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Factors Influencing Phenolic Compounds in Table Olives (*Olea europaea*)

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ABSTRACT: The Mediterranean diet appears to be associated with a reduced risk of several chronic diseases including cancer and cardiovascular and Alzheimer's diseases. Olive products (mainly olive oil and table olives) are important components of the Mediterranean diet. Olives contain a range of phenolic compounds; these natural antioxidants may contribute to the prevention of these chronic conditions. Consequently, the consumption of table olives and olive oil continues to increase worldwide by health-conscious consumers. There are numerous factors that can affect the phenolics in table olives including the cultivar, degree of ripening, and, importantly, the methods used for curing and processing table olives. The predominant phenolic compound found in fresh olive is the bitter secoiridoid oleuropein. Table olive processing decreases levels of oleuropein with concomitant increases in the hydrolysis products hydroxytyrosol and tyrosol. Many of the health benefits reported for olives are thought to be associated with the levels of hydroxytyrosol. Herein the pre- and post-harvest factors influencing the phenolics in olives, debittering methods, and health benefits of phenolics in table olives are reviewed.

KEYWORDS: *olive, Olea europaea, hydroxytyrosol, tyrosol, oleuropein, California-style black ripe, green ripe, Spanish, Greek, phenolics*

INTRODUCTION

Table olives are an important component of the Mediterranean diet, a diet linked with the reduction of certain chronic diseases including cardiovascular disease. Table olives contain a range of biologically active phenolic compounds. The predominant phenolic compound in fresh olive fruit is oleuropein. This phenolic compound is very bitter and must be removed to make olive fruit palatable. This is generally achieved through salt curing or alkaline hydrolysis. The main hydrolysis products of oleuropein include hydroxytyrosol and tyrosol. Many of the health benefits reported for olives are thought to be associated with the levels of hydroxytyrosol. Herein, the numerous factors that affect the phenolic composition of table olives are reviewed. These include the cultivar, degree of ripeness, and, importantly, debittering methods used for curing and processing table olives. In addition, the health benefits associated with phenolics in table olives are reviewed.

PHENOLIC COMPOUNDS IN OLIVES

The olive is fruit derived from a small evergreen tree in the family Oleaceae (*Olea europaea*). Olive fruit is classified as a drupe but differs from other drupes as it contains a much lower sugar content and higher concentration of oil. The oil content varies with cultivar and ripening degree, ranging from 3 to 38% on a fresh weight basis.[1,2] The primary cultivars planted in California include Ascolano, Barouni, Manzanillo, Mission (oil production), and Sevillano.[3] Manzanillo is the primary cultivar used in olive canning in California.[4]

An olive drupe is composed of three primary layers and the kernel: the skin or epicarp, the inner mesocarp layer composed of the pulp, and the inner endocarp layer that forms the stone wall around the kernel. Olive fruit is very bitter due to the presence of a high concentration of phenolic compounds and, in particular, the *o*-diphenol oleuropein and its derivatives, which include hydroxytyrosol. As a class of molecules, phenolics encompass a broad range of secondary plant metabolites that are characterized by the presence of at least one hydroxylated aromatic ring. Polyphenolic compounds appear to play important roles in human health via the modulation of pathways associated with inflammation, enzyme induction, and their ability to modulate oxidative stress.[5−9]

In olive leaf and fruit the primary bitter phenolics, oleuropein and ligstroside, are accumulated as a defense mechanism against pathogens and herbivores. In general, olive phenolics are localized in the skin and around seed structures. During fruit ripening, or when the olive tissue is injured by pathogens, herbivores, or mechanical damage, the enzyme β-glucosidase hydrolyzes oleuropein to produce the aglycone (Figure 1). The numerous phenolic compounds range from simple monophenolics to more complex phenolics with multiple aromatic rings. The more complex phenolics are often modified with sugars (i.e., glycosides). These sugar residues are often further modified by organic acids (e.g., synaptic, malic, coumaric). Although the chemical characteristics of olive phenolics vary, most have appreciable water solubility due to the sugar moieties and multiple sites of hydroxylation. Phenolic compounds in olives comprise 1−3% of the fresh pulp weight. The main classes include phenolic acids, phenolic alcohols, flavonoids, and the secoiridoids.[10−14] There are at least 36 structurally distinct olive oil phenolics that have been identified to date. They can be grouped according to their similar chemical structures as described below.

Phenolic Acids (Phenolcarboxylic Acids). Phenolic acids are the simplest forms of phenolics in olive fruit, containing a phenolic ring and an organic carboxylic acid function.

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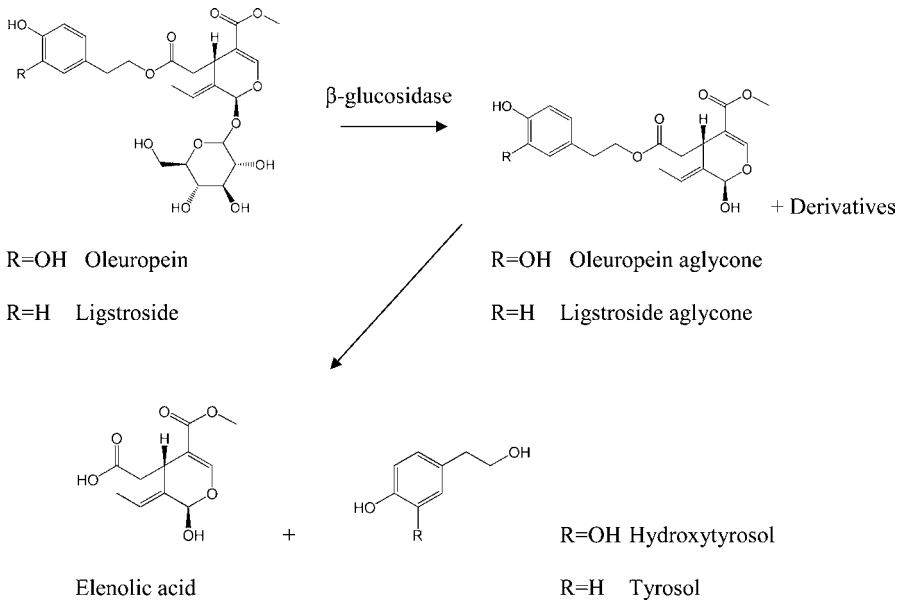

Figure 1. Hydrolysis of oleuopein and ligstroside.

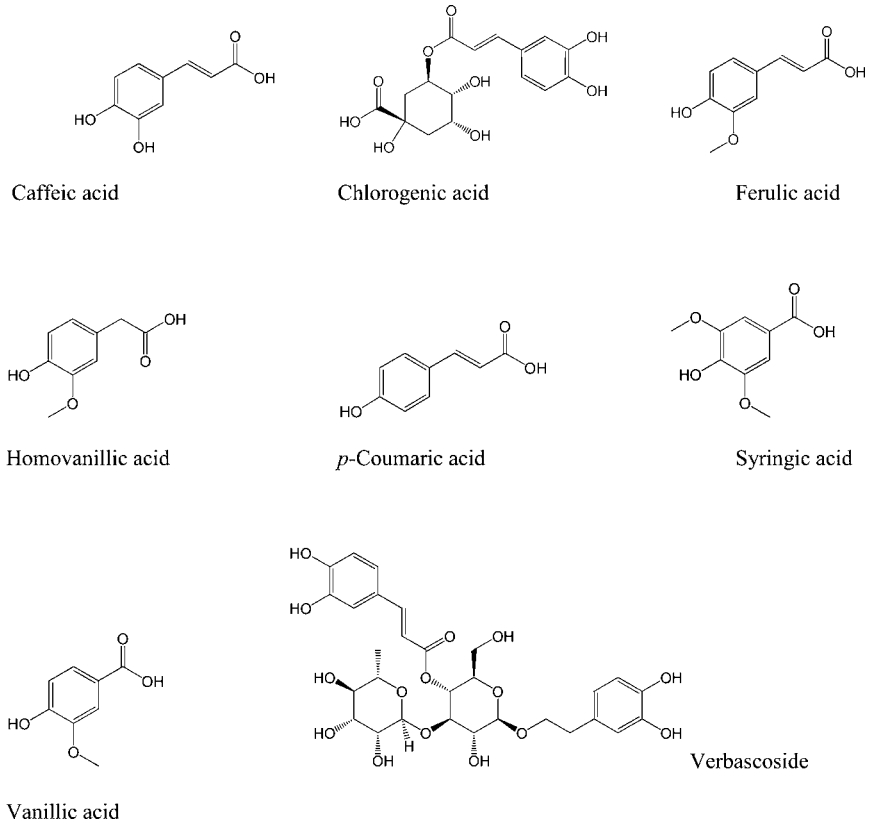

Figure 2. Primary phenolic acids present in *O. europaea* L.

These compounds can be divided into benzoic acid derivatives (C6−C1), cinnamic acid derivatives (C6−C3), and other phenolic acids and derivatives. The phenolic acids that predominate in olive fruit include caffeic acid, chlorogenic acids (ferulic, vanillic, coumaric, and syringic), and the more complex caffeic acid sugar ester verbascoside (Figure 2).[10,14−18] Levels of these compounds range depending upon cultivar and maturity and can be as high as ∼3 g/kg (dry weight) for verbascoside.[19]

Phenolic Alcohols. The primary phenolic alcohols found in olive fruit include hydroxytyrosol, tyrosol, and their glucoside forms (Figure 3).[16,17,20−22] The level of hydroxytyrosol in processed olives can be as high as ∼4 g/kg (dry weight).[20] Hydroxytyrosol is generated from the hydrolysis of oleuropein, whereas tyrosol is a hydrolysis product of ligstroside.[18] Hydroxytyrosol has a catechol moiety. It is a potent antioxidant that has numerous reported health benefits including immunostimulant, antioxidant, and antimicrobial activities and inhibition

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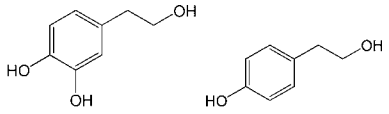

Hydroxytyrosol Tyrosol

Figure 3. Primary phenolic alcohols present in *O. europaea* L.

of atherosclerotic plaque formation. These actions are discussed in detail in the following sections.

Flavonoids. Flavonoids are the most common group of polyphenolic compounds in the human diet consisting of more than 1600 types. Many are reported to have potent antioxidant activity and the ability to decrease cardiovascular and cancer disease risk.[23−26] Flavonoids are composed of a C6−C3−C6 flavan nucleus as a basic unit. The predominant flavonoids in olives include luteolin-7-glucoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, rutin, apigenin-7-glucoside, quercetin-3-rhamnoside, and luteolin (Figure 4).[10]

Secoiridoids. These are found associated with only a few species of edible plants. Oleuropein, ligstroside, and demethyloleuropein are the most common and have related chemical structures (Figure 5). Oleuropein is an ester consisting of hydroxytyrosol and elenolic acid. Ligstroside is an ester consisting of tyrosol and elenolic acid. Other important derivatives include oleuropein aglycone, elenolic acid, oleoside-11-methyl ester, and dialdehydic form of elenolic acid linked to tyrosol (3,4-DHEA-EDA). Oleuropein is generally the most prominent phenolic compound in olive cultivars and can reach concentrations of up to 140 mg/g on a dry matter basis in young olives and 60−90 mg/g of dry matter in the leaves. It is suggested that demethyloleuropein may be a varietal marker because it is not present in all olive varieties.[13,19]

Phenolic Compounds in Olive Pulp. The phenolic composition of olives is very complex and depends upon many factors such as fruit maturation stage, part of the fruit (e.g., pulp or seed), cultivar, and season. The phenolic profile of pulp has been more extensively studied than that of other olive tissues. Oleuropein and hydroxytyrosol are the main phenolic compounds in olive pulp.[10,11] There are considerable differences in the levels of these phenolics among cultivars. Levels of oleuropein are generally above 3 g/kg (dry weight) and average ∼4.5 g/kg (dry weight). Levels of hydroxytyrosol are not consistent in the literature, ranging from 0.2 to ∼71 g/kg (dry weight).[10,17,19] Derivatives of oleuropein found in pulp include oleoside-11-methyl ester (elenolic acid glucose), demethyloleuropein, oleuropein aglycone, and oleuroside.[15,16] Demethylligstroside and ligstroside are also present in pulp.[19]

Hydroxytyrosol, tyrosol, and their glycosidic forms are the predominant phenolic alcohols in olive pulp.[10,27] Flavonoids and phenolic acids are present at low concentration (usually <100 mg/kg dry weight) and include luteolin-7-glucoside, rutin, apigenin-7-glucoside, luteolin-4-glucoside, luteolin-7-rutinoside, and quercetin-3-rhamnoside.[10,11,14,28,29] Phenolic acids such as *p*-coumaric acid,[18] chlorogenic acid,[10] vanillic acid,[16,17] syringic, ferulic, and homovanillic acid,[14] and caffeic acid[15] are also present in pulp. Again, levels are generally in the milligram per kilogram range. Verbascoside (0.7−209 mg/kg dry weight) is the major hydroxycinnamic acid derivative in olive pulp.[10]

Phenolic Compounds in Olive Leaf. Many phenolic compounds present in olive pulp are also found in leaf tissue. Oleuropein has been frequently reported as the major phenolic compound in olive leaves and can represent up to 9% of the dry weight matter.[15,30,31] In addition to oleuropein, leaves contain verbascoside,[18] demethyloleuropein, oleuropein diglucoside, ligstroside,[31] hydroxytyrosol, hydroxytyrosol glucoside, tyrosol, tyrosol glucoside, oleuroside, oleoside-11-methyl ester, and nuzhenide. The predominant phenolic acids in leaves include caffeic, chlorogenic, *p*-coumaric, homovanillic, and vanillic acid.[15] The flavonoids luteolin, luteolin-7-glucoside, luteolin-4-glucoside, luteolin-7-rutinoside, apigenin-7-rutinoside, rutin, quercetin, hesperidin, diosmetin, and apigenin-7-glucoside have also been described in olive leaves.[15,28,30−32]

Phenolic Compounds in Olive Seed. Several phenolic compounds of the secoiridoid class and the derivatives present in olive seeds include nuzhenide, nuzhenide oleoside, oleuropein, demethyloleuropein, oleuropein-aglycone dialdehyde (3,4-DHPEA-EDA), oleoside-11-methyl ester, and ligstroside. Nuzhenide (Figure 6), which is rarely found in other tissues of olive, is the main phenolic compound in seeds.[33] Seeds also contain the phenolic alcohols hydroxytyrosol, tyrosol, and their glucoside forms.[15] Other phenolics found in seeds are verbascoside, luteolin-7-glucoside, caffeic, chlorogenic, homovanillic, and vanillic acid.[15,33]

Phenolic Compounds in Stems and Small Branches. Several phenolic compounds including oleuropein, verbascoside, hydroxytyrosol, tyrosol, α-taxifolin, apigenin-7-glucoside, and luteolin-7-glucoside were identified in small olive branches.[30,34] Microwave-assisted extraction of phenolics from small branches indicates that high levels can be recovered. For example, 19 g/kg oleuropein, 2 g/kg tyrosol, 1 g/kg verbascoside, and 0.7 g/kg hydroxytyrosol were recovered from small branches.[34]

Phenolics Involved in Fruit Browning. Oleuropein is involved in the browning of olive fruit either after impact and wounding during harvesting or during subsequent processing treatments. This reaction is facilitated in part by β-glucosidases, esterases, and polyphenol oxidase (PPO). Initially, PPO is associated with the chloroplast membranes but becomes increasingly soluble during fruit maturation. Browning in olive fruit correlates with the oleuropein content and not with PPO activity, indicating that endogenous substrates are the main limiting factor.[35] A mechanism for olive fruit browning was described by Segovia-Bravo et al.[36] These authors suggest that there is first an enzymatic release of hydroxytyrosol, due to the action of the fruits' β-glucosidases and esterases on oleuropein and hydroxytyrosol glucoside; additional hydroxytyrosol can also be produced (in a markedly lower proportion) by the chemical hydrolysis of oleuropein. Subsequently, the oleuropein, hydroxytyrosol, and verbascoside are oxidized by PPO, which demonstrates maximum activity at pH 6.0. This activity is completely inhibited at a pH below 3.0.[37]

INFLUENCE OF CULTIVAR AND DEGREE OF RIPENESS ON THE COMPOSITION OF PHENOLICS

Cultivar Influence. The expression of phenolic compounds in olive fruit is predominately driven by genetic factors, and large differences exist between olive cultivars. In all cultivars, oleuropein and hydroxytyrosol are the major phenolic compounds present. The phenolic compounds in the pulp of several Portuguese cultivars (Bical, Bical De Castelo, Borreira, Borrenta, Branco, Madural, Madural Fina, and Madural Negra) were studied.[10] The concentrations of these phenolic compounds vary considerably between cultivars in fruits with similar degrees of ripeness. The average level of oleuropein was 4.549 g/kg (dry weight). The range reported was 0.388−21.681 g/kg (dry weight). The level

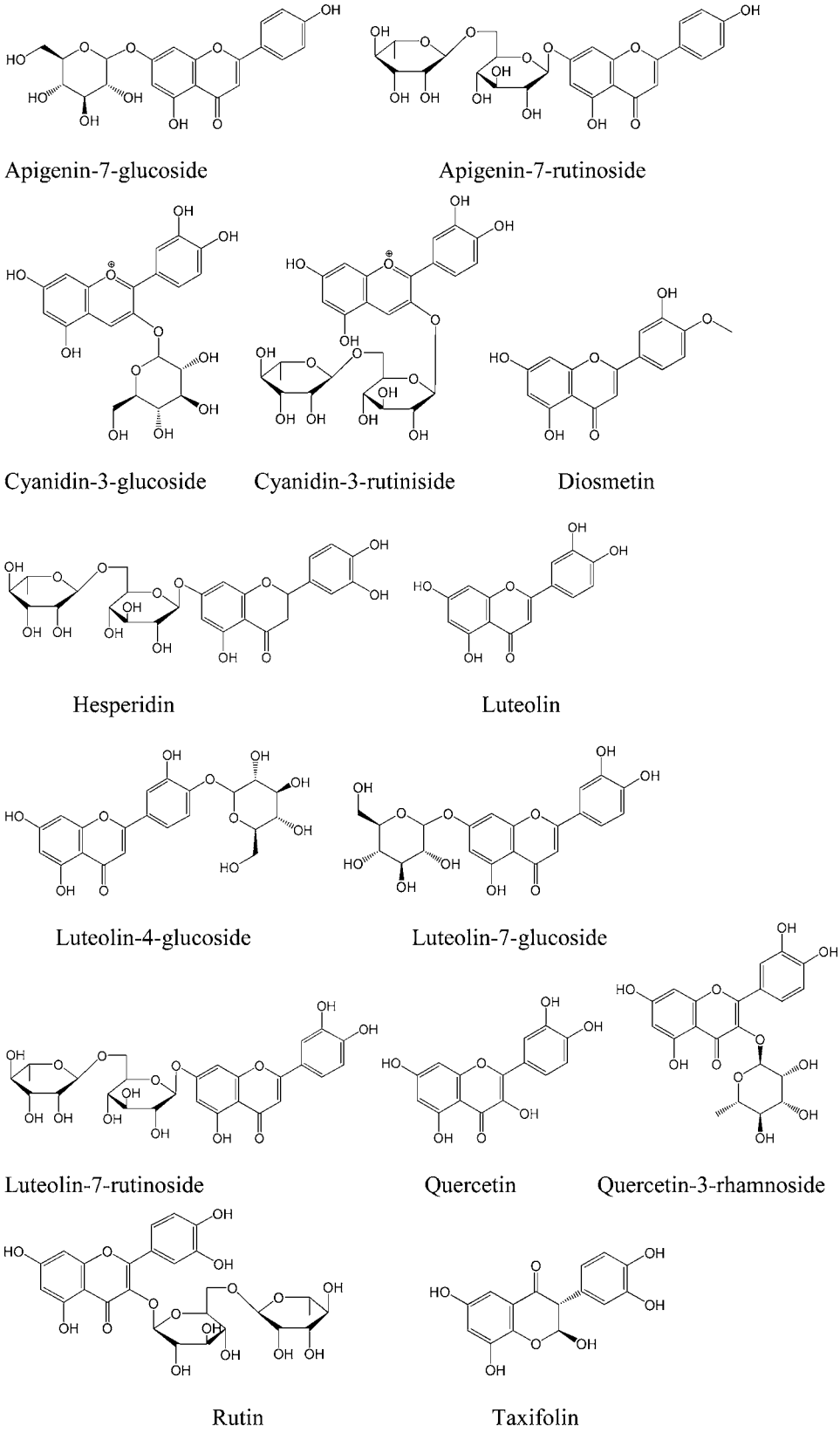

Figure 4. Primary flavonoids present in *O. europaea* L.

of hydroxytyrosol ranged from 1.477 to 15.763 g/kg (dry weight). In general, literature values reported for the range of hydroxytyrosol in olive pulp are between 0.3 and 8 g/kg (dry weight). Verbascoside levels are much lower, ranging from 1.9 to 164 mg/kg.[10]

Phenolic compounds in pulp of three Italian olive cultivars (Coratina, Carolea, and Cassanese) were studied by Sivakumar et al.[19] Oleuropein and ligstroside were the main phenolics present in these Italian olives. Coratina olives contain the highest levels of oleuropein and ligstroside (14.6 and 11.4 g/kg (dry

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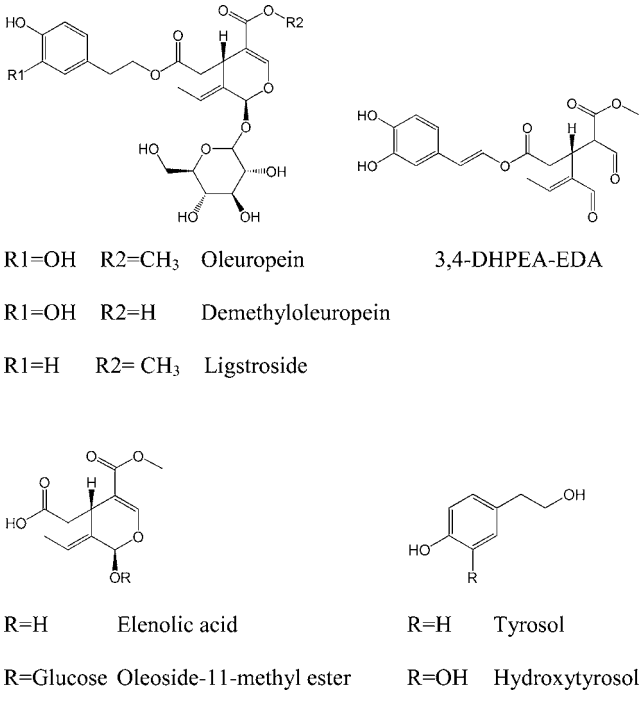

Figure 5. Chemical structures of common secoiridoids in *O. europaea* L.

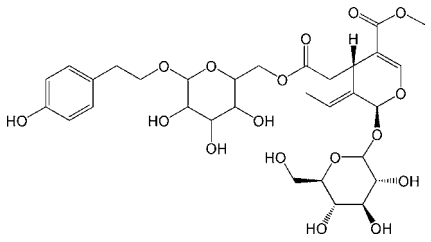

Figure 6. Chemical structure of nuzhenide in *O. europaea* L.

weight), respectively). The maximum oleuropein contents of Carolea and Cassanese olives were 12.7 and 10.1 g/kg, respectively, whereas the ligstroside levels were 11 and 9.3 g/kg, respectively. The maximum concentrations of verbascoside present in Carolea, Coratina, and Cassanese olives were identified as 1.9, 3.2, and 0.9 g/kg, respectively.[19] The concentration of verbascoside in the pulp of the Italian olive cultivars is higher than in Portuguese cultivars reported by Vinha et al.[10] Esti et al.[13] found levels of oleuropein between 0.31 and 3.45 mg/g fresh weight in Italian olives.

A study of the olive pulp taken from three Spanish cultivars (Gordal, Hojiblaca, and Lechín) with similar degrees of ripeness indicates that hydroxytyrosol-4-β-D-glucoside is the predominant phenolic with levels ranging from 20.54 to 22.12 g/kg dry weight.[27] Oleuropein and hydroxytyrosol levels ranged from 4.32 to 14.58 g/kg and from 0.62 to 2.6 g/kg, respectively. The verbascoside content ranged from 31.2 to 231 mg/kg, and low concentrations of ligstroside were found. Verbascoside and ligstroside in Spanish cultivars are much lower than levels found in the Italian cultivars as reported by Sivakumar et al.[19]

Demethyloleuropein appears to be very specific to some Leccino and Coratina cultivars. No trace of this compound was detected in pulp samples (obtained at several different degrees of ripeness) in Gentile Colletorto, Gentile Larino, Gentile Santacroce, Leccino, Paranzana, Rosciola, Saligna, Coratina, Carolea, and Cassanese cultivars.[13,19]

Cultivar selection can influence the content of phenolics in branches and leaves as well. Phenolic compounds in small branches and leaves from 13 olive cultivars (Alamenõ, Arbequina, Azulillo, Chorna, Hojiblanca, Lechín, Manzanillo, Negrillo, Nevadillo, Ocal, Pierra, Sevillano, and Tempranillo) collected in December 2005 were measured.[34] The highest levels of oleuropein and verbascoside were found in small branches (18.856 and 1.044 g/kg, respectively) and leaves (24.8 and 10.303 g/kg, respectively) of the Arbequina cultivar. Small branches and leaves of the Chorna cultivar contain the lowest levels of oleuropein (1.602 and 2.127 g/kg, respectively). Small branches of the Alamenõ cultivar have the lowest level of verbascoside (52 mg/kg), and leaves of the Ocal cultivar contain the lowest amount of verbascoside (284 mg/kg).

Degree of Ripeness. Three distinct phases are distinguishable in the development and ripening of olive fruit: a growth phase, during which accumulation of oleuropein occurs; a green maturation phase that coincides with a reduction in the levels of chlorophyll and oleuropein; and a black maturation phase that is characterized by the appearance of anthocyanins and flavonoids and during which the oleuropein levels continue to fall.[38] Oleuropein is very abundant in the early stages of fruit development; in young fruit it can reach 14% of dry matter. Studies of Cardoso et al. and Damak et al.[29,38] suggest that the disappearance of oleuropein in the pulp of the fruit is related to the formation of phenolic oligomers, identifying trimers of oleuropein. This reaction is presumably carried out by PPO (a diphenol oxidase; EC 1.10.3.2). These authors also note a similar loss in hydroxytyrosol. Earlier studies suggested that hydroxytyrosol increased as the fruit matured; however, these more recent studies provide significant evidence to suggest that this is not the case.

In general, levels of oleuropein decrease in olive pulp during maturation.[13,19,28,38,39] In contrast, the glucoside forms of flavonoids, luteolin-7-glucoside,[13,28] cyanidin-3-glucoside, cyanidin-3-rutinoside,[11,27] and quercetin-3-rutinoside,[13] are more abundant in the pulp of mature olive fruit. As maturation increases, levels of demethyloleuropein, hydroxytyrosol-4-β-D-glucoside, demethylligstroside, and oleoside-11-methyl ester increase.[11,13,19,39]

There is no significant difference found between phenolics in early-season leaves, collected when fruits are green, and those in late-season leaves (6 months older), collected when fruits are black-ripe.[15,30] Interestingly, Ortega-Garcia et al.[40] found significantly higher concentrations of oleuropein in olive leaves collected at the early stage of fruit maturation than in 40-day-older leaves. The oleuropein levels remained constant until olive leaves were 120 days old. However, Laguerre et al.[32] found significant changes in the phenolic profile between 1-year-old and 3-year-old Picholine olive leaves. Younger olive leaves contain higher levels of oleuropein, ligstroside, and flavonoid aglycones, whereas mature leaves have higher levels of verbascoside, oleuroside, and the glycosylated form of luteolin. This is in agreement with the results reported by Malik and Bradford,[28] who found higher levels of luteolin-7-glucoside, luteolin-4-glucoside, and verbascoside in mature olive leaves.

Phenolic compounds in olive seeds from immature green olives are different from those in mature black olives (Hardy's Mammoth cultivar). In seeds from immature olives, tyrosol glucoside is the major phenolic compound, whereas nuzhenide predominated in seeds from mature olives.[15]

PROCESSING METHODS USED WITH OLIVE FRUIT

The predominant types of commercialized table olives are Spanish-style green, California-style black ripe, and Greek-style naturally black olives. Processing methods used to create these different styles of table olives all act to debitter olives. The debittering process involves the hydrolysis of oleuropein (usually base-catalyzed) into less bitter forms that can leach out of the olive and be removed. Hydrolysis can be catalyzed either chemically (as with base or acid) or with enzymes (e.g., glycosidases). Because oleuropein and ligstroside are glycosides, they can be enzymatically hydrolyzed into glucose and the aglycone. Oleuropein and ligstroside are also esters of hydroxytyrosol and elenolic acid. The ester bond can be hydrolyzed into simpler nonbitter compounds, primarily elenolic acid and hydroxytyrosol.

Spanish-Style Green Olive Processing. For Spanish-style green olives, fruit is harvested with colors varying from green to straw yellow but having reached normal size. Olives in this stage are firm and resistant to a slight pressure when squeezed with the fingers. For this processing method, olives are put in a sodium hydroxide solution (lye; normally 1.3−2.6% w/v) until sodium hydroxide penetrates two-thirds or three-fourths of the distance between the surface of the olives and the stone. The sodium hydroxide concentration will depend upon the temperature, cultivar, and degree of fruit ripeness. The lye hydrolyzes the oleuropein into nonbitter hydroxytyrosol and oleoside-11-methyl ester. The olives are then washed to remove the excess lye and placed in a sodium chloride solution for a mild lactic fermentation. The fermentation rate can be influenced by the levels of oleuropein and other phenolics in the brine as they have antimicrobial activity. Generally, the initial salt concentration is held around 5−6% w/v. Dissolution of several nutrients from olives into surrounding brine allows lactic acid fermentation to occur, in particular, the hydrolysis of elenolic glucoside. As the fermentation progresses, the pH of the solution decreases to approximately 4, signaling the end of fermentation. Hydroxytyrosol diffuses into the brine solution rapidly, and levels remain constant throughout lactic fermentation. Levels of oleoside-11-methyl ester decrease during fermentation, as this is a substrate for lactic acid bacteria. Levels of oleuropein also decrease in brines throughout fermentation, presumably due to the hydrolysis of oleuropein into oleoside-11-methyl ester and hydroxytyrosol.[18,41] The concentration of salt is then increased to ≥8% to control the growth of *Propionibacterium* as it can result in spoilage. Finally, olives are packed in brine (≥8%);[42] however, a lower concentration of salt (5.53%) was found in the commercial products by López-López et al.[43] Addition of sorbic acid or its salt or pasteurization (at 62.4 °C for 15 min for bottled olives) may be used to prolong the shelf life of the olive products.[42]

Greek-Style Naturally Black Olive Processing. Greek-style naturally black olives are prepared from fully ripe or almost ripe olives. The color of the olives may be reddish black, violet black, deep violet, greenish black, or deep chestnut. For Greek-style black olive processing, the fruit is placed in a sodium chloride solution varying from 6 to 14%. Acetic acid may be added to prevent the growth of spoilage microorganisms. Fermentation (generally yeast) provides the characteristic flavors to olives, and oleuropein is eliminated during this step. This process takes around 6−9 months. After fermentation, olives are exposed to air to darken the skin color. Olives are then packed in fresh brine. The pH ranges from 3.6 to 4.5, and the sodium chloride content is around 8−10% for the packaged products.[42] However, 4.98% of salt is found in commercial products.[43] Preservation is carried out by the addition of sorbic acid, potassium sorbate, or pasteurization.[42]

California-Style Black and Green Ripe Olive Processing. California-style black and green ripe olive processing methods involve harvesting olives before complete maturity. The California-style black ripe olives are either directly treated with lye to remove bitterness or preserved in a brine solution (5−10% sodium chloride) until they can be lye treated and processed. A mild fermentation may occur during the initial brine storage. Brining for Californian-style black ripe olives is different from the Spanish-style green olive processing methods in that the olives are not treated with lye prior to brining. Therefore, the diffusion rate of fermentation substrates from olives into storage brine is slow in the California-style black ripe processing method. As a result, fermentation of Californian-style olives during storage brining is generally carried out by yeast. To prevent the growth of spoilage organisms (*Clostridium*, *Bacillus*, and Gram-negative bacteria), acids such as hydrochloric, acetic, or lactic acid are added to lower the pH of the brine solution to approximately 4. The methods for creating California-style black ripe olives are similar worldwide.

Oleuropein, ligstroside, and verbascoside in the fresh processed or brine-stored olives are hydrolyzed into the nonbitter components via successive treatments of a 1−2% sodium hydroxide solution for periods of 2−24 h over 3−7 consecutive days, during which time the lye penetrates the skin and reaches the pit. During the intervals between lye treatments, the fruit is suspended in water or a weak brine solution in which air is bubbled. The air is generally bubbled from the bottom of the storage tank to promote the circulation of the liquid and provide the oxygen required for the black color formation (an oxidation reaction). Sparging the tanks with air causes the oxidation and polymerization of *o*-diphenols, mainly hydroxytyrosol and caffeic acid.[44] Upon polymerization, these compounds form brown-black pigments, which are observed mainly on the surface and to a lesser extent in the flesh of the olive. Each successive lye treatment will progressively increase the shade and penetration of the color into the olive. The surface color obtained through this process is not stable and fades during the shelf life of the packed product. To prevent color deterioration, irons salts such as ferrous gluconate, ferrous sulfate, and ferrous lactate can be used to stabilize color. Iron salts fix the color as they form an iron (Fe)−phenol complex that is very stable. Typically, a ferrous gluconate (0.1% w/v) or lactate (0.06% w/v) solution is added for 1−12 h to fix the developed black. Olives are then rinsed with water to neutralize the solution. Lactic acid or carbon dioxide can be used to reduce neutralization time. Mild heat can be applied to hasten the removal of residual oleuropein. Olives are then packed in cans in a ∼3% sodium chloride solution. In the United States, canned olives are preserved with sterilization at 115.6 °C for 60 min or at 121.1 °C for 50 min.[42]

California-style green ripe processing methods differ from the California-style black ripe olive processing method, as they do not include air oxidation or ferrous gluconate treatment. Moreover, only fresh olives are processed as California green-ripe olives, whereas California-style black olives can be made from fresh or brine-stored olives.

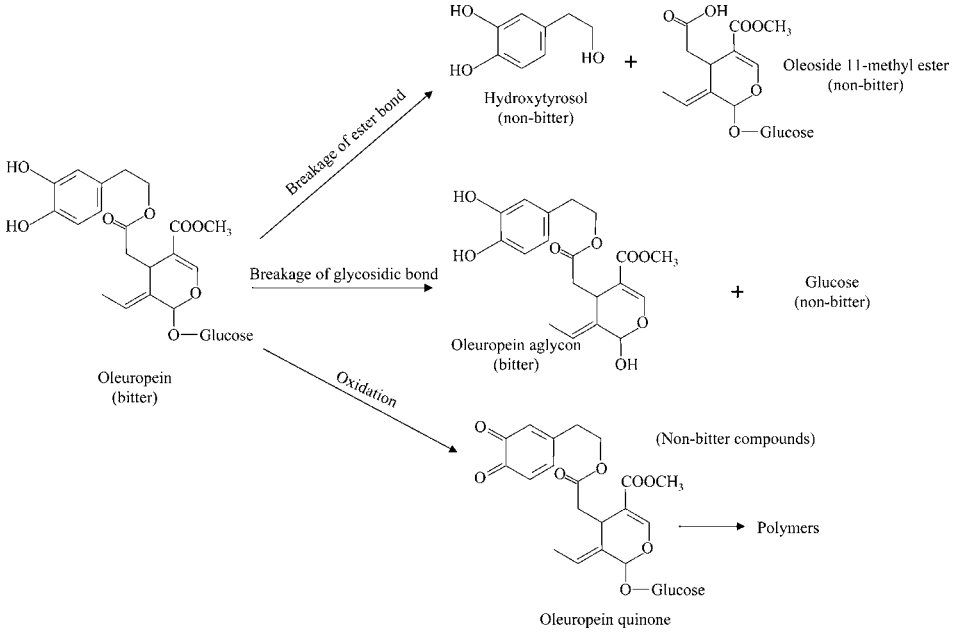

Figure 7. Bitterness of oleuropein and products of its chemical transformations.[69]

INFLUENCE OF PROCESSING ON OLIVE PHENOLICS

Spanish-, Greek-, and California-style processing methods are designed to remove oleuropein, the bitter phenolic compound in olives. These methods not only affect oleuropein levels but also the concentrations of other phenolics present in the finished olive products. Profiles of phenolic compounds in the end products are significantly different due to the various types of processing methods used. In general, the California-style processing method results in the lowest concentrations of phenolic compounds, especially hydroxytyrosol. Greek- and Spanish-style processing methods provide more appreciable levels of phenolic compounds in table olives.

The lye causes the hydrolytic cleavage of the ester bond on oleuropein between hydroxytyrosol and oleoside-11-methyl ester (elenolic acid glucoside), resulting in an increase of these two nonbitter compounds (Figure 7).[45] Acid hydrolysis of this bond is possible; however, this reaction rate is slower and is, therefore, usually not used in commercial olive processing.

Verbascoside is hydrolyzed via the same mechanisms, giving rise to nonbitter hydroxytyrosol and caffeic acid. Hydrolysis of ligstroside (a tyrosol ester of elenolic acid) produces tyrosol and the oleoside-11-methyl ester. Rutin and luteolin-7-glucoside levels decrease during lye treatment, presumably due to the hydrolysis of the glycosides. Rinsing lowers the concentration of phenolic compounds in olives due to diffusion of these compounds into rinsing water.

Phenolic Changes during Spanish-Style Green Olive Processing. Spanish-style green olive processing methods (SPM) consist of a sodium hydroxide treatment, rinsing, and brining in which lactic acid fermentation occurs. Sodium hydroxide cleaves oleuropein, resulting in an increase of concentrations of oleoside-11-methyl ester and hydroxytyrosol. Tyrosol, a hydrolysis product of ligstroside, is also formed during this step.[18] At the beginning of the fermentation, hydroxytyrosol, tyrosol, and oleoside-11-methyl ester diffused into the surrounding medium. After reaching equilibrium, the concentrations of hydroxytyrosol and tyrosol in fermenting olive brine remain constant. Consumption of oxygen by microorganisms may prevent further oxidation of hydroxytyrosol. Levels of oleoside-11-methyl ester decrease rapidly due to the conversion of this compound into elenolic acid and glucose through the acid conditions produced from microbial action (Figure 8). Elenolic acid is unstable and degrades in the acidic conditions of the brine solution. The glucose becomes a substrate for fermenting microorganisms.[18,41] The concentrations of luteolin-7-glucoside, caffeic acid, *p*-coumaric acid, and oleuropein decrease as fermentation progresses.[18]

Phenolic Changes during Greek-Style Black Olives Processing. Greek-style naturally black olive processing methods include a natural fermentation in brine and air oxidation for color improvement. This natural fermentation results in a higher retention of total phenolics than Spanish- or California-style processing methods. In the study by Marsilio et al.,[46] olives of the same variety and maturation stage were processed by Greek- and Spanish-style processing methods. The total phenolic content in fresh olives was 5138 mg/kg of wet weight. After 5 months of fermentation, levels had dropped to 2513 mg/kg in the Greek-style olives and to 448 mg/kg in the Spanish-style olives. The concentrations of individual phenolic compounds in fresh, Spanish-style, and Greek-style table olives followed similar trends and are shown in Table 1. Levels of hydroxytyrosol and tyrosol are higher as these components are formed during the lye treatment as in the Spanish-style processing.

Phenolic Changes during California-Style Black Ripe Olive Processing. Olives that cannot be processed right after harvest are preserved in a brine solution containing an acid to prevent the growth of spoilage organisms. This brine storage can influence levels of oleuropein. For example, after 4 months of brine storage, oleuropein levels were shown to decrease (from 1650 to 10 mg/100 g dry weight) (Table 2) due to microbial metabolism, acid hydrolysis, and/or diffusion of oleuropein and its hydrolysis products into the surrounding brine.[17] The levels of derivatives of oleuropein aglycone (Figure 9), hydroxytyrosol, and tyrosol increase significantly. The flavonoids luteolin-7-glucoside and rutin also decrease during brine storage.

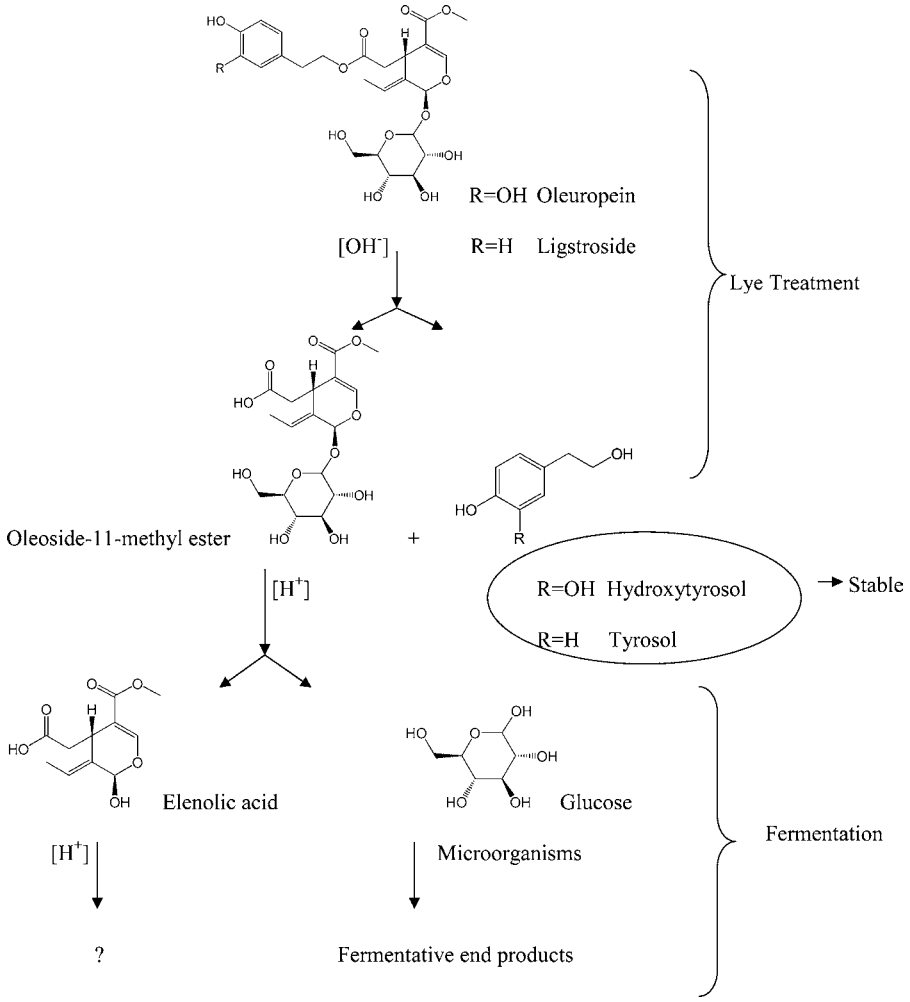

Figure 8. Influence of fermentation on oleuropein, eleolic acid glucoside, hydroxytyrosol, and tyrosol.

Table 1. Levels of Phenolics in Ascolana Tenera Olives before and after Processing (Modified from Reference 44)[a]

compound	fresh olives (mg/kg olive pulp)	SPM olives (mg/kg olive pulp)	GPM olives (mg/kg olive pulp)
tyrosol	189	51	89
vanillic acid	103	16	26
hydroxytyrosol	945	221	510
3,4-dihydroxy-phenylglycol	125	3	2
aglycone 1	65		1
aglycone 2	358		12
aglycone 3	117		12
oleoside-11-methyl ester	262	4	56
oleuropein	1028		2
luteolin-7-glucoside	88		
rutin	26		

[a]SPM, Spanish processing method; GPM, Greek processing method.

Table 2. Effect of NaOH and Air Oxidation on Phenolic Composition (Milligrams per 100 g Dry Weight) of Intosso Olives (Modified from Reference 17)

compound	fresh olives	brine-stored olives	lye-treated and air-oxidized olives
tyrosol	40	63	152
hydroxytyrosol	57	395	1030
vanillic acid	3		
oleuropein aglycone 1	2	70	
oleuropein aglycone 2	20	185	2
oleuropein aglycone 3	33	135	11
oleoside-11-methyl ester	140	120	tr
oleuropein	1650	10	
rutin	8		
luteolin-7-glucoside	2		

Verbascoside is more stable, and its concentration remains consistent throughout the brine storage.[16,47]

Recent studies indicate some yeast and lactic acid bacteria strains (e.g., *Lactobacillus pentosus* and *Lactobacillus plantarum*) are able to degrade oleuropein during the brining of fruits.[48,49] However, the predominant microorganism in brines is not always the inoculated starter, and the growth of lactic acid bacteria has been shown to be inhibited by phenolic compounds, especially oleuropein, hydroxytyrosol, elenolic acid, and an isomer of oleoside-11-methyl ester.[50,51] In fact, low lye penetration of Manzanillo olives as well as incomplete hydrolysis of oleuropein is thought to be responsible for inhibition of fermentation of Manzanillo olives.[50]

During the lye treatment, oleuropein is hydrolyzed into oleuropein aglycone, hydroxytyrosol, and elenolic acid glucoside.

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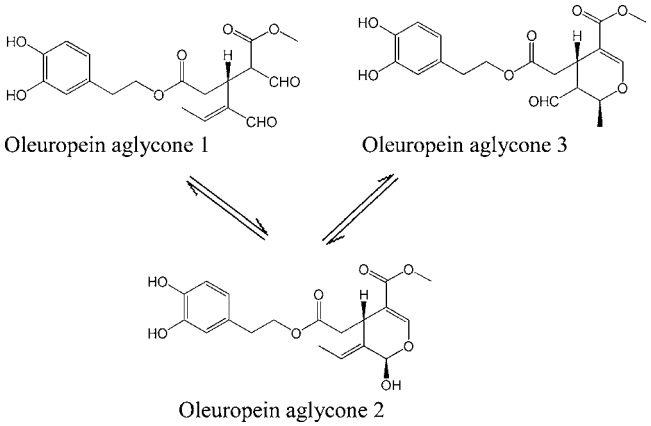

Figure 9. Oleuropein aglycones interconversion and their molecular structures.

Concentrations of hydroxytyrosol increase in the lye, and it becomes the major phenolic compound. Concentrations of hydroxytyrosol significantly decrease during the rinsing step due to diffusion into the rinsing water. Ferrous gluconate treatment causes a sharp decrease in hydroxytyrosol due to oxidation, whereas the tyrosol level remains unchanged. Concentrations of hydroxytyrosol and tyrosol do not change during sterilization. Tyrosol is the main phenolic compound in the end product. The changes of hydroxytyrosol and tyrosol levels in olive during California-style black ripe olive processing are shown in Table 3.[16] California-style black ripe olives contain much lower concentrations of total phenolic compounds than either Spanish- or Greek-style table olives (Table 4 as referenced in ref 52). The level of phenolics remains relatively constant in oil with all processing methods. At the end of processing, verbascoside and tyrosol glucoside are present only in Greek-style table olives.[52]

Stability of Hydroxytyrosol in Olives during Heat Processing. Olive phenolics can degrade during heating processes. The degradation rate depends on each phenolic compound, time, and temperature. Decomposition is faster at higher temperatures. For example, hydroxytyrosol completely disappears when olive oil is heated for 150 min at 220 °C, whereas approximately 30 and 50% of the initial hydroxytyrosol content are still present if the oil is heated to only 170 and 90 °C, respectively.[53] Normally, hydroxytyrosol and its derivatives are less stable than tyrosol as hydroxytyrosol is more susceptible to oxidative degradation. For example, after an extra virgin olive oil has been heated at 180 °C for 3 h, hydroxytyrosol is no longer detected, and only 6% of oleuropein aglycone is found, whereas >70% of the ligstroside and 30% of initial tyrosol content are still present. The decomposition rate of hydroxytyrosol in olive oil also depends on the type of olive. The loss of hydroxytyrosol is more rapid in oil containing high linoleic acid content and low amounts of phenolics (Arbequina variety) than in the oil richer in oleic acid and higher phenolic antioxidants (Picual variety).[54]

The method used to process table olives will have a major impact on the phenolic profile of table olives. California-style black ripe olive processing methods result in the lowest levels of total phenolics and have considerably lower (or absent) levels of hydroxytyrosol than other processed table olives.

Table 4. Total Phenolic Compounds (Micromolar) in Table Olives: Spanish-Style Green, California-Style Black Ripe, and Greek-Style Naturally Black Olives (Modified from Reference 50)

			total phenolic compounds (μM)	
sample name	processing method	cultivar	juice phase	oil phase
SG	Spanish-style green olive	Gordal	4252	777
SM	Spanish-style green olive	Manzanilla	8886	860
SH	Spanish-style green olive	Hojiblanca	4854	773
CH	California-style black ripe olive	Hojiblanca	1808	713
CC	California-style black ripe olive	Cacereña	1722	546
GT	Greek-style naturally black olive	Thassos	4792	720

EXTRACTING PHENOLICS FROM OLIVES

Numerous approaches have been developed for recovering phenolics from processing wastewaters and/or spent brines. The extraction/removal of phenolics is desired for both debittering purposes and for recovering valuable olive phenolics from coproduct materials. The use of ultrasound has been tested for the extraction of phenols from different plant materials employing various combinations of ultrasound power and frequency. The use of high-power ultrasonication (400−450 W)

Table 3. Phenolic Compounds in Fresh Green, Fresh Black, and Brine-Stored (Green and Black) California-Style Black Ripe (Douro Variety) Olives (Modified from Reference 16)

	mg/100 g of pulp DW[a]					
compound	1	2	3	4	5	6
tyrosol	2.00	49.65	41.07	1.36	37.12	38.78
vanillic acid	0.45	2.07	3.52	0.79	0.52	
hydroxytyrosol	**3.08**	**205.25**	**2.72**	**1.03**	**165.98**	**0.54**
4-(acetoxyethyl)-1,2-dihydroxybenzene	9.72	1.26	0.93	2.49	1.54	
3,4-dihydroxyphenylglycol	7.40	1.82	1.62	1.66	3.46	
decarbomethoxyoleuropein aglycone		2.30			0.31	0.13
oleuropein aglycones	**62.10**	**8.96**	**0.40**	**13.51**	**6.14**	
oleoside-11-methyl ester	**313.61**	**14.49**	**0.94**	**125.32**	**6.85**	
oleuropein	**504.13**	**6.29**		**113.67**	**1.00**	**4.63**
luteolin-7-glucoside	1.20			1.22		
rutin	8.14			3.00		1.85

[a] 1, fresh green olives; 2, green olives after 2 months of brine storage; 3, green olives after California-style black ripe processing; 4, fresh black olives; 5, black olives after 2 months of brine storage; 6, black olives after California-style black ripe processing.

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at 20−22 kHz was used for extraction of phenolics from olive leaves,[55] whereas lower ultrasound power (100−250 W) with 20−50 kHz was applied for extracting polyphenols from strawberries.[56] Ultrasound has been used to improve the extraction of oil from olive paste.[57] More recently, high-intensity ultrasonication was used to improve the extraction of phenolic compounds from olive fruit.[58] This study focused on the improved extraction of nine phenolics from freeze-dried olive fruit (including oleuropein). Absorptive resins have successfully been used to extract and adsorb olive phenolics from olive mill wastewater. For example, Agalias et al.[59] used a nonpolar polystyrene-based resin to recover phenolics from oil mill wastewater.

The use of enzymes and bacteria for debittering has also been considered. However, when using these approaches, the enzyme (and bacterial) stability and activity in brine solutions, the salt and pH of brines, and diffusion of hydrolysis products from the olive fruit are important factors that can influence the success of the debittering process. Although enzymatic treatments may successfully hydrolyze or polymerize surface oleuropein, they may be less effective at catalyzing reactions deep in the flesh. Most olive phenolics are localized in the vacuole and in drop-like inclusions associated with the cell wall or extracellular cuticle in a homogeneous distribution from epicarp through the inner mesocarp of the olive drupe.[60]

Esterases have been considered for debittering olives. A decrease of concentration of oleuropein in olive fruit during maturation coincides with the formation of demethyloleuropein and oleoside-11-methyl ester due to esterase activity.[40,61] U.S. Patent 5,998,641 describes the use of the enzymes Teazyme C (ex Quest) and Laccase TM (ex Novo) for debittering olive oil emulsions. The Teazyme enzyme displays pectinase activity, esterase activity, and also β-glucosidase activity. Teazyme has an optimum pH range between 3.0 and 4.0 and a temperature range of 15−50 °C. This enzyme works in the aqueous phase of the oil emulsion to reduce the bitterness of the olive oil to organolepically acceptable levels within 100 h. Laccase (EC 1.10.3.2, *p*-diphenol:oxygen oxidoreductase) is an enzyme produced in significant amounts by a white-rot fungi. It has been used in the successful treatment of olive oil wastewater.[62,63] U.S. patents exist for this application.

PPO is responsible for browning in damaged olive fruit by acting on *o*-diphenols (e.g., the catechol moiety of oleuropein).[64−67] The polymerization of *o*-diphenols is involved in the color development of olives.[17] The chemical and enzymatic oxidation of *o*-diphenols is rapid at alkaline and acidic pH values. Therefore, enzymatic oxidation of oleuropein in olives preserved in acidified brines (pH <4.5) is possible if oxygen is available. Early studies by Shasha et al.[68] indicated that the bitter component of olives could be removed by introducing a stream of air through an olive pulp in water. On the basis of these observations, a method was patented (2008) to debitter olives by keeping the fruits under an overpressure of oxygen (0.3 bar) for 1−3 days.[69] Brine-stored (4 months) yellow-green Manzanillo olives, placed in oxygen-pressurized jars, lost their bitterness and the color changed to brown (not black). Pitted olives required 12 h to become debittered, whereas whole olives required 30 h.

Bacterial hydrolysis of oleuropein during storage in brines has also been explored.[49] Oleuropein has antimicrobial activity, particularly against lactic acid bacteria.[50,51] *L. plantarum* appears to have the highest resistance and biodegradation capacities toward oleuropein and other phenolic compounds in olives;[49] however, other strains including *L. pentosus* demonstrate activity toward isolated oleuropein as well.[70] Studies of *L. plantarum* in conjunction with β-glucosidase during fermentation indicate that oleuropein levels were reduced to levels found in ripe olive fermentation in 7 days.[71]

HEALTH EFFECTS OF OLIVE PHENOLICS

The Mediterranean population has lower mortality rates from coronary heart disease and from several cancers (e.g., large bowel, breast, endometrium, ovary, and prostate). A key component of the Mediterranean diet is olive oil. Other important components of the Mediterranean diet are table olives and grapes. The rates of cardiovascular disease and cancer are lowest in Crete, where olives and olive oil are consumed in even greater quantities as compared to other Mediterranean countries. In the seminal Seven Countries Study, the 10-year incidence of myocardial infarction (fatal and nonfatal) in men aged 40−59 years was 26/10000 in Crete males as compared with 1074/10000 in men from eastern Finland. The health benefits of Mediterranean diets have been attributed to a high ratio of dietary monounsaturated to saturated fats from the high consumption of olive oil and potentially the phenolic compounds present in olive oil, olives, and grapes;[72] however, other studies indicate that it is difficult to isolate one individual dietary component as being a larger contributor toward the overall benefit of the Mediterranean diet.[73]

The concentration of phenols in extra virgin olive oil varies from 50 to 800 mg/kg oil[74] with a mean value for commercial olive oil of approximately 180 mg/kg. Intake of olive oil in the Mediterranean countries is estimated to be 30−50 g/day.[75] A daily consumption of 50 g of olive oil with a concentration of 180 mg/kg of phenols would result in an estimated intake of about 9 mg of olive oil phenols per day. Vissers et al.[75] found that absorption of administered ligstroside aglycone, hydroxytyrosol, tyrosol, and oleuropein aglycone was 55−66% in human subjects and that at least 5% was excreted as hydroxytyrosol and tyrosol. A daily consumption of 9 mg of olive phenolics would therefore result in approximately 4.5 mg of olive phenolics equivalents per day.

Oleuropein has several pharmacological properties attributed to it including antioxidant[7,76,77] anti-inflammatory,[78] antiatherogenic,[79] and anticancer[45,80] as well as antimicrobial[81] and antiviral activities.[82] More recently, oleuropein has been shown to be cardioprotective against acute doxorubicin cardiotoxicity[83] and has been shown to exhibit anti-ischemic and hypolipidemic activities.[84] Each of these actions is described in detail below.

General Antioxidant Activity. Although it is unclear how dietary antioxidants influence the redox status of plasma in humans, there is clear epidemiological evidence that increased consumption of fruits and vegetables containing antioxidants (e.g., flavonoids, phenolics vitamin C) is associated with improvements in heath in terms of cardiovascular disease risk and cancer. In humans, reactive oxygen species (ROS) including hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet -}$), nitric oxide ($NO^{\bullet}$), peroxyl ($RO_2^{\bullet}$), peroxynitrite ($ONOO^-$), singlet oxygen ($^1O_2$), and hydroperoxide (H_2O_2) are produced via normal metabolic processes. By definition, oxidative stress arises from an imbalance between the production of ROS and the body's ability to detoxify (quench) these species or repair the damage they produce. If these species are unquenched, they react with critical chemical components of cells such as lipids, proteins, and DNA and produce oxidative damage. Oxidative damage leads to protein dysfunction and damaged cells and tissues and, if left unchecked,

can result in localized inflammation and damage to DNA. In arteries, localized inflammation can lead to atherosclerotic plaque formation and vascular damage. Damaged DNA can lead to the progression of cancer.

Lipids are especially susceptible to oxidative damage and, once oxidized, promote the oxidation of nearby lipids unless an antioxidant is available to stop this cycle. ROS are involved in many human diseases including cancer, cardiovascular disease, inflammation, and aging. Phenolic compounds in olives can suppress oxidation caused by ROS in several ways. A key antioxidant activity of phenolics is related to their free radical scavenging ability.[78] The key phenolic compounds in olives have chemical structures that enable them to neutralize free radical species by donating a hydrogen atom to the ROS, reducing and stabilizing it. Once the phenolic compound donates the hydrogen atom, it becomes a free radical. However, it has an aromatic ring system that can stabilize the newly formed radical through resonance stabilization, making it essentially nonreactive. Oleuropein and hydroxytyrosol are potent and dose-dependent inhibitors of copper sulfate-induced oxidation of low-density lipoproteins.[6,85] In studies of De la Puerta et al.[7] oleuropein demonstrated both the ability to scavenge nitric oxide and to cause an increase in the inducible nitric oxide synthase (iNOS) expression in cells. Oleuropein and hydroxytyrosol were also shown to scavenge hypochlorous acid (HOCl),[86] an oxidative substance produced in vivo by neutrophil myeloperoxidase at the site of inflammation.[87] In studies of rabbits fed diets that contained olive oil and oleuropein, Coni et al.[88] demonstrated that the addition of oleuropein increases the ability of low-density lipoprotein (LDL) to resist oxidation and reduce the plasma levels of total, free, and esterified cholesterol.

Some phenolic compounds can act as antioxidants by chelating transition metals as a result of their *o*-dihydroxy structure. In fact, certain higher plants exude *o*-dihydroxyphenols into the root medium to react with ferrous, forming a complex that can be reabsorbed by the plant. Metal ions such as ferrous are important in the production of oxygen free radicals. Due to their *o*-dihydroxy structure, hydroxytyrosol and oleuropein can form complexes with transition metals, which inactivate the transition metal and suppress the oxidative reaction in which transition metals are involved.[8] Tyrosol, which lacks the *o*-diphenol structure, was found to be ineffective in scavenging free radicals.

Anti-inflammatory Activity. Inflammation occurs in response to injury or irritation, which can be mechanical, chemical, or pathogen induced. When inflammation occurs, white blood cells are recruited to the area. Activated phagocytes (e.g., macrophages and neutrophils) produce ROS to kill microorganisms but can also lead to tissue injury (chronic inflammation). Others produce chemicals that are potent mediators of inflammation, which include prostaglandins and leukotrienes. These compounds are involved in pain, swelling, and inflammation. Leukotreines are generated from arachidoinic acid by the activity of 5-lipoxygenase.

Hydroxytyrosol > oleuropein > caffeic acid > tyrosol elicit anti-inflammatory effects by inhibiting 5-lypoxygenase activity and the production of leukotriene B_4, and none inhibit cyclo-oxygenase.[5] Two recent human studies support the in vivo antithrombotic activity of olive phenolics. The administration of virgin olive oil providing 6.6 mg/day of hydroxytyrosol (7 weeks) to mildly hyperlipomic individuals decreased serum TXB_2 levels as compared with refined olive oil.[89] In type II diabetic patients, a hydroxytyrosol rich extract (equivalent to 25−50 g olives) was found to have potent antiaggregating platelet activity (5 males), decreasing serum thromboxane B_2 levels by 46%, but had no effect on prostaglandins.[90] In mice, oleuropein was also found to increase the functional response of macrophages stimulated with bacterial lipopolysaccharide (a endotoxic component of the outer membrane of Gram-negative bacteria that elicits an immune response[91]), as evaluated by a significant increase (58.7%) in the production of nitric oxide.[92] This increase is due to a direct effect of oleuropein, which increases both the activity and the expression of INOS.

Anticarcinogenic Activity. Damage to DNA is associated with an increased risk for cancer. Olive phenolics, especially hydroxytyrosol and oleuropein, exert strong anticancer effects by interfering at several steps of cancer development. The primary anticancer activities include (1) inhibition of arachidoinic acid metabolism and generation of pro-inflammatory compounds (as discussed above); (2) quenching ROS, which can cause damage to DNA;[93] and (3) modulation of pathways that lead to the proliferation, vascularization, and death of cancer cells.[79,94−100]

The activities of hydroxytyrosol toward chemically induced cytotoxicity were originally investigated by Manna et al.[94] These studies indicate that hydroxytyrosol counteracts ROS-induced cytotoxicity in human Caco-2 cells in a peroxide and xanthine oxidase/xanthine system. The activities of hydroxytyrosol toward chemically induced DNA damage were then investigated by Deiana et al.[93] in vitro. These studies indicate that low concentrations of hydroxytyrosol (50 mM) scavenge peroxynitrite and prevent $ONOO^{\bullet}$-dependent DNA damage and tyrosine nitration.

Antiproliferative and apoptotic activities have been demonstrated for numerous olive phenolics. In normal tissue, cell proliferation (i.e., multiplication) and programmed cell death (apoptosis) are balanced. Cancer occurs when there is an uncontrolled rapid proliferation of cells within a tissue. Inhibiting proliferation of cancer cells is a common goal of chemotherapies. Fabiani et al.[95] demonstrated that hydroxytyrosol inhibits cellular proliferation in HL60 and human adenocarcinoma (HT29) cells. It acts by interfering with the cell cycle of HL60 by blocking the G_1 phase of cell reproduction, resulting in a decrease of the percentage of cells in the S and G_2/M phases (active replication stages). Hydroxytyrosol was also found to induce apoptosis in HL60 cells after 24 h of incubation. The effect of caffeic acid on cellular proliferation and apoptosis on tumor cells was smaller than that of hydroxytyrosol, whereas tyrosol, which lacks the *o*-phenolic structure, had no antiproliferation activity and did not induce apoptosis.[96] More recently, studies indicate that 3,4-DHPEA at high concentrations (100 μM) to induces apoptosis in HL60 cells through oxidative stress caused by the extracellular production of hydrogen peroxide (H_2O_2).[97] Additionally, 200 μg/mL oleuropein was found to reduce the viability of MCF-7 cells (human breast cancer cells) and number of MCF-7 cells by inhibiting the rate of cell proliferation and inducing cell apoptosis.[98] Following this experiment, oleuropein and hydroxytyrosol were shown to inhibit proliferation of the MCF-7 cells by inhibiting estrogen-dependent receptors involved in uncontrolled tumor cell growth by Siranni et al.[99] In studies of Hamdi and Castellon[45] oleuropein was found to inhibit the proliferation and migration of advanced-grade tumor cell lines in a dose-responsive manner and regressed tumor in mice within 9−12 days. More recently, Abe et al.[100] demonstrated antiproliferation effects of oleuropein on bovine smooth muscle cells (SMCs). Interference of SMC proliferation occurs via a block in the cell cycle between the G1 and S phases and inhibition of extracellular signal-regulated kinase 1/2 (ERK 1/2). Menendez et al.[101]

showed that oleuropein and ligstroside aglycone induce strong tumoricidal effects within a micromolar range by selectively triggering high levels of apoptotic cell death in cultured breast cancer cells. Most recently, Bartoli et al.[102] demonstrated that olive oil consumption (5% olive oil diet) can prevent the formation of aberrant crypt foci (precursors to colorectal polyps) and colon carcinomas in rats.

Prevention of Cardiovascular Disease. Cardiovascular disease refers to a class of diseases that involve the heart or blood vessels (arteries and veins) and usually refer to arterial diseases (atherosclerosis). The oxidative modification of LDL (plasma proteins carrying cholesterol and triglycerides) is an important incident in the development of atherosclerosis. Oxidation of LDL triggers inflammatory responses resulting in the up-regulation of pro-inflammatory genes and modification of various adhesion molecules. This subsequently leads to the recruitment of leukocytes. Macrophages (a type of leukocyte) take up oxidized LDL and become foam cells. Accumulation of dying foam cells triggers the formation of atherosclerotic plaque (collection of macrophages, lipids, tissue, etc.). This results in localized oxidative stress and a shrinking of the diameter of the artery, leading to the decrease of blood flow and oxygen supply. Because LDL oxidation triggers the atherosclerosis process, inhibition of the LDL oxidation by antioxidants is hypothesized to prevent cardiovascular disease. LDL may be oxidized through both enzymatic and nonenzymatic (induced by free radicals) reactions. Lipoxygenase enzymes play an important role in the oxidation of LDL.

The benefit of consumption of olives to prevent cardiovascular disease is thought to come from the combined effect of the high monounsaturated fatty acids and olive phenolics. For example, Scaccini et al.[103] demonstrated that the LDL and very low density lipoprotein (VLDL) from rats fed diets supplemented with olive oil are more resistant to oxidative modification than those from the rats fed control diets, although both diets contain the same amounts of vitamin E and oleic acid. In another study, administration of hydroxytyrosol reduced the size of atherosclerotic lesions and plasma malondialdehyde (a marker for oxidative stress) in rats fed diets containing high saturated fat and cholesterol for 1 month.[104] Important risk factors related to cardiovascular disease include high plasma concentration of total cholesterol, high triglycerides, and low levels of high-density lipoprotein (HDL). Administration of hydroxytyrosol decreased total cholesterol and triglycerides by 41% level.[105]

Leukotriene (LT) B_4 is also involved in atherosclerosis by inducing accumulation of leukocytes at inflammatory sites. Hydroxytyrosol, tyrosol, caffeic acid, and oleuropein inhibit LTB_4 generation via inhibition of the 5-lipoxygenase level.[5]

Degenerative Disease Protection. Fibroblasts are a type of cell that synthesize the extracellular matrix and collagen and are involved in maintaining healthy tissues. Normal human fibroblasts undergo normal biological aging (cells usually die after 50 replications), which can be accelerated by environmental factors. The proteasome is an enzyme complex involved in the degradation of proteins. Its function becomes impaired during aging, whereas its increased expression delays the aging of human fibroblasts. Katsiki et al.[105] demonstrated that oleuropein enhances proteasome activity in cells in culture and extended cell life by 15%.

Alzheimer's disease is characterized by a loss of memory. Research indicates that the disease is associated with amyloid plaque formation in the brain and extensive loss of neurons.[106] A number of epidemiological studies have shown that diets rich in unsaturated fatty acids, polyphenols, vitamins, and antioxidants are preventive factors in the etiology of this disease. On the basis of this observation St. Laurent-Thibault et al.[107] investigated the influence of tyrosol and hydroxytyrosol in the protection of neuroblastoma cells from amyloid-β-induced toxicity and found them to attenuate the toxicity. Oxidative stress is thought to play an important role in the development of Alzheimer's disease.[108,109] Ju et al.[110] demonstrated that exposure of human neuroblastoma cells (SH-SY5Y) to H_2O_2 results in decreased cell viability. Fructus Ligustri Lucidi extract rich in hydroxytyrosol and tyrosol prevents H_2O_2-induced oxidative damage and apoptosis via its free radical scavenging activity and reverses the redox imbalance.

Taken together, the evidence is strong for a role of hydroxytyrosol and oleuropein in protection against chronic diseases associated with oxidative stress. This is likely due to their *o*-dihydroxy structure. There is less evidence for tyrosol in this regard. Most information has been derived from cell culture studies and from animal models. The majority of human data are anecdotal with respect to the consumption of the Mediterranean diet and improved health. To date, there are not enough human studies of large sample size to determine the conditions under which olive phenolics will provide health benefits. Human studies investigating the antithrombotic and antihypertensive properties of olive phenolics appear promising; however, more randomized, controlled trials are needed to strengthen this relationship and the role of olive phenolics in human health.

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Notes

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