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# Oleuropein hydrolysis in natural green olives: Importance of the endogenous enzymes

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ABSTRACT

The bitter taste of olives is mainly caused by the phenolic compound named oleuropein and the mechanism of its hydrolysis during the processing of natural green olives was studied. First, a rapid chemical hydrolysis of oleuropein takes place at a high temperature of 40 °C and at a low pH value of 2.8, but the chemical hydrolysis of the bitter compound is slow at the common range of pH for these olives (3.8–4.2). However, decarboxymethyl elenolic acid linked to hydroxytyrosol and hydroxytyrosol have been found in a high concentration during the elaboration of natural green olives. When olives were heated at 90 °C for 10 min before brining, these compounds are not formed. Hence, the debittering process in natural green olives is due to the activity of β-glucosidase and esterase during the first months of storage and then a slow chemical hydrolysis of oleuropein happens throughout storage time.



## 1. Introduction

Like many fruits and vegetables, olives contain a significant amount of phenolic compounds distributed in the skin, flesh and seed. Oleuropein is the major polyphenol in fresh olives and, due to its bitter taste; it must be completely or partially removed or transformed to make this fruit edible. This compound consists of a molecule of elenolic acid linked to the orthodiphenol hydroxytyrosol by an ester bond and to a molecule of glucose by a glycosidic bond (Panizzi, Scarpati, & Oriente, 1960).

There are very few studies on the presence of endogenous β-glucosidase enzymes in olives and scarce information on esterase enzymes (Briante et al., 2002; Ramírez, Medina, Brenes, & Romero, 2014), although they could be involved in the hydrolysis of oleuropein

The affinity of the β-glucosidase enzyme for oleuropein has been observed in numerous olive varieties from Italy, Tunisia and Spain (Briante et al., 2002; Gutiérrez-Rosales, Romero, Casanovas, Motilva, & Mínguez-Mosquera, 2010; Gutiérrez-Rosales, Romero, Casanovas, Motilva, & Mínguez-Mosquera, 2012; Jemai, Bouaziz, & Sayadi, 2009).

Moreover, researchers have demonstrated in model solutions that oleuropein is hydrolysed by the action of β-glucosidase, forming glucose and the corresponding aglycone (Capasso et al., 1997; De Leonardis, Macciola, Cuomo, & López, 2015; Romero-Segura, Sanz, & Pérez, 2009; Walter, Fleming, & Etchells, 1973). Recently, the enzymatic hydrolysis of oleuropein by an olive leaf protein extract has been proposed (De Leonardis, Testa, Macciola, Lombardi, & Iorizzo, 2016). It has also been reported that oleuropein is converted into the decarboxymethyl dialdehydic form of oleuropein aglycone, HyEDA (Fig. 1) by the action of the endogenous β-glucosidase enzyme during the elaboration of olive oil (Montedoro et al., 2002; Romero-Segura, García-Rodríguez, Sánchez-Ortiz, Sanz, & Pérez, 2012). In addition, endogenous esterase can hydrolyse the oleuropein ester bond, forming hydroxytyrosol and a derived glycosylate (Amiot, Fleuriet, & Macheix, 1989).

Among table olive elaborations, the most popular ones are the Spanish-style green and the California-style black olives. In both processes, oleuropein is chemically hydrolysed by treating the fruits with diluted sodium hydroxide solution; this alkaline solution produces the breakage of the ester bond oleuropein with the consequent formation of hydroxytyrosol and elenolic acid glucoside, both non-bitter compounds (Brenes & de Castro, 1998).

On the other hand, there are other trade preparations that involve the direct brining of olives, either green or purple fruits, which are called natural olives because the fruits are not subjected to any treatment with sodium hydroxide (Medina et al., 2010). The harvested fruits are currently put into an acidified brine (Romeo, Piscopo, & Poiana, 2010). Supposedly, the olives lose their bitterness slowly due to the diffusion of oleuropein from the pulp to the brine (Arroyo-López et al., 2005; Romero, Brenes, García, García, & Garrido, 2004). In addition, the bitter compound can be chemically hydrolysed by the acidic conditions of the solution,

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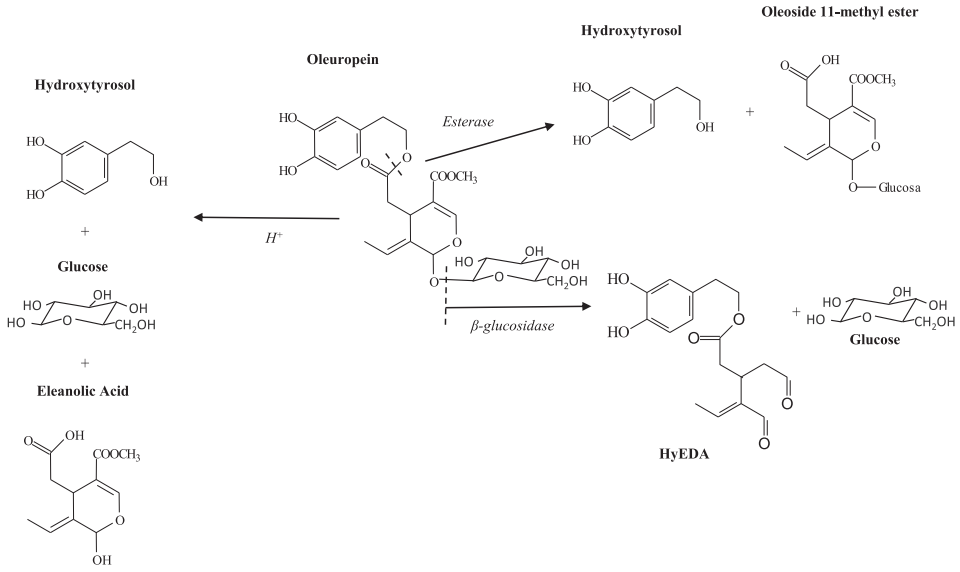


**Fig. 1.** Structure of oleuropein and products obtained by its acid hydrolysis and by the action of hydrolase enzymes.

giving rise to glucose, elenolic acid and hydroxytyrosol (Fig. 1), none of which are bitter.

It is assumed that the endogenous enzymes of olive are degraded or inactivated during the treatment with sodium hydroxide, due to the high pH of the solution (Pandey & Ramachandran, 2008). In contrast, in the case of natural olives, the penetration of sodium chloride and acetic acid into the pulp of the fruit causes a breakdown of the tissue and, consequently, the endogenous hydrolase enzymes (β-glucosidase and esterase) could act on the oleuropein molecule. Jemai et al. (2009) have proposed the possibility of synergism between esterases and β-glucosidases in the fresh fruit on the tree. Firstly, β-glucosidase enzyme breaks the bond between glucose and the rest of the oleuropein molecule; then the esterase enzyme hydrolyses the aglycone so that hydroxytyrosol and elenolic acid are formed. The formation of a high concentration of HyEDA in the brine of 17 olive varieties processed as natural olives in brine has been reported (Medina, García, Romero, de Castro, & Brenes, 2009). Although researchers have proposed many theories for the debittering of olives not treated with sodium hydroxide, such as simple diffusion of the glucoside to the surrounding brine, chemical hydrolysis or microbial action, until now this phenomenon remains unsolved.

The aim of this work was to elucidate the mechanisms by which natural green olives lose their bitterness. In particular, the roles of the endogenous hydrolase enzymes and the chemical conditions of the medium in the loss of bitterness in natural green olives have been investigated.

## 2. Materials and methods

### 2.1. Hydrolysis of oleuropein in model system

#### 2.1.1. Experiment A

Aliquots of 2 mL of a solution containing 6% sodium chloride, 0.2% acetic acid and 5 mM commercial oleuropein (Sigma–Aldrich, St Louis, MO) were stored under a nitrogen atmosphere at different temperatures (10, 22 and 40 °C). The phenolic composition was measured at 1.5, 3 and 5 months. The experiment was carried out in duplicate.

#### 2.1.2. Experiment B

Sterile brine from olives with an oleuropein concentration of 4.7 mM, 3.4% sodium chloride and 0.3% acetic acid was heated at 90 °C for 30 min. Aliquots of 2 mL were stored under a nitrogen atmosphere at different pHs (3.5, 3.8, 3.9 and 4.3 units) at room temperature. The phenolic composition was measured at 1 and 2 months. The experiment was carried out in duplicate.

### 2.2. Hydrolysis of oleuropein in olive elaboration on a laboratory scale

#### 2.2.1. Experiment A

Fruits of the Hojiblanca variety (*Olea europaea* L.) from the 2011–2012 season, at the ripening stage corresponding to a green-yellow colour on the surface, were supplied by local farmers. The olives (190 g) were put into a bottle of 250 mL capacity and covered with a 5% sodium chloride and 0.5% acetic acid solution (control, C).

To eliminate interference from the activity of microorganisms, the olives were also elaborated in aseptic conditions (S) in accordance with Medina, Brenes, Romero, García, and de Castro (2007). The fruits were selected to remove those with blemishes, cuts, and insect damage. After washing thoroughly with tap water to remove impurities, the olives were placed in a sodium hypochlorite solution (50 mg/L active chlorine) at 35 °C for 2 min and then they were washed with sterilised water twice to remove chlorine. Subsequently, 190 g of fruits were put in autoclaved bottles (250 mL capacity) and covered with a 5% sodium chloride and 0.5% acetic acid sterile solution. These manipulations were carried out in a laminar flow cabinet.

To eliminate interference from the activity of the endogenous enzymes and microorganisms, the olives were heated at 90 °C for 10 min and elaborated in aseptic conditions as explained above (S-P).

Finally, all the bottles were sealed and stored at room temperature for 1, 2, 4 and 6 months. After this time, the bottles were opened and checked for microbial growth by visual appearance and plate counts. Microorganisms were not detected in any aseptic brine. The phenolic composition was analysed both in olive pulp and brines. The experiment was carried out in duplicate.

#### 2.2.2. Experiment B

Fruits of the Manzanilla variety (*Olea europaea* L.) from the 2012–2013 season, at the ripening stage corresponding to a green-yellow colour on the surface, were supplied by local farmers. Olives (190 g) were put into bottles of 250 mL capacity and covered with a 5% sodium chloride and 0.5% acetic acid solution under