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Phenolic compounds change during California-style ripe olive processing

Vincenzo Marsilio *, Cristina Campestre, Barbara Lanza

Istituto Sperimentale per la Elaiotecnica, Contrada Fonte Umano n.37, 65013 Città S.Angelo (PE), Italy

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Abstract

Changes in phenolic composition of olive fruits (*Olea europaea* Intosso cv.) during California-style ripe olive processing were investigated. Hydroxytyrosol, tyrosol, oleuropein, oleuropein aglycones and oleoside-11- methyl ester were the main phenols identified by GC and GC-MS techniques. During the darkening process, only hydroxytyrosol decreased markedly and its diminution in flesh was directly related to the olive fruit browning development. Iron salts, used for colour fixation, seem to play a catalytic role in the oxidation of hydroxytyrosol and mechanisms involved in the browning are proposed. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Table olives; *Olea europaea* L.; Processing; Phenolic compounds; Ferrous salts; Browning

1. Introduction

Table olives (*Olea europaea* L.) and derivative products are an important part of the Mediterranean diet and are recognized as a valuable source of so-called "functional food" because of their natural phenolic antioxidant content. Phenolic compounds have been shown to possess antioxidative, antimutagenic, anticarcinogenic and antiglycemic beneficial properties (Benavente-García, Castillo, Lorente, Ortuño & Del Rio, 2000; Visioli & Galli, 1998). In addition, they appear to be involved in the defence of plants against invading pathogens, including bacteria, fungi and viruses (Koutsoumanis, Tassou, Taoukis & Nychas, 1998).

Phenolics, such as C_6-C_2 simple phenols, flavonoids and seicoiridoids have been found in olive fruit. Oleuropein, the bitter seicoiridoid principle that must be eliminated from olives before they can be edible, and hydrolytic derivatives, such as hydroxytyrosol, oleuropein aglycones and oleoside-11-methyl ester, are the predominant phenolic components in olive flesh (Panizzi, Scarpati & Oriente, 1960). Recently, many phenolic glycosides such as verbascoside, ligstroside (Çalis, Hosny, Khalifa & Nishibe, 1993) and demethyloleuropein (Ragazzi, Veronese & Guiotto, 1973) have also been described in the fruit. Other phenolic compounds which are quantitatively important and common in olive fruit are tyrosol and 3,4-dihydroxyphenylglycol (Bianchi & Pozzi, 1994) as well as some flavonol glycosides, such as rutin and luteolin-7-O-glucoside (Amiot, Fleuriet & Macheix, 1986). The phenolic fraction of olive fruit is very complex; moreover, seasonal and varietal changes, both in quality and in level of phenolic compounds are also well known phenomena accompanying the development and ripening of olives.

In table olives, phenols have a great relevance because of their contribution to the colour, taste and texture of the product. They play a crucial role in the browning process which occurs in ripe olive manufacturing (by darkening in an alkaline solution), representing the substrate for the activity of oxidative enzymes, essential for the formation of pigments responsible for the black colour of the marketable product. Nevertheless, the oxidative browning, occurring after impact and wounding of the fruits, is an undesiderable reaction, where maintenance of green colour is an important organoleptic requirement (Goupy, Fleuriet, Amiot & Macheix, 1991; Sciancalepore & Longone, 1994).

Ripe olive processing technology, also known as the Californian system, consists of three consecutive treatments of fruits (on three consecutive days) with dilute sodium hydroxide solutions penetrating the skin, 1–2

* Corresponding author. Tel.: +39-085-95212; fax: +39-085-959518.
E-mail address: elaiotec@unich.it (V. Marsilio).

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mm into the flesh and to the pit, respectively. During the intervals between NaOH treatments, the olives are immersed in water through which air is bubbled. Olives darken progressively, both in the flesh and on the surface and, once the colour is obtained, water washing and aeration continue until the pH is around 8. Then, iron salts (ferrous gluconate or lactate) are added to improve the development of the colour (Brenes, Romero, García & Garrido, 1995; García, Brenes & Garrido, 1986). Finally, the product is canned and sterilized.

The darkening process has been mainly related to enzyme-catalyzed or chemical oxidation browning reactions, involving the oxidation of natural *o*-diphenols in olives to quinones, followed by the transformation of quinones into different dark compounds (Romero, Brenes, García & Garrido, 1998). Phenolic browning reactions have been extensively studied in model systems (Cilliers & Singleton, 1989, 1991; Romero et al., 1998). However, the mechanism to explain the oxidation phenomena in ripe olives is not completely known since the behaviour of olive varieties is different and the dark colour obtained under alkaline conditions is not always satisfactory and homogeneous.

In the present paper, we report some investigations of the darkening process in California-style ripe olive processing of the Intosso cultivar, so far mainly used for Spanish-style green olive manufacturing. The aim was to study the phenolic composition of olives before the Californian-style processing took place, to know the transformation of such compounds during alkaline oxidation and to postulate the role of iron salts in the brown colour development. Hypothetical mechanisms concerning browning of ripe olives are also proposed.

2. Materials and methods

2.1. Olive samples and experimental design

Green olive fruits of the Intosso cultivar were harvested in early October from an orchard near Chieti-Abruzzo (central Italy). Olives were immediately stored under anaerobic conditions in 8% (w/v) NaCl brine for 4 months, before use. The processing procedures (Fig. 1) consisted of 0.5 M NaOH solution treatment of the olives on three consecutive days, until the lye penetrated the cuticle layers, 1–2 mm of the pulp and to the pit, respectively. In the intervals between the alkali treatments, water was added to cover the olives and air was bubbled for 24 h. Water addition and aeration continued until pH reached 8; then a 5 g/l ferrous gluconate solution was added and air was bubbled for another 24 h. Finally, the olives were canned in brine containing 30 g/l NaCl and 0.4 g/l ferrous gluconate and autoclaved at 121°C for 15 min.

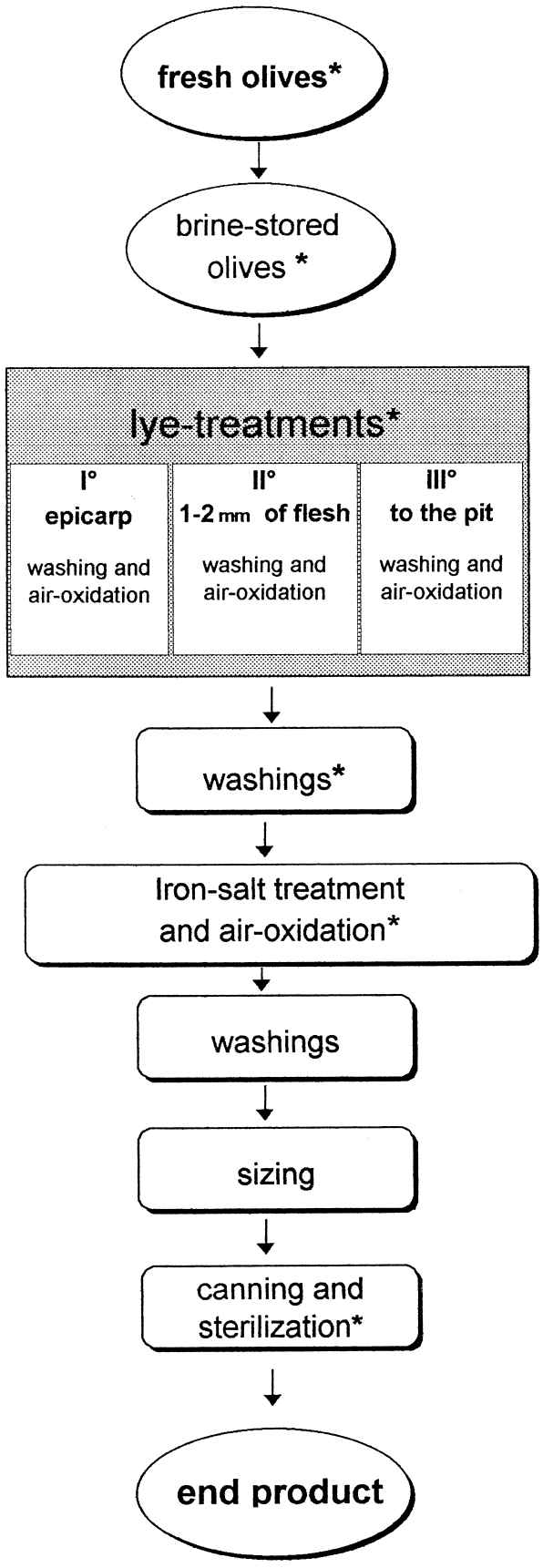

Fig. 1. Flow diagram of California-style ripe olive processing. *, Steps analysed in the present study.

2.2. Chemicals

Resorcinol was obtained from Merck (Darmstad, Germany). Tyrosol and vanillic acid were obtained from Sigma (St. Louis, MO). Oleuropein, rutin and luteolin-7-O-glucoside were purchased from Extrasynthèse (Z.I. Lyon-Nord, Genay, France). Hydroxytyrosol and oleoside 11-methyl ester were prepared by alkaline hydrolysis of oleuropein, according to the method described by García, Romero, Brenes and Garrido (1996). All other standards were of analytical grade.

2.3. Extraction of phenolic compounds

Fruits were destoned, immediately frozen in liquid nitrogen for 30 min and homogenized in a blender. Approximately 5 g of the powder obtained were mixed four times in 30 ml of 80% (v/v) ethanol, containing 0.5% (w/v) sodium metabisulfite, and centrifuged at 5000 *g* at 2–3°C for 20 min. An ethanolic solution of resorcinol (0.5 g/l) was added as internal standard.

The combined supernatants were concentrated under reduced pressure and washed with hexane in a 1:1 water/hexane mixture, to remove free fatty acids, oil and other lipid contaminants. The remaining aqueous solution was partitioned four times against ethyl acetate in a water to phase ratio of 1:1, then the ethyl acetate extract was filtered on sodium sulfate anydrous and evaporated to dryness at 30°C under vacuum. The extract was converted into trimethylsilyl ethers with a silylation mixture made up of pyridine, hexamethyldisilazane and trimethylchlorosilane (2:1:1) for 1 h at room temperature. The solvent was removed under a stream of nitrogen and TMS-derivatives were kept in isooctane for GC and GC–MS analyses.

2.4. GC and GC–MS analyses

Separation was achieved on a Carlo Erba GC-5160, equipped with a FID and a HP1 capillary column (Hewlett-Packard, Palo Alto, CA) of 30 m×0.32 mm (i.d.), 0.10 μm film thickness. The column temperature was programmed from 70 to 90°C at 20°C/min, from 90 to 300°C at 4°C/min and hold at 300°C for 40 min. Hydrogen was used as carrier gas at 35 kPa. The sample (0.3 μl) was injected by the "on column" mode.

For GC–MS analysis, a Hewlett Packard (HP) GC-5890 interfaced to a MSD-5970 was used. The chromatographic conditions were the same as described above. Analyses were performed, operating in the electron impact mode at 70 eV, using helium as carrier gas.

3. Results and discussion

The amounts of phenolic compounds in olive flesh of Intosso cultivar are shown in Table 1. The major components identified in fresh sample were oleuropein, oleoside-11-methyl ester, hydroxytyrosol and oleuropein aglycones.

Table 1
Effects of NaOH and air-oxidation on phenolic composition of Intosso cultivar[a]

Compound	Fresh olives	Brine-stored olives	Lye-treated and air-oxidized olives
Tyrosol	40	63	152
Hydroxytyrosol	57	395	1030
Vanillic acid	3	–	–
Oleuropein aglycon 1	2	70	–
Oleuropein aglycon 2	20	185	2
Oleuropein aglycon 3	33	135	11
Oleoside-11-methyl ester	140	120	tr.
Oleuropein	1650	10	–
Rutin	8	–	–
Luteolin-7-O-glucoside	2	–	–

[a] Data, expressed in mg/100 g of dry weight, are the means of three determinations; coefficient of variation was normally under 10% for all the compounds.

Oleuropein accounted for 84% of the fraction determined, oleoside-11-methyl ester for 7%, and hydroxytyrosol and oleuropein aglycones for about 3%. Vanillic acid and the flavonol glycosides, rutin and luteolin-7-O-glucoside, were present at lower concentrations.

After 4 months of preservation in brine, oleuropein content decreased markedly, because of bacterial metabolism of the fermenting brine. Parallel to the oleuropein reduction, an increase in its derivative aglycones (Fig. 2) and hydroxytyrosol was observed.

Vanillic acid and flavonoids, contents which were low in fresh olives, underwent a complete disappearance, whereas tyrosol increased, showing the same trend as hydroxytyrosol.

Alkaline aerobic oxidation, during ripe olive processing, induced significant changes in the total phenolic concentration. Oleuropein aglycones diminished considerably, while tyrosol and hydroxytyrosol increased markedly. During washings with water to remove the NaOH excess before iron salt treatments, a total disappearance of all the compounds reported in Table 1 was observed, and also tyrosol and hydroxytyrosol (Fig. 3) showed marked reductions, because of the effects of diffusion and dilution in the water. This influence was more evident for hydroxytyrosol, problably due to its higher water solubility. In the presence of ferrous ions with air bubbling, hydroxytyrosol content in flesh rapidly fell to a very low level, while tyrosol remained practically unchanged, with the end-product showing a content similar to that of fresh fruits. A phenolic oxidation process has been suggested as a major factor responsible for this behaviour. Therefore, according to the literature (Brenes, García & Garrido, 1992) only *o*-diphenols seem to be involved in oxidation browning reactions.

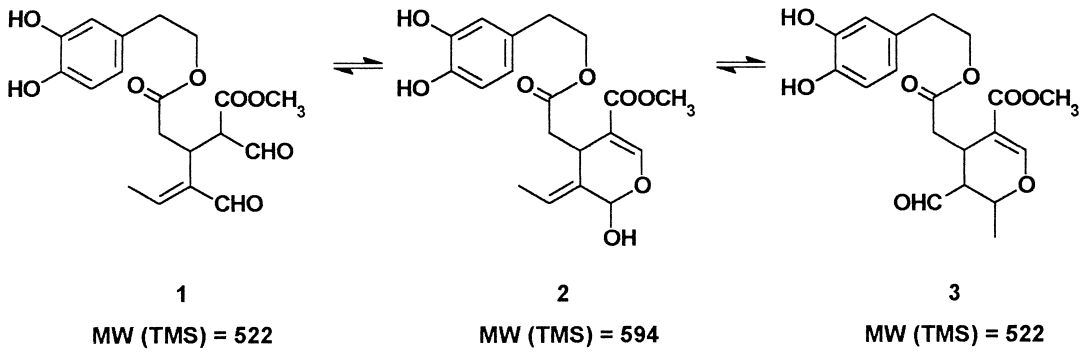

Fig. 2. Oleuropein aglycone interconversions and their molecular weight as trimethylsilylderivatives.

The sterilization step did not change tyrosol and hydroxytyrosol contents.

Throughout the alkali aerobic treatments, a progressive darkening of fruits occurred. While the oxidative browning of phenols in food generally results in a loss of nutritional value, in some processed foods, such as ripe olives, these reactions are a part of desirable changes, essential to the product, and could contribute to the colour complexity.

Two types of browning reaction could be involved. The first is due to enzymatic activity of polyphenol oxidase (PPO, EC 1.10.3.1), present in some fresh fruits and in juices (Sciancalepore & Longone, 1994). In processed foods, with the enzyme removed or inactivated, substantial nonenzymic autoxidation take place (Cilliers & Singleton, 1989). In either case, the final product of enzymic or non-enzymic oxidation of *o*-diphenols is the same quinone. In alkaline medium, where more of the phenols exist as the phenolate ions, autoxidation is extremely easy: the product of a single-electron oxidation of a phenolate ion is a semiquinone free-radical.

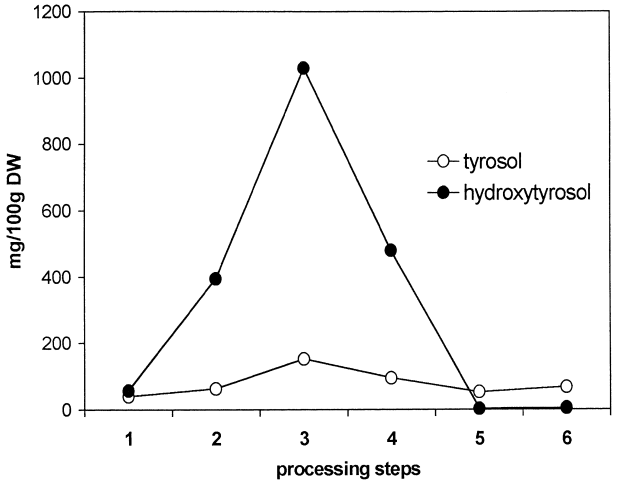

Fig. 3. Evolution of hydroxytyrosol and tyrosol content in olive flesh during Californian style processing steps: 1, fresh olives; 2, brine-stored olives; 3, lye-treated and air-oxidized olives; 4, washed olives; 5, iron-salts treated olives; 6, sterilized olives. Data, expressed on dry weight basis, are the means of three determinations.

This free-radical, while very reactive like all free-radicals, is stabilized by the same resonance delocalization of the unpaired electron around the ring as the phenolate ion. If the phenol is a derivative of 1,2-dihydroxybenzene, such as hydroxytyrosol or 3,4-dihydroxyphenylglycol, oxidative removal of a second electron leads to an *o*-quinone. The reactive electrophilic *o*-quinone intermediate can readily undergo attack by nucleophiles, such as proteins and other phenols. Fig. 4 shows the postulated nucleophilic addition reaction of protein functional groups to the quinoide system of hydroxytyrosol. Addition can take place at two positions of the quinone ring to produce the derivatives A and B. In addition, each of the trigonal carbon atoms with which P-X combines becomes tetrahedral and asymmetric, creating the possibility of two diastereoisomers in each case. Thus, loss of hydroxytyrosol during processing, shown in Fig. 3, may be due to these and related transformations.

The browning of fruits during processing could also be due to the polymerization of phenols to dark pigments, as shown in Fig. 5.

Our results, however, show a continued increase of hydroxytyrosol during alkali aerobic treatments, parallel with the surface fruit colour development. A sharp decrease of hydroxytyrosol was observed only in the final step, after ferrous ions addition, which caused an almost total disappearance of this *o*-diphenol after 24 h oxidation and a more intense browning of fruits. Although iron application in ripe olive processing is recommended only for colour fixation (García et al. 1996), in the present work, iron salts, acting as Lewis acids, seem to exert catalytic effects on the chemical oxidation of phenolic compounds (Lattanzio, Cardinali, Di Venere, Linsalata & Palmieri, 1994; Rigaud, Cheynier, Souquet & Moutounet, 1991). Thus, the oxidation of colourless ferrous-phenol complexes to dark ferric ones seems to play an active role in the browning development, leading to the formation of probably darker and more homogeneous polymers. Ionic interaction in the fruits, between iron and other compounds, such as proteins, polysaccharides, or combination with tannins to form iron tannate, also of black colour, could be important in the ripe olives colour formation and fixation.

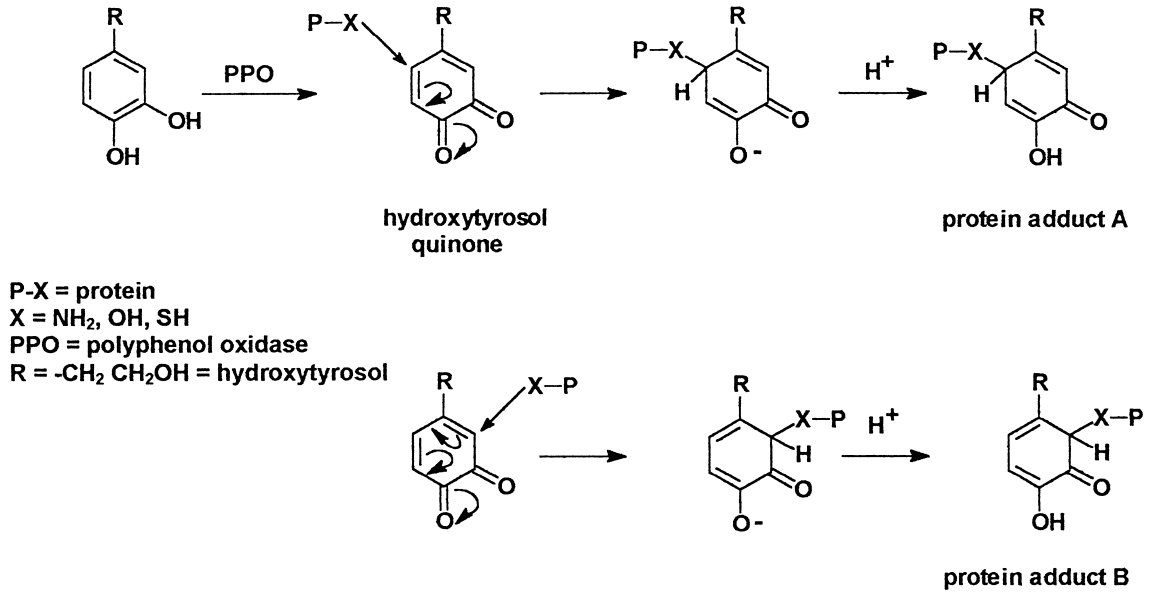

Fig. 4. Postulated nucleophilic addition reaction of protein functional groups to the conjugated system of hydroxytyrosol quinone.

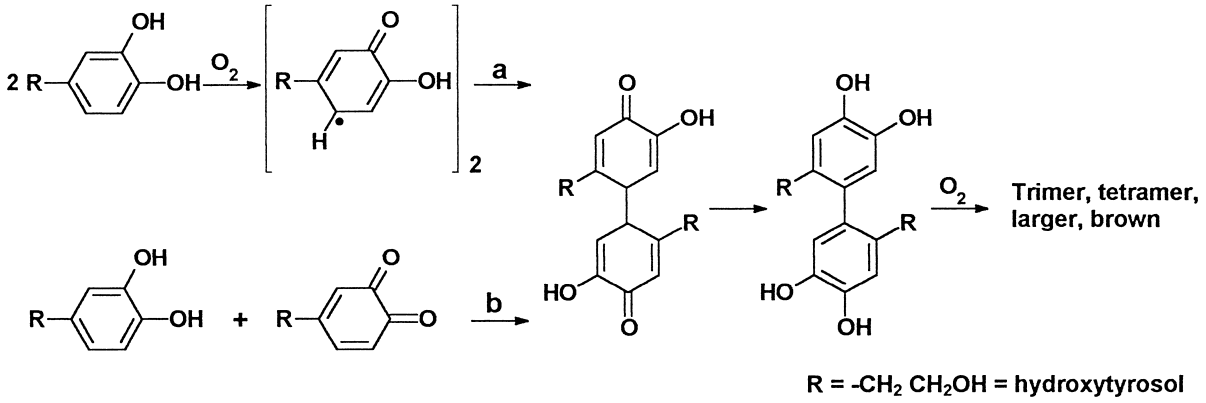

Fig. 5. Oxidative polymerization of *o*-diphenols: the reaction could take place by condensation between two semiquinones (a) or a quinone and an *o*-diphenol (b).

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