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Potential use of olive by-products

Extraction of interesting organic compounds from olive oil waste

By **Juan Fernández-Bolaños*, Guillermo Rodríguez, Rocío Rodríguez, Rafael Guillén** and **Ana Jiménez**

Food Biotechnology Departament, Instituto de la Grasa (CSIC)
Avda. Padre García Tejero 4, Apartado 1078, 41012 Sevilla, Spain
*Corresponding author: Tel: 34-954691054; Fax 34-954691262; E-mai l: jfbg@cica.es

RESUMEN

Obtención de compuestos orgánicos de interés a partir de los residuos de la extracción del aceite de oliva

Existe una gran cantidad de compuestos bioactivos y de alto interés presentes en la aceituna. Muchos de ellos se conocen por las cualidades beneficiosas que aportan al aceite de oliva virgen. La mayoría permanecen en mayor cantidad en el subproducto de la extracción del aceite. Aunque, el alpechín, el orujo y el nuevo subproducto de extracción del aceite en dos fases, alperujo, representan un problema potencial de vertido y contaminación, también son una prometedora fuente de compuestos de alto valor. Esta revisión resume lo último que se conoce sobre la utilización de estos residuos en el campo anteriormente mencionado, con más de 90 referencias que incluyen artículos y patentes. Todas estas investigaciones han sido clasificadas en cuanto a la recuperación de constituyentes naturalmente presentes o en cuanto a la bioconversión de los residuos en sustancias de interés.

PALABRAS-CLAVE: Alpechín - Alperujo - Compuestos bioactivos - Orujo de aceituna - Recuperación de sustancias valiosas.

SUMMARY

Extraction of interesting organic compounds from olive oil waste

In the olive fruits there is a large amount of bioactive compounds and substances of high interest. Many of them are known by owing health beneficial properties that contribute to protective effect of the virgin olive oil. During olive oil processing, most of them remain in the olive oil wastes. Although, olive-mill wastewater (OMWW) or “alpechin”, olive oil cake (OOC), and the new by-product, known as “alperujo” in Spain and generated by the two-phase extraction process, represent a major disposal and potentially severe pollution problem for the industry, they are also promising source of substances of high value. This review summarises the last knowledge on the utilisation of residual products, with more than 90 references including articles and patents, which are promising with regard to future application. All these investigations have been classified into two options, the recovery of valuable natural constituents and the bioconversion into useful products.

KEY-WORDS: “Alperujo” - Bioactive compounds - Olive mill wastewater - Olive oil cake - Recovery of valuable substances.

1. INTRODUCTION

During the last years the interest in the recovery, recycling and upgrading of residues from plant food processing has increased drastically (Laufenberg *et al.*, 2003). These food industries produce large volume of wastes both solid and liquid, which represent a disposal and potentially environmental pollution problem. Nevertheless they are also promising sources of compounds that can be recovered and used as valuable substances by developing of new processes. Particularly, the bioconversion of these wastes to useful products is receiving increased attention.

Olive oil and table olives are typical Mediterranean products, whose nutritional and economic importance is well-known. Indeed, consumption of olive oil and table olives has shown to be associated with a variety of health benefits, including a lower incidence of heart disease and certain types of cancer (Tuck and Hayball, 2002; Aruoma, 2003; Pérez-Jiménez, *et al.*, 2005). These findings have prompted considerable research into the composition of olive fruits, and the nature of the components of olive fruits responsible for the observed beneficial health effects.

The Mediterranean area provides 97 % of the total olive production of the world, being the olive oil industry an important activity, producing 95% of the world’ s olive oil (Aragón and Palancar, 2001). This olive oil industry generates large amounts and varieties of wastes, which remain most of potentially interesting compounds. For example, the virgin olive oil is of good quality with high resistance to autoxidation due to its great content of orthodiphenolic compounds. Nevertheless, the extraction system generates a liquid/solid waste rich in hydroxytyrosol, with a concentration 10-100 fold higher than olive oil (Lesage-Meesen *et al.*, 2001). Up to now the emphasis has been focused on detoxifying these wastes prior to disposal, feeding, fertilisation/composting, because they are not easy degradable by natural processes, or even used in combustion as fuel (Vlyssides *et al.*, 2004). However, the recovery of high value compounds or the utilization of these wastes as raw matter for new products is particularly attractive way to reuse it, always that the recovery process is of economic and practical interest. This, added to the alternative proposals to diminish the environmental impact, will allow the placement of olive market in high competitive position and these wastes should be considered as by-products (Niaounakis and Halvadakis, 2004).

The manufacturing process of olive oil has undergone evolutionary changes. The traditional discontinuous pressing process was initially replaced by the continuous centrifugation, using a three-phase system and later on a two-phase system. Depending on the different olive oil production method there are different kinds of wastes, being mainly one type of residue or another according to the most common extraction technology used in each country.

The classic production of olive oil generates three phases and two wastes: olive oil (20 %), solid waste (30 %) and aqueous liquor (50 %). The solid waste (olive oil cake (OOC) or "orujo") is a combination of olive pulp and stones. The aqueous liquor comes from the vegetation water and the soft tissues of the olive fruits, with water added during processing, so-called "alpechin or olive-mill waste water (OMWW)". The presence of large amounts of organic substances (oil, polyphenols, protein, polysaccharides, etc), responsible of the high COD values (up to 220 g/L) and minerals salts, represent a significant problem for the treatment of waste water (Borja *et al*., 1997; Niaounakis and Halvadakis, 2004).

The use of a modern two-phase processing technique in which no water is added, generates oil and a new by-product that is a combination of liquid and solid waste, called "alperujo, alpeorujo or two-phase olive mill waste". This by-product is high-humidity residue with thick sludge consistency that contains 80 % of the olive fruit, including skin, seed, pulp and pieces of stones, which is later separated and usually used as solid fuel (Vlyssides *et al*., 2004). In Spain, over 90 % of olive oil mills operate with this system, which means that annual production of this by-product is approximately 2.5-6 million of tons depending of the season (Aragon *et al*., 2000). However, the implementations in Italy and Grece are neglible (< 5%)

Several research groups have been working on the alternative use of these organic residues and the recovery of valuable substances. Despite most of the technologies reviewed in this study have been tested in laboratory and only some of them in industrial scale, we have evaluated the new strategies and the existing techniques, including our own investigations, which are promising with regard to future application in the olive waste management. By using an adequate technology, the olive-mill waste in general, and alperujo in particular, can be converted into value added products. The alternative use is represented by two possible applications:

1.1.Recovery of natural constituents.
1.2.Bioconversion into useful products.

1.1. Recovery of valuable natural constituents

A more recent approach to exploiting olive-mill waste has involved the use of processing technologies to fractionate potential high value components from residue. The recovered compounds may be broadly classified into insoluble, water-soluble and lipid-soluble. They can be also classified depending on their procedence from intracellular content or the cell wall material. In the present review, an extractable components-related categorization and their uses have been given preference since this has been considered more useful to the readers.

Phenolic compounds

The olive fruits contain a wide variety of phenolic compounds (Brenes *et al*., 1999; Mateos *et al*., 2001; Ryan *et al*., 2002; Bianchi, 2003; Owen *et al*., 2003). They are potent antioxidants and play an important role in the chemical, organoleptic and nutritional properties of the virgin olive oil and the table olives. The positive effect of olives and olive-derived products consumption on human health, well documented by a large number of epidemiological studies (Owen *et a*l., 2000; Pérez-Jiménez, *et al*., 2005), may be explained in part by the antioxidant effect of these phenolic compounds. As natural dietary antioxidants they may protect the organism against oxidative damage caused by oxidant agents (active oxygen, free radical, etc) that are involved in the etiology of chronic diseases such as cancer and atherosclerosis (Aruoma, 2003; Visioli *et al*., 2005). Also, they prevent the deterioration of food by inhibition of lipid oxidation. Furthermore, addition of natural antioxidants could be a strategy to develop functional food, so these natural additives improve, at the same time, the health-promoting properties and the storage period of food product. Therefore, it would be desirable to get a process for the extraction of antioxidant components from olive-based starting materials.

Olive fruits have a characteristic phenolic composition, which depends qualy and quantitatively on type of olives, stage of maturity, season and/or climatological conditions (Romero *et al*., 2004). Oleuropein is the major secoiroid compound of unripe olive fruit, which decreases with maturation, while demethyloleuropein and the dialdehydic form of elenolic acid (EDA) linked to 3,4-dihydroxyphenylethanol (3,4-DHPEA or hydroxytyrosol) increase. The glucoside of hydroxytyrosol is the predominant phenolic in ripe olives (Romero, *et al*., 2002 a). Besides, olive fruits contain other secoiroids such as verbascoside and ligustroside. Other groups of phenolic compounds are derived from cinnamic (p-cumaric, ferulic and cafeic acid) and benzoic acids (3,4-dihydroxyphenyacetic acid and 4-hydroxybenzoic acid). Other phenols found in olive pulp are catechol, methylcatechol, phenylalchols (tyrosol, hydroxytyrosol), relatively high concentrations of flavonoids (luteolin-7-

glucoside, apigenin-7-glucoside, rutin and quercitin) and several anthocyanin pigments (cyaniding-3-glucoside and cyaniding-3-rutinoside) that give intensive violet-dark color of ripe olives (Romero *et al.*, 2002 b).

The main phenolic compounds present in virgin olive oil are tyrosol, hydroxytyrosol, its secoiroids and conjugate forms (oleuropein, ligustroside, verbascoside) and lignans (pinoresinol and acetopinoresinol) (Brenes *et al.*, 2002).

During the olive oil mechanical process, the major proportion of the phenolic compounds are found in the aqueous phase, while only a minor percent (<1%) are located in the olive oil (Vierhuis *et al.*, 2001 a). This explains why a large fraction of them can be found in the alpechin (major obstacle in its detoxification). The use of two-phase centrifugal decanters makes that, despite the obtained virgin olive oil has a greater concentration of phenolic compounds that obtained by the three-phase mode, most of them (about 98%) remains in the alperujo (Vierhuis *et al.*, 2001 a). Therefore, both residues seem to be an affordable and abundant source of natural antioxidants. However up to date they have not been effectively exploited.

Hydroxytyrosol is one of major phenolic compounds present in olive fruit and it has been revealed to be the most interesting, because of its remarkable pharmacological and antioxidant activity (Fabiani *et al.*, 2002; Visioli *et al.*, 2004). Currently, many studies on bioavailability and metabolism in human are being conducted in order to establish its health-beneficial effects (Miró-Casas *et al.,* 2003; Visioli *et al.*, 2005). Nevertheless, the use of this compound has been limited, until present, because the product was not available commercially. Several patents using liquid-liquid extraction in counter-current (Calero et al., 1994), adsorbent resins (Cuomo and Rabovskiy, 1999), extraction with supercritical fluid with a column operating in the counter-current mode (Crea, 2002) or ultrafiltration and adsorption in non-ionic resins (Brenes and Castro, 2003) from olives and olive by-products have been developed. Also, numerous procedures of synthesis (Bai et al., 1998; Espin et al., 2001; Allouche et al., 2004; Allouche and Sayadi, 2005) and extraction (solid-liquid extraction, liquid-liquid extraction or adsorption technique using resins) from olive oil wastes have been published (Capasso et al., 1999; Visioli *et al*, 1999; Bouzid *et al.*, 2005). However, the only system of purification that has been successful for its industrial exploitation up to date it has been that developed by our research group. The patented system (Fernández-Bolaños *et al.*, 2002 a) allows to obtain, from any liquid source of hydroxytyrosol (from any olive production by-products), in a very simple, practical and economic way, two forms of purified hydroxytyrosol. The first of them, obtained to the end of the first phase of the procedure, with approximately 50% of purity in weight, is called Hytolive®2 , and the second one (Hytolive®1) can reach to 99.6% of purity at the final phase of the process. Both products are at the beginning of their commercial promotion. The system includes, at the first phase, passing the liquid source of hydroxytyrosol through an ion-exchange resin to trap the antioxidant and posterior elution with water. The second phase was a XAD-type adsorbent non-ionic resin. This matrix is washed with a mixture of methanol or ethanol and water (30-33%), a solution containing at least 75 % of hydroxytyrosol present in the olive by-product is obtained. The polar organic solvent is finally removed to produce a solid with about 95 % by weight hydroxytyrosol, plus significant fractions that reach until 99.6% of purity (Figure 1).

The extracted Hytolive®1 has an antioxidant activity characterized by increasing the oxidative stability of refined olive oil in 1.71-fold in presence of 100 ppm of hydroxytyrosol, measured by Rancimat method (Fernandez-Bolaños *et al.*, 2002 b). Also, antioxidant capacity was assessed by the oxygen radical absorbance capacity (ORAC) assay, revealed that the hydroxytyrosol (Hytolive®1) was more active (39.8 μmol of Trolox equivalent/mg) than ascorbic acid (4.36 μmol of Trolox equivalent/mg) and other extracts derived from grapes and wine (10 and 13 μmol/mg) (www.genosa.com/hytolive). The Hytolive®1 and Hytolive®2 have also been assayed by four "in vitro" test of antioxidant activity, showing hydroxytyrosol from both products a higher radical scavenging capacity that vitamin E and C. A decrease in DPPH radical of 45 % for 1 mM of hydroxytyrosol against a 27 % for 1mM of vitamin E and C was found. These compounds also shown equivalents values of ferric reducing power (0.102 quercitin equivalent for 1 mM hydroxytyrosol) than measured for 1 mM of vitamin E (0.107 quercitin equivalent), but higher than from 1 mM vitamin C (0.061 quercitin equivalent). In the assays that imply lipid oxidation (inhibition of primary oxidation, POIC, and secondary oxidation, TBARS), hydroxytyrosol had an intermediate activity between vitamin E, the most active, and vitamin C (Rodríguez *et al.*, in press).

It should be remarked that hydroxytyrosol produced by the method above described may be used for a variety of applications: as natural food antioxidant, preparation of functional foods, pharmaceutical solutions or cosmetics. Currently, the company Puratos is using Hytolive® as ingredient in bakery (www.genosa.com/hytolive).

Oleuropein has been considered a valuable component with certain antiviral, antibacterial, antifungal, antioxidant and anti-inflammatory properties (Aziz *et al.*, 1998 Visioli and Galli, 2002). Several methods for its recovery from olive vegetation water have been patented (Crea and Caglioti, 2000).

Formulations of antioxidant polyphenols derived from olive vegetation water, with effective amount of substantially purified hydroxytyrosol or a substantially purified mixture of hydroxytyrosol and oleuropein, are now being used as a therapeutic and/or an antioxidant for a variety of health purposes. For example, the CreAgi´s Olivenol polyphenol extract (Olivenol®) is useful for the protection of skin damage resulting from exposure to ultraviolet

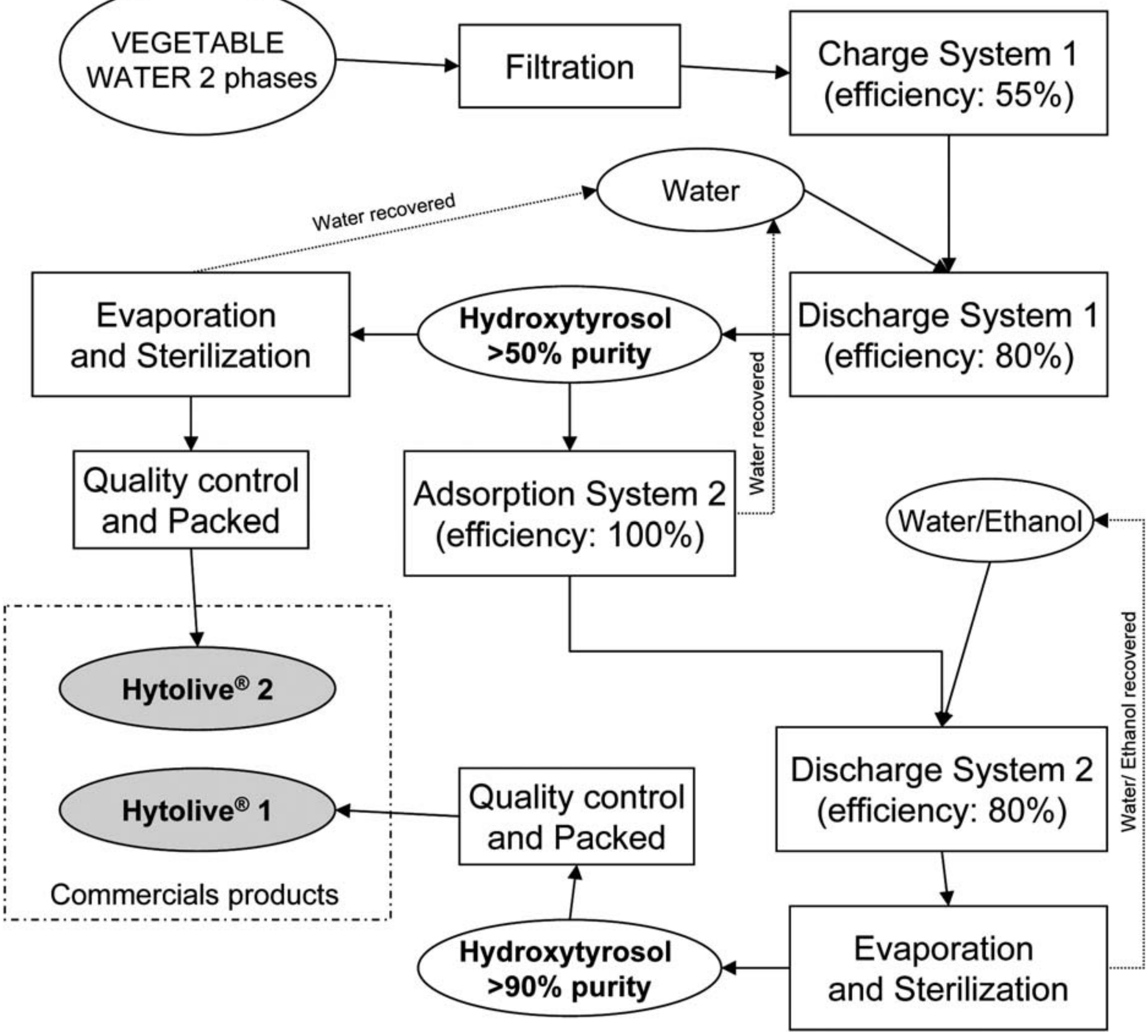

Figure 1
Flow chart of the system of purification of hydroxytyrosol (Hytolive®1 and Hytolive®2) developed to industrial level by the Genosa company .

radiation (Crea, 2004) and for the treatment of AIDS-associated neurological disorders, inflammation and inflammation-associated disorders (Crea, 2003). Also, the composition of certain foods, particularly of spreads, processed tomato products and dressing, are being fortified with an aqueous phase characterized by a content of tyrosol and hydroxytyrosol at least 15 ppm, which will enhance their nutritional value (Van der Boom and Zeelenberg-Miltenburg, 2000).

Squalene and tocopherols

Numerous, mainly apolar compounds from olive oil waste could be extracted and /or fractionated by utilizing supercritical fluid extraction technology. Oil deodorizer distillate and alpechin have been treated with supercritical carbon dioxide to analyze the possibility of obtained squalene (Bondioli *et al*., 1993; EU project FAIR2-CT95-1075). A process for the generation of tocopherols with supercritical fluid extraction from alperujo has also been performed (Ibañez *et al*., 2000). Both compounds are of great interest and required in large quantities in health, food and pharmaceutical industries owing to their antioxidant activity and high biological values (Rao *et al*., 1998; Mardones and Rigoti, 2004).

For the recovery of all valuable “minor components” present in olive oils, nowadays a new and promising process that involves distillation molecular during industrial deodorization in refining process from olive oil is being investigated in the Instituto de la Grasa (CSIC), (Spanish proyect AGL 2000-0420-P4-02).

Triterpenes

In olive by-products, exploitable amounts of triterpenes, such erythrodiol, oleanolic and maslinic acid have been found. It has been shown that all these compounds are concentrated in the skin of fruit (Bianchi, 2003). Process for the industrial recovery of oleanolic and maslinic acid from the solid waste resulting of olive oil production (from three-phase or two-phase) have been described by García-Granados (1998) and Kuno and Shirohara (2002). They comprise selective extractions and fractionation of resulting mixtures with the use of solvents. The latter authors have also converted the acids into physiologically acceptable salts by treatment with a basic medium.

The studies carried out with this pentaciclyc triterpenes have demonstrated that they have a wide variety of biological effects (Montilla *et al*., 2003), specially they have shown anti-inflammatory and antioxidant properties *in vitro* studies, as well as vasodilatatory activity in animal models (Rodríguez-Rodríguez *et al*., 2004). The use of maslinic acid as a protease inhibitor can be used for the treatment of HIV (García-Granados *et al*., 2000; Zhu *et al*., 2001) and for the treatment of diseases caused for parasites of the genus *Crytosporidium* (García-Granados *et al*., 1999).

Pectins and oligosaccharides

Cell wall material comprises a number of molecular components which are highly complex and provides a range of functional components. It consists of a framework of cellulose microfibrils which are made of glucose chains, many of which are closely associated in regions of crystallinity. Attached to these, there are a number of other complex molecules, including polysaccharides (pectins and hemicelluloses), proteins and phenolics. The cell wall of olive fruit contains considerable quantities of pectic polysaccharides and hemicellulosic polymers that are rich in xylans and xyloglucans (Jiménez *et al*., 1994; Jiménez *et al*., 2001; Vierhuis *et al*., 2001 b).

Exploitation of the residual cell wall from alperujo has been extracted *via* rheologically-active hydrocolloid. The gelation potential of pectins obtained from by-product, with 45 % galacturonic acid, 31 % arabinose and a degree of methylesterification of 43 %, was investigated (Cardoso *et al*., 2003). Quality evaluation revealed that this olive pectin has favourable gelling properties.

Cell wall-derivated components can also have nutritional and physiological benefits. Nondigestible oligosaccharides are usually considered to enhance the growth of bifidobacteria and lactic acid bacteria in the human large intestine, with certain evidence of a preventive effect against colon cancer and other intestinal dysfunctions (Roberfroid and Slavin, 2000). Oligosaccharides derived from alperujo hydrolysis, potential source of prebiotic, were isolated by size exclusion chromatography and partially characterized by HPLC (Fernández-Bolaños *et al*., 2004). In this study, we have developed a process that includes a hydrothermal treatment and an autohydrolysis of alperujo, for the recovery of the antioxidant hydroxytyrosol and of carbohydrates of low molecular weight from the water-soluble fraction. The total mixture of oligosaccharides was mainly constituted of xylose residues and relatively small amounts of rhamnose, arabinose and glucose, representing about 23 % of the total sugars. For every liter of hydrolysate, to a concentration of 30 g/L of neutral sugars, this would allow the recovery of approximately 6.9 g of oligosaccharides. Further studies are needed to verify their biological activity.

Mannitol

Another group of interesting compounds that could be obtained from olive oil waste are soluble sugars, including the sugar alcohol or polyol called mannitol. Glucose is the main soluble sugar present in olive pulp, together with smaller quantities of sucrose and fructose, and a significant amount of mannitol (Guillén *et al*., 1992). The recovery of mannitol from steam-treated alperujo, above mentioned, was almost complete with respect to the mannitol content of original alperujo. The further purification steps, illustrated in Figure 2, have achieved a mannitol with a high degree of purity (Fernández-Bolaños *et al*., 2004). Calero *et al*.,(1994) and García-Granados y Martínez (1994) have described another process to obtain mannitol from alpechin, olive twig, leaves or alperujo.

Mannitol is used as an exicipient in pharmacy, and as anticaking and free-flow agent, lubricant, stabiliser and thickener, and low calorie sweetener in the food industry. Due to its physicochemical properties, it is predominantly used in chewing gum and in bread products for diabetics (Alonso and Setser, 1994).

Polymerin

A dark and complex metal polymeric mixture, named polymerin, was recovered from olive waste water. It is composed of polysaccharides (54.4 %), melanin (26.1 %), protein (10.4 %) and minerals (11.06 %), mainly potassium. All the organic components were strongly linked in supramolecular structure *via* covalent and hydrogen bonds. These polymers could be used in agriculture as bioamendments, and/or metal biointegrador and as biofilter for toxic metals, due to their similarity with humic acids (Capasso *et al*., 2002, 2004).

1.2. Bioconversion into useful products

Biological conversion processes of olive oil waste into various value added products through liquid submerged and or/and solid-state fermentation (SSF) have been of interest to many laboratories. As shown in Table 1 most of research has been done in the last years.

Table 1
Value added products obtained by bioconversion of olive oil residues.

Residues	Description process/ Biocatalyst	Products	Reference
OMWW	*Clostridium* spp. (Medium with 50 % v/v OMWW)	Butanol (2.8-8 g/L)	Wähner *et al.*, 1988
OMWW	*Arthobacter* spp.	Indolacetic acid.	Tomati *et al.*, 1990
OMWW	*Pseudomonas aeruginosa* (OMWW as the sole carbon source)	Biosurfactant: rhamnolipid	Mercadè and Manresa (1994)
OMWW	*Propionibacterium shermanii,* on predigested OMWW with *Aspergillus niger*	Vitamin B_{12}	Muñoz, 1998
OMWW	Recombinant strain *Eschericha coli* P-260, by expression of the enzyme 4-HPA hydrolase of *Klebsiella pneumoniae*	Synthesis of pigments, colorants, alkaloids and polymers, which structure base is a quinone	Martin *et al.*, 1998
Olive oil cake (OOC)	SSF: *Rhizomucor pusillus, R. rhizopodiformis*	Lipase (applied in bakery, pharmaceuticals)	Cordova *et al.*, 1998
OOC	SSF: Delignification (with four fungi), saccharification with *Trichoderma* spp, and biomass formation with *Candida utilis* and *Saccharomyces cerevisiae*.	Crude protein enriched from 5.9 to 40.3%. Source for animal fodder	Haddadin *et al.*, 1999
OMWW	*Funalia trogii* ATCC200800 *Trametes versicolor* ATCC200801	Plant growth hormones: Gibberellic acid, abscisic acid and indolacetic acid and cytokinin	Yurekli *et al.*, 1999
OMWW	*Xanthomonas campestry*, in a medium with OMWW (50-60% v/v)	Xanthan gum, for food and non-food applications as thickener or viscosifier	López *et al.*, 2001
OMWW	*Paenibacillus jamilae* CP-7, in aerobic condition in a medium with OMWW (80% v/v)	Exopolysaccharide, antitumor agent with inmunomodulatory properties	Ruíz-Bravo *et al.*, 2001
OMWW	*Azotobacter chroococcum* (OMWW as the sole carbon source)	Bioplastic: Homopolymers of β-hydroxybutyrate and β-hydroxyvalerate	Pozo *et al.*, 2002
OMWW (undiluted)	*Botryosphaeria rhodina* mycelium growth	β-glucan β(1→3), β(1→6)	Crognale *et al.*, 2003
OMWW	SSF: *Panus tigrinus*, on OMWW-based media	Laccase and Mn-peroxidase with interest by ligninolytic activity	Fenice *et al.*, 2003
OOC	SSF: *Aspergillus oryzae*	α-amilase, used in bakery, breweries, textile industry, clinical sector	Ramachandran *et al.*, 2004
OMWW	*Lentinula edodes* mycelium growth	Xylan and β-glucan (lentinan), with pharmacological properties as antitumoral agent	Tomati *et al.*, 2004
OOC	SSF: *Ceratocystis moniliforms, Moniliella suaveolens, Thichoderma harzianum*	Flavor active δ- and γ-decalactones	Laufenberg *et al.*, 2004
Alperujo	Growth of six phenotypically distinct group of yeast, by a dynamic fed-batch microcosm system	Promising fermented product	Giannoutsou *et al.*, 2004
OMWW	Anaerobic fermentation to obtain volatile fatty acids, as substrate for polyhydroxyalkanoates production	Biodegradable polymers	Dionisi *et al.*, 2005
OOC	SSF: Aspergillus oryzae	Neutral protease	Sandhya et al., 2005

OMWW (alpechin) has been tested as growth medium for the production of different organic products from fungi, yeast or bacteria, at the same time that some of this fungal and microbial biomasses metabolise phytotoxics. Olive oil cake (OOC) due to its content in nutrients (carbohydrates and proteins), good holding capacity, and swelling capacity, can be excellent substrate for SSF with filamentous fungi and yeast (Laufenberg *et al.*, 2004). Alperujo seems to be a promising substrate for growth of yeast.

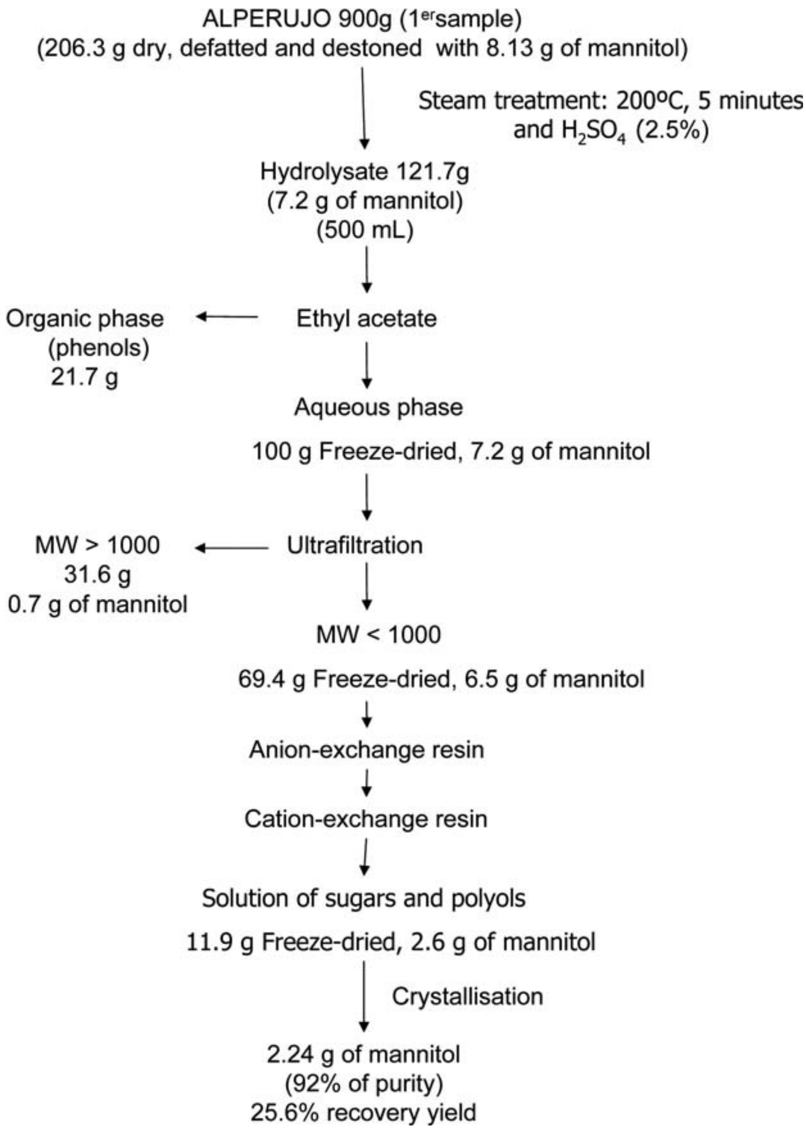

Figure 2
Scheme and masse balance of partial purification of mannitol in the hydrolysate obtained after hydrotermal treatment of alperujo (Fernández-Bolaños et al., 2004).

Nevertheless the polyphenolic fraction in all these olive waste is detrimental to microbial growth. In some cases, intensive pre-treatments (chemical, physical or biological) are needed and sometimes, it is also required a fermentation with selected microorganisms, single or mixed culture, as well as an adaptation processing. The posterior isolation, purifications or recovery of the products are also of high cost. Therefore, the bioconversion is economically attractive only if high value products are produced. Biotechnological production of natural aroma as lactones from fatty acids is of great interest because there is an increasing economic interest in natural flavours (Laufenberg *et al.*, 2004). Exopolysaccharides (Ruiz-Bravo *et al.*, 2001) and β–glucan (Tomati *et al.*, 2004) have recently been found to have important pharmacological properties.

2. FUTURE TRENDS

Valorisation of olive oil by-products will require exploitation of many components. Despite the studies cited and their potentially promising results, research on potential utilisation routes including all levels of value, from high-value (antioxidant, antiviral, anticarcinogenic,..) to relatively low-value (compost, feeding,..), has not been completed yet. It is clear that reaching an adequate exploitation of these by-products will require their whole utilisation, which will help to increase the value of all out puts (Waldron, 2005).

The recycling strategy applied to alperujo is presented schematically in Figure 3. We have developed a process that allows an integral recovering of this by-product, enhancing the field of the recovery of valuable compounds. We have already achieved the recovery and purification (under patent) of the most active phenol present in the olive oil, the hydroxytyrosol (strongly bound to the solid phase of alperujo), with antioxidant properties among other many health benefits (Tuck and Hayball, 2002; Carluccio *et al*., 2003). The system consists on a hydrothermal treatment (steam treatment to temperatures in the range of 160-240° C), where an autohydrolysis process occurs and the solid olive by-products is partially solubilised. This method makes easier the solid-liquid separation; it allows the recovery of others added-value compounds from the water-soluble fraction (mannitol, oligosaccharides and fermentable sugar) besides the hydroxytyrosol. At the same time, it makes very interesting the utilization of the final solid residue. This residue is considerably reduced after the treatment and several compounds such as oil, cellulose, and proteins are concentrated, that could be valued.

Despite of successful of the proposed technology, its practical implementation could consist on a simplification of system and/or a continue research on the recovery of great part of potentially interesting compounds from the olive oil by-products. The production, via bioconversion, of the new products from the solid-phase of steam-pretreated alperujo, is an innovative aspect. The resultant residue is a substrate that offers excellent possibilities to biotechnological processes due to its residual oil, sugars and proteins content, and overcoat to its important decrease of polyphenolic compounds with antimicrobial properties.

Nowadays, the system is being adapted in order to getting simpler and less severe operating conditions that will allow to employ a range of minor temperatures. It will make possible the recovery of other valuable compounds already identified in olive fruits or their by-products, and at the same time that are adapted conditions more familiar for olive oil producers.

After the goal in the industrial exploitation of hydroxytyrosol, the recovery of the other many bioactive and high value compounds, with potential importance to human health such as flavonoids, lignans and other phenolics compounds, sterols, tocopherols, squalene and triterpenic alcohols and acids (Newmark, 1999; Cassidy *et al*., 2000;

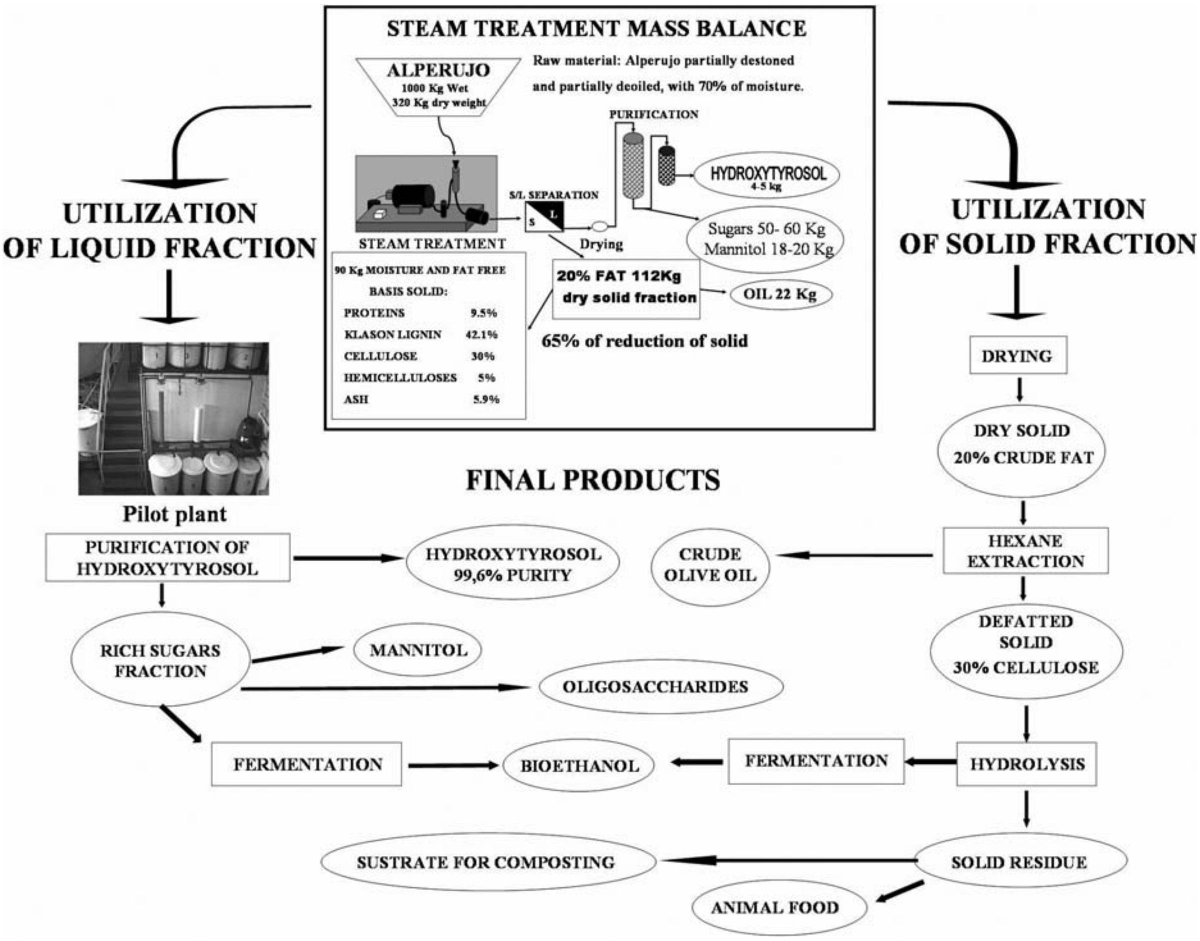

Figure 3
Strategy for an integral recovery and revalorization of alperujo.

Rodríguez-Rodríguez *et al.*, 2004), in aqueous or oil phases after the pretreatment would be investigated. This great variety of components will require different technologies for their isolation and purification, being undoubtedly, the supercritical fluid CO_2 extraction or molecular distillation some of the most desirable technologies.

The reactor could also incorporate a trap of volatiles that would allow the recovery of these valuable compounds. The natural volatile compounds produced from fresh fruits during the crushing and malaxation steps are incorporated in the olive oil, giving rise its priced aroma (Pérez *et al.*, 2003). This aroma is characterized by the presence of a broad spectrum of classes of volatiles (aldehydes, cetons, esters, alcohols and hydrocarbons), being the C_6 and C_5 metabolites (trans-2-henal, trans-2-hexen-1-ol, cis-3-hexyl acetate, cis-2-pentenal, etc), via lipoxygenase cascade, the major contributors to pleasant green and fruits (Ranalli *et al.*, 2004). Also, some flavour constituents (long-chain α,β unsaturated aldehydes) have been characterized as antimicrobial agents (Kubo *et al.*, 1995). The interest in the possible use of these natural compounds to prevent microbial growth or for flavours and fragrances has notably increased in response to the pressure of the consumer to reduce or eliminate chemically synthesised compounds. Their recovery from olive oil by-products will open the field of their use.

An interesting alternative will be the biotechnological production of aroma compounds from these residues; we believe that water-insoluble material of steam-treated alperujo, once eliminated the problems with phenolics compounds (Laufenberg *et al.*, 2004), will be an excellent substrate for the production of lactones by SSF due to its high content of oleic acid, which is a precursor substrate used by numerous moulds and yeasts.

Since cell-wall derived components can also have nutritional and physiological benefits, they have recently attracted interest. Indigestible oligosaccharides (oligofructans, xylo-oligosaccharides, galacto-oligosaccharides), currently considered as prebiotic (Roberfroid and Slavin, 2000) or oligogalacturonic acid with degree of polymerization of 2-7, with antimicrobial activity (Guggenbichler *et al.*, 1997), also show an important potential application. From the hydrolysate of steam-treated alperujo the oligosaccharides, already detected (Fernández-Bolaños *et al.*, 2004), should be chemically characterized and physiologically evaluated. The search of oligalacturonic acids also might be an interesting task. Even some additional treatment (chemical or enzymatic) could be performed to improve their functionality. The remaining insoluble residue from steam-treated alperujo could be further used as a medium to produce exopolysaccharides or β-glucans, which have been associated with immunological functionality and antitumoral effect (Ruiz-Bravo *et al.*, 2001; Tomati *et al.*, 2004).

In practice, for the complete utilization of olive by-products all these processes should not be separated but used in combination with each other. It is important to evaluate the economical conditions, to obtain reproducible and safety products (essential that the pesticides and other agrochemicals are excluded) and that can be developed on a large scale (Tomás-Barberán *et al.*, 2005; Waldron, 2005).

The olive oil by-products, as we have mentioned along all the paper, constitute an interesting source of phytochemicals and natural antioxidants. Their application in food, which increase their health-promoting properties, is a promising field. Functional foods is an area in rapid growing, where investigations on the bioactivity, bioavailability and toxicology of phytochemical and their stability and interactions with other food ingredients need to be carefully assessed by *in vitro* and *in vivo* studies (Schieber et al., 2001). In this context, obtaining isolated and purified compounds, with high degree of purity, will facilitate to solve these concerns and will help to demonstrate their biological activity.

Therefore, if the recovery and the production of new products from the olive oil by-products, added to alternative proposal, are successful, we will be capable to solve the environmental problems, as well as increase the competitiveness in the olive oil sector and the expansion in the food, pharmaceutical and cosmetic sectors.

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