Bibliography of Olive Polyphenols: summary

The extracts of the olive tree, obtained from the leaves, fruits and branches of it, contain about twenty polyphenols among which we highlight hydroxytyrosol, oleuropein and tyrosol, however, throughout this document we will refer to them generically as polyphenols of the olive tree. Products enriched with these polyphenols have been developed for the prevention or treatment of problems, such as Ulcerative Colitis, for their antioxidant, anti-inflammatory and regenerative capacity and protection of DNA.

The products developed do not contain any petroleum derivatives, nor actives such as silicones or paraffins, which would prevent the beneficial active components from penetrating the cellular system. In addition, they also do not contain dyes or fragrances because many of these substances are often the cause of allergies or extreme sensitivities. The products have been made only with elements of natural origin, isolated, purified and concentrated without altering their biological composition.

In addition, the invention affects the way of manufacturing the products in an ecological way, free of traces of pesticides and that achieves an assured stability of the unsaturated fatty acids and polyphenols of the olive tree, without degrading or oxidizing for long periods of time.

Oleuropein (OLE) is one of the most abundant phenolic compounds in olive leaves (*Olea europaea L*). Recently, the European Medicines Agency (EMA) has issued its own evaluation report on the health properties of this molecule in human health[11]. It is widely known for its potent antioxidant and anti-inflammatory activities that appear to be the basis of its multiple pharmacological activities: antiviral, anticancer, neuroprotective, antimicrobial, and gastroprotective, among others[12,13].

While a number of encouraging preclinical data have revealed the effects of polyphenols on intestinal inflammation as plant extracts or pure molecules, scarce studies are available on isolated oleuropein. Regarding IBD, OLE has particularly been shown to be effective in acute, chronic and colon cancer-associated colitis[14-16]. However, OLE is limited by its poor stability not only against external factors (light, oxygen) but also through the human organism (pH, enzymes). Several studies suggest that OLE undergoes a complex process of biotransformation during gastric digestion[17-19].

Because of this, the efficacy results recovered from oral administration of OLE could be lower than expected and too high doses have been used to date for in vivo studies, which could provide toxicity.

Since ancient times, the benefits of olive oil in relation to the protective and moisturizing action on the skin and other tissues have been known. This is because oleic acid (from the Omega-9 series), the main fatty acid in olive oil, and its derivative Hydroxy-oleic are able to help in the restructuring of lipid cell membranes.

Traditionally, the health effects of olive oil were attributed only high lipid content, however, today it is known that olive polyphenols also exert a cellular protective activity. The polyphenols of the olive tree are part of 1-3% of the unsaponifiable fraction of the oil. The composition of olive polyphenols varies in quantity and quality depending on the olive variety, the age of the tree, the agricultural techniques employed in cultivation, the degree of maturity, soil composition, climate, technical processing and storage (Amarowicz *et al*., 2009, Cicerale *et al*., 2009, Corona *et al*., 2009, Muzzalupo *et al*., 2011, Visioli & Galli, 1998). In general, there are four main classes of phenolic compounds found in these oils: flavonoids, lignans, simple phenols, and secoiridoids (Figure 1).

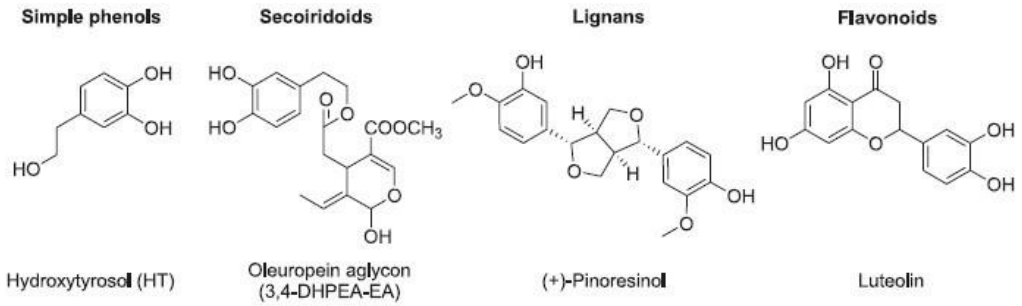

Figure 1. Main phenolic compounds of olive oil (Martin-Pelaez *et al*.,2013)

Apparently, chemical modifications occur in the leaves of the olive tree. Endogenous hydrolytic enzymes, mainly glycosidases, can be activated during leaf grinding or maceration processes in aqueous extracts and catalyze the hydrolysis of phenols such as oleuropein (Ryan *et al*., 2003) with the consequent production of aglycon, which is rapidly isomerized through the enolic form to a dialdehyde (Figure 2).

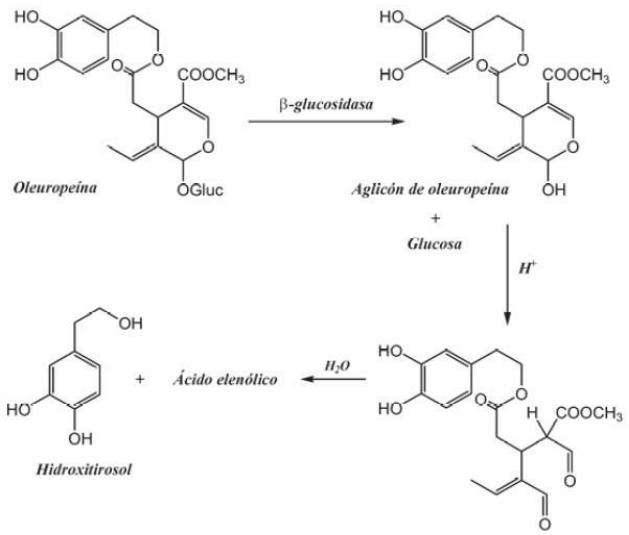

Figura 2. Hydrolysis and partial degradation of oleuropein in olive leaves (De la Fuente P. *et al.*, 2004)

Chemical modifications also occur in the fruit of the olive tree, in this way, the olives can contain mainly polar compounds such as the glycosides oleuropein and ligstroside that are the precursors of the aglycone derivatives, even more polar compounds. Oleuropein-aglycone is the ester of elenolic acid with 3,4-o-dihydroxyphenylethanol (hydroxytyrosol) and ligstroside-aglycone is the ester of elenolic acid with 4-hydroxyphenylethanol (tyrosol) and are formed when a glucose molecule of the corresponding glycoside is lost in the olive ripening process. Aglycones and their different derivatives are the most abundant phenols in ripe olives and oil. The derivatives differ in their chemical structures, being able to open or close the ring, being the polyphenols tyrosol and hydroxytyrosol the final products of the hydrolysis of the aglycone derivatives of oleuropein and ligstroside (Figure 3).

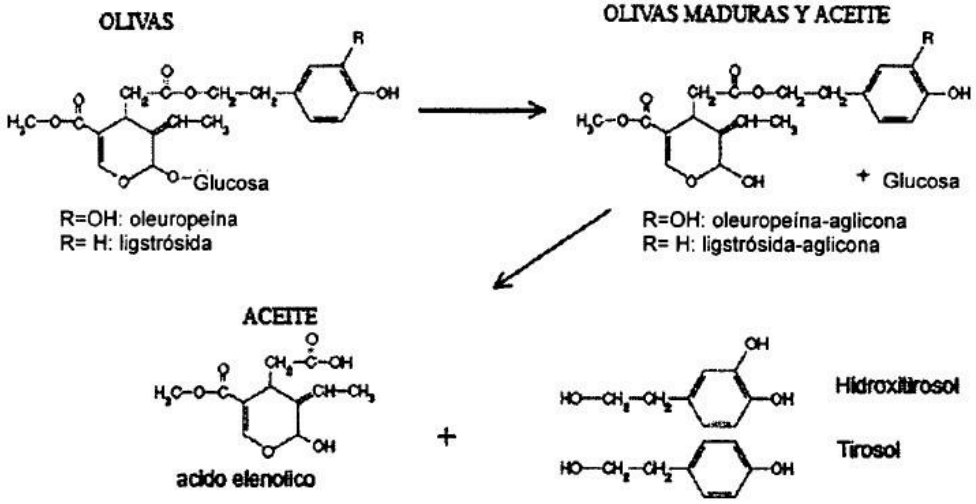

Figura 3. Hydrolysis and partial degradation of oleuropein in the fruit of the olive tree.

Oleuropein is a bitter glycoside found mostly in the leaves of the olive tree, *Olea europea*, but also in other parts of this tree, including the roots, bark and fruits. The medicinal use of olive leaf extracts dates back to the early 1800s, when they were used in liquid form as a treatment for malaria infections. Currently, it has achieved great popularity due to the growing number of scientific publications that highlight its healthy effects in the different fields of medicine. These compounds are characterized by a high capacity to curb oxidative stress and neutralize free radicals (Chimi H, *et al.,* 1991). Likewise, several studies have shown other biological activities, such as antiplatelet (Petroni *et al*., 1995), antioxidant of LDL lipoproteins (Manna *et al*., 2004, Visioli *et al*., 1995) and inhibitor of lipooxygenases (involved in the inflammatory process) (de la Puerta *et al*., 1999), improving the symptoms of skin disorders such as dermatitis, eczema, psoriasis, as well as ability to induce apoptosis in HL-60 cells (Della Ragione F, *et al*., 2000) and in vitro activity against Gram-negative and Gram-positive bacteria (Bisignano *et al*., 1999).

Some studies have shown that exposure of highly aggressive human breast cancer cells to phenolic extracts of extra virgin olive oil (EVOO) enriched with the secoiridoids oleuropein aglycone and decarboxymethyl oleuropein aglycone, can induce intracellular signaling pathways that could respond to biological stress at the molecular or cellular level. The strength of the evidence supporting the activity of these secoiridoids in xenohormesis has been tested assuming that they have tumoricidal activity resulting from paradoxical activation by cellular stress of anti-aging transcriptomic signals of cancer cells (Menéndez *et al*., 2013).

These authors confirmed that the pathways activated by the EVOO secoiridoids could defend cells and tissues in a similar way to hormesis, since they regulate energy metabolism in such a way that an increase in cell survival in times of stress would be expected. In addition, the anticancer activity of EVOO secoiridoids was related to cellular stress activation of anti-aging transcriptomic signals of cancer cells, including endoplasmic reticulum stress and response to unfolded proteins, spermidine and polyamine metabolism, and sirtuin-1 (SIRT1) and NRF2 signaling. EVOO secoiridoids activated the AMPK enzyme complex and inhibited crucial genes involved in the Warburg effect and the self-renewal capacity of "immortal" cancer stem cells.

So far the European Food Safety Agency (EFSA) has verified that "*the polyphenols in olive oil contribute to the protection of blood lipids against oxidative damage*", although in order to ensure this cause-effect in the protection of blood lipids against cholesterol oxidation, much more important than the cholesterol level itself, EFSA requires food products that want to advertise these effects, to provide a minimum of "*5 mg daily of hydroxytyrosol and its derivatives (for example, an oleuropein or tyrosol complex)*", which in the case of olive oil should be contained in 20 g (approximately two tablespoons of oil). "*In order for a product to bear this claim, the consumer shall be informed that the beneficial effect is obtained with a daily intake of 20 g of olive oil*, "says EFSA in its List of authorised health claims, Annex to Commission Regulation (EU) No 432/2012 of 16 May 2012, establishing a list of authorised health claims made on foods other than those relating to the reduction of the risk of disease and to the development and health of children.

With this, EFSA ratifies the results of numerous investigations and publications, which have shown that the polyphenols of the olive tree hydroxytyrosol, oleuropein and tyrosol help to:

- Exercise anti-inflammatory actions.
- Maintain a correct blood pressure.
- Contribute to defenses.
- Protect LDL cholesterol from oxidation.
- Maintain adequate levels of the protective HDL cholesterol.

En este documento se recogen algunas publicaciones y patentes de relevancia relacionadas con los ácidos grasos insaturados y los polifenoles del olivo.

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- ✓ PAT US 5998641 process of increase in the content of polyphenols in olive oil without increasing bitterness, using emulsified olive oil with an aqueous solution containing polyphenols and an enzyme to desamargar (i.e. β-glucosidase).
- ✓ PAT US-A-6162480 vegetable oil antioxidant enrichment method.
- ✓ PAT US-B1-6746706 method of enrichment of compositions of food (cream spreads, vinaigrette, tomato sauce), where they contain a 20-100% of an aqueous phase characterised by a content between 15 ppm and 50 ppm for together tyrosol and hydroxytyrosol.
- ✓ PAT US-B1-6942890 method to enrich foods by adding to these solid materials from the olive, which requires the incubation during a certain time of food that will enrich,
- ✓ PAT US-B-6361803 using organic solvents, in particular polar solvents, in order to produce an olive extract containing hydroxytyrosol with an acceptable purity.
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