Chapter 15

Microbiological Aspects of Table Olives

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Additional information is available at the end of the chapter

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1. Introduction

Table olives are the most important fermented vegetables because of their worldwide economic importance. The three main techniques for table olive production used in Italy concern 82% green olives, 16% black olives and 2% processed at the cherry ripened stage (UNAPROL, 2008). There are three main trade preparations of table olives: Spanish-style olives, Californian-style olives and naturally black or turning colour olives (Garrido-Fernández et al., 1997). The Spanish processing method includes treatment with sodium hydroxide solution, washing, brining, fermentation and packaging. The Greek-style method is milder and includes washing, natural fermentation in brine, air-oxidation for colour improvement, and packing. The Californian method includes lye treatment, washing, iron-salt treatment and air-oxidation, canning and heat treatment. This last method includes a final sterilization, so it is usually considered as a more safe production. Besides these most prominent preparations, there are many other traditional table olive elaboration recipes that are less known in the international market (Panagou et al., 2003) but very relevant at local market level, where they are frequently sold in in glass jars or plastic pouches.

In the South of Italy the main traditional process method for table olive production is the natural olive process, according which untreated, generally green, olives are washed, put into containers and then filled with freshly prepared brine. Both treated and natural (un-treated) olives have to fermented, and in order to enhance their safety extent, the current practice requires the reduction of pH to a value of 4.5 or below. The fruits are maintained in the brine until they lose their natural bitterness, at least partially (Arroyo-López et al., 2008a), and where they undergo the fermentation process whose characteristics depend on the cultivar and on the applied conditions. At the end of the process the olives acquired typical characteristics of final products.

Olives contain a significant amount of oil, ranging from 12% to 30%, depending on the considered cultivar. The fermentable carbohydrates of flesh olives generally ranges from 2% to 6%, however, when the olives are washed or lye treated sugars are also lost along with

other soluble compounds. Olive fermentation is considered to be over when the sugars are totally consumed by microorganisms. The months necessary for this process might change depending on several factors, such as the variety and olive size, the salt concentration and temperature (Cardoso et al., 2010). Nowadays there are neither physico-chemical nor microbiological controls to objectively determine the end of fermentation and producers decide, according to personal criteria, when olives are ready to eat (Hurtado et al., 2008).

Other important components of olives are polyphenols. The most important classes of phenolic compounds in table olives are phenolic acids, phenolic alcohols, flavonoids, and secoiridoids (Sousa et al., 2006). The olive phenols are nutritionally interesting due to their antioxidant activities, moreover, these compounds are determinant in the shelf-life of olive oil and sensory qualities of both oil and table olives. Some of these, such as oleuropein and its hydrolysis derivatives, have antimicrobial activities against a wide variety of microorganisms, including lactic acid bacteria (LAB). The inhibiting effect of many polyphenols on LAB growth has been widely studied (Fleming et al., 1973; Ruiz-Barba et al., 1993). Moreover, the increase of oleuropein content in the growth medium reduces the activity of bacteria to hydrolyse this glycoside (Romeo & Poiana, 2007). Recently several studies on antimicrobial activity of olive products have been carried out, namely with olive leaves, olive fruits and their pure compounds, such as oleuropein, hydroxytyrosol and aliphatic aldehydes (Sousa et al., 2006) and it has been found that ferulic acid exhibits toxicity effects toward several microorganisms (Sayadi et al., 2000).

Compared to the relatively few microbial species employed in other fermented foods, microorganisms evolved in vegetable fermentations are many and different. In olive fruits the epiphytic microbial population consists of yeast, fungi, and both Gram positive and Gram negative bacteria but throughout the fermentation process, *Enterobacteriaceae*, LAB and yeasts are the most relevant microorganisms (Garrido-Fernández et al., 1997).

It has been generally established that LAB are responsible for the fermentation of treated olives. While LAB and yeasts compete for the fermentation of untreated olives, and in some cases yeasts can be exclusively responsible for fermentation on untreated olives.

LAB usually isolated from fermentation brines of treated olives include both heterofermentative and homofermentative species (Hutkins, 2006), and *Lactobacillus plantarum* is considered essential to produce the lactic acid needed for preservation and typical flavour. *L. plantarum* generally coexists with a yeast population until the end of the fermentation process and during storage, although other microorganisms may be involved depending on the applied parameters of the process. Organic acids, such as lactic acid, and sodium chloride are primary preservatives for table olives. Olives show a water activity greater than 0.85 and a final pH close to 4.6 or below, which represent the most important hygienic limit to avoid microbiological risks for consumers.

The control of temperature during the fermentation steps often led to beneficial effects, especially in those region where the fermentation temperature follows environmental fluctuations. Unfortunately, in most companies the temperature control is not applicable because it is an expensive procedure.

The control of salt, temperature, anaerobiosis (or low oxygen percentage) and process hygiene is necessary for successful fermentation. Under appropriate conditions, most non-lactic acid bacteria will grow slower than LAB that are less affected and that will grow and rapidly produce acid compounds, mainly lactic acid. These acids, along with CO_2 that may also be produced, create an even more stringent environment for competitors. So the competitors disappear while LAB overcome the lactic acid fermentation.

In the past, fermentation was always considered to be an economical means for temporary preservation of different kind of foods. Nowadays, the consumption of fermented foods is also promoted because of their health benefits, nutritional value, sensorial properties and functionality. This last aspect has been studied for some foods, such as yogurt and fermented milk (Lavermicocca et al., 2005), but it must be improved with regards to vegetable fermentation. The challenge of the next years must be the enhancement of this research field and the design of new functional foods.

2. Microorganisms associated to table olives

2.1. Role of yeasts

The positive role of yeasts in table olive fermentation has recently been reconsidered. Yeasts are especially relevant in directly brined green and black natural olive fermentations, where fruits are not treated with NaOH solutions. In these conditions, in the first fermentation step the LAB growth is slow because of the presence of phenolic compounds in brine. Growth of oxidative yeasts and molds may occur in brine surfaces if the tanks are open. To prevent this growth, the air layer between the liquid and the top of tank must be reduced as much as possible.

The main roles of yeasts in the processing of fermented olives, are associated with the production of alcohols, ethyl acetate, acetaldehyde and organic acids, compounds that are relevant for the development of taste and aroma and for the preservation of the typical characteristics of this fermented food (Alves et al., 2012).

Some yeast species seem to improve the growth of LAB. Yeasts are able to synthesize substances such as vitamins, amino acids and purines, or breakdown complex carbohydrates, which are essential for the growth of *Lactobacillus* species that request a nutritionally rich environment for optimal growth (Viljoen, 2006). However, in table olive processing yeasts can also produce spoilage such as off-flavour production, clouding of brines and softening of fruits (Arroyo-López et al., 2008a).

Recently, molecular methods have been applied for the identification of yeast associated with table olives. These techniques confer a higher degree of accuracy in the final identification than classical biochemical methods. Deiana et al. (1992) observed that the species found depended on the degree of maturation of the olive fruits. The genera *Candida, Pichia, Rhodotorula, Saccharomyces, Debaryomyces, Kluyveromyces, Kloeckera, Torulopsis, Trichosporon* and *Cryptococcus* were found by several authors (Arroyo-López et al., 2008a; Rodríguez-Gómez et al., 2010). While the main frequently isolated species, in both naturally

black and Spanish-style olive brines, are *Candida boidinii*, *Candida diddensiae*, *Pichia anomala*, *Pichia kluyveri*, *Pichia membranifaciens* and *Saccharomyces cerevisiae* (Oliveira et al., 2004; Coton et al., 2005; Arroyo-López et al., 2006). Recently Rodríguez-Gómez and co-workers (2012) drawn up a list of isolates representative of the yeasts of table olives, and the most suitable yeasts to be used as starters, alone or in combination with LAB.

The interrelationships between *Lactobacillus* species and yeasts in table olives may also play an essential role in product preservation. Several authors have recently focused their attention on yeast biodiversity associated with the different types of olive processes with particular regard to their enzymatic activities, in order to propose yeast as starters (Bautista-Gallego et al., 2011).

2.2. The microbiological hazards in table olives

Fermented vegetable technology is based on lactic acid and alcoholic fermentations, that convert sugars to different end-products, and the obtained food products take on new and different characteristics (Hutkins, 2006). To enhance the quality of final product is one of the main scientific and technological challenges for table olive production together with the reduction of cost of harvesting, of spoilage occurrence, and of environmental pollution (Brenes, 2004).

The olive-ecosystem is influenced by the indigenous microbial population, by the intrinsic factors related to the olives (pH, aw, phenols, sugar content, etc.) and extrinsic factors (temperature, oxygen and salt levels). The microbial population characterizing the first days of fermentation seem to be always the same: *Enterobacteriaceae*, lactic cocci, *Bacillaceae* and yeasts, whose evolution is strongly related to the pH value. In addition to these populations, Nychas and co-workers (2002) also reported the presence of *Pseudomonas* spp. at the start of fermentation, that decreased as other Gram-negative bacteria, within the first two weeks of fermentation.

Among the three main commercial preparations of table olives, there are some processing parameters affecting the fermentation process. The most important is the pH. The fermentation process in Spanish-style olives (treated olives) begins at an alkaline pH, higher than 9–10, because the fruits are previously treated with NaOH to hydrolyse the oleuropein (Medina et al., 2010). In this case, the microbial population is mainly composed of *Enterobacteriaceae*, lactic cocci and other epiphytic microorganisms which are able to drop the pH value below 7.0, creating the optimal conditions for LAB growth. Regarding the other two main preparations, Greek-style and California-style, the fermentation is influenced since the first steps by the processing conditions. In this case, the microbial population starts to grow at an acidic pH because organic acid (acetic, citric, lactic) are added to prevent the growth of Gram-negative bacteria.

No official microbiological criteria for table olives are available. However, the Standards of the Codex Alimentarius prescribes the minimum requirements related to hygiene for table olives. The final product shall be free from microorganisms and parasites in amounts which

may represent a hazard to health and shall not contain any substance originating from microorganisms in amounts which may represent a hazard to health (Pereira et al., 2008). To reduce the risk of food-borne illness and spoilage phenomenon, good practices in agriculture (GAP), hygiene (GHP) and manufacturing (GMP) must be applied.

Although heat treatments have some negative effects such as alterations in consistency and colour (Romeo et al., 2009), the correct use of temperature during pasteurization and/or sterilization is essential to ensure microbiological safety and stability, inactivating enzymes, and lessens the oxidizing processes. If heat sterilization is applied to olives, the treatment must be sufficient both in time and temperature, to destroy spores of *Clostridium botulinum* (COI, 2004). While olives preserved by salt and acidification or natural fermentation, are usually *C. botulinum* and its toxin free, only if the pH is constantly monitored and maintained below 4.6. Clostridial bacteria are relatively common in the environment because they are spore-forming. Spores of *C. botulinum* were detected both in pasteurized and sterilized olives (Pereira et al., 2008) indicating a poor attention to the application of sterilisation parameters. The occurrence of *C. botulinum* appears, however, to be rare. The sulphite reducing *Clostridium* spores are indicators of remote faecal contamination. Their presence in pasteurized olives is due to the occurrence of anaerobic fermentations or to the resistance of spores to pasteurization. However, the spores should be destroyed by sterilisation as its presence in a sterilised product indicates either inadequate heat treatment or post-sterilisation contamination.

The occurrence of *Listeria monocytogenes* in green table olives has been assessed, demonstrating that the product, despite its low pH and high salt concentration, can support *Listeria* survival for which an appropriate heat treatment must be applied (Caggia et al., 2004). Another hazard in table olives is *Escherichia coli* O157:H7, a pathogenic bacterium responsible for hemorrhagic colitis and hemolytic uremic syndrome. Its presence may be particularly associated to the Spanish-style method because the drop in the pH is slower than in natural fermented olive brine. The death rate of *E. coli* could be affected by using starter strain (Spyropoulou et al., 2001). More recently, the species *Enterobacter cloacae*, an opportunistic pathogen for humans, has been recovered in spontaneously fermented table olives (Bevilacqua et al., 2010a). The occurrence of *Listeria, Salmonella*, *Escherichia coli*, *Yersinia* pathogen strains and others are extensively reported in the scientific report of the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), issued on 21 February 2012.

Other than the pH value, a parameter which strongly influences the storage and quality of table olives is NaCl concentration. Its level is important for achieving stability of the products because it prevents spoilage and growth of pathogens. During recent years, consumers have developed an attitude on low sodium intake principally because a diet rich in sodium leads to higher blood pressure. So, several scientific studies (Arroyo-López et al., 2008b; Romeo et al., 2009; Bautista-Gallego et al., 2010; Bautista-Gallego et al., 2011; Panagou et al., 2011) have focalized on the viability, application and consequences of replacement of sodium with calcium or potassium in table olive fermentation. Apparently, NaCl may be substituted in diverse proportions with KCl or $CaCl_2$ without substantially altering the

usual fermentation profiles and producing good sensorial characteristics. In particular, a mixture of NaCl, $CaCl_2$ showed the ability to reduce both bacterial and yeast growth, while KCl showed similar effect of NaCl. Moreover, using different mixed salts, Tsapatsaris and Kotzekidou (2004) showed that the replacement of NaCl by KCl in Kalamon olives resulted in a strong synergy between calcium lactate and calcium acetate with higher growth rates of starter cultures of *Lactobacillus plantarum* and *Debaryomyces hansenii*.

The replacement of NaCl with other chlorides could be important in those productions traditionally processed in a high salt concentration, such as Greek-style olives, because this action could lower the NaCl concentration without reaching the lowest limits necessary to obtain a safe product. Therefore, besides the pH decrease and the NaCl concentration, several actions have been proposed in order to overcome all the fermentation problems: pasteurization, addition of sugars (glucose and sucrose), extra salt addition and use of starter cultures. Sugar supplements increase the pH drop rate reducing the dangerous early stage and ensuring the safety of the final product (Chorianopoulos et al., 2005).

2.3. LAB starter cultures

At industrial level LAB play a positive role in the production of wines and beers, but therefore they represent major spoilage organisms for such products (Bamforth, 2005). Table olive processing is still based on empirical methods despite its growing economical value. However, interest in developing starter cultures to be used in table olives is increasing. LAB have long been employed in fermentation as a food preservation technique owing to their progressive acidification of the fermenting brine with a consequent pH decrease (Marsilio et al., 2005). In addition, the use of LAB could standardize olive fermentation and reduce the use of highly polluting chemicals as NaOH (lye solution), contribute significantly to storage preventing microbial spoilage, and improve flavour.

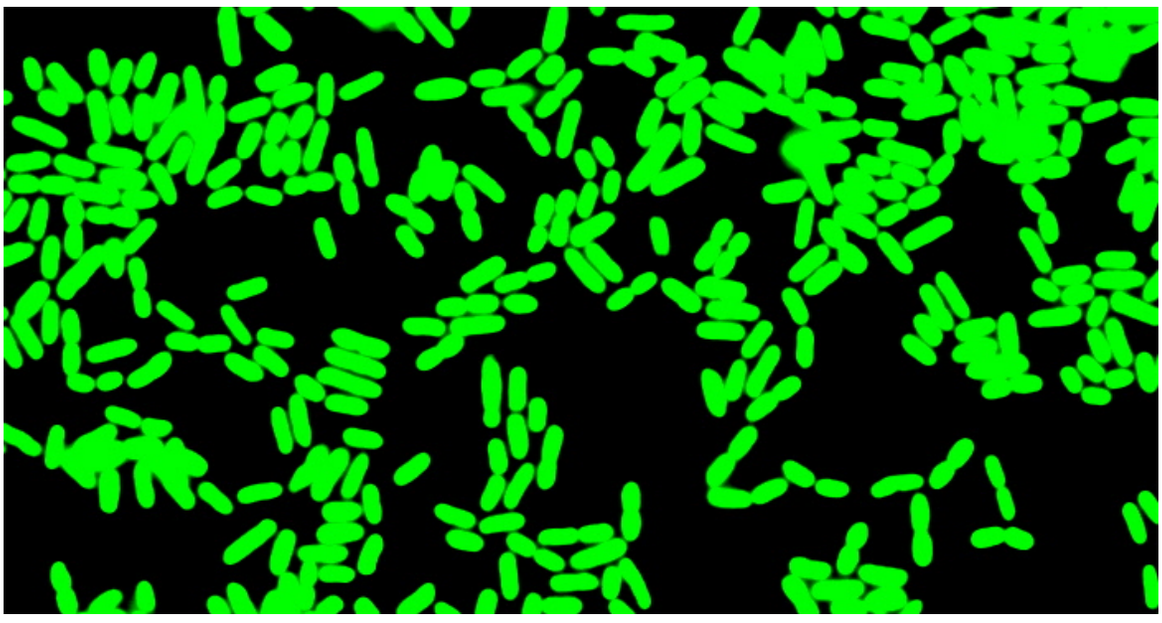

Figure 1. *L. plantarum* strain detected by fluorescence microscopy.

In table olive processing, starter cultures must have some properties such as good resistance to the inhibitory effect of polyphenols, good survival against wild strains of related species, rapid acid production, complete utilization of fermentable sugars, good tolerance against high levels of salt and low pH, and a possibly inhibitory effect against undesirable organisms. The latter effect is due to the production of bacteriocin, peptides that were found to be active against a number of natural competitors of *L. plantarum* in the fermentation brines and also against bacteria that can cause olive spoilage (Leal-Sánchez et al., 2003). This property is considered of importance in the development of new preservation technologies of foods (Devlieghere el al., 2004).

Moreover, lactobacilli are important members of the healthy human microbiota and exert several beneficial physiological effects, such as antimicrobial and antitumorigenic activities (Nguyen et al., 2007; Bevilacqua et al., 2010b). The reduction of cholesterol by LAB has been demonstrated in human, mouse, and pig studies (Nguyen et al., 2007). Nowadays, foods fortified with health-promoting probiotic bacteria are mainly produced with milk derivatives, so functional food industries are focusing on new non-dairy foods that can contribute to a regular assumption of probiotics (Lavermicocca et al., 2005).

The species of the genus *Lactobacillus* are widely occurring in many natural environments often playing important roles in fermentation processes and in the regulation of relationships among species of complex ecosystems. In particular, *L. plantarum* and *L. pentosus* are regarded as the main species leading this process (Table 1) often being used as a starter in guided olive fermentation (Sánchez et al., 2001; Leal-Sánchez et al., 2003; Hurtado et al., 2009). *L. pentosus* and *L. plantarum* are also the most frequently isolated species in table olives; the other species used as inocula, with little exception, have always been studied in conjunction with them (Hurtado et al., 2012).

However, a significant occurrence of *Leuconostoc* spp. on olive fruits and leaves was highlighted in the study of Ercolini and co-workers (2006), suggesting that *Lactobacillus* spp. may also originate from the environment or tools of production and not exclusively from the olives. Lavermicocca and others (2005) used table olives as a vehicle for delivering probiotic bacterial species, such as *Lactobacillus rhamnosus*, *L. paracasei*, *Bifidobacterium longum* and *B. bifidum*, but these strains are not involved in spontaneous fermentation and so they are not well adapted to the environmental conditions of table olives (Perricone et al., 2010).

Isolation from olive brines of *Enterococcus* strains has been reported by several authors, so mixed starters of *E. faecium* and *L. plantarum* (Lavermicocca et al., 1998) or *E. casseliflavus* and *L. pentosus* have been studied. The suggestion to inoculate *E. casseliflavus,* isolated from fermenting olives, is due to its good tolerance to the initial high pH (in case of lye treatment), without the drawback of transmissible antibiotic resistance shown by *E. faecium* (de Castro et al., 2002).

The selection of starters is based on diverse criteria including homo- and hetero-fermentative metabolism, acid production, salt tolerance, flavour development, temperature range growth, oleuropein-splitting capability and bacteriocin production (Panagou et al.,

2008). Furthermore, the ability to grow at low temperatures must be considered essential in cold regions, since heating brine is complex and expensive (Durán Quintana et al., 1999).

Genus	Species	Authors & year
Lactobacillus	*plantarum*	Randazzo et al., 2011 Ruiz-Barba et al., 2010 Perricone et al., 2010 Hurtado et al., 2010 Kumral et al., 2009 Sabatini et al., 2008 Panagou et al., 2008 Saravanos et al., 2008 Romeo & Poiana, 2007 Marsilio et al., 2005 Chorianopoulos et al., 2005 Lamzira et al., 2005 Caggia et al., 2004 Leal-Sánchez et al., 2003 Sánchez et al., 2001
	pentosus	Aponte et al., 2012 Hurtado et al., 2010 Medina et al., 2009; 2008 Panagou et al., 2008 Peres et al., 2008 Romeo & Poiana, 2007 Servili et al., 2006 Caggia et al., 2004 De Castro et al., 2002 Sánchez et al., 2001
	casei	Randazzo et al., 2011 Caggia et al., 2004
	paracasei	De Bellis et al., 2010 Saravanos et al., 2008
	paraplantarum	Romeo & Poiana, 2007
	brevis	Kumral et al., 2009 Romeo & Poiana, 2007
	coryniformis	Aponte et al., 2012
Leuconostoc	*cremoris*	Kumral et al., 2009
	paramesenteroides	Kumral et al., 2009
Pediococcus	*pentosaceus*	Ruiz-Barba et al., 2010
Enterococcus	*faecium*	Ruiz-Barba et al., 2010
	casseliflavus	De Castro et al., 2002

Table 1. LAB tested as starter cultures in table olives (references are shown for the last twelve years)

3. Enumeration of microorganisms in fermented olives: Methods of analysis

Each microorganism should grow and form a separate colony when the sample is plated in a solid medium during plate count procedures. Unfortunately, some organisms may not be capable to grow under the conditions used. Moreover, some chains of organisms could appear as a single colony (Swanson et al., 2001).

Up to now, methods available for detection and identification of microbial population involved in table olive fermentation have been very limited and generally culture-dependent, not providing reliable information on the composition of the entire microbial community (Randazzo et al., 2012). A culture-independent method, such as the denaturing gradient gel electrophoresis (DGGE), has the potential to study microbial population quickly and economically, avoiding the use of selective cultivation and isolation of bacteria (Rantsiou et al., 2005). So, plate count techniques are still the major but not the most representative method. Using aseptic techniques, brine samples may be taken and used directly to prepare serial dilutions. In order to analyse the whole olive, for example dry salted olives, the containers should be shaken and after mixing the olives, a sample should be taken aseptically with a sterile spoon, weighed and transferred to a sterile container such as plastic bag. After adding a sterile volume of quarter-strength Ringer's solution or 0.9% NaCl, the bag is then pummelled in a stomacher to prepare dilutions. Each mL collected by this bag represents 1 g of olive sample. The decimal dilutions are usually used for the calculation of results. Different ranges to obtain a readable number of colonies on plates may be necessary depending on the microorganism, procedures and initial contamination of olives. After pipetting the diluted sample into the petri plate, about 15 mL of each liquefied medium is added (the temperature must not exceed 45°C) into the plate. The medium is mixed with inoculum by carefully rotating. Several dilutions or replicate plates for each dilution should be prepared for each sample, making sure that the sample is tested, at least, in triplicates. After solidification, the petri plates should be inverted and placed in the incubator at specific temperature shown in the next sections. After incubation, the plates should contain between 25 and 250 colonies for the best count accuracy (Swanson et al., 2001). If microbial changes during fermentation are to be followed, the sampling must start at time zero, when the olives are salted or brined, and samples should be collected at regular intervals up to the end of fermentation. The sampling intervals may be of 1-3 days during the first month of fermentation, then these intervals may be extended up to 7 days. The brine samples, such as olives, should be examined as soon as possible, but if it is temporarily impossible, the samples must be refrigerated and analysed within 24 hours.

3.1. Lactic acid bacteria

The simply enumeration of LAB may be carried out on MRS agar. It is probably the most commonly used medium for the cultivation of lactobacilli and other LAB (Schillinger and Holzapfel, 2003). This medium must be supplemented with nystatin (50 mg/L) or

cycloheximide (100 mg/L) as inhibitors of eukaryotic organisms (to prevent yeasts and molds growth) or with sodium azide (200 mg/L) as Gram-negative bacteria inhibitor. A number of differential and selective media were created for the isolation and characterization of certain groups of LAB. HHD medium is the most used for the differential enumeration of homofermentative and heterofermentative LAB. This medium contains fructose which is reduced to mannitol by heterofermentative but not by homofermentative LAB. Differences in the colour of the colonies are based on differences in the amounts of acids produced by these bacterial groups (Schillinger & Holzapfel, 2003). So in this medium, homofermentative LAB are blue to green, while heterofermentative LAB colonies are white (Fleming et al., 2001). M17 medium should be used for lactococci isolation, but isolation of these LAB is more frequent when analysing dairy products rather than fermented vegetables. All LAB isolation requires anaerobic conditions and incubation at 30-32°C for 48-72 hours, depending on the medium used.

3.2. Mesophilic aerobic bacteria

The mesophilic count, or standard plate count, is generally obtained on Plate Count agar (PCA) which is a generic medium for aerobic microorganisms that grow at mesophilic temperatures. Aerobic plate counts are poor indicators of safety in some products such as those fermented which commonly show a high aerobic count. However, this count gives information about the hygienic and sensorial quality, about the adherence to good manufacturing practice and shelf life of the product (Morton, 2001).

Alternatively to PCA, similar generic media are available as Nutrient agar. The growth conditions are 25-30°C for 24-48 hours, aerobically.

3.3. Yeasts and molds

Yeasts and molds are widely diffused eukaryotic microorganisms because of their adaptation to different environmental conditions. Yeasts and molds can cause various degrees of food decomposition. Invasion and growth may occur on virtually any type of food (Beuchat & Cousin, 2001). Their contamination of food can lead to product losses but, in particular, the highest risk is due to the mycotoxin production by the molds. Several yeasts genera may be important for the sensorial properties of fermented products and for their interrelation with *Lactobacillus* bacteria (see section 2.1) in fermented olives. However, film-forming yeasts as *Debaryomyces, Candida, Pichia* and *Endomycopsis*, are often associated with pickled products and vegetable brines (Fleming et al., 2001), representing the cause of olive defects and consequent product losses.

Available media for yeasts and molds count are several: Rose Bengal Chloramphenicol agar, YM agar, Oxytetracycline Glucose Yeast Extract agar, Sabouraud agar and others. When not included in the medium, the addition of 100 mg/L chloramphenicol is recommended to inhibit the growth of bacteria. For simple enumeration of yeasts and molds, the plates should be incubated aerobically at 25°C for 48 hours.

3.4. *Enterobacteriaceae* and coliform bacteria

The *Enterobacteriaceae* family consists of Gram-negative, facultative anaerobic rods widely distributed in the environment, but which are usually associated with intestinal infections.

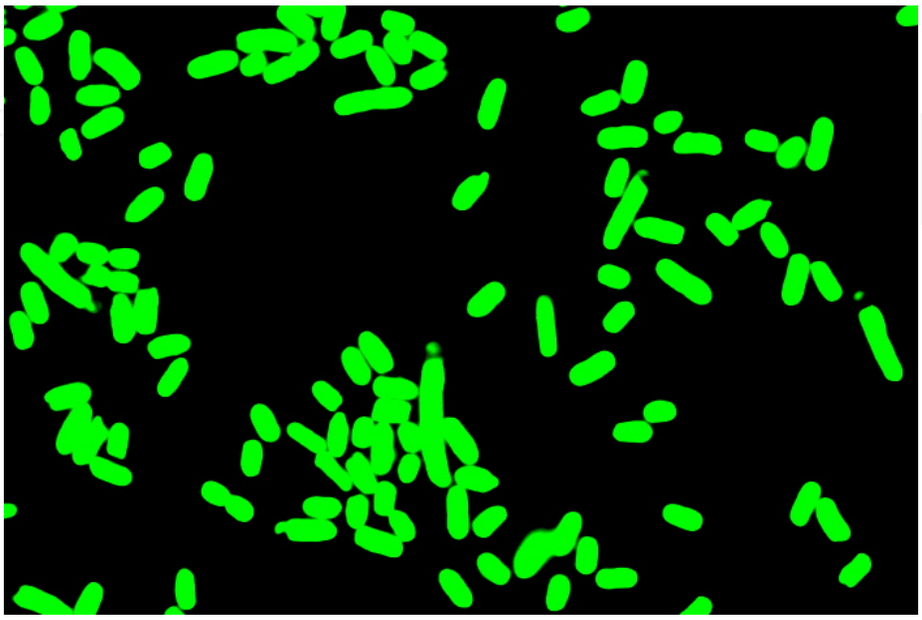

Figure 2. *E. coli* strain detected by fluorescence microscopy.

Numerous studies have determined that *Escherichia coli*, coliforms, faecal coliforms and *Enterobacteriaceae* are unreliable when used as index of pathogen contamination of food. So National and International advisory committees invalidated the prediction of food safety based on levels of *Enterobacteriaceae*, coliforms, faecal coliforms and *E. coli* (Kornacki & Johnson, 2001). The most important application of *Enterobacteriaceae* and coliforms is their enumeration to assess if pasteurization has been adequately performed for example in pasteurized milk, because a proper pasteurization under appropriate conditions inactivates *E. coli* cells present in raw material.

Total coliform bacteria belong to the *Enterobacteriaceae* family, and include *E. coli* as well as various members of the genera *Enterobacter, Klebsiella* and *Citrobacter*. All ferment lactose with gas and acid production in 48 hours at 35-37°C. While faecal coliforms are coliforms which can ferment lactose to acid and gas within 48 hours at 45°C and they are so called because are more closely associated with faecal pollution (Manafi, 2003). The term coliform is based only on lactose hydrolysis and has no taxonomy validity. In order to perform the best examination of olive samples, it is preferable enumerate both *Enterobacteriaceae* and coliforms, because some lactose-negative bacteria, such as *Salmonella,* are pathogens.

The most probable number (MPN) is a method which indicates the most likely number of microorganisms present in the analysed sample. This method, used for several years, has now been replaced with techniques on agar media because the MPN is based on a statistical approximation. So, in addition to Violet Red Bile agar or Violet Red Bile Glucose agar, other differential media based on a chromatic response were developed.

Mac Conkey's MUG agar and Eosin Methylene Blue agar (EMB) are differential and selective media, suitable to obtain at the same time the isolation of *Salmonella, Shigella* and coliform bacteria, in particular *E. coli*. In the first medium, lactose-negative colonies are colourless, lactose-positive colonies are red and often surrounded by a turbid zone due to the precipitation of bile acids. *E. coli* can be identified by fluorescence in UV due to its β-D-glucoronidase production. In EMB agar, lactose-fermenters form colonies with dark-blue centres (*E. coli* may also have a green metallic sheen) while the non-lactose fermenters form completely colourless colonies.

3.5. *Staphylococcus aureus*

The foods most associated with staphylococcal poisoning are meat products, dairy products and cream filled bakery products. In processed foods in which *S. aureus* is destroyed by processing, its presence usually indicates post-processing contamination from human skin, mouth, nose or food handlers (Lancette & Bennett, 2003). Due to the high salt tolerance of *S. aureus*, it can grow in table olives even though the low pH and the olive phenols may represent natural inhibitors (Tassou & Nychas, 1994). However, it may be isolated and enumerated in table olives for the same above mentioned reason (as contamination index).

A variety of coagulase-positive and coagulase-negative staphylococci are able to produce enterotoxins, but *S. aureus* still plays a predominant role in staphylococcal food poisoning.

Baird-Parker agar and Rabbit Plasma Fibrinogen agar are the media recommended by the International Organisation for Standardisation (ISO). Moreover, Baird-Parker agar is also used in the official AOAC method in the United States (Zangerl &Asperger, 2003). Tellurite reduction, egg yolk reaction and a high level of sodium chloride are the most applied selective chemicals added in media for *S. aureus* isolation and enumeration.

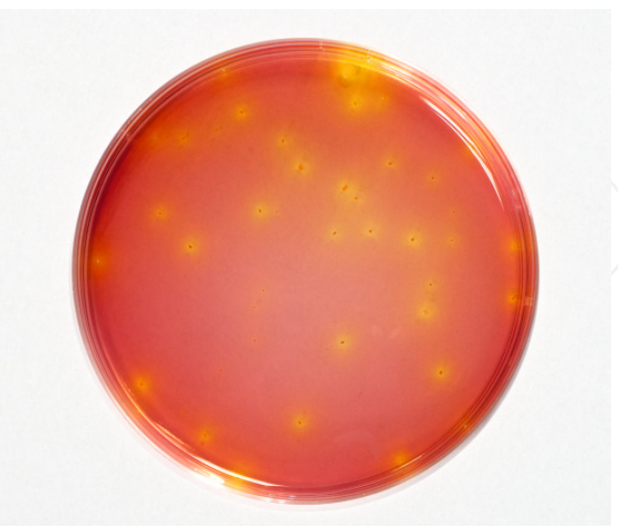

Figure 3. *S. aureus* colonies on Mannitol Salt Agar.

S. aureus colonies in Baird-Parker agar are black, with an opaque zone around the colony because of the egg yolk reaction. Another medium frequently used is the Mannitol Salt agar,

which contains mannitol. Coagulase-positive staphylococci grow and produce acid from mannitol showing a yellow colony and halo in a red medium. For all these media, the chemical inhibitors usually used are not completely selective, so additional diagnostic tests may be necessary to identify *S. aureus* colonies. Microscopic examination, catalase test and coagulase test may be rapidly executed to identify *S. aureus* from isolates. Common MPN procedures may be used also for enumeration of *S. aureus*. In most cases, the methodologies need a liquid enrichment procedure to detect low numbers of staphylococci (<100 UFC/g).

Culture conditions are usually 37°C for 48 hours aerobically.

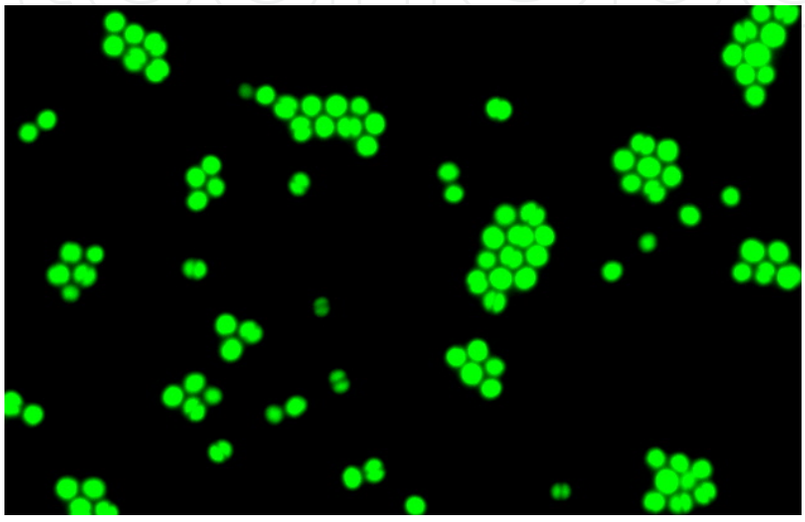

Figure 4. *S. aureus* strain detected by fluorescence microscopy.

3.6. Sulphite reducing clostridia

Clostridia are a widespread heterogeneous group of bacteria showing metabolic and nutritional differences. They easily contaminate foods because they produce resistant spores which can survive under mild processing conditions. The group of sulphite reducing clostridia may be used as marker of raw material quality and hygiene of manufacturing practices. Of particular concern for public health are *C. botulinum*, which forms a deadly toxin in foods, and *C. perfringens* which causes enteritis when present in high numbers. Other species or strains are also known to be toxinogenic or neurotoxinogenic (Bredius & Ree, 2003). *C. perfringens* is the agent of a food poisoning usually associated with consumption of cooked meats or poultry products. Sporulating cells of *C. perfringens* produce a heat-labile enterotoxin which appears to be released *in vivo* in the intestine (Labbe, 2001).

Most isolation media include sulphite and an appropriate iron salt. Sulphite is reduced to sulphide by the clostridial enzyme sulphite reductase; the sulphide will then precipitate as a black deposit in the presence of iron salt. This causes a blackening of the liquid media and clostridia will appear as black colonies. To obtain a higher selectivity for *C. perfringens* isolation, the Oleandomycin Polymixin Sulphadiazine Perfringens (OPSP) agar medium was performed. It contains selective antibiotics and utilises sodium metabisulphite and liver

extract as sources of H_2S with ferric ammonium citrate as the indicator. In any case, subsequent confirmation tests such as motility, reduction of nitrate, lactose fermentation, gelatin liquefaction (Labbe, 2001) should be necessary. The plates are incubated anaerobically at 37°C for 18-48 hours.

3.7. *Listeria monocytogenes*

Listeria species are ubiquitous organisms widely distributed in the environment, especially in plant matter and soil. The microorganism is established as an important foodborne pathogen, which can grow at high salt concentration (up to 10% of NaCl) and at refrigerated temperatures. A study carried out by the U.S. Food and Drug Administration, U.S. Department of Agriculture (USDA, 2001), indicated that vegetables are able to support *L. monocytogenes* growth and, after a few years, Caggia and co-workers (2004) demonstrated that *L. monocytogenes* can survive and grow in green table olives. As indicated by the most recent scientific report of the European Food Safety Authority (2012), the number of listeriosis cases in humans in the EU slightly decreased, and 1,601 confirmed human cases were reported in 2010.

The minimum infective dose of *L. monocytogenes* has not yet been established and many authorities require that the organism must be absent in 25 g of product. This has led to the development of methods following the sequence of pre-enrichment, selective enrichment and diagnostic plating. The whole procedure may take about five days. Then, presumptive positive results need to be confirmed adding further time to complete the examination (Beumer & Curtis, 2003).

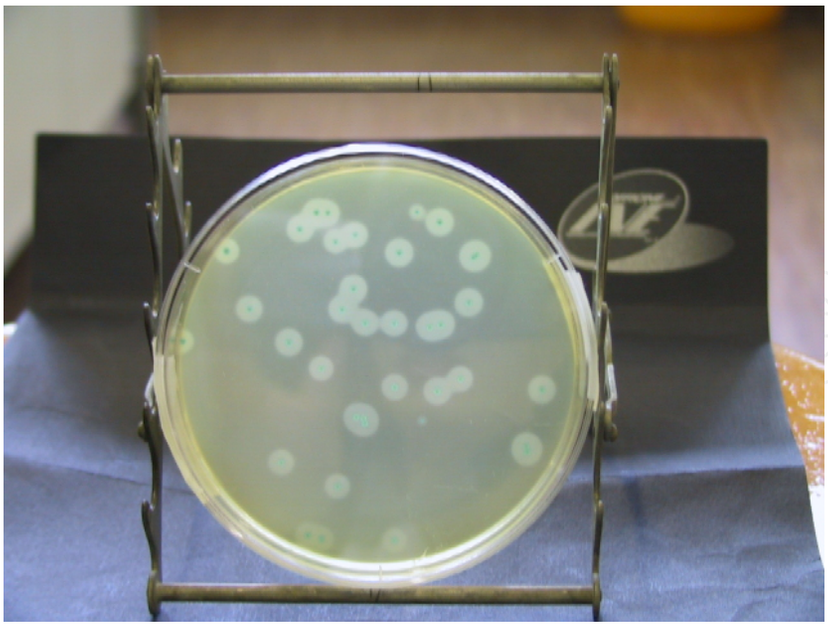

Figure 5. *L. monocytogenes* colonies on ALOA medium.

Most of the isolation media differentiate *Listeria* spp. by means of aesculin hydrolysis which, in the presence of iron, forms a black phenolic compound. According to the USDA method

(McClain & Lee, 1988) 25 mL of brine sample are added to 225 mL of Listeria Enrichment broth base for the resuscitation of stressed cells. After blending, the bag is incubated at 30°C and then, at intervals from 4 hours to 7 days, an aliquot is plated for enumeration in Listeria Selective agar base and incubated at 37°C for 24 h. Black colonies on selective agar base medium were considered presumptive *Listeria* colonies.

The presumptive *Listeria* isolates are to be tested for sugar fermentation, tumbling motility, hemolytic reaction and growth at different salt concentrations (Caggia et al., 2004). Alternatively to this medium, ALOA agar contains the chromogenic compound for the detection of β-glucosidase, common to all *Listeria*, which appear as blue coloured colonies. While *L. monocytogenes,* which possesses a specific phospholipase, hydrolyses the specific substrate added to the medium producing an opaque halo around the colonies (Beumer & Curtis, 2003). Another chromogenic medium used is the Rapid' L. mono agar.

Where counts < 100 CFU/g are expected, it is necessary to use the Most Probable Number (MPN) technique (USDA, 2001).

In order to rapidly perform additional confirmation tests, systems of strips are commercially available for *Listeria* (API Listeria) as for other pathogens.

3.8. Propionibacteria

Propionibacteria are often associated with food spoilage. The "zapateria" spoilage, which can occur in brined olives, is characterized by a malodorous fermentation due to propionic acid produced by certain species of *Propionibacterium* (Jay, 2000), alone or together with *Clostridium* spp.. The propionibacteria may be considered as marker of the end of shelf life of table olives, because the growth of these bacteria cause an increase in pH values creating conditions for the growth of spoilage or pathogen bacteria and the arising of off-odors (Plastourgos & Vaughn, 1957).

Unfortunately, propionibacteria are difficult to isolate because they grow very slowly on solid media. The complex medium usually used is not able to suppress competing organisms. This medium is Sodium Lactate agar (1% typticase, 1% yeast extract, 1% sodium lactate, 0.025% dipotassium phosphate, 1.5% agar) in which the propionibacteria growth appears in 5-7 days at 32°C under anaerobic or microaerophilic conditions (Richter & Vedamuthu, 2001).

4. Conclusion

A successful olive treatment depends on different factors. The olive cultivars show different fermentation behaviours when directly brined. In fact, besides the correct application of manufacturing practices, the knowledge of the chemical and physical characteristics of olive cultivar used and its attitude to treatments are decisive. A more complete knowledge of the olive cultivars is necessary to bring fermentation to a successful condition and attain a good final product. A correlation between each method and chemical-physical composition of

each cultivar is needed. Moreover, the presence of potential pathogens detected in all olive treatments by several authors suggests the necessity of pH control, following good hygienic practices throughout the process, and the necessity of a heat treatment of traditional products which are often empirically performed. Control and verification systems should be employed in order to guarantee a safe and hygienic product.

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5. References

Alves, M.; Gonçalves, T. & Quintas, C. (2012). Microbial quality and yeast population dynamics in cracked green table olives' fermentations. *Food Control*, Vol.23, pp. 363-368.

Aponte, M.; Blaiotta, G.; Croce, F.L.; Mazzaglia, A.; Farina, V.; Settanni, L. & Moschetti, G. (2012). Use of selected autochthonous lactic acid bacteria for Spanish-style table olive fermentation. *Food Microbiology*, Vol.30, No.1, pp. 8-16.

Arroyo López, F.N.; Durán Quintana, M.C.; Ruiz Barba, J.L.; Querol, A. & Garrido Fernández, A. (2006). Use of molecular methods for the identification of yeast associated with table olives. *Food Microbiology*, Vol.23, pp. 791–796.

Arroyo-López, F.N.; Querol, A.; Bautista-Gallego, J. & Garrido-Fernández, A. (2008 a). Role of yeasts in table olive production. *International Journal of Food Microbiology*, Vol.128, pp. 189-196.

Arroyo-López, F.N.; Bautista-Gallego, J.; Durán-Quintana, M.C.; Rodríguez-Gómez, F.; Romero-Barranco, C. & Garrido-Fernández, A. (2008 b). Improvement of the storage process for cracked table olives. *Journal of Food Engineering*, Vol.89, pp. 479-487.

Bamforth, C.W. (2005). The Science Underpinning Food Fermentations, In: *Food, Fermentation and Micro-organisms*, Blackwell Publishing (Ed.), 1-2, ISBN-13: 978-0632-05987-4, Oxford, UK.

Bautista-Gallego, J.; Arroyo-López, F.N.; Durán-Quintana, M. C. & Garrido-Fernández, A. (2010). Fermentation profiles of Manzanilla-Aloreña cracked green table olives in different chloride salt mixtures. *Food microbiology*, Vol.27, No.3, pp. 403-12.

Bautista Gallego, J.; Arroyo López, F.N.; Romero Gil, V.; Rodríguez Gómez, F.; García García, P. & Garrido Fernández, A. (2011). Chloride salt mixtures affect Gordal cv. green Spanish-style table olive fermentation. *Food Microbiology*, Vol.28, pp. 1316-1325.

Beuchat, L.R. & Cousin, M.A. (2001). Yeasts and Molds, In: *Compendium of methods for the Microbiological examination of Foods*, Fourth edition, F.P. Downes & K. Ito (Eds.), American Public Health Association, 209-215, ISBN: 0-87553-175-x, Washington DC.

Beumer, R.R. & Curtis, G.D.W. (2003). Culture media and methods for the isolation of *Listeria monocytogenes*, In: *Handbook of Culture Media for Food Microbiology*, J.E.L. Corry, G.D.W. Curtis & R.M. Baird (Eds.), Elsevier Science, 79-90, ISBN: 0-444-51084-2, Amsterdam, Netherlands.

Bevilacqua, A.; Cannarsi, M.; Gallo, M.; Sinigaglia, M. & Corbo, M.R. (2010 a). Characterization and implications of *Enterobacter cloacae* strains, isolated from Italian table olives "Bella di Cerignola". *Journal of Food Science*, Vol.75, pp. 53-60.

Bevilacqua, A.; Altieri, C.; Corbo, M.R.; Sinigaglia, M. & Ouoba, L.I.I. (2010 b). Characterization of lactic acid bacteria isolated from Italian Bella di Cerignola table olives: selection of potential multifunctional starter cultures. *Journal of Food Science*, Vol.75, No.8, pp. 536-544.

Bredius, M.W.J. & de Ree, E.M. (2003). Media for the detection and enumeration of clostridia in foods, In: *Handbook of Culture Media for Food Microbiology*, J.E.L. Corry, G.D.W. Curtis & R.M. Baird (Eds.), Elsevier Science, 49-60, ISBN: 0-444-51084-2, Amsterdam, Netherlands.

Brenes, M. (2004). Olive fermentation and processing: scientific and technological challenges. *Journal of Food Science*, Vol.69, No.1, pp. 33-34.

Caggia, C.; Randazzo, C.L.; Di Salvo, M.; Romeo, F.V. & Giudici, P. (2004). Occurrence of *Listeria monocytogenes* in green table olives. *Journal of food protection*, Vol.67, No.10, pp. 2189-94.

Cardoso, S.M.; Mafra, I.; Reis, A.; Nunes, C.; Saraiva, J.A. & Coimbra M.A. (2010). Naturally fermented black olives: Effect on cell wall polysaccharides and on enzyme activities of Taggiasca and Conservolea varieties. *LWT - Food Science and Technology*, Vol.43, pp. 153-160.

Chorianopoulos, N.G.; Boziaris, I.S.; Stamatiou, A. & Nychas, G.-J.E. (2005). Microbial association and acidity development of unheated and pasteurized green-table olives fermented using glucose or sucrose supplements at various levels. *Food Microbiology*, Vol.22, No.1, pp. 117-124.

COI (2004). Trade Standard Applying to Table Olives. *International Olive Oil Council*, COI/OT/NC no. 1, Dezembro de 2004.

Coton, E.; Coton, M.; Levert, D.; Casaregola, S. & Sohier, D. (2005). Yeast ecology in French cider and black olive natural fermentations. *International Journal of Food Microbiology*, Vol.108, pp. 130–135.

De Bellis, P., Valerio, F., Sisto, A., Lonigro, S. L., & Lavermicocca, P. (2010). 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*, Vol.140, No.1, pp. 6-13.

De Castro, A.; Montaño, A.; Casado, F.-J.; Sánchez, H. & Rejano, L. (2002). Utilization of *Enterococcus casselinflavus* and *Lactobacillus pentosus* as starter cultures for Spanish-style green olive fermentation. *Food Microbiology*, Vol.19, pp. 637-644.

Deiana, P.; Farris, G.A.; Catzeddu, P. & Madan, G. (1992). Impiego di fermenti lattici e lieviti nella preparazione delle olive da mensa. *Industrie Alimentari,* Vol.31, pp. 1011–1023.

Devlieghere, F.; Vermeiren, L. & Debevere, G. (2004). New preservation technologies: possibilities and limitations. *International Dairy Journal*, Vol.14, 273-285.

Durán Quintana, M.C.; García García, P. & Garrido Fernández, A. (1999). Establishment of conditions for green table olive fermentation at low temperature. *International Journal of Food Microbiology*, Vol.51, No.2-3, pp. 133-143.

EFSA & ECDC (2012). The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010. *EFSA Journal*, Vol.10(3):2597, pp. 1-442, Available from: http://www.efsa.europa.eu/efsajournal.

Ercolini, D.; Villani, F.; Aponte, M. & Mauriello, G. (2006). Fluorescence in situ hybridisation detection of *Lactobacillus plantarum* group on olives to be used in natural fermentations. *International Journal of Food Microbiology*, vol.112, No.3, pp. 291-296.

Fleming, H.P.; Mcfeeters, R.F; Thompson, R.L. & Sanders, D.C. (1973). Storage stability of vegetables fermented with pH control. *Journal of Food Science*, Vol.48, pp. 975-981.

Fleming, H.P.; Mcfeeters, R.F. & Breidt, F. (2001). Fermented and acidified vegetables, In: *Compendium of methods for the Microbiological examination of Foods*, Fourth edition, F.P. Downes & K. Ito (Eds.), American Public Health Association, 521-532, ISBN: 0-87553-175-x, Washington DC.

Garrido Fernández, A.; Fernández Díaz, M.J. & Adams, R.M. (1997). *Table Olives. Production and Processing*, Chapman & Hall, London, UK.

Hurtado, A.; Reguant, C.; Esteve-Zarzoso, E.; Bordons, A. & Rozès, N. (2008). Microbial population dynamics during the processing of Arbequina table olives. *Food Research International*, Vol.41, pp. 738-744.

Hurtado, A.; Reguant, C.; Bordons, A. & Rozès, N. (2009). Influence of fruit ripeness and salt concentration on the microbial processing of Arbequina table olives. *Food Microbiology*, Vol.26, No.8, pp. 827-833.

Hurtado, A.; Reguant, C.; Bordons, A. & Rozès, N. (2010). Evaluation of a single and combined inoculation of a *Lactobacillus pentosus* starter for processing cv. Arbequina natural green olives. *Food Microbiology*, Vol.27, No.6, pp. 731-740.

Hurtado, A.; Reguant, C.; Bordons, A. & Rozès, N. (2012). Lactic acid bacteria from fermented table olives. *Food microbiology*, Vol.31, No.1, pp. 1-8.

Hutkins, R.W. (2006). Fermented vegetables, In: *Microbiology and Technology of fermented foods*, Blackwell Publishing (Ed.), 233-260, ISBN-13: 978-0-8138-0018-9, Oxford, UK.

Jay, J.M. (2000). Fruit and Vegetable Products: Whole, Fresh-Cut, and Fermented In: *Modern Food Microbiology*, Sixth Edition, Aspen Publishers, Inc., 131-161, ISBN 0-8342-1671-X, Gaithersburg, Maryland.

Kornacki, J.L. & Johnson, J.L. (2001). Enterobacteriaceae, coliforms and *Escherichia coli* as quality and safety indicators, In: *Compendium of methods for the Microbiological examination of Foods*, Fourth edition, F.P. Downes & K. Ito (Eds.), American Public Health Association, 69-82, ISBN: 0-87553-175-x, Washington DC.

Kumral, A.; Basoglu, F. & Sahin, I. (2009). Effect of the use of different lactic starters on the microbiological and physicochemical characteristics of naturally black table olives of Gemlike cultivar. *Journal of Food Processing and Preservation*, Vol.33, pp. 651-664.

Labbe, R.G. (2001). *Clostridium perfringens*, In: *Compendium of methods for the Microbiological examination of Foods*, Fourth edition, F.P. Downes & K. Ito (Eds.), American Public Health Association, 325-330, ISBN: 0-87553-175-x, Washington DC.

Lamzira, Z.; Asehraou, A.; Brito, D.; Oliveira, M.; Faid, M. & Peres, C. (2005). Bloater spoilage of green olives. *Food Technology and Biotechnology*, Vol.43, pp. 373-377.

Lancette, G.A. & Bennett, R.W. (2001). *Staphylococcus aureus* and Staphylococcal enterotoxins, In: *Compendium of methods for the Microbiological examination of Foods*, Fourth edition, F.P. Downes & K. Ito (Eds.), American Public Health Association, 387-403, ISBN: 0-87553-175-x, Washington DC.

Lavermicocca, P.; Gobbetti, M.; Corsetti, A. & Caputo, L. (1998). Characterization of lactic acid bacteria isolated from olive phylloplane and table olive brines. *Italian Journal of Food Science*, Vol.10, pp. 27–39.

Lavermicocca, P.; Valerio, F.; Lonigro, S.L.; De Angelis, M.; Morelli, L.; Callegari, M.L.; Rizzello, C.G. & Visconti, A. (2005). Study of Adhesion and Survival of Lactobacilli and Bifidobacteria on Table Olives with the Aim of Formulating a New Probiotic Food. *Applied and Environmental Microbiology*, Vol.71, No.8, pp. 4233–4240.

Leal-Sánchez, M.V.; Ruiz-Barba, J.L.; Sánchez, A.H.; Rejano, L.; Jiménez-Díaz, R. & Garrido, A. (2003). Fermentation profile and optimization of green olive fermentation using *Lactobacillus plantarum* LPCO10 as a starter culture, *Food Microbiology*, Vol.20, pp. 421-430.

Manafi, M. (2003). Media for detection and enumeration of 'total' Enterobacteriaceae, coliforms and Escherichia coli from water and foods, In: *Handbook of Culture Media for Food Microbiology*, J.E.L. Corry, G.D.W. Curtis & R.M. Baird (Eds.), Elsevier Science, 167-194, ISBN: 0-444-51084-2, Amsterdam, Netherlands.

Marsilio, V.; Seghetti, L.; Iannucci, E.; Russi, F.; Lanza, B. & Felicioni, M. (2005). Use of a lactic acid bacteria starter culture during green olive (*Olea europaea* L cv Ascolana tenera) processing. *Journal of the Science of Food and Agriculture*, Vol.85, No.7, pp. 1084-1090.

McClain, D. & Lee, W.H. (1988) Development of USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poutry. *Journal of the Association of Official Analytical Chemists*, Vol.71, pp. 660-664.

Medina, E.; Romero, C.; de Castro, A.; Brenes, M. & García, A. (2008). Inhibitors of lactic acid fermentation in Spanish-style green olive brines of the Manzanilla variety. *Food Chemistry*, Vol.110, No.4, pp. 932-937.

Medina, E.; García, A.; Romero, C.; de Castro, A. & Brenes, M. (2009). Study of the anti-lactic acid bacteria compounds in table olives. *International Journal of Food Science & Technology*, Vol.44, No.7, pp. 1286-1291.

Medina, E.; Gori, C.; Servili, M.; De Castro, A.; Romero, C. & Brenes, M. (2010). Main variables affecting the lactic acid fermentation of table olives. *International Journal of Food Science & Technology*, Vol.45, No.6, pp. 1291-1296.

Morton, R.D. (2001). Aerobic Plate Count, In: *Compendium of methods for the Microbiological examination of Foods*, Fourth edition, F.P. Downes & K. Ito (Eds.), American Public Health Association, 63-67, ISBN: 0-87553-175-x, Washington DC.

Nguyen, T.D.T.; Kang, J.H. & Lee, M. S. (2007). Characterization of *Lactobacillus plantarum* PH04, a potential probiotic bacterium with cholesterol-lowering effects. *International Journal of Food Microbiology*, Vol.113, No.3, pp. 358-61.

Nychas, G.-J.E.; Panagou, E.Z.; Parker, M.L.; Waldron, K.W. & Tassou, C.C. (2002). Microbial colonization of naturally black olives during fermentation and associated biochemical activities in the cover brine. *Letters in Applied Microbiology*, Vol.34, pp. 173-177.

Oliveira, M.; Brito, D.; Catulo, L.; Leitao, F.; Gomes, L.; Silva, S.; Vilas-Boas, L.; Peito, A.; Fernandes, I.; Gordo, F. & Peres, C. (2004). Biotechnology of olive fermentation of 'Galega' Portuguese variety. *Grasas Y Aceites*, Vol.55, pp. 219–226.

Panagou, E.Z.; Tassou C.C. & Katsaboxakis, C.Z. (2003). Induced lactic acid fermentation of untreated green olives of the Conservolea cultivar by *Lactobacillus pentosus*. *Journal of the Science of Food and Agriculture*, Vol.83, pp. 667-674.

Panagou, E.Z.; Schillinger, U.; Franz, C.M.A.P. & Nychas, G.-J.E. (2008). Microbiological and biochemical profile of cv. Conservolea naturally black olives during controlled fermentation with selected strains of lactic acid bacteria. *Food microbiology*, Vol.25, No.2, pp. 348-58.

Panagou, E.Z.; Hondrodimou, O.; Mallouchos, A. & Nychas, G.-J. E. (2011). A study on the implications of NaCl reduction in the fermentation profile of Conservolea natural black olives. *Food microbiology*, Vol.28, No.7, pp. 1301-1307.

Pereira, A.P.; Pereira, J.A.; Bento, A. & Estevinho, M.L. (2008). Microbiological characterization of table olives commercialized in Portugal in respect to safety aspects. *Food and Chemical Toxicology*, Vol.46, pp. 2895-2902.

Peres, C.; Catuloa, L.; Brito, D. & Pintadoa, C. (2008). *Lactobacillus pentosus* DSM 16366 starter added to brine as freeze-dried and as culture in the nutritive media for Spanish style green olive production. *Grasas y Aceites*, Vol.59, pp. 234-238.

Perricone, M.; Bevilacqua, A.; Corbo, M.R. & Sinigaglia, M. (2010). Use of *Lactobacillus plantarum* and glucose to control the fermentation of "Bella di Cerignola" table olives, a traditional variety of Apulian region (southern Italy). *Journal of Food Science*, Vol.75, pp. 430-436.

Plastourgos, S. & Vaughn, R.H. (1957). Species of *Propionibacterium* associated with zapatera spoilage of olives. *Applied Microbiology*, Vol.5, pp. 267-271.

Randazzo, C.L.; Fava, G.; Tomaselli, F.; Romeo, F.V.; Pennino, G.; Vitello, E. & Caggia, C. (2011). Effect of kaolin and copper based products and of starter cultures on green table olive fermentation. *Food Microbiology*, Vol.28, No.5, pp. 910-919.

Randazzo, C.L.; Ribbera, A.; Pitino, I.; Romeo, F.V. & Caggia, C. (2012). Diversity of bacterial population of table olives assessed by PCR-DGGE analysis. *Food Microbiology*, In Press, 18.06.2012, Available from: http://dx.doi.org/10.1016/j.fm.2012.04.013.

Rantsiou, K.; Urso, R.; Iacumin, L.; Cantoni, C.; Cattaneo, P.; Comi, G. & Cocolin, L. (2005). Culture dependent and independent methods to investigate the microbial ecology of

Italian fermented sausages. *Applied and Environmental Microbiology*, Vol.71, pp. 1977-1986.

Richter, R.L. & Vedamuthu, E.R. (2001). Milk and milk products, In: *Compendium of methods for the Microbiological examination of Foods*, Fourth edition, F.P. Downes & K. Ito (Eds.), American Public Health Association, 483-495, ISBN: 0-87553-175-x, Washington DC.

Rodríguez-Gómez, F.; Arroyo-López, F.N.; López-López, A.; Bautista-Gallego, J. & Garrido-Fernández, A. (2010). Lipolytic activity of the yeast species associated with the fermentation/storage phase of ripe olive processing. *Food Microbiology*, Vol.27, pp. 604-612.

Rodríguez-Gómez, F.; Romero-Gil, V.; Bautista-Gallego, J.; Garrido-Fernández, A. & Arroyo-López, F.N. (2012). Multivariate analysis to discriminate yeast strains with technological applications in table olive processing. *World Journal of Microbiology and Biotechnology*, Vol.28, pp. 1761-1770.

Romeo, F.V. & Poiana, M. (2007). Ability of commercially available *Lactobacillus* strains as starters in brining and debittering of table olives. *Acta Alimentaria*, Vol.36, No.1, pp. 49-60.

Romeo, F.V.; De Luca, S.; Piscopo, A.; Perri, E. & Poiana, M. (2009). Effects of post-fermentation processing on the stabilisation of naturally fermented green table olives (cv Nocellara Etnea). *Food Chemistry*, Vol.116, pp. 873-878.

Ruiz-Barba, J.L.; Brenes-Balbuena, M.; Jiménez-Díaz, R.; García-García, P. & Garrido Fernández, A. (1993). Inhibition of *Lactobacillus plantarum* by polyphenols extracted from two different kind of olive brine. *Journal of Applied Bacteriology*, Vol.74, pp. 15-19.

Ruiz-Barba, J.L.; Caballero-Guerrero, B.; Maldonado-Barragán, A. & Jiménez-Díaz, R. (2010). Coculture with specific bacteria enhances survival of *Lactobacillus plantarum* NC8, an autoinducer-regulated bacteriocin producer, in olive fermentations. *Food Microbiology*, Vol.27, No.3, pp. 413-417.

Sabatini, N.; Mucciarella, M.R. & Marsilio, V. (2008). Volatile compounds in uninoculated and inoculated table olives with *Lactobacillus plantarum* (*Olea europaea* L., cv. Moresca and Kalamata). *LWT - Food Science and Technology*, Vol.41, No.10, pp. 2017-2022.

Sánchez, A.H.; Rejano, L.; Montaño, A. & de Castro, A. (2001). Utilization at high pH of starter cultures of lactobacilli for Spanish-style green olive fermentation. *International journal of food microbiology*, Vol.67, No.1-2, pp. 115-22.

Sayadi, S.; Allouche, N.; Jaoua, M. & Aloui, F. (2000). Determinal effects of high molecular-mass polyphenols on olive mill wastewater biotreatment. *Process Biochemistry*, Vol.35, pp. 725–735.

Saravanos, E.; Kagli, D.; Zoumpopoulou, G.; Panagou, E.Z. & Tassou, C.C. (2008). 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.

Schillinger, U. & Holzapfel, W.H. (2003).Culture media for lactic acid bacteria, In: *Handbook of Culture Media for Food Microbiology*, J.E.L. Corry, G.D.W. Curtis & R.M. Baird (Eds.), Elsevier Science, 127-140, ISBN: 0-444-51084-2, Amsterdam, Netherlands.

Servili, M.; Settanni, L.; Veneziani, G.; Esposto, S.; Massitti, O.; Taticchi, A.; Urbani, S.; Montedoro, G.F. & Corsetti, A. (2006). 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*, Vol.54, pp. 3869-3875.

Sousa, A.; Ferreira, I.C.F.R.; Calhelha, R.; Andrade, P.B.; Valentão, P.; Seabra, R.; Estevinho, L.; Bento, A. & Pereira, J.A. (2006). Phenolics and antimicrobial activity of traditional stoned table olives 'alcaparra'. *Bioorganic & Medicinal Chemistry*, Vol.14, pp. 8533–8538.

Spyropoulou, K. E.; Chorianopoulos, N. G.; Skandamis, P. N. & Nychas, G. J. (2001). Survival of *Escherichia coli* O157:H7 during the fermentation of Spanish-style green table olives (Conservolea variety) supplemented with different carbon sources. *International journal of food microbiology*, Vol.66, No.1-2, pp. 3-11.

Swanson, K.M.J.; Petran, R.L. & Hanlin, J.H. (2001). Culture methods for enumeration of microorganisms, In: *Compendium of methods for the Microbiological examination of Foods*, Fourth edition, F.P. Downes & K. Ito (Eds.), American Public Health Association, 53-62, ISBN: 0-87553-175-x, Washington DC.

Tassou, C.C. & Nychas, G.J.E. (1994). Inhibition of *Staphylococcus aureus* by Olive Phenolics in Broth and in a Model Food System. *Journal of Food Protection*, Vol.57, No.2, pp. 120-124.

Tsapatsaris, S. & Kotzekidou, P. (2004). 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*, Vol.95, pp. 157-168.

UNAPROL (2008). Filiera olivicola - Analisi strutturale e monitoraggio di un campione di imprese. Campagna 2007-2008, Reg. CE 2080/2005, 05/12/2011, Available from http://www.unaprol.it/Pubblicazioni/FILIERA%20OLIVICOLA%20CAMP.%202007-08.PDF

U.S. Food and Drug Administration, and Center for Food Safety and Applied Nutrition, Department of Health and Human Services (2001). Bacteriological analytical manual online, 18.06.2012, Available from: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm.

Viljoen, B.C. (2006). Yeast ecological interactions. Yeast–yeast, yeast bacteria, yeast–fungi interactions and yeasts as biocontrol agents, In: *Yeasts in Food and Beverages*, Querol, A. & Fleet, H. (Eds.), 83–110, Springer–Verlag, Berlin.

Zangerl, P. and Asperger, H. (2003). Media used in the detection and enumeration of *Staphylococcus aureus*, In: *Handbook of Culture Media for Food Microbiology*, J.E.L. Corry, G.D.W. Curtis & R.M. Baird (Eds.), Elsevier Science, 91-110, ISBN: 0-444-51084-2, Amsterdam, Netherlands.