

## Use of solid residue from the olive industry

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### RESUMEN

#### Uso de residuos sólidos a partir de la industria del olivo

La investigación de nuevos aprovechamientos del olivar y en particular de los subproductos del proceso de producción del aceite, tiene gran relevancia tanto en la economía como en el medio ambiente de los pueblos donde se desarrolla este cultivo. Son numerosos los trabajos publicados sobre la composición química de la aceituna y el aceite de oliva, en cambio sólo existen algunos estudios sobre el aislamiento e identificación de los compuestos de la hoja de olivo, en esta contribución se revisan los conocimientos sobre la composición química de la hoja de olivo, así como, las utilidades de los residuos sólidos -hoja de olivo y hueso de aceituna- que se originan en la producción del aceite de oliva y de aceitunas de mesa, como energía renovable y para la obtención de compuestos de alto valor añadido. Estos últimos, compuestos bioactivos dirigidos a los mercados de los aditivos alimentarios naturales, farmacéutico y cosmético, todos ellos muy receptivos en la actualidad a los productos de origen natural.

**PALABRAS-CLAVE:** Aprovechamiento de subproductos - Biomasa del olivar - Compuestos bioactivos - Hoja de olivo - Residuo sólido.

### SUMMARY

#### Use of solid residue from the olive industry

Research into finding new uses for olive products, particularly by-products of olive oil production, is of great relevance not only to the economy, but also to the environment, in the towns where olives are grown. A large number of research articles has been published dealing with the chemical composition of olives and olive oil; however, only a few studies have centered on isolating and identifying compounds in the olive leaf. In this article an overview of the present body of knowledge on the chemical composition of the olive leaf will be presented. Also to be discussed is the use of solid residue, namely, the olive leaf and the olive stone. Both of these types of residue result from olive oil and table olive production, and can be used as a renewable energy source, as well as to obtain high added-value compounds. The latter, bioactive compounds are directed towards the cosmetic, pharmaceutical, and natural food supplements markets, all of which are currently highly receptive to products of natural origin.

**KEY-WORDS:** Bioactive compounds - Olive biomass - Olive leaf - Solid residue - Use of by-products.

### 1. INTRODUCTION

Olive tree cultivation in the Mediterranean goes back to ancient times, ever since the Roman Age

(Melillo, 1994), when olive cultivation spread throughout the entire Mediterranean basin. This longevous tree (*Olea europaea*, Oleaceae) integrates and identifies economically, socially, and culturally the inhabitants of this basin; olive oil constitutes the main source of nutritional fats and is a very valuable product for exportation. Today, Mediterranean countries account for around 98% of the world's olive cultivation (approximately ten million hectares); they produce about 1,9 million metric tonnes per annum of olive oil, in addition to 1,1 million t of table olives (mean production during the four-season reference period from 1999/00 to 2002/03, <http://www.internationaloliveoil.org/>).

The use of solid residue, such as leaves and stones, is of great economic and social importance for the Mediterranean area, as these by-products accumulate in large amounts during olive oil production and the elaboration of table olives.

At present, it is possible to obtain activated carbon from olive stones (Rivera-Utrilla *et al.*, 1986; Galiatsatou *et al.*, 2001; Feng-Chin and Ru-Ling, 2000; Khalil *et al.*, 2001; Blázquez *et al.*, 2005), and the new biomass boiler system designs facilitate the use of olive stones as fuel for the generation of heat and electricity (Ortega-Jurado *et al.*, 2004; Vlyssides *et al.*, 2004). However, olive leaves, which are produced in large amounts, render scarce profits at present. Nevertheless, the market for natural ingredients and additives is rapidly growing, with such products obtaining high prices.

In the last few years, the possible toxicity of synthetic compounds (Fukushima and Tsuda, 1985; Fukushima *et al.*, 1986; Stone, 2003), has increased interest in natural products and the food additives, cosmetics and pharmaceutical industries have improved their efforts in preparing bioactive compounds from natural products by extraction, fractionation, and purification.

### 2. BIOACTIVE COMPOUNDS IN OLIVE LEAVES

Olive leaves are agricultural residue resulting from the pruning of olive trees. The leaves may also be considered an easily available industrial by-product, as they find their way to the olive mills in appreciable quantities. Olive leaves represent around 10% of the total weight of olives arriving to the mill (Espínola, 1996). Currently this by-product

is not very profitable, given that in many countries the leaves have traditionally been used only as feed for livestock (Gómez-Cabrera *et al.*, 1992; Sánchez *et al.*, 2002; Ruíz *et al.*, 2004). Often the leaves are disposed of by simply burning them together with the branches from pruning.

## 2.1. Phenolic Components

The leaves of *Olea europaea* L. have been studied, among other reasons, for their therapeutic effects. Interest in the potential health benefits of olive leaf extract dates back to the mid-nineteenth century, when reports were made of the ability of an extract (made from boiling the leaves) to reduce fever, including the ability to prevent or cure malaria symptoms even more effectively than quinine (Hanbury, 1854; Capretti and Bonaconza, 1949).

This singular physiological effect also encouraged identification of the principal agent involved. In the early 20<sup>th</sup> century, the phenolic compound which is most abundant and characteristic in the fruit of the olive was isolated by Bourquelot and Vintilesco (1908). In 1960 Panizzi *et al.* established that the compound was a heterosidic ester of elenolic acid and hydroxytyrosol. Oleuropein is the principal member of the family of secoiridoids in the olive tree (*Olea europaea* L.). In 1974, Inouye *et al.* confirmed its structure by mass spectrometry.

For over thirty years since the isolation of oleuropein, extracts from various parts of the olive tree have been studied. The glucoside has been found in all of them: leaves, shoots, olive (pulp, husk and stone), wood, bark and roots (Pasquale *et al.*, 1991; Juven, 1968). Le Tutour and Guedon (1992) point out that oleuropein content in olive leaves is very high, around 60-90 mg per gram (dry material), although they also contain other compounds. Through hydrolysis, other phenolic compounds have been isolated and identified in olive leaves, although they are present in lesser quantity. These compounds include demethyloleuropein, ligstroside (Ragazzi *et al.*, 1973), verbascoside (Fleuriet *et al.*, 1984; Soler-Rivas *et al.*, 2000) oleoside dimethylester (Panizzi *et al.*, 1960), a non-glucosidic secoiridoid (Gariboldi *et al.*, 1986), and oleuroside, an isomer of oleuropein (Kuwajima *et al.*, 1988).

Additionally, olive leaves contain a significant amount of flavonoids. Found in the leaf are luteolin-7-O-glucoside, luteolin-7-O-rutinoside, apigenin-7-O-glucoside, rutin, luteolin, and apigenin, all of which have been identified by HPLC (De Laurentis, 1997; Ficarra and Ficarra 1991; Heimler *et al.*, 2002). The purpose of this group of phenolic compounds present in the olive leaf seems to be, by virtue of their absorption spectrum, that of limiting the effects of UV light and the entailing oxidative damage (Galli and Visioli, 1999). Flavonoids also have the capacity to inhibit oxidation caused by free radicals (Bors *et al.*, 1990 a and b).

The complexity of the chromatographic profiles of the fraction extracted with polar solvents from the olive leaf clearly indicates the presence of other

phenolic derivatives, which may be present in the natural matrix. On the other hand, it can not be ruled out that the procedures of extraction and treatments of the sample may alter the structure of some of the secondary metabolites originally present in the plant tissue. Therefore, the need exists for the development of reliable methods for the analysis of the alcoholic extract of the leaf, prior to any stage of purification. De Nino *et al.* (1997) employ a method of direct analysis of the leaf extract, whose objective is the direct characterization of metabolites of the olive leaf using mass spectrometry (MS). This methodology would permit the "chemical characterization" of a particular variety, or the identification of compounds of great biological value.

Among the group of phenolic compounds present in the olive leaf, oleuropein and the products of its hydrolysis are those of the greatest biological interest (Fleming *et al.*, 1973).

Oleuropein has a vasodilator effect, increases blood flow in the coronary arteries and improves arrhythmia (Zaruelo *et al.*, 1991; Manna *et al.*, 2004). This compound has proven to be a potent antioxidant with anti-inflammatory properties (Visioli and Galli, 2002). In 1970 several articles presented the antiviral and antibacterial activity of calcium enolate (obtained by hydrolysis from oleuropein) (Elliot *et al.*, 1975; Soret, 1969). Subsequent publications show that oleuropein also engages in antimicrobial activity against viruses, retroviruses, bacteria, yeasts, fungus, molds and other parasites (Juven *et al.*, 1972; Tassou and Nychas, 1995; Aziz *et al.*, 1998; Zanichelli *et al.*, 2005). Other clinical effects of oleuropein are the fortification of cellular and organism protection through macrophage response (Visioli *et al.*, 1998), the inhibition of plaque aggregation and eicosanoid production (Petroni *et al.*, 1995), and a reduction by nearly 30% in the level of low density lipoproteins (LDL) (Visioli and Galli, 1994; Andrikopoulos *et al.*, 2002).

## 2.2. Terpenic components

Vázquez-Roncero and Janer (1969) contributed diverse qualitative results of the presence of terpenic acids in the olive leaf. Later, Mussini *et al.* (1975) isolated alpha-amyrine and confirmed the presence of maslinic acid. Bianchi *et al.* (1993, 1994) analyzed these compounds and some of those found by Vázquez-Roncero and collaborators, in three Italian varieties.

Among the terpenic compounds in the olive leaf, one to take notice of is oleanolic acid (3-beta-hydroxy-28-carboxyolean). This compound is found extensively throughout the plant kingdom, and in the olive leaf it occurs in free acid form in considerable proportions, accounting for  $\approx$  3% of dry leaf weight (Albi *et al.*, 2001). Oleanolic acid is biologically active with antiabortive, anti-cariogenic, anti-fertility, anti-hepatotoxic, anti-inflammatory, antisarcomic, cancer-preventive, cardiogenic, diuretic, hepatoprotective and uterotonic properties. Numerous studies have been published on the possible biological activity of

this acid and its glucosides. It has been studied in its activity as an inhibitor of the proliferation of leucemic cells (Es-Saady *et al.*, 1994), as a hypoglycemic (Yoshikawa *et al.*, 1994), as an anti-tumoral agent (Gerhauser *et al.*, 2003), as a hepatoprotective agent (Yim *et al.*, 2001), as an anti-inflammatory (Ismaili *et al.*, 2002; Takeoka *et al.*, 2000 and Choi *et al.*, 2002), as an anti-bacterial (Sasazuka *et al.*, 1995), as an inhibitor of the production of nitric acid (Honda *et al.*, 1997), as an anti-HIV agent (Mengoni *et al.*, 2002), in its anti-atherogenic activity (Somova *et al.*, 2003) and as a cardiogenic (Somova *et al.*, 2004). Also in existence are publications and patents for the preparation of functional foods with oleanolic acid (Hassel *et al.*, 1998; Putte, 2000; Guinda *et al.*, 2004).

### 2.3. Liposoluble compounds

In 1973, Janer isolated by Thin Layer Chromatography some liposoluble compounds present in olive leaf extract (hexane extract) and analyzed the fatty acids of the triacylglycerols from leaves. In recent years, Guinda *et al.* (2002) have isolated, identified and quantified numerous components of a hexane extract of olive leaves, consisting of: saturated hydrocarbons, squalene, ester waxes, alpha-tocopherol, triglycerides, beta-carotene, linear alcohols, alpha- and beta-amyrine, and beta-sytosterol. These compounds have been found in five Spanish varieties (Picual, Hojiblanca, Empeltre, Arbequina and Cornicabra). The concentration (ppm) of the compounds ranged from the following: 050-3400 (saturated hydrocarbons), 38-152 (squalene), 915-1874, 28-100 (beta-carotene), 832-1396 (triglycerides), 41-125 (alpha-tocopherol), 714-2500 (beta- sytosterol), 95-311 (alcohols) and 342-837 (terpenic dialcohols). The most numerous hydrocarbons in all of the varieties studied are C29, C31 and C33. The waxes are mainly constituted of fatty acids and the terpenic alcohols of the olive leaf (palmitic, oleic, and linoleic; alpha- and beta-amyrine). The terpenic dialcohols found in the olive leaf are uvaol and erythrodiol, with the uvaol content always higher than that of erythrodiol, which is the opposite case in olive oil. A large part of the compounds analyzed in the hexane extract of the olive leaf, such as alpha-tocopherol, beta-carotene, alpha- and beta-amyrine, squalene and beta-sytosterol, are bioactive compounds capable of modulating human physiological systems. Therefore, these compounds have multiple applications in the pharmaceutical, cosmetics and food additives industries. Numerous articles have been published on the antioxidant activity of alpha-tocopherol, as well as on the protective effects of alpha-tocopherol and of beta-carotene with regard to atherogenesis (Devaraj *et al.*, 1998) and cancer (Virtamo *et al.*, 2000; 2003). Other publications examine the potential use of squalene (Kelly, 1999) and its preventive effect on colon cancer (Newmark, 1999; Rao *et al.*, 1998). Still others examine the potential health benefits of the phytosterols: beta-sytosterol reduces cholesterol levels in the blood, as

well as being effective at in vitro reduction of breast cancer cells (Awad *et al.*, 2001; 2003). Another area of study has been the anti-inflammatory activity of the triterpenic dialcohols of the olive leaf (alpha- and beta-amyrine) (Martín-Cordero *et al.*, 2001; Puerta *et al.*, 1997).

### 2.4. Other compounds

The olive tree, like other members of the family of Oleaceas, is distinguished by its capacity for the biosynthesis of mannitol and oligosaccharides of the raffinose family (raffinose and, above all, stachyose), as well as sucrose, as direct products of photosynthesis (Flora and Madore, 1993; Stoop *et al.*, 1996). Mannitol, a hexitol derivative of mannose, offers a series of properties with advantageous applications in the food and pharmaceutical industries. Its sweetening potency is equivalent to 70% that of sucrose; it has a low caloric value (2 kcal/g), it does not cause caries and its metabolism in humans is not dependent on insulin, which makes it suitable for consumption by diabetics. Additionally, it has healthful effects as an antioxidant (Wisselink *et al.*, 2002) and is of therapeutic use in severe head injuries, as it is effective in the reduction of intracranial pressure (Brain Trauma Foundation, 1996; Filipo *et al.*, 1997; Unterberg *et al.*, 1997). Its industrial preparation is achieved by means of catalytic hydrogenation of sugars. As this chemical process yields a scant quantity of mannitol, in recent years research has been conducted into alternative biotechnological processes. The use of olive leaves as raw material in obtaining mannitol offers a promising alternative, as they contain between 10 and 20 mg per gram of plant tissue of this carbohydrate (Flora and Madore, 1993; Sánchez, 1998).

Recently, Campeol *et al.*, 2004, have isolated and identified through mass spectrometry (EIMS) and nuclear magnetic resonance (H and C RMN), a new compound: 1.5-Anhydroxylitol. This compound, never before detected in the plant kingdom, was obtained in the range of 0.1 to 1%.

For the purpose of obtaining basic data for the processing and manufacture of foods, Ok-Hwan *et al.* (2005) have analyzed the contents of the leaf as to moisture, ash, crude protein, fat, carbohydrates, the principal amino acids (glutamic and aspartic) and principal free sugar (sucrose), as well as the concentration of Ca and vitamins A and C. Neither vitamins B6 nor B12 were detected.

## 3. USES OF OLIVE LEAVES

### 3.1. The Benefits of extracts

At present, there is a growing body of evidence for the beneficial role that the phytonutrients play in relation with the prevention of serious diseases (Arai, 1997; Ovesen, 1997). The enormous interest in these substances in recent years as additives or

natural food ingredients has gone beyond food preparation and conservation, into the new area known as nutraceuticals. This science studies the therapeutic effects of the components of foods. It is known that "functional foods" can modulate human physiological systems, such as the immunological, endocrinal, nervous, circulatory and digestive systems, with this last a proven object of study (Hassel, 1998). At present, several natural extracts, among them that of the olive leaf, are used in the manufacture of functional foods (Micol *et al.*, 2003).

Diverse epidemiological studies have proven the association between diets rich in antioxidant compounds and a reduction in the risk of contracting certain types of cancer, cardiovascular and ocular diseases in which oxidative stress plays an important part in their development (Garewall, 1997; Visioli *et al.*, 2000). As a result, nutritional recommendations generally include the consumption of food and drink rich in antioxidant compounds (Packer *et al.*, 1999). It is also becoming more frequent to design foods with antioxidant functions (Hollingsworth, 1998). Among the antioxidants present in the olive leaf, the phenolic compounds are notable. Phenolic compounds are one of the main products of the secondary metabolism and comprise a wide range of structural classes and biological functions.

Olive leaf extracts are well-known for their antioxidant properties (Susnik-Rybarski, *et al.*, 1983; Benavente-García *et al.*, 2000; Briante *et al.*, 2001; De la Fuente *et al.*, 2005; Bouaziz and Sayadi, 2005; Coll *et al.*, 2000). Other significant qualities associated with olive leaf extracts include that of being a hypotensor (Khayyal *et al.*, 2002), a hypoglucemiant (Manceau *et al.*, 1942; González *et al.*, 1992; Trovato *et al.*, 1993; Komaki *et al.*, 2003), a hypocholesterolemiant (De Pasquale *et al.*, 1991), cardioprotective (Cicosta *et al.*, 1990), radioprotective (Benavente-García *et al.*, 2002), anti-HIV (Lee-Huang *et al.*, 2003) and anti-viral (Micol *et al.*, 2003), antimutagenic (Yahiaoui *et al.*, 1994), anticarcinogenic (Pieroni *et al.*, 1996; Onderoglu *et al.*, 1999; Hamdi and Castellon, 2005; Kosak and Stern, 1962), hypotensive (Kosak and Stern, 1960), and anti-inflammatory (Fehri *et al.*, 1996). Of special interest is the extract's antimicrobial activity, in the possibility of utilizing them as alternative food additives (Markin *et al.*, 2003; Tassou and Nychas, 1994). Similarly, various authors have reported on the antibacterial activity of the phenolic compounds of the olive tree (Tassou and Nychas, 1995; Tranter, 1993; Rodríguez *et al.*, 1988; Paster *et al.*, 1988; Nychas *et al.*, 1990). Other studies include one conducted on the effect of an aqueous extract of olive leaf on the thyroid gland in laboratory animals (Al-Qarawi *et al.*, 2002), and another on the antioxidant activity of an olive leaf extract against sunflower oil oxidation (Frag *et al.*, 2002).

In the olive tree, phenolic compounds are more abundant in the fruit than in the leaf; even so, they are present in significant amounts (Le Tutour and Guedon, 1992; Paiva-Martins and Gordon, 2001), which makes utilization of the leaves worthwhile. In

addition, the leaf contains a considerable amount of terpenic compounds, whose biological activity is well-known. A specific review of the pharmacological activity of oleanolic acid has been published (Tian *et al.*, 2002), as well as a study of the anticarcinogenic effect of ursolic acid (Es-Saady *et al.*, 1994, 1996a y 1996b). Basically, the leaf represents a by-product whose potential for use is still under development. Its use would facilitate the obtaining of natural products at a competitive cost.

### 3.2. Uses of the extracts

Up to now, use of the olive leaf has been made principally through formulations which include an extract of the same, without any previous separation of the active compounds. There are numerous preparations of this type on the market; they are used as complements to the treatment of certain diseases or as nutritional supplements. New patents appear daily for functional foods or cosmetics based on olive leaf extracts. The patents are quite varied in their formulation; for example, there are liquid preparations with alcohol and with glycerin (Oliveda Network SL, 2005 a y b). Equally diverse are the applications to which these formulations are destined: manufacture of dietetic bread for diabetics (Scheneder, 1985); preparations which contain lycopene and olive leaf extract for use in food or cosmetics (Coll *et al.*, 1999); use of olive leaf extracts in dietetic cookies (Shtukatur, 2003); the manufacture of a nutritional supplement with physiological effects containing olive leaf extract (Stueckler 1998); an extract of olive leaves to combat free radicals, and their use in dietetic foods and in cosmetics (Amari 1998).

## 4. OBTAINING HIGH ADDED-VALUE COMPOUNDS FROM THE OLIVE LEAF

Works on obtaining bioactive compounds from the olive leaf are recent and not very numerous. The techniques employed range from biotransformation (Ryan *et al.*, 2002; Verhe *et al.*, 1992; Briante *et al.*, 2001), solid/liquid extraction and subsequent fractionation of the extract (Albi *et al.*, 2001), and supercritical fluid extraction (SFE) (Lucas *et al.*, 2002; Tabera *et al.*, 2004b).

Briante *et al.*, (2001, 2002 and 2004) have proposed a bioreactor for obtaining large quantities of hydroxytyrosol of high purity (91-94% in weight), starting from olive leaf extract. The process consists of extraction with ethanol/water (1:1) from the leaf and subsequent biotransformation of the extract, by means of a hyperthermophilic beta-glucosidase, partially purified and immobilized in a support of chitosan (polysaccharide obtained from the shells of crustaceans). The bioreactor is separated into two chambers; the first column is filled with the matrix of the chitosan enzyme and is used for the hydrolysis of oleuropein into aglucone. The second column of the bioreactor is used as a second reaction chamber at high temperature and under pH-controlled

conditions, permitting total hydrolysis of the aglucone of oleuropein into hydroxytyrosol. Lucas *et al.*, (2002), obtain concentrates of alpha-tocopherol directly from the leaves, by means of supercritical fluid extraction (SFE). Tabera *et al.*, (2004b), have patented a process for obtaining high added-value compounds from the olive leaf, consisting mainly of a first stage of solid/liquid extraction with solvents of decreasing polarity, and subsequent fine fractionation of the different extracts through extraction with supercritical CO<sub>2</sub>, in countercurrent (CC-SFE). With this method they have obtained concentrates of alpha-tocopherol (Tabera *et al.*, 2004a) and hydroxytyrosol, among others. Guinda and collaborators, have developed a procedure for obtaining terpenic acids from the leaves of *Olea europaea* by means of extraction from the leaf with an organic polar solvent, and separation of this acid by crystallization. This patented process (Guinda *et al.*, 2000) allows for obtaining oleanolic acid with approximately 93% purity. Subsequent purification by means of a simple thermal treatment allows for this acid to be obtained with a purity of 98-99%. The advantage of this procedure is even greater when taking into account that even though this triterpenic acid is found extensively throughout the plant kingdom, in the olive leaf, oleanolic acid occurs in free acid form in considerable proportions ( $\cong$  3% dry leaf weight). Additionally, there is a considerable amount of leaves available annually, when including those coming from pruning and those separated from the olives in the mills.

At the present time, is being investigated in the Instituto de la Grasa (CSIC), (Spanish project AGL 2003-02795), a new and encouraging process that involves a continuous separation procedure, by means of molecular distillation (Short Path Distillation), of the natural compounds present in alcoholic olive leaf extract.

## 5. FINAL CONSIDERATIONS

To increase the economic performance of the olive and related industries, it is of great importance to make use of a by-product like the olive leaf. Bioactive compounds obtained from the leaves have dietetic, pharmaceutical and cosmetic applications. Using this residue not only meets with the requirement of its effective and innocuous elimination: as well as preventing environmental problems, it also creates new sources of wealth which contribute to higher profitability in the initial industrial process. Moreover, it should be taken into account that making the management of residue profitable generates all sorts of new industries, with the resulting social gains.

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