



Technological process of fermented olive

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Abstract

The natural fermentation process of green olives occurs in environment characterized by high polyphenols (i.e. Oleuropein) and sodium chloride contents, and low nutrients contents, while Lactic acid bacteria (LAB) (i.e. *L. plantarum*) and yeasts (i.e. *Candida*) were the dominant microorganisms. LAB and yeasts are reported to assure essential role in the natural fermentation process of green olives, and demonstrated various technological beneficial effects, mainly the production of organic acids, aroma, vitamins, amino acids, enzymes (i.e. β -glucosidase) Moreover, selected strains of these microorganisms (LAB and yeasts) can be used as starter in the controlled fermentation process, in order to increase the shelf life of fermented olives, and to improve the nutritional and sensorial characteristics of the product. This review focuses on the technological and beneficial properties of LAB and yeasts in the natural fermentation process of green olives.

Keywords: Olive, Fermentation, Lactic acid bacteria, Yeast, Technological properties

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Introduction

The olive tree is an important plant in the agricultural system of many countries with a Mediterranean climate, it occupies a world area of about 11,193,000 hectares. In Morocco, the olive tree is the main cultivated fruit species, with an area of about 1,000,000 hectares, covering the main agricultural areas of the country (Fez, Boulmane, Taounate, Meknes, Tafilalet, Marrakech, Beni Mellal, Tadla, Azilal, Oriental) [1].

The variety "Moroccan Picholine" represents more than 90% of the Moroccan olive heritage [2]. Several other local clones of the Moroccan Picholine (Dahbia, Menara Haouzia and Mesllala, etc.), and foreign varieties (Picholine of Languedoc, Picual, Frantoio, Manzanilla, Arbéquina, Arbosana, Koroneiki, etc.) are newly introduced. The Moroccan Picholine is characterized by its strong adaptation to the Moroccan pedoclimatic conditions and is also considered as a dual-purpose variety for olive oil and table olives.

The world production of table olives is approximately 2.65 million tons on 2015/2016. In Morocco the production is estimated to 120,00 tons, representing 4.6% of the world production [3], where 70% of this production is exported, while the rest (30%) is absorbed by the local market. Morocco is classified as the 4th world exporter with 10.7% of the world market, during the 2015-2016 campaign, after Egypt (11.3%), Argentina (13.1%) and the European Union (44.4%) [3].

The olive is a drupe that contains a bitter ingredient, oleuropein, a low sugar content (2.6 to 6%), and high oil content (12 to 30%) [3]. These characteristics make the olive a fruit that cannot be eaten directly. It must be subjected to treatments that vary considerably from one region to another and also depend on the olive variety [4].

The oleuropein from the olive must be eliminated because of it has a very pronounced bitter taste, although it is not harmful to health. The fruits are usually subjected to treatments with sodium or potassium hydroxide, brine, or successive washes according to local systems or habits [4].

Recently, the phenomenon of oleuropein tolerance and biodegradation has been demonstrated in certain species of lactic acid bacteria [5-11].

However, very few studies have been done on the effect of this phenolic compound on other species of lactic acid bacteria associated with the natural fermentation process of green olives, including *L. pentosus*, *L. brevis* and *L. pentosaceus* [9]. Especially since it has been shown that green olives of the Moroccan picholine variety are fermentable without the need for fruit debittering treatment [12]. This indicates the presence of strains of oleuropeinolytic bacteria in Moroccan green olives in natural fermentation.

This process of natural fermentation of olives is characterized by the coexistence of bacteria and yeast [13, 14]. However, the role of yeasts in this process of oleuropein biodegradation is not well elucidated. Thus, the in-vitro study of the interactions between lactic acid bacteria and yeasts, particularly

the biodegradation of oleuropein, would allow a better understanding of this phenomenon which takes place in the traditional process.

The objective of this paper is the study of the technological fermentation process of green olives and the conditions of biodegradation of oleuropein by lactic acid bacteria and yeasts, in order to allow the selection of starter capable of ensuring the biological debittering of green olives.

1. Green table olive

Table olive is prepared from the fruits of cultivated olive tree varieties (*Olea europaea*) and subjected to bitterness removal treatments and preserved by natural fermentation, or by heat treatment, with or without preservatives [15] (page 5).

Green table olives are the most popular fermented vegetable food in Morocco. They constitute a major agro-industrial sector in the economic development of the country. Among the industrial preparations of table olives are the Spanish method for the preparation of green olives, the Californian style for oxidized black olives, the Greek preparation for black olives called Greek style [13].

2. Nutritional richness of the olive

Olive pulp contains 60 to 68% of water, 12 to 28% of oil, 8 to 12% of carbohydrates and 0.7 to 2% of protein content [16]. Sugars, consisting of glucose, fructose, galactose and sucrose, are the most important fraction in the process of olive fermentation [17]. Organic acids, representing 0.1 to 2.1% of the pulp, include malic, citric, oxalic and tannic acids [18]. Phenols, which constitute 1 to 3% of the pulp, are dominated by oleuropein [19, 20]. Olives also contain vitamin C, tocopherol, thiamine and β -carotene [21, 22], and are also rich in K, Ca, Mg, P, Fe, Zn and Mn [23].

3. Green olive process

Table olives can be produced by three methods, depending on the stage of ripening of the fruit: the Spanish (or Sevillana) method (**Figure 1**), used as the main industrial process for the production of green olives [16]. In Morocco, the transformation process of table green olives is the Spanish style. It is based on the alkaline treatment of the olives (debittering) with a concentration between 2% and 4% of NaOH, to eliminate oleuropein, the responsible agent for the bitterness of olive fruits. The olives must be kept in this solution until it reaches two-third of the pulp (5 to 6 hours). After removal of the bitterness, the olives are washed successively to remove the residual soda. Then the debittered olives are put in brine with 11% NaCl, where they undergo natural fermentation [16, 24].

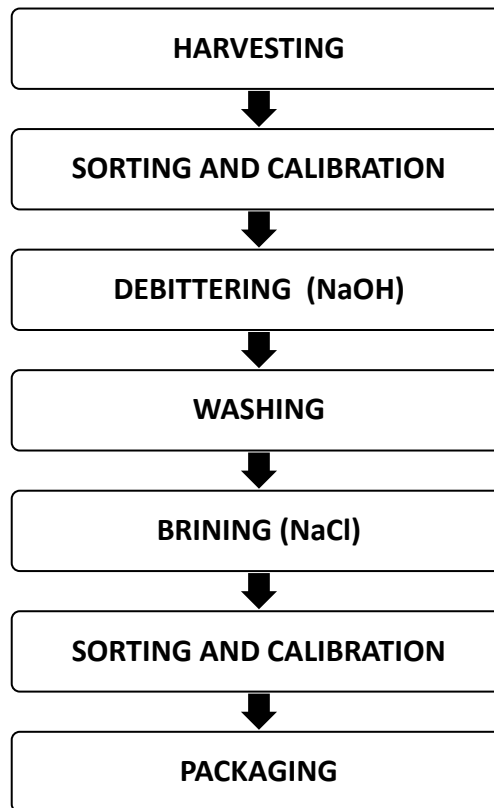


Figure 1: General process of elaboration of green table olives by the Spanish Style

4. Fermentation process of green olives

The natural fermentation is a spontaneous process used in the preservation of green table olives. The microbial evolution in the spontaneous fermentation process of the olive is generally characterized by three distinct phases [24].

The initial phase is characterized by the growth of LAB, Gram-negative bacteria (*Enterobacteriaceae*), yeasts, molds and all the other microorganisms naturally associated with olive fruits and fermenters. The LAB species, present at this stage, include species belong to *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Pediococcus*, genera [12, 25].

The second phase is the most important stage in the olive fermentation process. This phase, which begins when the pH is around 6, is characterized by the growth of LAB of the genera *Leuconostoc* and *Pediococcus* and the progressive decrease in the population Gram-negative bacteria [12, 25]. This second phase should be short to promote the rapid dominance of LAB over the other undesirable microorganisms [26]. At a pH close to 5 lactobacilli, including *Lactobacillus brevis*, *Lactobacillus fermentis*, *Lactobacillus buchneri*, develop rapidly, however, *Lactobacillus* species, particularly *L. plantarum*, tolerate high acidity and continue the production of organic acids in the medium and decrease the pH to values below 4 [27].

The third and longest phase can take up to two months. During this phase, *L. plantarum* and *Lactobacillus pentosus* dominate in the medium (**Table 1**), while the other *Lactobacillus* species are inhibited, because of the high acidity of the medium [12, 24, 28]. The duration of this phase varies depending the initial concentration of sugars, salt, temperature and the dominance of *L. plantarum* and *L. pentosus* [25]. During this phase, *Lactobacillus* strains proliferate and assure the development of the flavour and texture of the final product [18, 24].

5. Role of lactic acid bacteria and yeasts in the fermentation of green olives

5.1. Lactic acid bacteria

Lactic acid bacteria are the most important group of bacteria in the olive fermentation process (**Table 1**); they convert fermentable sugars into lactic acid and other organic acids according to their metabolic pathways. Homofermentative LABs, including some *Lactobacillus*, *Streptococcus*, and *Pediococcus*, and heterofermentative LABs, including *Leuconostoc* and some species of *Lactobacillus*, have been detected in fermented olives [29, 30].

Lactobacillus spp. play a major role in the olive fermentation process [31, 32]. *Lactobacillus pentosus* was the predominant species in their study (96.4%), while *L. plantarum* was rare. In another work, *Lactobacillus pentosus* was also the predominant species (81.9%), followed by *Leuconostoc pseudomesenteroides* (10.4%) and *Pediococcus parvulus* (7.6%), in green olive, of the Spanish variety (cv *Alorena*), fermented during 6 months [29]. In addition to *L. pentosus* and *Leuconostoc mesenteroides* are also reported as the dominant species in natural black olives (cv. *Conservolea* and *Kalamata*) of Greek origin [33]. Heterofermentative LABs produce volatile compounds and small amounts of ethanol that are very important for the final organoleptic characteristics of fermented olives [34]. Homofermentative LABs also produce many volatile compounds that make a significant contribution to the final taste of table olives [35]. In addition, LABs can produce antimicrobial molecules that allow the preservation of table olives such as organic acids, hydrogen peroxide and bacteriocins [36-39].

5.2. Yeasts

The most frequently isolated strains, from different olive varieties, belong to *Candida*, *Pichia*, *Saccharomyces*, *Debaryomyces*, *Issatchenkia*, *Zygorululasporea* and *Wickerhamomyces* (**Table 2**) [12, 40-44]. Their main role is associated with the production of alcohols, ethyl acetate, acetaldehyde and organic acids, which are relevant for the development of taste and aroma and the preservation of the typical characteristics of this fermented food [44]. Yeasts are capable of synthesizing substances such as vitamins, amino acids, and purines, or carbohydrates complex, which are essential for the growth of *Lactobacillus* species, requiring a nutritionally rich environment for their optimal growth [45].

Table 1: Lactic acid bacteria isolated from natural fermented olives.

Lactic acid Bacteria	Processed table olives	Reference
<i>Lactobacillus. plantarum</i>	Green olive, Spanish style	[12, 46]
	Green olive, Spanish style	[27, 47].
	Green olives, Spanish style	[48].
	Green olives, Spanish style	[10, 49]
	Green olives, Spanish style	[50].
	Untreated green olives	[51].
	Black Olives Greece Style	[52].
<i>L. casei</i>	Untreated green olives	[53].
<i>L. brevis</i>	Untreated green olives	[53].
	Green olives, Spanish style	[10].
<i>L. pentosus</i>	Green olives, Spanish style	[54].
	Green olives, Spanish style	[10].
	Olives marketed	[55].
	Green olives, Spanish style	[50].
<i>Leuconostoc mesenteroides</i>	Green olives, Spanish style	[54].
	Olives marketed	[55].
<i>Pediococcus acidilactici</i>	Olives marketed	[55].
<i>P. pentosaceus</i>	Green olives, Spanish style	[10].
<i>L. coryniformis</i>	Green olives, Spanish style	[50]
<i>L. paraplantarum</i>	Untreated green olives	[51]
<i>P. ethanolidurans</i>	Black Olives Greece Style	[52]

6. Main factors affecting natural fermentation

6.1. Physicochemical parameters

Several physicochemical parameters act on the growth of lactic acid bacteria during the fermentation process. They are at the origin of the variation of the microbiological and organoleptic quality of the finished product. These parameters are the variety of olives, the content and nature of polyphenols, temperature, salt and soda concentration, the initial pH of the brine ... [56-58].

a. Temperature

Temperature plays a crucial role in the success of the olive fermentation process. However, most of the fermentation process takes place in winter, when the ambient temperature is low, which delays the activity of lactic acid bacteria and may even inhibit their growth [59], thus slowing down the fermentation process. A temperature between 30 and 40°C speeds up fermentation and considered as the optimal temperature for the growth of lactic acid bacteria, in particular *Lactobacillus plantarum*. Above this temperature, the olives lose their consistency and flavor [60].

b. pH and acidity

Among the determining factors in the success of the olive fermentation process are the pH and the acidity, its evolution depends essentially on the initial conditions of the brine [58]. When the physicochemical conditions of the brine (temperature, fermentable matter and anaerobiosis, with a high load of lactic acid bacteria in the raw material) are favorable, lactic acid bacteria grow rapidly by metabolizing sugars into lactic acid and carbon dioxide [61-63]. This rapidly decreases the pH to values below 3.8 [64]. Other acids can also be produced but in small quantities, such as acetic, citric, malic, succinic, tartaric and traces of formic acid [18, 28, 56, 65-69]. Their further accumulation decreases the pH and contributes to the specific flavour of the finished product [59, 66].

On the other hand, if the initial conditions of the brine are not favourable for the growth of lactic acid bacteria, a flora such as *Enterobacteriaceae* and *Clostridium* can settle and consequently malodorous fermentations can take place [70, 71]. These two parameters limit the development of microorganisms, especially coliforms [58], and subsequently improve the sensory characteristics and hygienic quality of the finished product [72].

c. Salt content

The salt content of the brine is also a decisive factor in the control of the lactic fermentation process of table olives [73]. Thus, an adequate NaCl concentration controls microbial populations [74] and selects the species of lactic acid bacteria [68, 75]. On the other hand, a concentration of less than 6% NaCl can allow the growth of spore-forming microorganisms such as *Clostridium* during the first days of fermentation when the pH is still high [76]. A concentration between 6 and 8% NaCl inhibits the growth of microorganisms responsible for malodorous fermentations [68], and stimulates the growth of lactic acid bacteria that dominate yeast growth [75, 77]. This is considered as a desirable trait to limit the formation of gas pockets and the softening of olives by the yeasts [75]. On the other hand, a high concentration of NaCl (10 to 14%) retards growth and inhibits the metabolism of lactic bacteria, especially the production of lactic acid [58]. Under these high salinity conditions, halo tolerant yeasts dominate [75, 78] and favour the formation of gas relatives and wrinkles in the pulp [79]. The finished product is thus characterized by low acidity with a pH between 4.5 and 4.8 [68, 80].

d. Lye content

The alkaline treatment of green olives with NaOH is the key step in the Spanish style process. It affects the physicochemical and organoleptic characteristics of the processed olives, as it affects the growth and metabolism of the *Lactobacillus* genera involved in fermentation. This chemical treatment increases the permeability of the olive pulp by destroying the cytoplasmic membrane, hemi-cellulosic polysaccharides

and pectin of the cell wall [81]. This enriches the brine with nutrients used by lactic acid bacteria in their growth [82, 83].

Knowing that the NaOH concentration/duration ratio of olive treatment is variable and depends on the temperature, variety and ripening stage of the olives [23], the alkaline treatment of olives with adequate concentrations is of great importance. Indeed, olives treated with low NaOH concentration remain partially debittered and rigid, this treatment slows down the release of nutrients, which delays fermentation [84]. On the other hand, prolonged treatment or a high NaOH concentration causes the softening of the olives, which alters the quality of the finished product [58]. In addition, it is responsible for the high pH at the beginning of fermentation, thus promoting the growth of *Enterobacteria* and the incidence of gaseous deterioration [85].

e. Fermentable material

The fermentable matter found in the brine of fermenting olives diffused from the olive pulp, consists of soluble sugars such as glucose, fructose, sucrose, mannitol and inositol [17]. It constitutes the most important fraction in the fermentation process, since it is indispensable for the development of microorganisms. The rate of diffusion of soluble sugars depends on several factors, namely the permeability of the epidermis and fruit pulp, temperature, salt content, degree and duration of debittering, and increases along with the elevation of the concentration of NaOH used [86]. In addition, during the washing process, a large part of these sugars is eliminated, and their content increases from approximately 74.94 mg/Kg to 18.3 mg/Kg after a treatment with 3% NaOH and two rinses of six hours duration each [86].

f. Content and nature of polyphenols

The content and nature of polyphenols in olives can have an inhibiting effect on the growth of lactic acid bacteria in the brine. The polyphenol content of olives can exceed 80mg/100g [87]. It depends on the variety, origin and degree of ripening of the olive fruit [88]. Oleuropein, the responsible agent for the bitter taste of olive fruits [16, 89, 90] is the majority phenolic glucoside found in non-bittered olives [20, 91-93]. However, hydroxytyrosol dominates in table olives treated in the Spanish style and represents 60.7 to 85.9% of total polyphenols [94, 95]. The maximum concentration of hydroxytyrosol found in olive brine is around 1.15g/l [96].

Oleuropein and its acid hydrolysis products inhibit the growth of gram-positive bacteria involved in the fermentation of olives. Oleuropein aglycone is more inhibitory than oleuropein [97, 98], and its activity is enhanced by the presence of salts in the medium [99]. Indeed, it has been reported that a concentration of 200µg/ml of oleuropein has no inhibitory effect on the growth of lactic acid bacteria involved in the fermentation of olives. Moreover, oleuropein is not responsible for the inhibition of lactic acid bacteria

in NaOH-treated olives, because it is hydrolyzed during the alkaline treatment in the process of olives debittering [100, 101]. On the other hand, a concentration of 125µg/ml of aglycone and/or elenolic acid is largely sufficient to completely inhibit the growth of *L. plantarum*, especially in the presence of 5% NaCl [102].

Hydroxytyrosol inhibits the growth of lactic acid bacteria (Lactic Acid Bacteria: LAB) in brine [96]. While in olives not undergoing chemical debittering treatment, inhibition of LAB has been attributed to the isomer of Oleoside -11 methyl ester and decarboxymethyl elenolic acid-tyrosol [103, 104]. In addition, some authors have previously reported that inhibition of LAB may also be due to the synergistic effect between phenolic compounds [87, 96]. However, the presence of non-phenolic compounds such as sugars, salts, amino acids and lipids may significantly decrease or mask their inhibitory effect [87, 105].

6.2. Microbiological factors

Microbial populations that coexist during the natural fermentation process, depending on the physicochemical parameters of the environment and the interactions between them, are at the origin of the variation in the microbiological and organoleptic quality of the finished product. There are two groups of microbial populations; the desirable population consisting of lactic acid bacteria and homofermentative bacteria, in particular *Lactobacillus plantarum* and *Lactobacillus pentosus*, and the undesirable population consisting of certain sporulating or non-sporulating Gram-positive bacteria, Gram-negative bacteria, yeasts and moulds.

7. Alterations associated with green table olives

During the process of elaboration of table olives, they are often confronted with numerous alterations leading to enormous economic losses. The main alterations are related to microbiological and physicochemical factors. Olives can be injured or damaged during harvesting, transportation and processing. Typical alterations in table olives are softening, putrid and butyric malodorous fermentation and gaseous deterioration [23]. The latter type of alteration is most common in Spanish style Moroccan green olives. It is due to the fermentation by gas-producing microorganisms such as coliforms, heterofermentative lactic acid bacteria, fermentative yeasts and butyric bacteria [23].

8. Inoculation of green table olives by starter cultures (LAB and yeast)

The starter cultures consist of live indigenous microorganisms (LAB and yeasts) mainly isolated from fermenting olives that can be added to fermenting olives in high cell numbers to accelerate and improve the fermentation process [106, 107]. The species of LAB and yeasts isolated from table olives are shown in **Table 1** and **Table 2**. The use of LAB and indigenous yeasts allowed to shorten the fermentation time, standardize the process and improve the organoleptic and nutritional properties of the olives [108].

Table 2: Yeasts isolated from fermenting table olives

Yeast	Preparation of table olives	Reference
<i>Aureobasidium pullulans</i>	Conservolea Black Olives	[42]
<i>Candida aaseri</i>	Conservolea Black Olives	[42]
<i>C. apicola</i>	Broken green olives pickled directly	[40]
<i>C. boidinii</i>	Seasoned fermented green olives	[40]
	Black olive in brine	[41]
	Broken green olives pickled directly	[44]
	Green olives in brine	[44]
	Conservolea Black Olives	[42]
<i>C. diddensiae</i>	Leccino Olives	[44]
	Seasoned green table olives	[40]
	Broken Green olives pickled directly	[31, 44]
<i>C. oleophila</i>	Green olives in brine	[109]
	Broken green olives pickled directly	[44]
<i>C. etchellsii</i>	Green olives in storage	[110]
<i>C. versatilis</i>	Green olives in storage	[110]
<i>C. quersitrusa</i>	Broken green olives pickled directly	[44]
<i>C. silvae</i>	Conservolea Black Olives	[42]
<i>C. sorbosa</i>	Directly pickled green olive	[52]
<i>C. tropicalis</i>	Green olive, Spanish style	[109]
<i>C. tartarivorans</i>	Cellina di Nardo Olives	[111]
<i>Citeromyces matritensis</i>	Broken green olives pickled directly	[44]
<i>Cystofilobasidium capitatum</i>	Conservolea Black Olives	[42]
<i>Debaryomyces hansenii</i>	Conservolea Black Olives	[42]
	Cellina di Nardo Olives	[111]
	Conservolea Black Olives	[112]
	Kalamata olives	[109]
<i>D. ethcellsii</i>	Leccino Olives	[111]
	Directly pickled broken green olives, Spanish style	[41]
	Green olives, Spanish style	[109]
	Conservolea Black Olives	[42]
<i>Issatchenkia occidentalis</i>	Green olive in brine	[42]
<i>Saccharomyces cerevisiae</i>	Broken green olives pickled directly	[109]
	Green olive in storage	[110]
<i>Rhodotorula mucilaginosa</i>	Broken green olives pickled directly	[44]
<i>Rh. glutinis</i>	Green olive in storage	[110]
<i>Pichia guilliermondii</i>	Conservolea Black Olives	[42]
<i>P. kluyveri</i>	Conservolea Black Olives	[42]
<i>P. anomala</i>	Green olive in storage	[110]
<i>P. manshurica</i>	Conservolea Black Olives	[42]
<i>P. membranifaciens</i>	Conservolea Black Olives	[42]
	Directly pickled green olives	[109]
	Cellina di Nardo Olives	[111]
	Kalamata olive	[112]
	Leccino Olives	[112]

According to Tufariello, Durante [108], the role of microbial starters, selected for specific biotechnological and safety characteristics, is considered as an agent of:

- Controlling fermentation process;
- Monitoring the progress of the fermentation process;
- Improvement of the sensory properties of table olives;
- Maintenance and/or improvement of the nutritional characteristics of the product;
- Protection against undesirable deterioration and pathogenic microorganisms;
- Fortification of table olives with microorganisms with probiotic potential;
- Improving product stability and increasing shelf life.

9. Selection of starters for green olive fermentation

The criteria used to select the microorganisms to be used as starter cultures for the fermentation of green olives are listed in **Table 3**. The microorganisms should be easily adapted to the fermentation environment and should have non-pathogenic, probiotic, and technological characteristics [113]. Also, they should improve the nutritional properties and health aspects of the product and develop better flavors [32]. The number of bacteria is also important for its beneficial effects. It is necessary that they are present in sufficient numbers [114, 115]. In addition, the culture starter must be able to grow in the presence of oleuropein [25]. Otherwise, this natural inhibitor would limit the adaptation of LAB to the brine environment [69]. Technological characteristics such as survival in brine, production of large amounts of lactic acid during fermentation, tolerance to high pH values and high concentrations of saline solution is also extremely important as well as the adhesion to the olive surface, these factors are important for the final selection of the starter [25, 116-119].

Microorganisms must also possess specific enzymes that contribute positively to the organoleptic properties of the final product [113]. Among LAB, the strains mostly used as starter cultures in olives are *Lactobacillus pentosus*, *L. plantarum* and *L. paracasei*, which are potential probiotic bacteria [54, 120, 121].

Table 3: Desirable and undesirable characteristics of microorganisms used as starter cultures in foods [113].

Properties	Desirable	Undesirable
Technological	Ability to grow at high salt concentrations, high and low pH values; Capacity of adhesion on the skin of the olive	Production of CO ₂ ; Assimilation of lactic acid; Production of biogenic mycotoxins and amines
Functional	Degradation of oleuropein Production of vitamins Antimicrobial activity	
Enzymatic activity	Esterase, Lipase, Catalase, Phytase, Alkaline/acid phosphatase, β -glucosidase	Proteolytic and xylanolytic activity
Probiotics	Survive under digestive tract conditions Ability to adhere and colonize epithelial cells; Antimicrobial activity against pathogens;	

10. Degradation of Oleuropein

10.1. Structure and characteristics of oleuropein

Oleuropein is the most abundant phenolic compound in olives fruits and leaves, responsible of their bitter taste [122]. The structure of oleuropein has been specified as that of a heterosidic ester of elenolic acid and dihydroxyphenylethanol [123, 124] (**Figure 2**). Oleuropein has shown both beneficial health effects and adverse effects. Omar [122] showed that oleuropein has antioxidant, anti-inflammatory, anticancer, anti-atherogenic, antiviral, antimicrobial, hypolipidemic and hypoglycemic effects. In addition, the antimicrobial activity of oleuropein on lactic acid bacteria is classified as an adverse effect [84, 98, 99, 104, 125]. Lactic acid bacteria are the microorganisms responsible for the lactic fermentation process of green olives [23, 126], their inhibition affects negatively the olive fermentation process.

10.2. Chemical hydrolysis of oleuropein

Oleuropein is chemically hydrolysable under acid or basic pHs,—by hydrolysing the ester bond. Oleuropein can be slightly hydrolyzed at acidic pH [92], and the major products are hydroxytyrosol, hydroxytyrosol glycoside, oleuropein aglycone, elenolic acid, glucose and tyrosol [92, 102, 127]. Under alkaline pH, oleuropein is fully hydrolyzed in 5 minutes with a 1M solution of NaOH at room temperature, generating hydroxytyrosol, tyrosol, and their derivatives as hydrolysis products [127]. Ryan, Lawrence [92] reported that the oleuropein is strongly hydrolyzed at basic pH, releasing hydroxytyrosol, elenolic acid glucoside, elenolic acid and glucose as hydrolysis products.

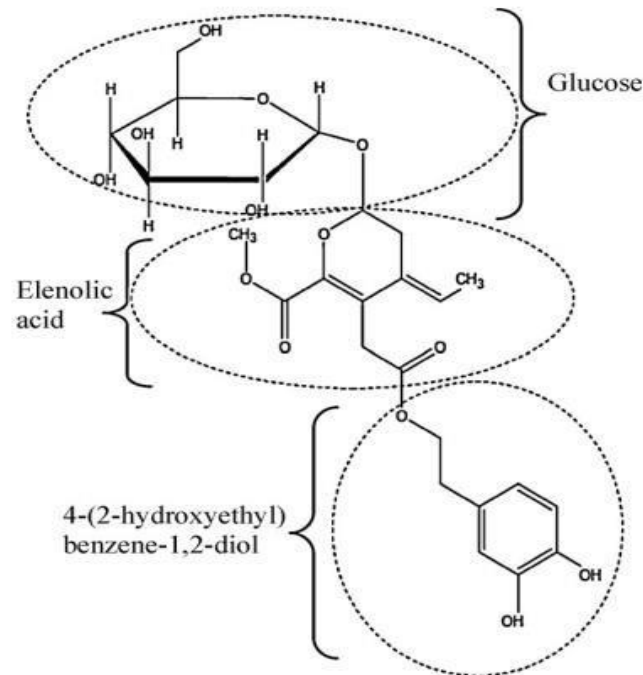


Figure 2: Molecular structure of oleuropein [124].

11. Enzymatic hydrolysis of oleuropein

Oleuropein can be enzymatically converted to hydroxytyrosol by β -glucosidase and esterase [128]. Several researchers have studied the *in vitro* degradation of oleuropein by different microorganisms. Hydrolysis of oleuropein by β -glucosidase releases glucose and oleuropein aglycone [129, 130]. This latter is hydrolyzed by esterases to simpler non-bitter compounds, namely hydroxytyrosol and elenolic acid [87, 129, 131].

There are many studies on β -glucosidases of microbial origin for the substitution of NaOH in the table olives processing [130, 132]. Table olives processed with β -glucosidase and esterase have a higher organoleptic and nutritional values than those treated with NaOH, due to their high contents of sugars and proteins [6].

12. Biodegradation of oleuropein by lactic acid bacteria

Biodegradation of oleuropein (OLP) has been achieved through the use of LABs by Cifardini, Marsilio [5] and Marsilio, Lanza [6]. This latter reported that strains of *Lactobacillus plantarum* degrade oleuropein in olive fruits (Table 4). *L. plantarum* initially hydrolyses oleuropein by its β -glucosidase to produce its aglycone form. In the second step, the esterase activity of *L. plantarum* gives rise to hydroxytyrosol and elenolic acid [8, 133] (Figure 3). *L. pentosus*, *L. brevis*, and *Pediococcus pentosaceus* have been isolated from fermented olives with the ability to degrade oleuropein through the

production of β glucosidase [10]. The biodegradation of OLP by some strains of LAB was demonstrated in the absence of stress factors, including salt and acid pH. In the presence of NaCl (5%), pH 4, these strains were unable to degrade this polyphenol compound [10]. *L. plantarum*, *L. paracasei*, *L. casei*, *Bifidobacterium lactis*, *Enterococcus faecium*, and the *L. acidophilus* strain were tested for their ability to convert oleuropein by-products in olives and olive oil into hydroxytyrosol [128]. Among these strains, *L. plantarum*, due to its auxotrophic characteristics, was the most efficient with a hydroxytyrosol production yield of 30% [128].

Table 4: Study of the biodegradation of oleuropein by lactic acid bacteria

Substrates	Products	Strains used	References
Oleuropein	Hydroxytyrosol	<i>L. plantarum</i> 6907 and <i>L. paracasei</i> 9192	[128]
Oleuropein	Hydroxytyrosol	<i>L. plantarum</i> (B17, B20, and B21)	[5]
Oleuropein / 5- bromo-4-chloro3-indolyl β -D-glucuronide	Hydroxytyrosol	<i>L. plantarum</i> , <i>L. pentosus</i> , <i>L. brevis</i> , and <i>Pediococcus pentosaceus</i>	[10]
Oleuropein	Hydroxytyrosol +Elenolic acid	<i>L. plantarum</i> B21	[8]

13. Interactions between yeasts and lactic acid bacteria

LAB and yeasts play an important role in the natural fermentation process of green olives [134]. *Lactobacillus spp.* coexist in brine with a diverse yeast population during the fermentation process [14, 24]. *L. plantarum* and *L. pentosus* appear to be the most relevant LAB species in starter selection for natural and treated olives, respectively [135].

Yeast metabolism, in the early stages of fermentation, can produce growth essential factors for the development of LAB, such as vitamins [136]. The use of yeast as a starting adjuvant promotes the dominance of LAB [137]. It has been suggested that LAB competes with wild yeasts in natural black olives from *Conservolea* [138]. Co-inoculation of *Candida diddensiae* and *L. pentosus* leads to a better microbial development profile than simple inoculations [135]. Interrelationships between *Lactobacillus* and yeast species in table olives can also play a key role in product preservation process [139].

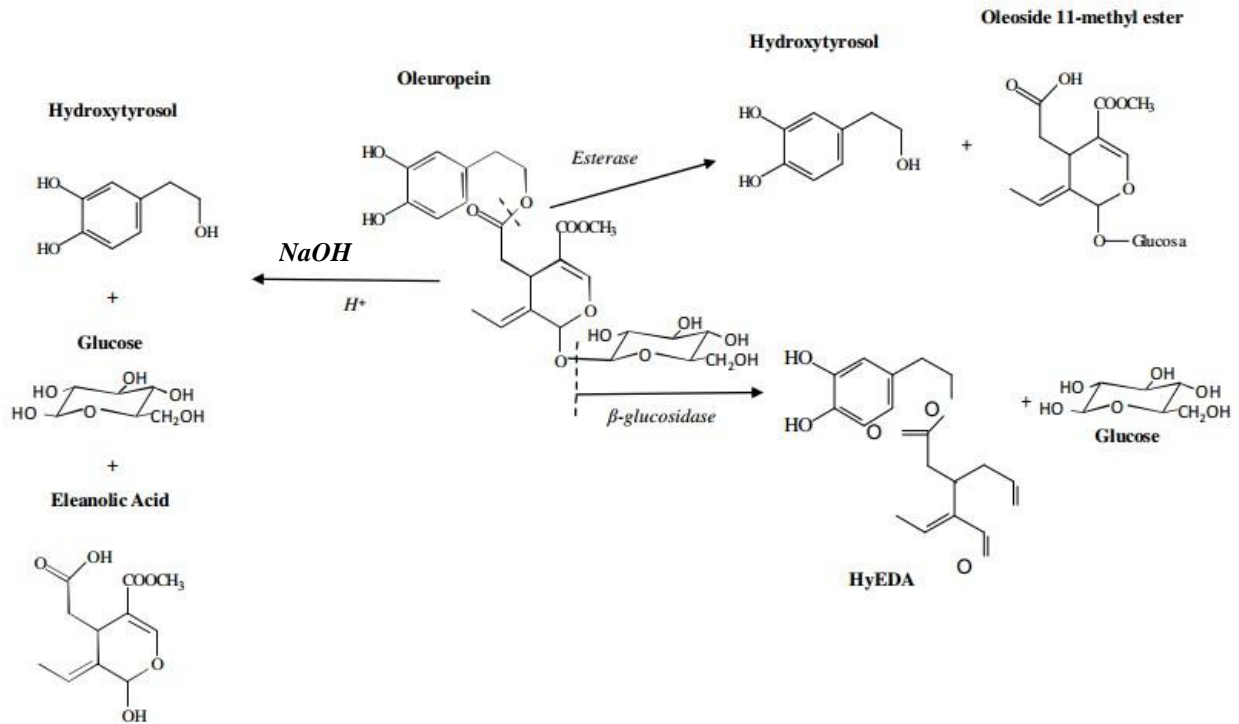


Figure 3: Structure of oleuropein and the products obtained by chemical hydrolysis and enzymatic hydrolysis [140]

Some interactions between yeasts and LAB in green olive fermentations have already been described [14, 67, 134, 141]. *L. pentosus* and *Saccharomyces cerevisiae* is a good example of positive interaction [142]. *L. pentosus* and yeast populations were able to form mixed biofilms (from day 7 of the process) throughout the fermentation process on glass slides and olive skin [14]. *G. candidum*, *P. galeiformis* and *C. sorbosa* are the main yeast species isolated from mixed biofilms containing *L. pentosus* [134]. *C. boidinii* has also shown an ability to adhere and colonize olive skin [134]. *Enterococcus casseliflavus*, has been tested as starter for Spanish style green olives with *Lactobacillus* [143], the results showed that fermentation was faster, with a decrease in the time during which the growth of spoilage microorganisms could occur and the survival of lactobacilli was higher.

Some studies have provided evidence of interactions between yeasts and LAB cultures [144-146]. Other studies on olives have shown that at least some yeast strains can have direct quantitative effects on LAB, potentially by promoting the release of nutrient compounds [141]. Conversely, the addition of the LAB had no apparent effect on the total number of yeasts or their diversity.

Table olives are a product fermented mainly by LAB and yeasts. The interactions of bacteria and yeasts are different in natural black and green olives and in olives treated with NaOH. Spontaneous fermentation of table olives may lead to high olive spoilages and/or fermented olive with variable quality,

the interest in the development and use of starter cultures for the production of table olives is gaining more and more interest. Indeed, *L. pentosus*, *L. paracasei* and *L. plantarum* have been used as starters in some parts of the world [115, 147-160]. The negative and positive aspects of yeasts in lactic fermentation of table olives have not been fully understood. Therefore, the activity and role of yeasts alone or in combination with LAB in olive fermentation need to be thoroughly investigated. *C. boidinii* seems to be the most important species as a possible starter culture in fermented olives [161-167]. Starter cultures are therefore an essential part of table olive fermentation to control the safety and quality of the final product. The selection of strains to be used as starter cultures is a complex process that starts from the isolation stage followed by the characterization and determination of their technological properties and eventually, the validation at the plant level. The probiotic potential of the selected strains should also be considered.

Conclusion

LAB and yeasts isolated from natural fermentation process of olives should have important physiologic and technological properties, mainly the production of organic acids, aroma compounds, enzymes (i.e. β -glucosidase), antimicrobial activity...etc. LAB and yeast strains degrading oleuropein, may be of great interest in biological debittering of olive fruits and in increasing the functional properties of fermented olives, due to the accumulation of hydroxytyrosol. The main LAB strains demonstrating high potential to be used belonged to *L. plantarum*, *L. pentosus* and *L. casei*; while yeast strains belonged to *Candida*. Therefore, these microbial strains can be used to assure the biological debittering of olives and to control their fermentation process, which may lead to the increase of the shelf life of fermented olive.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgments

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