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## New Olive-Pomace Oil Improved by Hydrothermal Pre-Treatments

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### 1. Introduction

The health properties of virgin olive oil (VOO) are well known in the Mediterranean Diet, in which VOO is the main source of fat (Boskou, 2000). The Mediterranean area provides 97% of the total olive production of the world and represents a major industry in the region (Aragon & Palancar, 2001). The fatty acid composition is not the only healthy component of olive oil; in addition, minor components have high biological activities (Pérez-Jiménez et al., 2007). From the olive oil by-product, the olive-pomace oil (OPO) is obtained. Recent studies have demonstrated the positive benefits of OPO on health, and these effects are due mainly to the presence of minor components (Ruiz-Gutiérrez et al., 2009). The new olive oil extraction processes in the olive mills make the extraction of OPO and the general utilisation of wastes more difficult. New thermal systems are proposed to pre-treat the olive oil wastes to facilitate their utilisation and OPO extraction.

#### 1.1 Olive oil extraction systems

The manufacturing process of olive oil has undergone evolutionary changes. The traditional discontinuous pressing process was initially replaced by continuous centrifugation, using a three-phase system and later a two-phase system. Depending on the different olive oil production method, there are different kinds of wastes. 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 cake 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”). The presence of large amounts of organic substances (oil, polyphenols, protein, polysaccharides, etc.) and mineral salts represents a significant problem for the treatment of wastewater (Borja et al., 1997).

The use of a modern two-phase processing technique to 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 a high-humidity residue

with a 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 use this system, which means that the annual production of this by-product is approximately 2,5-6 million tons, depending on the season (Aragon & Palancar, 2001).

### 1.2 Utilisation of olive oil wastes

Alperujo presents many environmental problems due to its high organic content and the presence of phytotoxic components that make its use in further bioprocesses difficult (Rodríguez et al., 2007a). Most of these components mainly phenolic compounds, confer bioactive properties, to olive oil. The extraction of the phenolic compounds has a double benefit: the detoxification of wastes and the potential utilisation as functional ingredients in foods or cosmetics, or for pharmacological applications (Rodríguez et al., 2007a). Although olive mill wastes represent a major disposal problem and potentially a severe pollution problem for the industry, they are also a promising source of substances of high value. In the olive fruits, there is a large amount of bioactive compounds, many of them known to have beneficial health properties. During olive oil processing, most of the bioactive compounds remain in the wastes or alperujo (Lesage- Meessen et al., 2001). Therefore, new strategies are needed for the utilisation of this by-product to make possible the bioprocess applications and the phase separation of alperujo.

Until now, efforts focussed on detoxifying these wastes prior to disposal, feeding, or fertilisation/composting, because they are not easy degradable by natural processes, or even used in combustion as biomass or fuel (Vlyssides et al., 2004). However, the recovery of high value compounds or the utilisation of these wastes as raw matter for new products is a particularly attractive way to reuse them, provided 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 the olive market in a highly competitive position, and these wastes should be considered as by-products (Niaounakis & Halvadakis, 2004).

### 1.3 Olive-pomace oil

After VOO extraction, the residual oil, or crude olive-pomace oil (COPO), is extracted by organic solvent extraction or centrifugation from olive oil wastes. After the COPO refining step, the refined olive-pomace oil (ROPO) is blended with VOO, obtaining OPO for human consumption. Currently, the growing interest in OPO is due to its biological active minor constituents (Ruiz-Gutiérrez et al., 2009). The concentration of these components in OPO is higher than the concentration in VOO, with the exception of polar phenols (Perez-Camino & Cert, 1999). Today, new processes for COPO refining are studied in order to diminish the loss of minor components (Antonopoulos et al., 2006). Some of these components are recovered in the refining steps.

Alperujo is treated by the OPO extractors for crude olive-pomace oil extraction (**Figure 1**). First, the major part of the stone present is removed, with the initial stone concentration of about 45% and, after the stone extraction, less than 15%. The stone is easily commercialised for numerous uses, such as in combustion materials, activated carbon, liquid and gas production from stone pyrolysis, an abrasive for surface preparation or for cosmetics, in

addition to others (Rodríguez et al., 2008). The pitted alperujo is frequently centrifuged in the OPO extractor because the new decanter technology allows treating low-fat material for oil extraction, through which crude olive-pomace oil is obtained. After this mechanical extraction, a partially defatted and pitted alperujo is obtained, with a humidity close to 50%. This material is dried to no more than 10% humidity for both solvent extraction and combustion. Drying consumes much energy, therefore attempts are continuously to reduce energy costs and to avoid the appearance of undesirable compounds in pomace-olive oil formed by the high temperatures (up to 500 °C) such as polycyclic aromatic hydrocarbons (PAHs) (León -Camacho et al., 2003) or oxidised compounds (Gomes and Caponio, 1997).

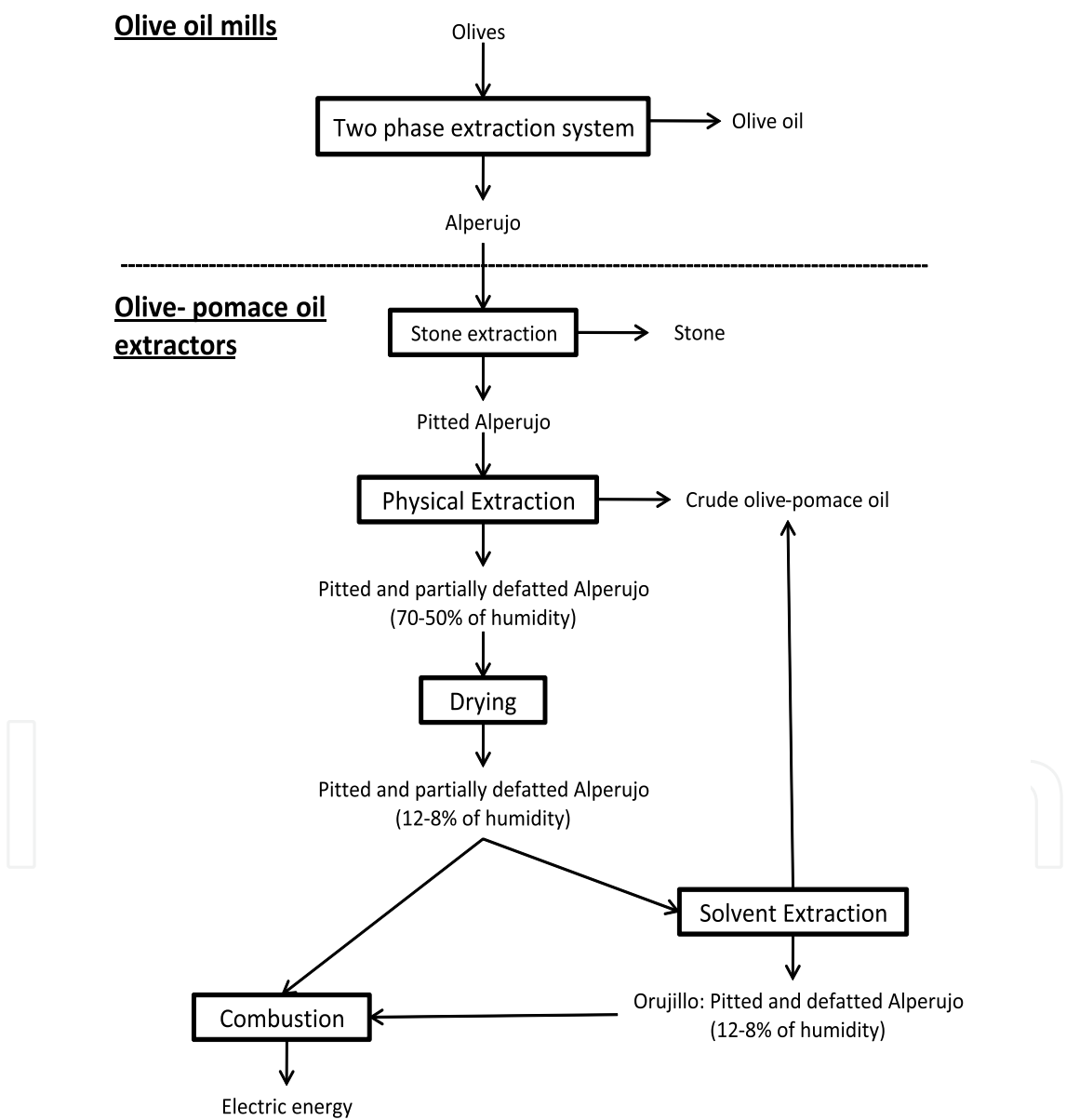


Fig. 1. General scheme of industrial olive oil and olive-pomace oil extraction and by-product processing.

The drying process is usually carried out in rotary heat dryers (Espínola-Lozano, 2003) in which alperujo and hot gases obtained from orujillo, olive stones or exhausted gases from co-generation systems (Sánchez & Ruiz, 2006) are introduced at 400 to 800 °C. The high temperatures have negative effects on the final composition of COPO. After drying, the pitted and partially de-fatted alperujo, with humidity close to 10%, is extracted with organic solvents. After the extraction, the organic solvent is removed for COPO production. The COPO obtained by physical or chemical methods has to be refined for human consumption. The final solid, called orujillo, is commonly used as a biomass for energy production.

The apparition of alperujo was supposed to be a great advantage for olive oil mills because the liquid waste (alpechin) was removed, but it was a serious inconvenience for COPO extractions with regard to the high humidity of the new semi-solid waste, or alperujo. Previous to the two-phase extraction system, the solid waste, or orujo, from the three-phase extraction system was treated with lower humidity (50%) than the alperujo (70%). Nowadays, despite the use of the final solid as biomass, the extraction of olive-pomace oil does not, in many cases, have economic advantages. In addition, the olive oil mills are improving the centrifugation systems in order to increase the quantity of olive oil, producing alperujo with lower oil concentrations. Consequently, the higher humidity in addition to the high organic content of alperujo complicate the COPO extraction, higher temperatures in heat dryers or alperujo with lower oil content. Therefore, pre-treatment alternatives are necessary to properly process the alperujo in the OPO extractor and improve the oil extraction balance and quality, while at the same time obtain new components and add value to the product.

#### 1.4 Minor components in OPO

Interest in olive-pomace oil is growing due to its economic advantages. It is cheaper than olive oil, and contains many minor components with bioactivities. OPO contains all of the functional compounds found in virgin olive oil, except for the polyphenols, in addition to other biologically active components (De la Puerta et al., 2009; Ruiz-Gutiérrez et al., 2009) that could be solubilised from leaves, skin or seeds of olives, depending on the extraction systems.

Phytosterols, tocopherols, aliphatic alcohols, squalene and triterpenic acid are some of the most important compounds that make the minor components an interesting fraction from the point of view of bioactive compounds that are agents for disease prevention.

The phytosterol's structure is similar to cholesterol, and they are a powerful agent in the cholesterol-lowering effects in human blood (Jiménez-Escrig et al., 2006) and as a cytostatic agent in inflammatory and tumoral diseases (Sáenz et al., 1998).

Tocopherols ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -form) are present in high concentrations in OPO.  $\alpha$ -tocopherol is an essential micronutrient involved in several oxidative stress processes related to atherosclerosis, Alzheimer's disease, accelerated aging and cancer (Mardones & Rigotti, 2004). Recently, biological activities against diseases like cancer in animal models have been also attributed to  $\gamma$ -form (Ju et al., 2010).

There is also squalene in olive-pomace oil. This compound has a beneficial effect on atherosclerotic lesions (Guillén et al., 2008, Bullon et al., 2009), dermatitis (Kelly et al., 1999)

and cellular proliferation and apoptosis in skin and intestinal cancers (Rao, 1998). After being absorbed by the human skin surface, squalene acts as a defence against oxidative stress due to the exposure to ultraviolet (UV) radiation from sunlight.

Aliphatic alcohols with long-chain fatty alcohols (C26 or hexacosanol, C28 or octacosanol and C30 or triacontanol) obtained from OPO have shown activity in reducing the release of different inflammatory mediators (Fernández-Arche et al., 2009), reducing platelet aggregation and lowering cholesterol (Taylor et al., 2003, Singh et al., 2006).

Uvaol and erythrodiol are the triterpenic alcohol fraction present in OPO. They are active antioxidant agents in the microsomal membranes of rat liver (Perona et al., 2005), with positive effects on the inflammatory process (Márquez-Martín et al., 2006), or in the prevention and treatment of brain tumours and other cancers (Martín et al., 2009).

### 1.5 Thermal pre-treatments

Alperujo is a high humidity solid that needs special pre-treatments to obtain a viable utilisation of all its phases. Only a few pre-treatments have been proposed for the total utilisation of alperujo, extracting the main interesting fractions. One of the more attractive processes is based on thermal pre-treatments that allow the recovery of all of the bioactive compounds and valuable fractions, making possible the utilisation of alperujo (Fernández-Bolaños et al., 2004). Thermal treatments produce the solubilisation of bioactive compounds to the liquid phase, leaving a final solid enriched in oil, cellulose and proteins. From the liquid, it is possible to extract and purify the bioactive compounds that confer healthy properties to olive oil, mainly phenols such as hydroxytyrosol (HT). HT is one of the more important phenols in the olive oil and fruit because it has excellent activities as a pharmacological and antioxidant agent (Fernández-Bolaños et al., 2002). HT has been recently commercialised by a patented system (Fernández-Bolaños et al., 2005). In addition to other important compounds, a novel phenol has been isolated and purified for the first time: 3,4-dihydroxyphenylglycol (DHPG). DHPG has never been studied as a natural antioxidant or functional compound with a higher antiradical activity and reducing power than the potent HT (Rodríguez et al., 2007b). After the thermal treatment and the solid-liquid separation, a solid that is rich in cellulose and oil is obtained. The cellulose is easy to extract and use as a source of fermentable sugar, animal feed or fertiliser (Rodríguez et al., 2007a). The thermal reaction improves the concentration in oil of minor components with functional activities. In addition, phenols increase in the liquid due to the solubilisation, with this fraction a rich source of interesting phenols, sugar and oligosaccharides, all of them with a potential use in the food or nutraceutical industry.

This alternative pre-treatment not only increases the concentration of oil in the final solid, but also the content of minor components in COPO prior to the refining process. The thermal treatment improves the functional profile, enhancing the quality and healthy properties of this oil (Lama-Muñoz et al., 2011). The application of thermal pre-treatment to alperujo makes the extraction of olive-pomace oil easier, improving its functional composition. On the other hand, it is important to note that all chemical changes of fats and oils at elevated temperatures result in oxidation, hydrolysis, polymerisation, isomerisation or cyclisation reactions (Quiles et al., 2002, Valavanidis et al., 2004). All of these reactions may be promoted by oxygen, moisture, traces of metal and free radicals (Quiles et al., 2002). Several factors, such as contact with the air, the temperature and the length of heating, the



type of vessel, the degree of oil unsaturation and the presence of pro-oxidants or antioxidants, affect the overall performance of oil (Andrikopoulos et al., 2002). In this work, the effect of two different thermal pre-treatments on COPO composition has been individually studied to balance the positive and negative factors in the final COPO.

### 1.5.1 Steam explosion system (SES)

The SES is commonly used as a hydrolytic process for lignocellulosic material utilisation (McMillan, 1994). This process (**Figure 2**) combines chemical and physical effects on lignocellulosic materials. The material is treated with high-pressure saturated steam for a few minutes and then the pressure is swiftly reduced, causing the materials to undergo an explosive decompression. The process causes hemicellulose degradation and lignin transformation due to high temperature, increasing the solubilisation of interesting compounds not only into the aqueous phase but also into the oil fraction. It is used mainly for the treatment of bagasse, such as wheat or rice straw, sugar cane, etc. The pre-treatment can enhance the bio-digestibility of the wastes for bioprocess applications to obtain, for instance, ethanol or biogas, and to increase the accessibility of the enzymes to the materials (De Bari et al., 2004; Palmarola-Adrados et al., 2004; Kurabi et al., 2005). High pressures (10-40 Kg/cm<sup>2</sup>) and temperatures (180-240 °C) are applied with or without the addition of acid in a short period of time, followed by explosive depressurisation. The SES makes it possible to obtain a final solid that is rich in COPO from alperujo. The thermal treatment solubilises a high proportion of solid, leaving behind components such as oil, proteins and cellulose. All these components are concentrated in the final solid.

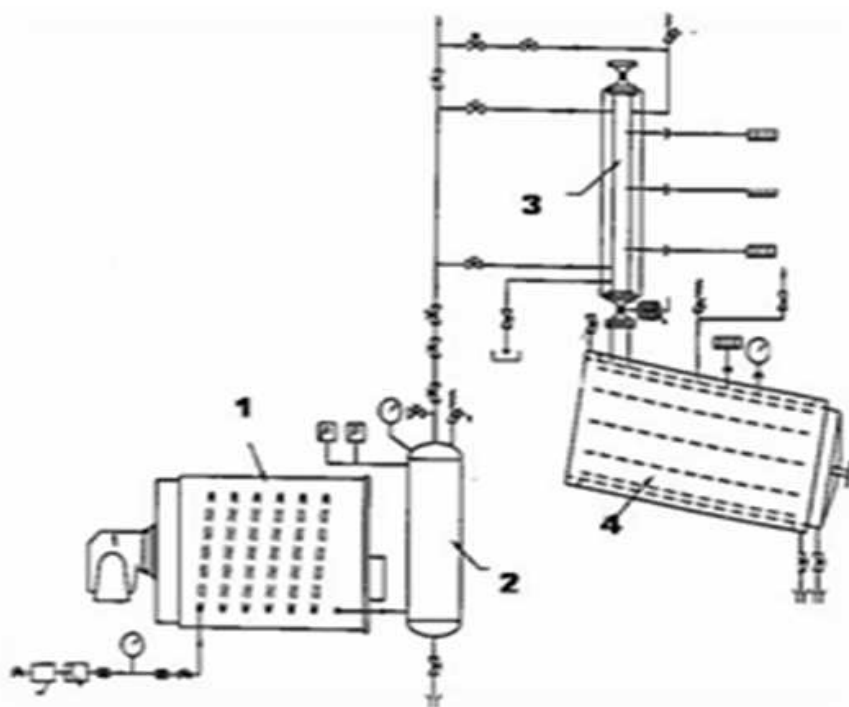


Fig. 2. Steam Explosion System scheme. Laboratory pilot unit designed in the Instituto de la Grasa (Seville, Spain), equipped with: 1) steam generator, 2) steam accumulator, 3) 2 L reactor stainless-steel and 4) expansion chamber.

### 1.5.2 New steam treatment (ST)

The system scheme is shown in **Figure 3**. A lower range of pressure and temperatures (3-9 Kg/cm<sup>2</sup> and 140-180 °C) than SES is applied for a longer period of time (15-90 min) in the novel system. The conditions and the contact of steam with the sample have been successfully improved, avoiding the technical complications and the high costs of the SES. This treatment has been recently patented to treat olive oil wastes, and the first tests have been carried out to assess its industrial viability for alperujo utilisation (Fernández-Bolaños et al., 2011).

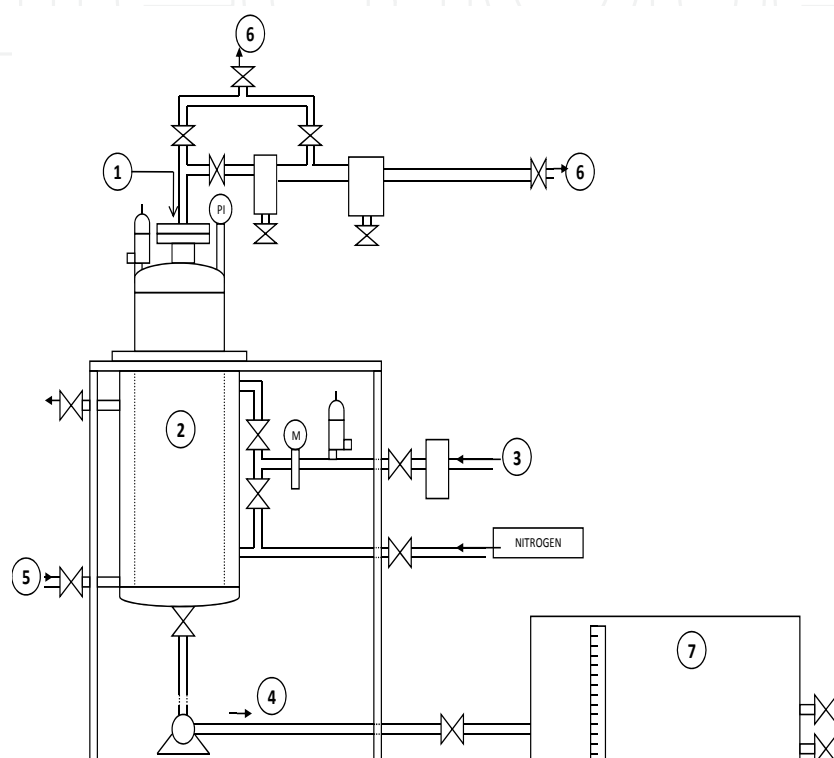


Fig. 3. New steam treatment (ST) reactor scheme designed in the Instituto de la Grasa (Seville, Spain) with: 1) sample entrance, 2) reactor chamber (100 L), 3) water steam, 4) sample exit, 5) cold water for refrigeration system, 6) vacuum and 7) solid-liquid separation.

The sample is introduced into the reaction chamber together with water steam. The sample temperature is increased up to 190°C for 30-60 minutes. After the reaction time, the sample is cooled with indirect water as a refrigerant. The liquid and solid phases of the treated sample are separated and the solid is finally extracted to obtain the crude olive-pomace oil.

The advantages of both systems are based on the important solubilisation of the initial solid to the liquid phase that occurs during the thermal treatment, leaving a final solid in which several components like oil, cellulose and protein are concentrated. The humidity of the final solid is also easier to remove by centrifugation or filtration, simplifying the drying process and the undesirable compounds that are formed at high temperatures.

In addition, the liquid phase is rich in bioactive compounds that are easy to extract. All these factors make possible the total utilisation of olive oil wastes, diminishing their environmental impact (Rodríguez et al., 2007a).



First, the application of SES on alperujo in order to obtain pomace olive oil was studied with or without acid as a catalytic agent. Due to the technical disadvantages of SES and to make the use of thermal pre-treatment in OPO mills easier and more convenient low severities, no depressurisation or acid addition were used in the new system (ST).

## 2. Experimental procedures

Samples of stored olive pomace or alperujo were collected from the COPO extraction factory Oleícola El Tejar (Córdoba, Spain) with 70% humidity. This by-product is generated as a waste from the two-phase olive oil extraction system.

### 2.1 Thermal treatments

The thermal treatments were carried out in the Instituto de la Grasa (CSIC) pilot plant by the steam explosion system and a new thermal system:

- a. The SES was carried out using a flash hydrolysis laboratory pilot unit with a 2 L reactor. The reactor was equipped with a quick-opening ball valve for the final explosion into the expansion chamber. Alperujo samples of 250 g were treated with saturated steam in a 2 L reactor with a maximum operating pressure of 40 Kg/cm<sup>2</sup>. The reactor was equipped with a quick-opening ball valve and an electronic device programmed for the accurate control of steam time and temperature for the final steam explosion. Prior to the treatment, some of the samples were acidified with H<sub>3</sub>PO<sub>4</sub>. The acid was added to the moist sample so as to reach a final concentration of 2,5 % (v/v). After the treatment, the samples were collected and filtered in vacuo through filter paper using a Buchner funnel.
- b. The new ST reactor has recently been patented (Fernández-Bolaños et al., 2011). It has a 100 L capacity stainless steel reservoir that can operate at temperatures between 50 and 190 °C by direct heating and at a maximum pressure of 9 Kg/cm<sup>2</sup>. The system allows the appropriate treatment of alperujo without steam explosion or high pressures and temperatures. The wet treated material was filtered by centrifugation at 4700 g (Comteifa, S.L., Barcelona, Spain) to separate the solids and liquids.

After solid separation, the solid phase was dried in a stove at 50 °C, and the reduction (%) in the mass of the solid phase was determined.

### 2.2 Analytical determinations

Oil was extracted from the treated solid obtained by SES and ST with n-hexane using a Soxhlet apparatus. The obtained oils were filtered and stored at -20 °C until analysis. Oil content and fat enrichment (pitted dry matter) were determined and compared with the values for untreated alperujo samples.

Determination of the concentrations of aliphatic alcohols, sterols and triterpenic dialcohols (erythrodiol and uvaol) was performed according to the Commission Regulation (EEC) No 2568/91 for olive oil and pomace oil. After the silylation reaction, 1 mL of heptane was added to the mixture, and 1 µL of the solution was injected into an Agilent 7890A gas chromatograph system (Agilent Technologies, Palo Alto, USA) equipped with an FID detector. The analytical column was an HP-5 (5%-phenyl)-methylpolysiloxane column (30 m x 0,32 mm i.d., 0,25 µm film thickness). The results were expressed as mg/kg of oil.

Tocopherols were evaluated using the IUPAC 2.432 method. Results were expressed as mg/kg of oil.

The wax and squalene compositions were determined according to the European Regulation EEC/183/93, by separation on a silica gel 60 (70-230 mesh ASTM) chromatographic column (Merck KGaA, Darmstadt, Germany) using hexane/ether (98:2) as the eluent with a few drops of Sudan I as a colorant. Dodecyl arachidate (Sigma) and squalane (Fluka) were added as internal standards. The results were expressed as mg/kg of oil.

Polar compounds, triglycerides and their derivatives oxidise and hydrolyse were prepared using solid-phase extraction and size-exclusion chromatography and monostearin as internal standard (Márquez -Ruiz et al., 1996). An aliquot (20  $\mu$ L) of the final solution was injected into a Hewlett Packard Series 1050 HPLC system equipped with a refractive index detector (LaChrom L-7490 Merck) and a 100-Å PL gel column (5  $\mu$ m) (Agilent). Elution was performed at 0,6 mL/min, with tetrahydrofuran as the mobile phase.

Determination of fatty acid, free acidity and peroxide value (PV) was carried out according to the Official Methods described in the European Community Regulation EEC/2568/91. The results were expressed as the percentage of oleic acid. The peroxide value was expressed in milliequivalents of active oxygen per kilogram of oil (mequiv O<sub>2</sub>/kg oil).

The indexes K<sub>232</sub>, K<sub>270</sub> and  $\Delta K$  were determined using the European Communities official methods (European Union Commission, 1991). Oil samples were diluted in isooctane and placed into a 1 cm quartz cuvette; for values calculation, each solution was analysed at 270 and 232 nm, with isooctane as a blank.

### 3. Results and discussion

Both systems allow the utilisation of the final solid for crude olive-pomace oil extraction. These COPOs have been characterised to assess the positive and negative effects of both treatments on its composition. The SES was studied as a commonly used method for lignocellulosic materials, and the ST was designed to simplify the first system and to diminish the negative effects of SES on crude olive-pomace oil. The lipid fraction of POO extracted from solids treated with either treatment was evaluated, and the minor components were also characterised, in the case of the ST.

#### 3.1 Steam explosion system (SES)

An average temperature of 200 °C and a time of 5 minutes were used with or without acid impregnation of alperujo. The acid increases the severity of the treatment, enhancing the oxidation of the samples. A vacuum was applied to one of the treatments, with acid addition in order to diminish the possible oxidative effect of oxygen at high temperatures and pressures. The results showed (**Table 1**) an important solubilisation of the solid in all treatment. In addition, the oil was concentrated in the final solid from 8,3 up to 19,9 % with respect to the dry final solid. Despite the high level of acidity in the initial sample after the treatment, these values decreased.

K<sub>232</sub> and K<sub>270</sub> are simple and useful parameters for assessing the state of oxidation of olive oil. The coefficient of specific extinction at 232 nm is related to the presence of products of

the primary stage of oxidation (hydroperoxides) and conjugated dienes, which are formed by a shift in one of the double bonds. The extinction coefficient at 270 nm is related to the presence of products of secondary oxidation (carbonylic compounds) and conjugated trienes (the primary oxidation products of linolenic acid).

The  $K_{232}$  values of all treated samples were lower than the untreated alperujo, unlike the  $K_{270}$  values in which only the sample treated with vacuum and without acid presented a similar absorbance at 270 nm. Only when vacuum and acid were applied to the SES did the value of  $K_{270}$  exceed the maximum concentration in ROPO (2,0), with all the  $\Delta K$  values lower than the maximum in ROPO (0,2). All these oxidised compounds diminished after the refining process.

The polar compound values that show the alteration level by the non-volatile compounds of oil are practically the same in all treatments, except when the acid and the vacuum are used simultaneously. Polar compounds provide an idea not only of oxidative reactions, but also of hydrolytic degradation, because they are partial constituents of FFA and glycerides.

Curiously, the concentration of oxidised triglycerides and polymers are lower after the SES treatments. This result could be explained by their partial solubilisation during the thermal treatment in the liquid phases that are previously separated by the oil extraction.

	Untreated sample	SES (200° C, 5 min)	SES (200° C, 5 min, 2,5% H <sub>3</sub> PO <sub>4</sub> )	SES (200° C, 5 min, vacuum)	SES (200° C, 5 min, 2,5% H <sub>3</sub> PO <sub>4</sub> , vacuum)
% of solid reduction	-	51,7	54,6	52,1	58,5
% of oil in final solid	8,3	17,2	18,0	16,9	19,9
Acidity (% oleic acid)	6,76	4,97	4,44	5,08	5,48
$K_{232}$	5,72	4,57	3,23	5,18	5,21
$K_{270}$	1,27	1,74	1,44	1,24	2,37
$\Delta K$	-0,03	0,00	0,04	0,06	0,07
Polar compounds mg/g	114,3	116,59	112,81	111,37	129,87
Oxidised Triglycerides <sup>a</sup>	1,61	1,25	1,34	1,41	1,36
Diglycerides <sup>a</sup>	3,10	4,47	4,83	4,37	5,13
Monoglycerides <sup>a</sup>	0,42	0,53	0,40	0,49	0,56
FFA (% as oleic acid)	6,10	5,28	4,49	4,66	5,73
Polymers <sup>a</sup>	0,23	0,14	0,23	0,21	0,21

<sup>a</sup> % with regard to the oil sample.

Table 1. Solid reduction, oil concentration in final solid and chemical characteristics of crude olive-pomace oil treated or untreated by SES in several conditions.

The quantity of triglycerides decreased after the SES treatment, with an increased presence of diglycerides and monoglycerides as an unmistakable sign of hydrolytic degradation.

The oxidative effects do not seem to be the main cause of the COPO alteration during the SES treatment, while hydrolysis seems to be an important effect on the triglyceride loss.

The composition of triglycerides was determined and the results are shown in the **Table 2**. The main triglyceride peaks in all samples were oleic-oleic-oleic (OOO), oleic-oleic-palmitic (OOP) and linoleic-oleic-oleic (LOO).

Despite the low quality of the initial oil, the relation of triglycerides was not altered by SES. As expected, in the crude olive-pomace oil obtained after SES treatment, the total content of triglycerides decreased up to 22% compared to the oil obtained from the untreated alperujo. Despite the high severity, only 22% of triglyceride composition was lost, with the rest susceptible for refining. The oxidative conditions of SES treatment were minimised using a vacuum or avoiding the acid addition.

The great advantages of the SES application on alperujo are based, in addition to other reasons, on the solid reduction (up to 58%) and oil concentration in the final solid (up to 20%). Because the triglycerides (TG) are concentrated, their loss is not a significant or negative factor to limit the use of this system. These reasons make technically possible the extraction of the crude olive-pomace oil from one sample of alperujo treated by SES. Therefore, the application of SES to this kind of alperujo allows for the obtaining of a final solid rich in oil in a high concentration that is susceptible for further refining processes for olive-pomace oil production. However, the technical inconveniences of SES such as high temperatures and pressures or the explosive decompression make it an inadequate system for olive-pomace oil extractors.

Triglycerides	Untreated sample		SES (200° C, 5 min) treated sample	
	Peak area	% of total glycerides Mean ± SD	Peak area	% of total glycerides Mean ± SD
LLL	18039	0,7 ± 0,05	11076	0,6 ± 0,02
LnLO	15271	0,6 ± 0,03	5886	0,3 ± 0,01
OLL+PoLO	99759	4,0 ± 0,10	75243	3,9 ± 0,77
PLL+LnOO	62860	2,5 ± 0,15	32818	1,7 ± 0,26
POLn	23935	1,0 ± 0,07	0	0,0 ± 0,00
LOO	404446	16,1 ± 1,02	321011	16,6 ± 1,23
LOP	154422	6,2 ± 0,81	126669	6,5 ± 0,87
LPP	4000	0,2 ± 0,01	6064	0,3 ± 0,02
OOO	959632	38,3 ± 1,67	788016	40,7 ± 2,03
OOP	448959	17,9 ± 1,11	364371	18,8 ± 1,30
POP	57959	2,3 ± 0,90	39691	2,0 ± 0,11
SOO	157822	6,3 ± 1,43	108877	5,6 ± 0,95
POS	28350	1,1 ± 0,03	21116	1,1 ± 0,05
AOO	49158	2,0 ± 0,16	26398	1,4 ± 0,06
Área total	2504443		1938316	

P, palmitic, Po, palmitoleic, M, margaric, S, stearic, O, oleic, L, linoleic, Ln, linolenic, and A, arachidic acids

Table 2. Triglycerides composition of crude olive-pomace oil obtained from alperujo untreated and treated by SES.

3.2 Effects of the new steam treatment

The ST effect on POO composition was determined by characterisation of the fatty acid fraction. After the thermal treatment in the range of 150-170°C for 60 min (Table 3), the final treated solid had an increase in oil yield up to 97%, with a reduction in solids up to 35,6-47,6% by solubilisation. The oxidative damage was lower in the new treatment. The analysis of the polar fraction showed that oxidised triglycerides and peroxide values increased slightly and that no polymerisation reactions occurred. The hydrolytic process is shown in the diglycerides increasing from 2,5 to 6,6%, with the FFA and the unsaponifiable matter for all treatments remaining constant.

	Untreated sample	ST (150° C, 60 min)	ST (160° C, 60 min)	ST (170° C, 60 min)
% of solid reduction	-	35,6	47,1	47,6
% of oil in final solid	8,1	11,8	14,3	16,0
Acidity (% oleic acid)	3,6	4,7	4,9	5,1
Peroxide Values (meq/Kg)	8,7	9,4	10,9	12,3
Oxidised Triglycerides <sup>a</sup>	0,7	1,1	1,1	1,6
Diglycerides <sup>a</sup>	2,5	5,2	6,6	6,6
Monoglycerides <sup>a</sup>				
FFA (% as oleic acid)	3,3	2,8	2,9	2,8
Unsaponifiable matter (%)	2,53	3,02	2,50	2,54

Table 3. Solid reduction, oil concentration in final solid and chemical characteristics of crude olive-pomace oil treated or untreated by ST at 150, 160 and 170°C for one hour. <sup>a</sup> % with regard to the oil sample.

The concentration of minor components (Table 4) was significantly increased by ST. Sterols, aliphatic alcohols, triterpenic alcohols, and squalene increased up to 33%, 57%, 23% and 43%, respectively. In addition, the content of tocopherols increased up to 57% compared to untreated POO. This increase is due to solubilisation during the thermal treatment. The waxes level is also increased because of the high solubilisation from the external cuticle of the olive fruit and the leaves. Waxes are easily removed by the refining of COPO.

The samples of alperujo had been stored for a long time and the oil was partially extracted by centrifugation in OPO extractors just before pitting. The alperujo was chosen because its low fat concentration makes the COPO extraction economically unviable. In this condition, the initial oil has a very low quality, as previously shown in the tables. The analysed oils showed, despite the low quality of initial oil present in the alperujo studied, that the effect of thermal treatment increases slightly the values of oxidised components and hydrolytic degradation. All COPOs obtained after the thermal treatments are susceptible for a posterior refining process for OPO obtention.



Components	Untreated sample	ST (150°C, 60 min)	ST (160°C, 60 min)	ST (170°C, 60 min)
Sterols	4927 (104)	5687 (291)	6546 (216)	6555 (298)
Aliphatic alcohols	4065 (77)	5532 (603)	5880 (283)	6389 (68)
Triterpenic alcohols	992 (58)	1054 (34)	1220 (124)	1189 (107)
Waxes	1535 (3)	2971 (5)	3124 (94)	3461 (110)
Squalene	2404 (36)	2472 (11)	2729 (109)	3439 (171)
Tocopherols	425 (33)	460 (6)	668 (14)	533 (20)

Table 4. Total minor component composition (mg/kg ± SD) of oils from steam-treated and untreated alperujo. Numbers between parentheses indicate the standard deviation of three replicates.

For human consumption, the refining process of OPO is necessary. The refining (physical or chemical) process eliminates undesirable compounds (peroxides, degradation products, volatile compounds responsible for off-flavours, free fatty acids, etc.) but also results in the loss of valuable bioactive compounds and natural antioxidants (Ruiz-Méndez et al., 2008). The new trends of refining systems involve losing as few minor components as possible to obtain a final OPO that is rich in the minor components. The thermal treatments increase the minor component of COPO that help to obtain a final olive-pomace oil rich in interesting compounds, whose concentrations might be higher after the refining process, mainly using the new physical systems. Moreover, some of these minor components are recuperated during the refining, such as squalene, the concentration of which is increased up to 43 % after the pre-treatment. After extraction, the defatted solid is lacking in phenols and then in phytoxic compounds for further bio-utilisation and rich in components like cellulose and protein.

**Figure 4** shows the main aspects of both thermal systems. The high difference between temperatures and pressures together with the absence of explosive decompression makes ST more appropriate and economically viable for industrial applications. A longer period of reaction time is necessary to treat with ST, but is easily applicable in an industrial continuous reactor. The percentage of solid reduction and, consequently, the final oil concentration show that despite the high severity difference between both treatments, there is not a significant difference in the results. Then, the new ST provides the major advantages of SES without technical complications.

Thus, the positive effect of a novel thermal treatment for the extraction of crude olive-pomace oil that could improve the commercial value of OPO and its bioactivities by increasing the concentrations of minor components concentration has been demonstrated. This treatment also significantly reduces the cost of oil extraction by centrifugation or solvent extraction because the starting solid is more concentrated in oil and is drier than untreated alperujo.



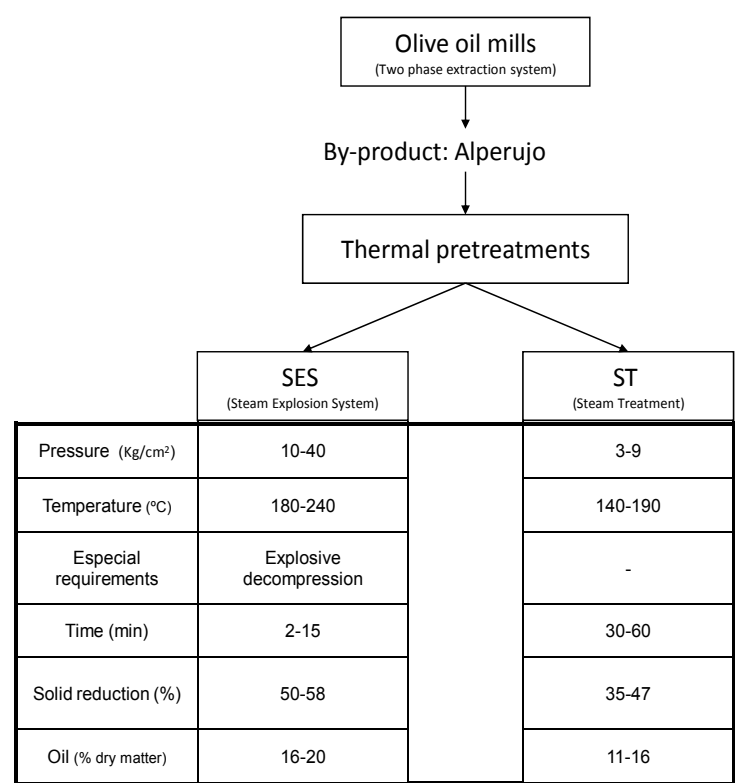


Fig. 4. Comparative scheme of two thermal pre-treatments used for alperujo utilisation.

4. Conclusion

The new treatment ST not only maintains the advantages of the SES with regard to the concentration of oil in the final solid and phase separation, but also diminishes the oxidation and significantly improves the concentration of the most interesting components of the minor fractions of POO. Thus, the application of ST enhances the functional properties of this new POO, increasing the oil extraction yield and the total recovery of bioactive compounds from the refining process.

The steam treatment offers not only serious advantages in terms of the oil but also in terms of the total recovery of alperujo as described above. The application of ST to treat olive oil wastes allows the phase separation and the concentration of interesting compounds and components in each phase. In the liquid phase, bioactive compounds like phenols and oligosaccharides are solubilised and are easy to extract. In the solid fraction, the oil and cellulose are concentrated. After the oil extraction, the solid has a low content of phytotoxics that are in the liquid phase, and therefore, it is susceptible for bio-treatment for the total utilisation of this fraction.

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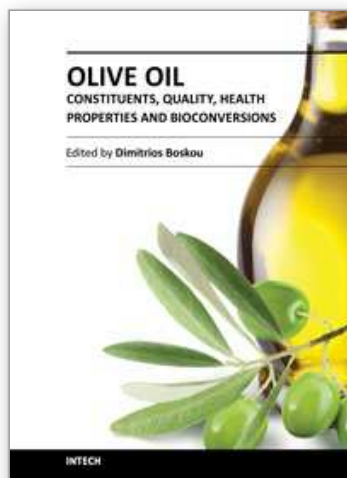
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