, responsible for sequestering nutritional divalent minerals; the capability to synthesize natural folates, essential cofactors in the biosynthesis of nucleotides and crucial for cellular replication and growth [111]; the ability to produce a number of bioactive compounds. Anyway, in order to obtain probiotics and healthy olives, it is necessary that yeasts adhere to olive skin and survive during storage/packaging. The copresence of yeasts

and LAB in the biofilm associated to epidermis of natural black olives (Greek-style fermented Conservolea) and Spanish-style olives (Gordal and Manzanilla) indicate that the coinoculation of yeasts and LAB as multifunctional starter is a good strategy for carrying probiotics by table olives [35, 58].

3.5. Influence of starters in bioremediation of table olive processing wastewaters (TOPW)

During table olives processing, clean water is used and a large quantity of wastewater is produced depending on cultivar, maturity, and type of treatment from 0.5 l/kg to 6 l/kg. In 2013/2014, 2.7 million tons of table olives were produced in the world. The production of 1.2–14 million tons of wastewaters can be estimated. The volumes of wastewaters depends on the different table olives processing methods: for Spanish style 2–3.5 l/kg of olives; for California green ripe olives 1.5–3.5 l/kg of olives, for California black ripe olives 2–6.5 l/kg of olives, for naturally black olives (Greek style) 1 l/kg of olives [112]. The availability of the water as a resource and the environmental impact deriving from its use are very important matters for many table olives producer countries. The main problems associated to TOPW are their high chemical oxygen demand (COD) up to 35 g/l, biological oxygen demand (BOD) ranging from 0.6 to 38.3 g/l, different pH values (alkaline, up to 9–13, for waters deriving by lye-treatment and acidic, 3.6–4.4, for fermentation brines), the presence of several water-soluble phenols and polyphenols, the high salt levels (56–77 g/l) in Greek-style olives.

The different composition of TOPW (produced by different table olives processing methods) requires the development of diverse approaches for their management and treatment. The contemporary presence of high organic matter content (reducing sugars, organic acids), suitable to be used by microorganisms, and of compounds that affect microbial growth and metabolism (phenols), renders biological approaches for their remediation very difficult.

There are different methods based on the use of aerobic and anaerobic processes of TOPW [113, 114]. There are also strategies that combine chemical and biological processes, using a pretreatment with *Aspergillus* sp. in order to degrade phenols, very toxic, and able to limit the activities of anaerobic digestion [115, 116]. Several studies demonstrated that the use of aerobic and anaerobic biodegradation of wastewaters can significantly reduce organic load expressed by COD (aerobic treatment between 50 and 70% and anaerobic between 81 and 94%) [116–119].

The use of starter microorganisms in table olive processing can represent a useful system to mitigate the presence of chemical pollutants in TOPW. They are phenols (such as oleuropein and derivates, anthocyanins), NaOH in the lye and sodium content in brine. Selected microorganisms, by their metabolic activities can reduce the presence of phenols. They can also efficiently dominate the spontaneous microflora and facilitate reduction of NaCl concentration in brines. Concerning NaOH in lye, a future challenge can be to develop new systems able to reduce or eliminate the use of NaOH in lye. These strategies can allow to perform a more natural transformation process by the combination of technological and biotechnological approaches.

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References

[1] http://www.internationaloliveoil.org/estaticos/view/132-world-table-olive-figures [Accessed: 04/03/2016].

[2] International Olive oil Council (2004). Trade Standard Applying to Table Olives. Resolution No. RES-2/91-IV/04.

[3] Garrido-Fernandez A, Fernandez Diez MJ, Adams MR. Olives and table olives. In: Garrido A, Fernandez MJ, Fernandez D, Adams MR, editors. Table Olives: Production and Processing. London: Chapman and Hall; 1997. p. 10–22, 289–367.

[4] Lanza B. Nutritional and sensory quality of table olives. In: Muzzalupo I, editor. Olive Germplasm – The Olive Cultivation, Table Olive and Olive Oil Industry in Italy, 2012. InTech. ISBN 978-953-51-0883-2. DOI: 10.5772/51723.

[5] 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: 248–253. DOI: 10.3389/fmicb.2012.00248.

[6] Medina-Pradas E, Arroyo-López FN. Presence of toxic microbial metabolites in table olives. Frontiers in Microbiology. 2015; 6: 1–6. DOI: 10.3389/fmicb.2015.00873.

[7] García A, Romero C, Medina E, García P, De Castro A, Brenes M. Debittering of olives by polyphenol oxidation. Journal of Agricultural and Food Chemistry. 2008; 56: 11862–11867. DOI: 10.1021/jf802967y.

[8] Blana VA, Grounta A, Tassou CC, Nychas GJ, Panagou EZ. Inoculated fermentation of green olives with potential probiotic Lactobacillus pentosus and Lactobacillus plantarum starter cultures isolated from industrially fermented olives. Food Microbiology. 2014; 38: 208–218. DOI: 10.1016/j.fm.2013. 09.007.

[9] Panagou EZ, Schillinger U, Franz CMAP, Nychasa GJE. Microbiological and biochemical profile of cv. Conservolea naturally black olives during controlled fermentation with selected strains of lactic acid bacteria. Food Microbiology. 2008; 25: 348–358. DOI: 10.1016/j.fm.2007.10.005.

[10] Servili M, Settanni L, Veneziani G, Esposto S, Massitti O, Taticchi A, Urbani S, Montedoro GF, Corsetti A. The Use of Lactobacillus pentosus 1MO to shorten the debittering process time of black table olives (Cv. Itrana and Leccino): a pilot-scale application. Journal of Agricultural and Food Chemistry. 2006; 54: 3869–3875. DOI: 10.1021/jf053206y.

[11] 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. DOI: 10.1016/j.ijfoodmicro.2008.08.018.

[12] Segovia Bravo KA, Arroyo Lopez FN, García García P, Duran Quintana MC, Garrido Fernandez A. Treatment of green table olive solutions with ozone. Effect on their polyphenol content and on Lactobacillus pentosus and Saccharomyces cerevisiae growth. International Journal of Food Microbiology. 2007; 114: 60–68. DOI: 10.1016/j.ijfoodmicro.2006.09.032.

[13] Bevilacqua A, Beneduce L, Sinigaglia M, Corbo MR. Selection of yeasts as starter cultures for table olives. Journal of Food Science. 2013; 78: M742–M751. DOI: 10.1111/1750-3841.12117.

[14] Bonatsou S, Benítez A, Rodríguez-Gómez F, Panagou EZ, Arroyo-López FN. Selection of yeasts with multi functional features for application as starters in natural black table olive processing. Food Microbiology. 2015; 46: 66–73. DOI: 10.1016/j.fm.2014.07.011.

[15] Tufariello M, Durante M, Ramires FA, Grieco F, Tommasi L, Perbellini E, Falco V, Tasioula-Margari M, Logrieco AF, Mita G, Bleve G. New process for production of fermented black table olives using selected autochthonous microbial resources. Frontiers in Microbiology. 2015; 6: 1007–1022. DOI: 10.3389/fmicb.2015.01007.

[16] Bleve G, Tufariello M, Durante M, Perbellini E, Mita G, Ramires AF, Grieco F, Logrieco AF. Metodo per la Produzione di Olive da Tavola Fermentate. 2015. European Patent Application No. 14197402.2. Munich: European Patent Office (EPO).

[17] Lavermicocca P, Valerio F, Lonigro SL, De Angelis M, Morelli L, Callegari ML, Rizzello GC, Visconti A. Study of the adhesion and survival of lactobacilli and bifidobacteria on table olives with the aim of formulating a new probiotics food. Applied Environmental Microbiology. 2005; 71: 4233–4240. DOI: 10.1128/AEM.71.8.4233-4240.2005.

[18] Argyri A, Zoumpopoulou G, Karatzas KA, Tsakalidou E, Nychas GJE, Panagou EZ, Tassou CC. Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. Food Microbiology. 2013; 33: 282–291. DOI: 10.1016/j.fm.2012.10.005.

[19] Barbaro B, Toietta G, Maggio R, Arciello M, Tarocchi M, Galli A, Balsano C. Effects of the Olive-Derived Polyphenol Oleuropein on Human Health. International Journal of Molecular Sciences. 2014; 15: 18508–18524. DOI: 10.3390/ijms151018508.

[20] Charoenprasert S, Mitchell A. Factors influencing phenolic compounds in table olives (Olea europaea). Journal of Agricultural and Food Chemistry. 2012; 60: 7081–7095. DOI: 10.1021/jf3017699.

[21] Boskou D, Camposeo S, Clodoveo ML. Table olives as sources of bioactive compounds. In: Boskou D, editor. Olives and Olive Oil Bioactive Constituents,; 2015.Urbana, IL, USA: AOCS Press. p 179–216, 217—260. ISBN: 978-1-630670-41-2.

[22] Steensels J, Verstrepen KJ. Taming wild yeast: potential of conventional and nonconventional yeasts in industrial fermentations. Annual Review of Microbiology. 2014; 68: 61–80. DOI: 10.1146/annurev-micro-091213-113025.

[23] Heperkan D. Microbiota of table olive fermentations and criteria of selection for their use as starters. Frontiers in Microbiology. 2013; 4: 1–11. DOI: 10.3389/fmicb.2013.00143.

[24] Tassou CC, Panagou EZ, Nychas GJE. Microbial colonization of naturally fermented olives. In: Preedy VR, Watson RR, editors. Olives and Olive Oil in Health and Disease. Singapore: Academic Press is an imprint of Elsevier; 2010. p. 397–406.

[25] Randazzo CL, Ribbera A, Pitino I, Romeo FV, Caggia C. Diversity of bacterial population of table olives assessed by PCR-DGGE analysis. Food Microbiology. 2012; 32: 87–96. DOI: 10.1016/j.fm.2012.04.013.

[26] Alves M, Gonçalves T, Quintas T. Microbial quality and yeast population dynamics in cracked green table olives fermentations. Food Control. 2012; 23: 363–368. DOI: 10.1016/j.foodcont.2011.07.033.

[27] Jalava K, Selby K, Pihlajasaari A, Kolho E, Dahlsten E, Forss N, Bäcklund T, Korkeala H, Honkanen-Buzalski T, Hulkko T, Derman Y, Järvinen A, Kotilainen H, Kultanen L, Ruutu P, Lyytikaïnen O, Lindström M. Two cases of food-borne botulism in Finland caused by conserved olives. Eurosurveillance. 2011; 16: 1–3.

[28] Cocolin L, Alessandria V, Botta C, Gorra R, De Filippis F, Ercolini D, Rantsiou K. NaOH-debittering induces changes in bacterial ecology during table olives fermentation. PLoS One. 2013; 8: 1–11. DOI: 10.1371/journal.pone.0069074.

[29] Bleve G, Tufariello M, Durante M, Perbellini E, Ramires FA, Grieco F, Cappello MS, De Domenico S, Mita G, Tasioula-Margari M, Logrieco AF. Physico-chemical and microbiological characterization of spontaneous fermentation of Cellina di Nardò and Leccino table olives. Frontiers in Microbiology. 2014; 5: 570, 1–18. DOI: 10.3389/fmicb.2014.00570.

[30] Bleve G, Tufariello M, Durante M, Grieco F, Ramires FA, Mita G, Tasioula-Margari M, Logrieco AF. Physico-chemical and microbiological characterization of natural fermentation process of Conservolea and Kalamata table olives and developement of a protocol for the pre-selection of fermentation starters. Food Microbiology. 2015; 46: 368–382. DOI: 10.1016/j.fm.2014.08.021.

[31] Nisiotou AA, Chorianopoulos N, Nychas GJ, Panagou EZ. 2010. Yeast heterogeneity during spontaneous fermentation of black conservolea olives in different brine

solutions. Journal of Applied Microbiology. 2010; 108: 396–405. DOI: 10.1111/j. 1365-2672.2009.04424.

[32] Heperkan D, Erol-Meriç B, Sismanoglu G, Dalkiliç G, Güler FK. Mint: mycobiota, mycotoxigenic fungi and citrinin production in black olives. Advances in Experimental Medicine and Biology. 2006; 571: 203–210. DOI: 10.1007/0-387-28391-9_13.

[33] De Castro A, Montano A, Casado FJ, Sanchez AH, Rejano L. Utilization of Enterococcus casselifavus and Lactobacillus pentosus as starter cultures for Spanish-style green olive fermentation. Food Microbiology. 2002. 19: 637–644. DOI: 10.1006/fmic.2002.0466.

[34] Abriouel H, Benomar N, Lucas R, Gálvez A. Culture-independent study of the diversity of microbial populations in brines during fermentation of naturally fermented Aloreña green table olives. International Journal of Food Microbiology. 2011; 144: 487–496. DOI: 10.1016/j. ijfoodmicro.2010.11.006.

[35] Arroyo-López FN, Romero-Gil V, Bautista-Gallego J, Rodriguez-Gómez F, Jiménez-Díaz R, García-García P, Querol A, Garrido-Fernandez A. Yeasts in table olive processing: desirable or spoilage microorganisms? International Journal of Food Microbiology. 2012; 160: 42–49. DOI: 10.1016/j.ijfoodmicro.2012.08.003.

[36] Leal-Sánchez MV, Ruiz-Barba JL, Sánchez AH, Rejano L, Jiménez-Díaz R, Garrido A. Fermentation profile and optimization of green olive fermentation using Lactobacillus plantarum LPCO10 as a starter culture. Food Microbiology. 2003; 20: 421–430. DOI: 10.1016/S0740-0020(02)00147-8.

[37] De Angelis M, Campanella D, Cosmai L, Summo C, Rizzello CG, Caponio F. Microbiota and metabolome of un-started and started Greek-type fermentation of Bella di Cerignola table olives. Food Microbiology. 2015; 52: 18-30. DOI: 0.1016/j.fm.2015.06.002.

[38] Martorana A, Alfonzo A, Settanni L, Corona O, La Croce F, Caruso T, Moschetti G, Francesca N. An innovative method to produce green table olives based on "pied de cuve" technology. Food Microbiology. 2015; 50: 126–140. DOI: 10.1016/j.fm.2015.03.008.

[39] Arroyo-López FN, Romero-Gil V, Bautista-Gallego J, Rodriguez-Gómez F, Jiménez-Díaz R, García-García P, Querol A, Garrido-Fernández A. Potential benefits of the application of yeast starters in table olive processing. Frontiers in Microbiology. 2012; 3: 161–164. DOI: 10.3389/fmicb.2012.00161.

[40] Garrido Fernández A, Garcia P, Brenes M. Olive fermentations. In: Remand HJ, Reed G, editors. Biotechnology. Weinheim: VCH Press; 1995. p. 539–627.

[41] Tsapatsaris S, Kotzekidou P. Application of 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: 157–163. DOI: 10.1016/ j.ijfoodmicro.2004.02.011.

[42] Ettayebi K, Errachidi F, Jamai L, Tahri-Jouti MA, Sendile K, Ettayebi M. Biodegradation of polyphenols with immobilized Candida tropicalis under metabolic induction. FEMS Microbiology Letters. 2003; 223: 215–219. DOI: 10.1016/S0378-1097(03)00380-X.

[43] Hurtado A, Ben Othman N, Chammem N, Hamdi M, Ferrer S, Reguant C, Bordons A, Rozés N. Characterization of Lactobacillus isolates from fermented olives and their bacteriocin gene profiles. Food Microbiology. 2011; 28: 1514–1518. DOI: 10.1016/j.fm.2011.07.010.

[44] Garcia PG, Barranco CR, Quintana MC, Fernandez AG. Biogenic amine formation and "zapatera" spoilage of fermented green olives: effect of storage temperature and debittering process. Journal of Food Protection. 2004; 67: 117–123. DOI: 10.1016/j.fm.2007.10.005.

[45] Spano G, Russo P, Lonvaud-Funel A, Lucas P, Alexandre H, Grandvalet C, Coton E, Coton M, Barnavon L, Bach B, Rattray F, Bunte A, Magni C, Ladero V, Alvarez M, Fernández M, Lopez P, de Palencia PF, Corbi A, Trip H, Lolkema JS. Biogenic amines in fermented foods. European Journal of Clinical Nutritional. 2010; 64: 95–100. DOI: 10.1038/ejcn.2010.218.

[46] Tristezza M, Vetrano C, Bleve G, Spano G, Capozzi V, Logrieco A, Mita G, Grieco F. Biodiversity and safety aspects of yeast strains characterized from vineyards and spontaneous fermentations in the Apulia Region, Italy. Food microbiology. 2013; 36: 335–342. DOI: 10.1016/J.FM.2013.07.001.

[47] Nychas GJN, Panagou EZ, Parker ML, Waldron KW, Tassou CC. Microbial colonization of naturally black olives during fermentation and associated biochemical activities in the cover brine. Letters in Applied Microbiology. 2002; 34: 173–177. DOI: 10.1046/j.1472-765x.2002.01077.x.

[48] Chorianopoulos NG, Boziaris IS, Stamatiou A, Nuchas 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. DOI: 10.1016/j.fm.2004. 04.010.

[49] Sabatini N, Marsilio V. Volatile compounds in table olives (Olea europaea L., Nocellara del Belice cultivar). Food Chemistry. 2008; 107: 1522–1528. DOI: 10.1016/j.foodchem.2007.10.008.

[50] Angerosa F, Servili M, Selvaggini R, Taticchi A, Esposto S, Montedoro G. Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. Journal of Chromatography A. 2004; 1054: 17–31.

[51] Fleming HP, Etchells JL, Bell TA. Vapor analysis of fermented Spanish-type green olives by gas chromatography. Journal of Food Science. 1969; 34: 419–422. DOI: 10.1111/j.1365-2621.1969.tb12794.x.

[52] Torrens J, Urpi P, Montserrat RA, Vichi S, López-Tamames E, Buxaderas S. Different commercial yeast strains affecting the volatile and sensory profile of cava base wine.

International Journal of Food Microbiology. 2008; 124: 48-57. DOI: 10.1016/j.ijfoodmicro.2008.02.023.

[53] Mallouchos A, Skandamis P, Loukatos P, Komaitis M, Koutinas A, Kanellaki M. Volatile compounds of wines produced by cells immobilized on grape skins. Journal of Agricultural and Food Chemistry. 2003; 51: 3060–3066. DOI: 10.1021/jf026177p.

[54] McFeeters RF. Fermentation microorganisms and flavor changes in fermented foods. Journal of Food Science. 2004; 69: 35–37. DOI: 10.1111/j.1365-2621.2004.tb17876.x.

[55] Damascelli A, Palmisano F. 2013 Sesquiterpene fingerprinting by headspace SPME–GC–MS: preliminary study for a simple and powerful analytical tool for traceability of olive oils. Food Analytical Methods. 2012; 6: 900–905. DOI: 10.1007/s12161-012-9500-9.

[56] Gilbert-López B, Robles-Molina J, García-Reyes JF, Molina-Díaz A. Rapid determination of BTEXS in olives and olive oil by headspace-gas chromatography/mass spectrometry (HS-GC-MS). Talanta. 2010; 83: 391–399. DOI: 10.1016/j.talanta.2010.09.052.

[57] Sabatini N, Mucciarella M, Marsilio V. Volatile compounds in uninoculated and inoculated table olives with Lactobacillus plantarum (Olea europaea L., cv. Morescaand Kalamata). Food Science and Technology. 2008; 41: 2017–2022. DOI: 10.1016/j.lwt.2007.12.002.

[58] Grounta A, Doulgeraki AI, Nychas GJE, Panagou EZ. Biofilm formation on Conservolea natural black olives during single and combined inoculation with a functional Lactobacillus pentosus starter culture. Food Microbiology. 2016; 56: 35–44. DOI: 10.1016/j.fm.2015.12.002.

[59] 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. DOI: 0.1016/j.fm.2006.02.005.

[60] Stiti N, Triki S, Hartmann MA. Formation of triterpenoids throughout Olea europaea fruit ontogeny. Lipids. 2007; 42: 55–67.

[61] Bianchi G. Lipids and phenols in table olives. European Journal of Lipid Science and Technology. 2003; 105: 229–242. DOI: 10.1002/ejlt.200390046.

[62] Guinda Á, Rada M, Delgado T, Gutiérrez-Adánez P, Castellano JM. Pentacyclic triterpenoids from olive fruit and leaf. Journal of Agriculture of Food Chemistry. 2010; 58: 9685–9691. DOI: 10.1021/jf102039t.

[63] Ismaili H, Milella L, Fkih-Tetouani S, Ilidrissi A, Camporese A, Sosa S, Altinier G, Della Loggia R, Aquino R. In vivo topical anti-inflammatory and in vitro antioxidant activities of two extracts of Thymus satureioides leaves. Journal of Ethnopharmacology. 2004; 91: 31–36. DOI: 10.1016/j.jep.2003.11.013.

[64] Liu J. Pharmacology of oleanolic acid and ursolic acid. Journal of Ethnopharmacology. 1995; 49: 57–68. DOI: 10.1016/0378-8741(95)90032-2.

[65] Tsai SJ, Yin MC. Antioxidative and anti-inflammatory protection of oleanolic acid and ursolic acid in PC12 cells. Journal of Food Science. 2008; 73: 174–178. DOI: 10.1111/j.1750-3841.2008.00864.x.

[66] Horiuchi K, Shiota S, Hatano T, Yoshida T, Kuroda T, Tsuchiya T. Antimicrobial activity of oleanolic acid from Salvia officinalis and related compounds on vancomycin-resistant Enterococci (VRE). Biological and Pharmaceutical Bulletin. 2007; 30: 1147–1149.

[67] Parra A, Rivas F, Lopez PE, Garcia-Granados A, Martinez A, Albericio F, Marquez N, Muñoz E. Solution- and solid-phase synthesis and anti-HIV activity of maslinic acid derivatives containing amino acids and peptides. Bioorganic & Medicinal Chemistry. 2009; 17: 1139–1145.

[68] Allouche Y, Beltran G, Gaforio JJ, Uceda M, Mesa MD. Antioxidant and antiatherogenic activities of pentacyclic triterpenic diols and acids. Food and Chemical Toxicology. 2010; 48: 2885–2890. DOI: 10.1016/j.fct.2010.07.022.

[69] Rodriguez-Rodriguez R, Perona JS, Herrera MD, Ruiz-Gutierrez V. Triterpenic compounds from "orujo" olive oil elicit vasorelaxation in aorta from spontaneously hypertensive rats. Journal of Agricultural and Food Chemistry. 2006; 54: 2096–2102. DOI: 10.1021/jf0528512.

[70] Liu J, Sun H, Duan W, Mu D, Zhang L. Maslinic acid reduces blood glucose in KK-Ay mice. Biological and Pharmaceutical Bulletin. 2007; 30: 2075–2078. DOI: 10.1248/bpb.30.2075.

[71] Liu J, Sun H, Wang X, Mu D, Liao H, Zhang L. Effects of oleanolic acid and maslinic acid on hyperlipidemia. Drug Development Research. 2007; 68: 261–266. DOI: 10.1002/ddr.20187.

[72] Sato H, Genet C, Strehle A, Thomas C, Lobstein A, Wagner A, Mioskowski C, Auwerx J, Saladin R. Anti-hyperglycemic activity of a TGR5 agonist isolated from Olea europaea. Biochemical and Biophysical Research Communications. 2007; 362: 793–798.

[73] Hsum YW, Yew WT, Hong PLV, Soo KK, Hoon LS, Chieng YC, Mooi LY. Cancer chemopreventive activity of maslinic acid: suppression of COX-2 expression and inhibition of NF-κB and AP-1 activation in Raji cells. Planta Medica. 2011; 77: 152–157. DOI: 10.1055/s-0030-1250203.

[74] Juan ME, Planas JM. Effects of pentacyclic triterpenes from olives on colon cancer. In: Watson RR, Preedy VR, editors. Bioactive Foods and Extracts: Cancer Treatment and Prevention. New York: CRC Press; 2010. p. 403–413.

[75] Li C, Yang Z, Zhai C, Qiu W, Li D, Yi Z, Wang L, Tang J, Qian M, Luo J, Liu M. Maslinic acid potentiates the anti-tumor activity of tumor necrosis factor a by inhibiting NF-κB signaling pathway. Molecular Cancer. 2010; 9: 1–13. DOI: 10.1186/1476-4598-9-73.

[76] Reyes FJ, Centelles JJ, Lupianez JA, Cascante M. (2Alpha, 3beta)-2,3-dihydroxyolean-12-en-28-oic acid, a new natural triterpene from Olea europea, induces caspase

dependent apoptosis selectively in colon adenocarcinoma cells. FEBS Letters. 2006; 580: 6302–6310. DOI: 10.1017/S0007114508882979.

[77] Reyes-Zurita FJ, Rufino-Palomares EE, Lupianez JA, Cascante M. Maslinic acid, a natural triterpene from Olea europaea L., induces apoptosis in HT29 human colon cancer cells via the mitochondrial apoptotic pathway. Cancer Letters. 2009; 273: 44–54. DOI: 10.1016/j.canlet.2008.07.033.

[78] Romero C, García A, Medina E, Ruíz-Méndez Mª V, de Castro A, Brenes M. Triterpenic acids in table olives. Food Chemistry. 2010; 118: 670–674. DOI: 10.1016/j.foodchem.2009.05.037.

[79] Owen RW, Haubner R, Würtele G, Hull WE, Spiegelhalder B, Bartsch H. Olives and olive oil in cancer prevention. European Journal of Cancer Prevention. 2004; 13: 319–326. DOI: 10.1097/01.cej.0000130221.19480.7e.

[80] Sakouhi F, Harrabi S, Absalon C, Sbei K, Boukhchina Kallel H. α-Tocopherol and fatty acids contents of some Tunisian table olives (Olea europea L.): changes in their composition during ripening and processing. Food Chemistry. 2008; 108: 833–839. DOI: 10.1016/j.foodchem.2007.11.043.

[81] Kastorini CM, Milionis HJ, Goudevenos JA, Panagiotakos DB. Mediterranean diet and coronary heart disease: is obesity a link—a systematic review. Nutrition, Metabolism and Cardiovascular Diseases. 2010; 20: 536–551. DOI: 10.1016/j.numecd.2010.04.006.

[82] Ünal K, Nergiz C. The effect of table olive preparing methods and storage on the composition and nutritive value of olives. Grasas Aceites. 2003; 54: 71–76. DOI: 10.3989/gya.2003.v54.i1.280.

[83] Wood JD, Richardson RI, Nute GR, Fisher AV, Campo MM, Kasapidou E, Sheard PR, Enser M. Effects of fatty acids on meat quality: a review. Meat Science. 2003; 66: 21–32. DOI: 10.1016/S0309-1740(03)00022-6.

[84] Lopez A, Montano A, Garcia P, Garrido A. Fatty acid profile of table olives and its multivariate characterization using unsupervised (PCA) and supervised (DA) chemometrics. Journal of Agricultural and Food Chemistry. 2006; 54: 6747–6753. DOI: 10.1021/jf0612474.

[85] Uylaser V, Yildiz G. Fatty acid profile and mineral content of commercial table olives from Turkey. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2013; 41: 518–523.

[86] Kalman A, Mujahid C, Mottier P, Heudi O. Determination of α-tocopherol in infant foods by liquid chromatography combined with atmospheric pressure chemical ionisation mass spectrometry. Rapid Communications in Mass Spectrometry. 2003; 17: 723–727. DOI: 10.1002/rcm.970.

[87] Bogani P, Galli C, Villa M, Visioli F. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. Atherosclerosis. 2007; 190: 181–186. DOI: 10.1016/j.atherosclerosis. 2006.01.011.

[88] Macheix, JJ, Fleuriet A, Billot J. Fruit phenolics, vol. 92.Boca Raton, FL: CRC Press; 1990. p.111–112.

[89] Ryan D, Robards K. Phenolic compounds in olives. Analyst. 1998; 123: 31R–44R.

[90] Borzillo A, Iannotta N, Uccella N. Oinotria table olives: quality evaluation during ripening and processing by biomolecular components. European Food Research and Technology. 2000; 212: 113–121. DOI: 10.1007/s002170000178.

[91] Uccella N. Olive biophenols: novel ethnic and technological approach. Trends Food Science and Technology. 2001; 11: 328–339. DOI: 10.3989/gya.095811.

[92] Saija A, Ucella N. Olive biophenols; functional effects on human wellbeing. Trends Food Science and Technology. 2001; 11: 357–363.

[93] Panizzi L, Scarpati ML, Oriente G. The constitution of oleuropein, a bitter glucoside of the olive with hypotensive action. Gazzetta Chimica Italiana. 1960; 90: 1449–1485.

[94] Bianchi G, Pozzi N. 3,4-Dihydroxyphenylglycol, a major C6–C2 phenolic in Olea europaea fruits. Phytochemistry. 1994; 35: 1335–1337.

[95] Blekas G, Psomiadou E, Tsimidou M, Boskou D. On the importance of total polar phenols to monitor the stability of Greek virgin olive oil. European Journal of Lipid Science and Technology. 2002; 140: 340–346. DOI: 10.1002/1438-9312(200206)104:6<340.

[96] Pistarino E, Aliakbarian B, Casazza AA, Paini M, Cosulich ME, Perego P. Combined effect of starter culture and temperature on phenolic compounds during fermentation of Taggiasca black olives. Food Chemistry. 2013; 138: 2043–2049. DOI: 10.1016/j.foodchem.2012.11.021.

[97] Leenen R, Roodenburg AJC, Vissers MN, Schuurbiers JAE, van Putte KPAM, Wiseman SA, van de Put FHMM. Supplementation of plasma with olive oil phenols and extracts: influence on LDL oxidation. Journal of Agricultural and Food Chemistry. 2002; 50: 1290–1297. DOI: 10.1021/jf010968u.

[98] Doulgeraki AI, Hondrodimou O, Iliopoulos V, 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. DOI: 10.1016/j.foodcont.2012.01.006.

[99] FAO/WHO. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria.Cordoba (Argentina): American Córdoba Park Hotel; 2001.

[100] Parvez S, Kang M, Chung HS, Cho C, Hong M-C, Shin M-K, Bae H. Survey and mechanism of skin depigmenting and lightening agents. Review. Phytotherapy Research. 2006; 20: 921–934. DOI: 10.1002/ptr.1954.

[101] De Bellis P, Valerio F, Sisto A, Lonigro SL, Lavermicocca P. Probiotic table olives: microbial populations adhering on olive surface in fermentation sets inoculated with

the probiotic strain Lactobacillus paracasei IMPC2.1 in an industrial plant. International Journal of Food Microbiology. 2010; 140: 6–13. DOI: 10.1016/j.ijfoodmicro.2010.02.024.

[102] Mena B, Aryana KJ. Influence of ethanol on probiotic and culture bacteria Lactobacillus bulgaricus and Streptococcus thermophilus within a therapeutic product. Journal of Medical Microbiology. 2012; 2: 70–76. DOI: 10.4236/ojmm.2012.23010.

[103] Tamime AY, Saarela M, Korslund Sondergaard A, Mistry VV, Shah NP. Production and maintenance of viability of probiotic micro-organisms in dairy products. In: Tamime AY, editor. Probiotic Dairy Products. London: Blackwell Publishing Ltd; 2005. p. 39–72.

[104] Lavermicocca P, Valerio F, Lisa Lonigro S, De Angelis M, Morelli L, Callegari ML, Rizzello CG, Visconti A. Study of adhesion and survival of Lactobacilli and Bifidobacteria on table olives with the aim of formulating a new probiotic food. Applied Environmental Microbiology. 2005; 71: 4233–4240. DOI: 10.1128/AEM.71.8.4233-4240.2005.

[105] Saravanos E, Kagli D, Zoumpopoulou G, Panagou EZ, Tassou CC. Use of probiotic lactic acid bacteria as starter cultures in Spanish-style green olive fermentation and determination of their survival using PFGE. Food Microbiology. 2008; 1–4 September, Aberdeen, UK.

[106] Valerio A, D'Antona G, Nisoli E. Branched-chain amino acids, mitochondrial biogenesis, and healthspan: an evolutionary perspective. Aging. 2011; 3: 464–478.

[107] Psani M, Kotzekidou P. Technological characteristics of yeast strains and their potential as starter adjuncts in Greek-style black olive fermentation. World Journal of Microbiology and Biotechnology. 2006; 22: 1329–1336. DOI: 10.1007/s11274-006-9180-y.

[108] Silva T, Reto M, Sol M, Peito A, Peres CM, Peres C, Malcata XF. Characterization of yeasts from Portuguese brined olives, with a focus on their potentially probiotic behavior. LWT Food Science and Technology. 2011; 44: 1349–1354. DOI: 10.1016/j.lwt.2011.01.029.

[109] 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: 194–202. DOI: 10.3389/ fmicb.2012.00194.

[110] Kourelis A, Kotzamanidis C, Litopoulou-Tzanetaki E, Scouras ZG, Tzanetakis N, Yiangou M. Preliminary probiotic selection of dairy and human 227 yeast strains. Journal of Biological Research Thessaloniki. 2010; 13: 93–104.

[111] Moslehi-Jenabian S, Lindegaard Pedersen L, Jespersen L. Beneficial effects of probiotic and food borne yeasts on human health. Nutrients. 2010; 2: 449–473. DOI: 10.3390/nu2040449.

[112] Cappelletti GM, Nicoletti GM, Russo C. Wastewater from table olive industries. In: Fernando Sebastian Garcia Einschlag, editor. Waste Water – Evaluation and Manage-

ment. InTech; 2011. ISBN: 978-953-307-233-3. Available from: http://www.intechopen.com/books/waste-water-evaluation-and-management/wastewater-from-table-olive-industries.

[113] Aggelis GG, Gavala HN, Lyberatos G. Combined and separate aerobic and anaerobic biotreatment of green olive debittering wastewater. Journal of Agricultural Engineering Research. 2001; 80: 283–92.

[114] Rivas FJ, Beltran FJ, Alvarez P, Frades J, Gimeno O. Joint aerobic biodegradation of wastewater from table olive manufacturing industries and urban wastewater. Bioprocess Engineering. 2000; 23: 283–286.

[115] Borja R, Martin A, Alonso V, Garcia I, Banks CJ. Influence of different aerobic pretreatments on the kinetics of anaerobic digestion of olive-mill wastewater. Water Research. 1995; 29: 489–495.

[116] Kyriacou A, Lasaridi KE, Kotsou M, Balis C, Pilidis G. Combined bioremediation and advanced oxidation of green table olive processing wastewater. Process Biochemistry. 2005; 40: 1401–1408.

[117] Beltran J, Gonzalez T, Garcia J. Kinetics of the biodegradation of green table olive wastewaters by aerobic and anaerobic treatments. Journal of Hazardous Materials. 2008; 154: 839–845.

[118] Brenes M, Garcìa P, Romero C, Garrido A. Treatment of green table olive wastewaters by an activated-sludge process. Journal of Chemical Technology and Biotechnology. 2000; 75: 459–463.

[119] Kotsou M, Kyriacou A, Lasaridi K, Pilidis G. Integrated aerobic biological treatment and chemical oxidation with Fenton's reagent for the processing of green table olive wastewater. Process Biochemistry. 2004; 39: 1653–1660.