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**The use of industrial thermal techniques to improve the bioactive compounds extraction and the olive oil solid waste utilization.**

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## **ABSTRACT**

The development of thermal treatments is helping to optimize the management of waste generated by the two-phase olive oil extraction system. A new treatment reactor, based on the steam explosion system, has been scaled up to the industrial level and offers several physical and chemical advantages. The reactor has been adapted to work into the pomace oil extractors. Chemical advantages include the efficient solubilization of simple phenols, like hydroxytyrosol, and sugars in the liquid fraction, and a significant reduction of humidity and toxicity of the residual solid fraction, in which cellulose and oil are concentrated, making the solid residual waste more suitable for subsequent bioprocess applications. All of these parameters will lead to the widespread use of steam treatment in the near future to improve the management and obtain the maximum exploitation of this byproduct.

Keywords: alperujo, hydroxytyrosol, olive oil, phenolic compounds, steam treatment.



## **1. INTRODUCTION**

The olive oil industry represents an important productive sector in the Mediterranean basin countries. Olive oil production using a three-phase extraction system generates two by-products: a very pollutant liquid waste, or alpechin, and solid waste or pomace. The use of a new two-phase system, which is predominantly used in Spain, produces a waste called alperujo, which is a mixture of vegetable water and pomace. Alperujo creates serious problems for olive pomace factories responsible for its receipt and management. In particular, the drying of alperujo – commonly by physical drying processes in rotary dryers – demands higher amounts of energy than the pomace produced by the three-phase extraction system and can present several problems to the driers because of its high moisture and sugar content (Niaounakis & Halvadakis, 2006). Moreover, alperujo provides a lower yield of olive pomace oil and has the additional risk of the development of polycyclic aromatic hydrocarbons that can be transferred to the oil (Ergönül & Sánchez, 2013). Because of the addition of waste water generated during the olive oil extraction to the alperujo, mainly from the vertical centrifuge and the olive washing stages, the final humidity of alperujo is very high, up to 70–80%. Thus, the energy costs of the drying process are quite high as well as the technical problems associated with reactions such as caramelization and oxidation, and the presence of high concentration of sugars which promote agglomerations increasing adhesion to the drier walls (Gómez-de la Cruz et al., 2014). After drying, the pomace is extracted from the alperujo by solvent, and finally, the defatted and dried solid phase of alperujo is used as biomass for the generation of electric energy in the co-generation plants, resulting in significant CO<sub>2</sub> emissions. The energy generation in Spain from olive oil waste biomass was enhanced by government subsidies, but these grants have

been drastically reduced. In addition, the olive mills are obtaining oil from the second extraction step, reducing the final concentration of oil that remains in alperujo. Thus, the pomace oil extractor industry that receives this byproduct must treat a high humidity alperujo with a low concentration of oil, meaning that the use of alperujo for energy production is no longer economically viable. These facts have forced the olive pomace oil industry to seek ways to diminish alperujo treatment costs and/or identify better, more efficient alternative uses of alperujo. Different methods have been proposed to reduce the environmental impact of this byproduct. For example, alperujo can be used as agricultural biofertilizer (Baddi, Cegarra, Merlina, Revel, & Hafidi, 2009), an additive in animal feed (Yáñez-Ruiz, & Molina-Alcaide, 2007), and in the production of biogas (Serrano et al., 2017a) and ethanol (Ballesteros, Oliva, Negro, Manzanares, & Ballesteros, 2002) since it is rich in carbohydrates. However, all these methods are associated with certain drawbacks that have led to only limited success. An interesting, environmentally friendly and economically attractive alternative for the olive oil sector has been developed, with a focus on the recovery and isolation of bioactive compounds from alperujo (Rubio-Senent, Rodríguez-Gutiérrez, Lama-Muñoz, & Fernández-Bolaños, 2012). There is an increasing trend in the utilization of bioactive compounds present in fruits and vegetables in the food, nutraceutical, and cosmetic industries due to the healthy properties of these natural constituents. According to epidemiological studies, the regular consumption of olive fruits and olive oil reduces the risk of the onset and development of cardiovascular diseases, atherosclerosis, and some types of cancer (López Gallego, Rodríguez, Espejo, Sanz, & Cortés, 2003; Visioli et al., 2005). The olive's well-known beneficial impacts on human health are not only due to its peculiar unsaturated fatty acid composition, but also to its minor components, among them antioxidant phenolic compounds (Viola, & Viola, 2009). *In vitro* studies have

demonstrated that phenolic compounds have a wide variety of biological activities (Cicerale, Lucas, & Keast, 2010). However, only 2% of olive phenolic compounds are transferred to the oil, while alperujo typically contains 98% of the total phenols in the olive fruit (Rodis, Karathanos, & Mantzavinos, 2002). The phenols found in alperujo include hydroxytyrosol (HT) and 3,4-dihydroxyphenylglycol (DHPG) (Rodríguez, Lama, Trujillo, Espartero, & Fernández-Bolaños, 2009). HT is used as a cosmetic and pharmaceutical product because of its antioxidant and anti-inflammatory activities, among many other properties (Tsagaraki, Lazarides, & Petrotos, 2006), while DHPG is a simple phenol with a higher antioxidant capacity than hydroxytyrosol (Rodríguez, Rodríguez, Jiménez, Guillén, & Fernández-Bolaños, 2007a).

Despite the importance of its phenolic compounds, alperujo is considered to be a very polluting waste, when in fact it is a good source of these phenols given the large amounts of alperujo that are generated in Spain (2–5 million metric tonnes per year) by the olive oil industry (Serrano, Fermoso, Rodríguez-Gutiérrez, Fernández-Bolaños, & Borja, 2017b). Different extraction techniques have been proposed for the recovery and isolation of phenols from olive fruit and olive oil by-products. Researchers have studied liquid-liquid extraction (Fki, Allouche, & Sayadi, 2005), chromatographic methods (Fernández-Bolaños et al., 2005; Johannissbauer, Bonakdar, & Richard-Elsner, 2003), supercritical fluid extraction (Crea, 2002), and membrane separation methods (Ibarra, & Sniderman, 2005). However, the use of organic solvents decreases the economic viability of these techniques.

Thermal treatments of alperujo were studied because the extraction of natural compounds from alperujo poses a problem as its components are not easily accessible. The integral use of alperujo requires a pretreatment step to breakdown its complex structure, resulting from the close association between celluloses, hemicelluloses, and

lignins, thus making it possible to separate or fractionate the solid and liquid phases as well as increase the accessibility and solubilization of compounds. Among the different existing pre-treatment methods, *steam explosion* initially represented an excellent alternative (Felizón, Fernández-Bolaños, Heredia, & Guillén, 2000). The steam explosion pretreatment is carried out at high temperatures (160–240 °C) and pressures (0.69–4.46 MPa) with saturated steam for short periods of time (a maximum of a few minutes) followed by an explosive decompression. As a result, alperujo suffers the disaggregation of its structure, the partial depolymerisation of cellulose, and autohydrolysis, due to the self-ionization of water and acid compounds that are generated *in situ* (Garrote, Falqué, Domínguez, & Parajó, 2007; Ramos, 2003).

Steam explosion has the advantage of facilitating the separation of the solid and liquid phases by centrifugation (operation virtually impossible without previous pretreatment), and causes the high solubilization of phenolic compounds and fermentable sugars, and the reduction of the solid fraction, leading to an increase of the fat content and other components in the final solid (Fernández-Bolaños, et al., 2001, 2004; Fernández-Bolaños, Rodríguez, Lama, & Sánchez, 2010a). Despite the numerous advantages of steam explosion, this thermal treatment has not been developed at an industrial level because of the use of such high temperatures and pressures and the technical complexity of explosive decompression. As the olive pomace industry commonly uses pressures of up to about 9 kg/cm<sup>2</sup> (0.88 MPa), a new pilot-scale reactor or discontinuous steam treatment system was designed to function at lower temperatures and pressures and without the necessity of explosive decompression (Fernández-Bolaños et al., 2010b). This thermal pre-treatment has been scaled up for industrial use, in which it is utilized at temperatures up to 170 °C and pressures of 8–9 kg/cm<sup>2</sup>. Two of the most attractive advantages of the new discontinuous steam treatment are the reduced humidity of the

thermally treated alperujo and the obtention of a liquid phase that is rich in phenols and can be commercialized as a source of natural antioxidants. With this method, the extractors already employed by the pomace oil industry only have to dry an alperujo with 30–50% of humidity instead of 70–80%. The benefits for the industrial utilization of this thermal treatment are evaluated in the present work in comparison with application of the steam explosion treatment. This evaluation is made in terms of the physical and chemical parameters, such as the release and solubilization of phenolic compounds (DHPG and HT) and carbohydrates, and the reduction of the solid fraction and its oil enrichment, which permit the subsequent use and valorization of alperujo.

## 2. MATERIALS AND METHODS

### 2.1. Reagents

Phenolic standards used were 3,4-dihydroxyphenylglycol from Sigma-Aldrich (St. Louis, MO, USA) and hydroxytyrosol, which was isolated from olive oil by-products and further purified (Fernández-Bolaños et al., 2005). HPLC-grade acetonitrile, methanol, chloroform and tetrahydrofuran were purchased from Merck (Darmstadt, Germany). Milli-Q water (Millipore, Milford, MA, USA) was used in all analytical work. Aldoses and uronic acid standards: glucose, galactose, xylose, arabinose, fucose, rhamnose, D-glucuronic and D-galacturonic, ABEE, sodium cyanoborohydride, sodium citrate, and citric acid monohydrate were obtained from the Sigma Chemical Co.; and acetic acid from Fluka (Switzerland). Hexane used for the oil extraction was of analytical reagent grade.

### 2.2. Alperujo

Alperujo (a very wet semisolid olive oil byproduct) from a two-phase oil extraction decanter was supplied by a Spanish olive pomace oil extractor plan, Oleícola El Tejar (Córdoba, Spain). Alperujo samples were also freshly collected from an olive oil experimental plant at the Instituto de la Grasa, CSIC (Seville, Spain) in a different season. Alperujo samples had high moisture content (70%). All samples were stored at -20 °C prior to treatment.

### 2.3. Thermal treatments

#### *Hydrothermal treatment*

The hydrothermal treatment has been patented (Fernández-Bolaños et al., 2010a) and was performed using a new steam treatment reactor prototype designed by our research group at the Instituto de la Grasa (Seville, Spain). The reactor has a 100 L capacity stainless steel reservoir that can operate at temperatures up to 190 °C and at a maximum pressure of 1.2 MPa. Heating of the olive pomace was performed by direct injection of high-pressure and high-temperature steam, increasing the contact between the steam and the alperujo to be heated. The reactor is equipped with a jacket for water-mediated cooling. Alperujo samples (20 kg) were loaded in the reactor and uniformly distributed. The treatments were done at three different temperatures (150°, 160°, and 170 °C) for 15 to 90 min. The reactor display indicates the pressure, time, and temperature values, which can be recorded and the data exported. The desired treatment temperature is reached quickly. Heating from ambient temperature to a range of 150–170 °C requires between 15 and 20 min. Temperature and pressure remain approximately constant over time treatment. The period of treatment was counted from the time the selected temperature was reached. After the treatment period, the cooling stage is also quick, taking 15 to 20 min for the temperature to decrease below 50 °C, depending on the

initial temperature conditions. Steam-treated alperujo is recovered without the necessity of an explosive decompression by opening a discharge valve. After thermal treatment, alperujo samples were centrifuged at 4700 g/1450 rpm (Comteifa, S. L., Barcelona, Spain) to separate the liquid and solid fractions.

#### *Steam explosion treatment*

The steam explosion treatments of alperujo were performed in a pilot-scale reactor, which was designed by our research group, built by the company Nusim, S.A. (Madrid, Spain), and is installed in the Department of Food Biotechnology at the Instituto de la Grasa (CSIC), Seville, Spain. The unit is equipped with a stainless steel container and has a volume of 2 L. The steam explosion reactor was loaded with 250 g of alperujo. Alperujo was heated at a temperature of 180–240 °C with high-pressure saturated steam (with a corresponding pressure of 0.78–4.27 MPa) for 5 min. To evaluate the total HT content, steam treatments were performed in the presence of an acid catalyst (2.5% sulphuric acid). An electronic computing device controls the time and the temperature in a pre-programmed manner. In this system, treated alperujo is exposed to atmospheric pressure by a quick-opening ball valve, which makes the material undergo an explosive decompression in an expansion chamber. After each treatment, the wet solid was filtered on a Buchner funnel using Whatman filter paper discs to separate the solid and liquid phases.

The solid residues were stove-dried at 50 °C for determination of the solid yield or solid reduction. The solid reduction was evaluated in terms of dry matter loss by difference between steam-treated and untreated dry alperujo samples. Dry alperujo samples were refluxed with n-hexane using a Soxhlet apparatus. The extraction time was 4 h at 70 °C. The solvent was removed in a vacuum rotary evaporator at 35 °C. The oils obtained

were recovered by filtering, and the oil content and fat enrichment were determined and compared with control values of untreated alperujo samples. Aliquots from all of the liquid phases (or liquors) were stored at -20 °C for further analyses of phenolic compounds and sugars.

To compare the effect of both thermal treatments, Overend et al. (1987) defined the severity parameter ( $R_0$ ) which depends on the time and the temperature.

#### 2.4. Analytical methods

##### *Phenolic compounds*

The identification of phenolic compounds was performed by high performance liquid chromatography (Hewlett-Packard model 1100, Palo Alto, CA, USA) equipped with an array detector monitoring at 280 nm and a C18 reverse-phase column (Spherisorb ODS-2; 250 x 4.6 mm i.d. and 5 µm particle size) supplied by Teknokroma (Barcelona, Spain) kept at 25 °C with a C18 guard column. All aliquots of liquors from hydrothermal treatments were filtered through 0.45 µm membranes and injected directly into the HPLC instrument. A flow rate of 1.0 mL/min and an injection volume of 20 µL were used. Separation was achieved using a linear gradient of two solvents: solvent A (Milli-Q water, pH 2.5 adjusted with 20 mM trifluoroacetic acid) and solvent B (acetonitrile). A linear gradient analysis was used as follows: Starting with 95% solvent A and 5% solvent B, this was increased to 25 % solvent B over 30 min. The system was equilibrated between runs for 5 min using the starting mobile phase composition. The identification of each phenol was based on the comparison of retention times with those of reference compounds and the UV spectra was recorded in the range of 200–360 nm. Quantification was performed using a regression curve in triplicate for four different concentrations ( $r^2 \geq 0.99$ ).

### *Sugars and uronic acids*

Sugars and uronic acids were derivatized with p-aminobenzoic ethyl ester (ABEE) for UV detection. The procedure employed was carried out according to the method of Gomis, Tamayo, and Alonso (2001). To a 5 mL liquid phase solution was added 400 µL of 1.4 M NaBH<sub>3</sub>CN in distilled water, 400 µL of glacial acetic acid, and 2 mL of 0.6 M ABEE in methanol, and the mixture was heated at 80 °C for 10 min. After cooling to ambient temperature, 2 mL of distilled water was added. The aqueous phase was extracted with 4 mL of chloroform to remove excess ABEE and the aqueous layer was subjected to HPLC analysis. HPLC analyses were performed on a Shimadzu SCL-10A (Shimadzu Corporation, Kyoto, Japan) system equipped with two LC-10AD pumps, a SPD-10A UV spectrophotometric detector, and a SIL-10A auto-injector. An Ultradase C8-bonded silica column (Análisis Vínicos, 150 mm x 4.6 mm i.d., 3 µm) was used. The analysis was monitored at 307 nm. The column was operated at 45 °C. The separation was achieved with 100 mM sodium citrate buffer, pH 5.5/tetrahydrofuran (THF), 88:12 v/v (solvent A) and acetonitrile (solvent B). The gradient mode was used, beginning with solvent A/solvent B (99:1) until 20 min, and then the elution strength was gradually increased with solvent B (20–28 min, 20% B), and returned to the initial conditions from 36 min. The flow rate was maintained at 0.5 mL/min. A second sample of liquors or liquid phases was subjected to quantitative hydrolysis with 2 N TFA at 120 °C for 2 hours before HPLC analysis. The increase in the concentrations of monosaccharides caused by hydrolysis provided a measure of the oligomer and polymer concentration (linked sugars).

## 2.5. Statistical analysis

Results were expressed as mean values  $\pm$  standard deviations. To assess the differences between samples, a comparison was performed using the Statgraphics Plus program version 2.1. Multivariate analysis of variance (ANOVA) followed by Duncan's comparison test was performed. Results were considered statistically significant for  $P < 0.05$ .

### **3. RESULTS AND DISCUSSION**

#### **3.1. Effects of steam treatment (temperature and time) on the solubilisation of phenolic compounds**

The main factors that affect steam pretreatment are time and temperature (Romero-García et al., 2016). The key aim of this study was to evaluate the effects of these parameters on the properties of alperujo treated with the new discontinuous steam treatment reactor. Different steam treatment conditions were examined, including three temperatures (150, 160, and 170 °C) and various treatment times (15–90 min). Treatment conditions of low severity ( $R_0 < 3.25$ ), such as 150° C for 45 min, were not used because they did not permit the easy separation of solid and liquid phases. Steam treatment of alperujo solubilized both the phenolic compounds DHPG and HT in the liquid phase. The amount of released phenolic compounds, HT and DHPG, increased gradually with the severity of the treatments in the new reactor (**Figure 1**). The higher the treatment temperature and time, the higher the concentration of HT obtained (**Figure 1A**). As can be observed, the concentration of solubilized hydroxytyrosol reached a maximum value of 9.65 g/kg raw dry alperujo at 170 °C for 60 min, similar to the value obtained (13.2 g/kg) using a steam explosion reactor at 230 °C for 5 min under

acidic conditions (2.5% H<sub>2</sub>SO<sub>4</sub>), the optimal conditions for solubilizing hydroxytyrosol in its free and conjugated forms (Fernández-Bolaños et al., 2002). The released HT levels were highest with the most severe pre-treatment at 170 °C for 60 min. This result is in accordance with the increased phenolic yields reported with the increased severity of the steam explosion of olive stones, olive waste and olive leaves (Romero-García et al., 2016; Fernández-Bolaños et al., 2002, 1999).

One temperature was selected to evaluate the influence of treatment time on phenolic compound extraction, 160° C (**Table 1**). In this experiment, the alperujo used was different than the used in the previous ones in which both parameters, temperature and time, were evaluated in **Figure 1**. When treatment time was prolonged from 15 to 90 min at 160 °C, phenolic compound solubilization showed significant increases for HT. In the case of DHPG, which is susceptible to thermal instability and degradation at high temperatures, the prolongation of steam explosion caused a negative effect on yield (Rodríguez et al., 2009). However, during prolonged treatments in the new reactor, high values of recovery of DHPG were obtained at 75 and 90° C, close to those extracted in optimal conditions (**Table 1**). The effects of time and temperature of the steam treatment can be represented by the severity parameter (R<sub>0</sub>). In this study, the treatments carried out with the new reactor were compared with those of the same severity performed in the steam explosion reactor. Three sets of treatment conditions were investigated (with R<sub>0</sub> 3.25, 3.64, and 3.83) for their effect on the extraction of phenolic compounds (**Table 2**). Steam pretreatment performed in the new reactor was the most effective pretreatment (in the absence of an acid catalyst). At the highest treatment severity (R<sub>0</sub> = 3.84), HT solubilization was approximately 40% higher in the new reactor. These results indicate that lower temperature and longer treatment time are more favourable for the extraction of phenolic compounds from alperujo. The results

are also indicative of the efficacy of the new steam pretreatment of alperujo for removing phenolic compounds, which are considered to be fermentation inhibitors (Ballesteros et al., 2002).

### **3.2. Effect of steam treatment (temperature and time) on the extraction of sugars**

The steam treatment of alperujo was also able to solubilize sugars in the liquid fractions. The sugars are released and hydrolyzed from the cell walls of the olive fruit as monomers (free sugars) and oligomers and/or polymers (linked sugars). The values of solubilized sugars are the result of two competitive effects, a balance between solubilization and losses due to the thermal degradation of solubilized sugars. The percent recovery of total linked sugars increased when the severity of treatment was increased (**Table 3**). Analysis of the sugar content in the liquid fractions revealed the release of glucose, galactose, xylose, and arabinose from steam-treated alperujo and an increased solubilization with increased treatment time, indicating that sugars released could be mainly hemicellulose-type xylans or xyloglucans, which are the major hemicelluloses present in olive fruit (Vierhuis, Schols, Beldman, & Voragen, 2001). However, efficient hemicellulose solubilization does not always correspond to high sugar recovery in the liquid fractions since a decrease in free sugars was observed with increasing severity owing mainly to the decomposition of sugars to furfural and hydroxymethylfurfural (Ballesteros et al., 2002).

The hemicellulose-derived sugars (arabinose, glucose, and xylose) were recovered in the liquid fractions in the form of oligomers and polymers. The treatment time contributed significantly to the hydrolysis and solubilization of hemicelluloses, as

shown by the increased values of glucose, xylose and arabinose in liquid phases in oligomer and polymer forms (**Figure 2**). Considerable amounts of oligomers were solubilized as a result of steam treatment, as reported by other authors, including from olive tree residues (Cara, Ruiz, Ballesteros, Negro, & Castro, 2006). However, when the treatment time was prolonged above 60 min, a decrease was observed in the percent of oligomeric glucose recovered, probably due to its degradation to hydroxymethylfurfural.

Experiments performed with steam explosion showed that a significant amount of hemicellulose was hydrolyzed Comparison of the solubilization of total sugars for the two treatment methods, steam explosion versus the new reactor, using the same conditions of severity, according to the  $R_0$  parameter, revealed that sugar solubilization was higher for the samples treated by steam explosion (**Table 3**). This significant difference is probably due to the better solubilization of the hemicellulosic material and possibly a part of the cellulosic fraction of the alperujo. It was also apparent that at longer treatment times of steam treatment in the new reactor, there is substantial degradation of the monomeric sugars (**Figure 2**), which greatly reduces the recovery of the total sugars. This fact is supported by the increasing concentration of hydroxymethylfurfural and furfural, degradation products of hexoses and pentoses, respectively, in the liquid fraction following steam treatment (Serrano et al., 2017b).

### **3.3. Effects of steam treatment (temperature and time) on oil content and reduction of residual solid fraction**

The effect of temperature on the oil content and reduction of the residual solid fraction was also investigated. For 60-min treatments, there was a significant reduction of solid

with increased severity, resulting in a dry weight loss that ranged from 29.0% at 150° C to 38.8% at 170° C (**Figure 3**). The high dry matter loss during pretreatment indicated that a large amount of the raw alperujo is dissolved. The largest reductions of the residual solid fraction were obtained for the severest conditions of treatment (at 170 °C for 60 min). The dry weight loss or solid reduction is caused by the solubilization of phenolic compounds as well as hemicellulose hydrolysis. Previously, it was shown that hemicellulose is one of the major constituents extracted during steam treatment (Fernández-Bolaños et al., 2001). As a result of solid reduction, treated alperujo had a higher oil content, increasing from 8.1% to 16.0% and resulting in enrichment from 45.7% to 97.5%, at 150° C and 170° C, respectively. Steam treatment breaks down cell walls by the combined effect of high temperature and pressure, and residence time releases the oil contained in the vacuoles and in the olive seed, causing the oil enrichment.

The effect of treatment time on oil content and reduction of the solid fraction was also studied (at 160 °C for 15 to 90 min) and the same trend was observed, with treatment time increasing both these parameters (**Figure 3**). At 160 °C, measurement of weight loss revealed significant solid reduction when treatment time was prolonged from 15 to 90 min, with a reduction of 36.1%. The effects of steam treatment in the new reactor are similar to the results obtained with the steam explosion reactor, with similar values of solid reduction when treatment severity was increased, with a solid reduction or weight loss of about 55% and an oil enrichment in the solid phase of around 122% reported for steam explosion treatment at 200 °C for 5 min without catalyst (Rodríguez, Rodríguez, Fernández-Bolaños, Guillén, & Jiménez, 2007b).

The moisture content of treated alperujo after solid-liquid separation by filtration and/or centrifugation ranged from 30% to 40% (results not shown), a decrease of

approximately one-third compared with raw alperujo. At 160° C for 60 and 90 min, the moisture content decreased from the initial 70% in raw alperujo to 39 and 35%, respectively.

#### **4. CONCLUSION**

Different steam treatment conditions were evaluated, confirming that the new discontinuous steam treatment reactor provides the same advantages as the steam explosion reactor in terms of the extraction of bioactive compounds (simple phenols and sugars in an oligomeric form) and the oil enrichment and reduction of the residual solid phase. The new steam treatment reactor represents a good alternative to resolve the economic and environmental problems associated with the generation of alperujo, a byproduct from the two-phase olive oil process. The recovery of phenolic compounds from alperujo would not only improve the economic value of this waste, but could also provide an important source of natural and beneficial phenolic compounds for further applications, as HT and DHPG are confirmed to have phytotoxic and antimicrobial effects. Solubilization of the sugars contained in alperujo leads to the production of a final solid residue that is less sticky and much easier to dry, by reducing caramelization. The considerable reduction of the residual solid fraction by the new reactor, resulting from the effective fractionation of the liquid phase with a high solubilization of phenols and sugars, would produce a final alperujo solid fraction that is rich in oil. From an industrial point of view, the increased yield of oil extraction would be a significant benefit for this reactor and reduce the costs of waste management. Thus, the current industrial application of this steam treatment should focus not only on the energetic advantages of a drier alperujo, but also consider the improvements in the concentration

of oil, a final solid with lower toxicity, and a liquid phase that is a natural source of bioactive compounds. Thus, alperujo treated by the new discontinuous steam treatment reactor is much more attractive for subsequent bioprocess applications or bioremediations.

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## **Figure captions**

**Figure 1.** Effect of I) time and I) temperature on HT and DHPG content obtained in the liquid phases of steam pretreated alperujo. The values are given in g/kg of raw dry matter. Means with the same letter were not significantly different,  $p<0.05$ .

**Figure 2.** Effect of different times of steam treatment at 160 °C on the oligomeric sugar composition of the liquid fraction. Data are expressed as a percent of the raw untreated dry matter (g/100g raw dry alperujo). Standard deviations are shown.

**Figure 3.** Effects of A) temperature and B) time on oil content and solid reduction for 60-min treatments of alperujo. The error bars indicate the standard deviation of the mean of three replicates.

## Tables

**Table 1.** Effect of time of steam treatment (at 160 °C) on the extraction of phenolic compounds from the liquid phase of alperujo using the new steam reactor. Values are expressed in g/kg of raw dry alperujo.

Compound	Control	Time (min)					
		15	30	45	60	75	90
<b>DHPG</b>	1.43 <sup>*a</sup>	0.75 c (0.12)	0.94 b (0.01)	0.79 c (0.02)	0.84 bc (0.03)	0.98 b (0.01)	0.81 bc (0.04)
	(0.12)	(0.02)	(0.01)	(0.02)	(0.03)	(0.01)	(0.04)
<b>HT</b>	3.87 <sup>** A</sup>	2.34 D (0.09)	2.84 CD (0.07)	3.26 BC (0.10)	3.35 B (0.07)	3.76 A (0.08)	3.08 BC (0.08)
	(0.09)	(0.07)	(0.10)	(0.07)	(0.08)	(0.11)	(0.08)

<sup>\*</sup>Steam explosion treatment at 230 °C for 5 min with 2.5% sulfuric acid, the optimal conditions for evaluating the total hydroxytyrosol content (free and conjugated) in alperujo (Fernández-Bolaños et al., 2002a).

<sup>\*\*</sup>Thermal treatment at 90 °C for 2 h, the optimal conditions for quantifying the maximum recovery of DHPG (Rodríguez et al., 2009).

Mean (standard deviation) of three determinations. Means with the same letter were not significantly different, p<0.05.

**Table 2.** Effects of the two steam treatment methods, steam explosion and the new reactor (steam pretreatment) on the solubilisation and/or recovery of phenolic compounds (HT and DHPG) and total sugars from alperujo.

Treatment	Conditions T/t	Severity/ $R_0$ <sup>a</sup>	HT (%) <sup>b</sup>	DHPG (%) <sup>c</sup>	Total sugars (% w/w of raw dry alperujo)
Steam explosion	200 °C/2 min	3.25	24.85 d	n.d.	15.33 i
New reactor	150 °C/60 min		44.24 c	11.95 B	4.43 iii
Steam explosion	200 °C/5 min	3.64	41.79 c	n.d.	10.96 ii
New reactor	160 °C/75 min		97.16 a	68.53 A	5.71 iii
Steam explosion	220 °C/2 min	3.83	34.32 cd	n.d.	10.35 ii
New reactor	170 °C/60 min	3.84	73.11 b	17.70 B	4.87 iii

<sup>a</sup> The severity parameter combines time (t) and temperature (T) in the form of  $R_0 = te^{[(T-100)/14.75]}$ .

<sup>b</sup> Percent of solubilised HT calculated for the maximum value obtained in optimal conditions (steam explosion treatment at 230 °C for 5 min with 2.5% sulphuric acid).

<sup>c</sup> Percent of DHPG calculated for the quantified maximum value by treatment at 90° C for 2 h.

n.d. not detected.

Means with the same letter (lower case for HT, capital letter for DHPG and (i) for total sugars) were not significantly different, p<0.05.

**Table 3.** Effect of temperature on the solubilisation of sugars (free and linked) in the liquid fractions following 60-min steam pretreatment (data are expressed as % g/100 g of raw dry alperujo).

Sugars	Alperujo		150 °C				170 °C					
	(Control) <sup>a</sup>	45 min	60 min	90 min	15 min	45 min						
	F <sup>b</sup>	L <sup>c</sup>	F	L	F	L	F	L	F	L	F	L
<b>GlcA</b>	0.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<b>GalA</b>	0.26	0.38	0.15	0.34	0.14	0.42	0.21	0.52	n.d.	0.42	0.16	0.41
<b>Glc</b>	13.6	0.50	5.87	0.45	4.77	1.02	2.08	4.78	0.37	2.10	0.50	2.81
<b>Gal</b>	n.d.	n.d.	n.d.	1.55	n.d.	1.63	0.45	0.51	n.d.	0.35	0.11	0.42
<b>Xyl</b>	n.d.	0.26	0.10	0.16	0.17	0.68	0.11	0.83	n.d.	0.74	0.10	1.54
<b>Ara</b>	n.d.	0.28	0.08	0.46	0.27	0.75	0.20	0.85	0.19	0.89	0.25	0.72
<b>Fuc</b>	n.d.	nd	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<b>Rha</b>	n.d.	0.28	n.d.	0.26	0.09	0.25	0.14	0.55	n.d.	0.47	0.16	0.45
<b>Total</b>	13.98	1.7	6.2	3.22	5.44	4.75	3.19	8.04	0.56	4.97	1.28	6.35

<sup>a</sup> Aqueous extract (40% p/v) at ambient temperature.

<sup>b</sup> Free sugars in a monomeric form.

<sup>c</sup> Linked sugars in a oligomer and/or polymeric forms.

n.d., not detected.

F: free; L: linked;

## Figures

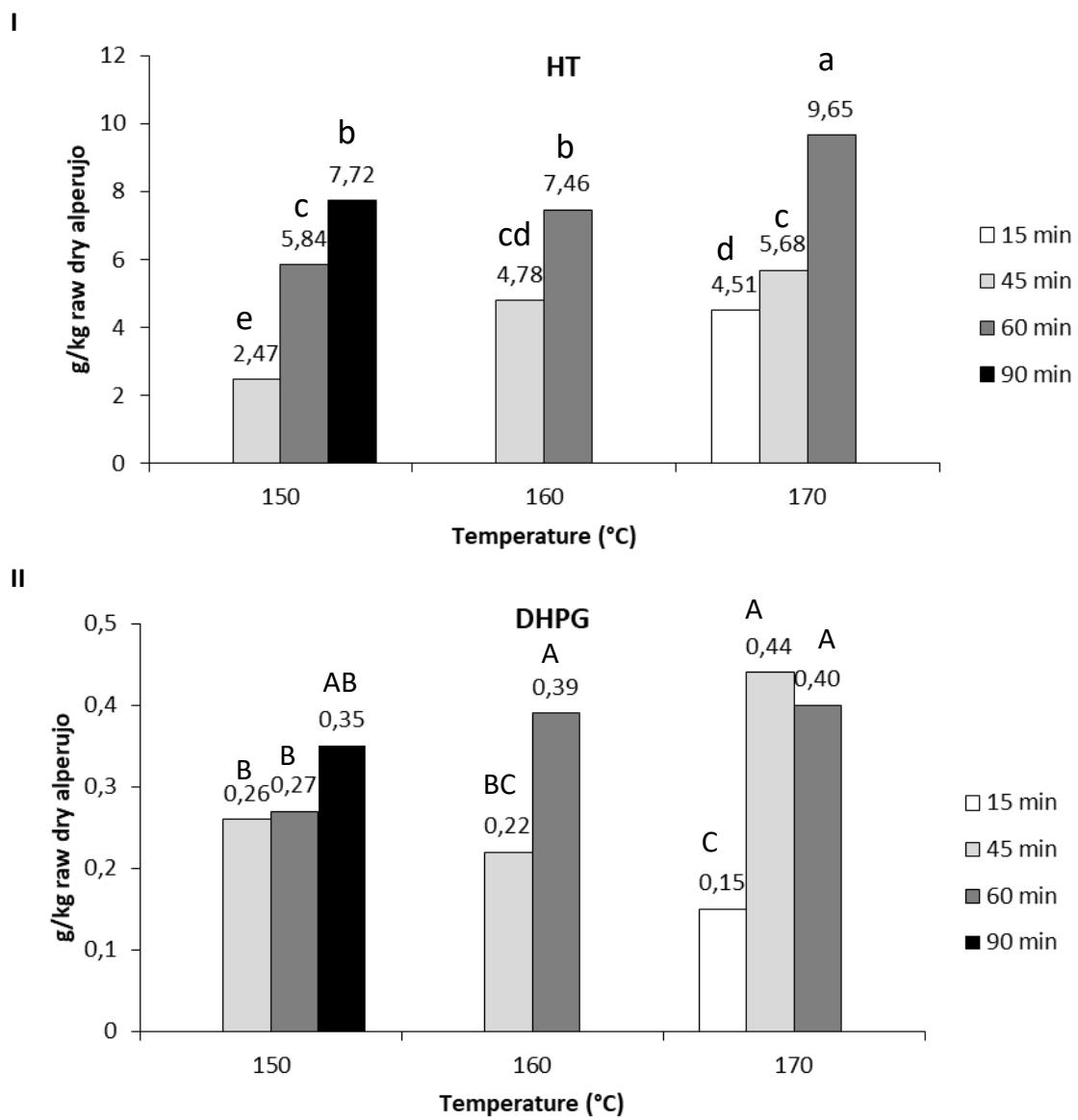
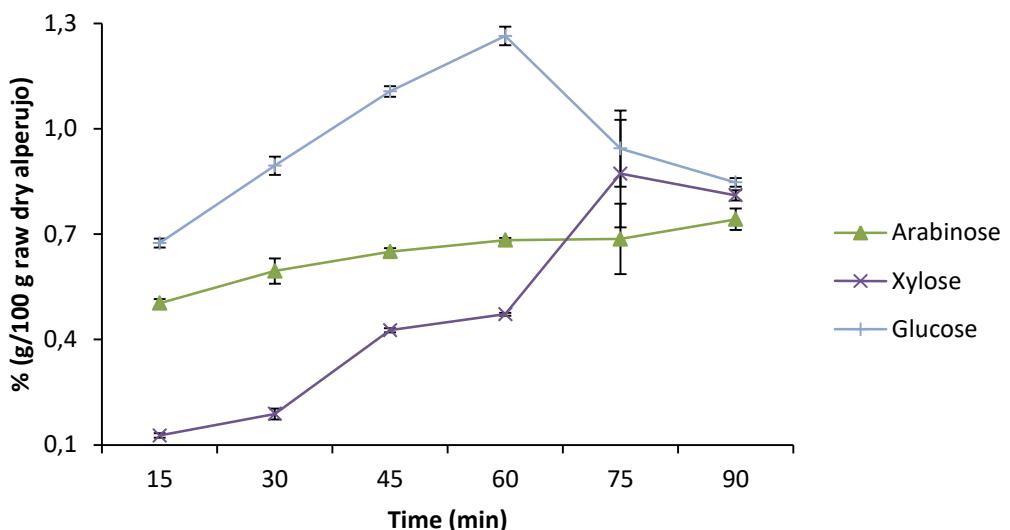
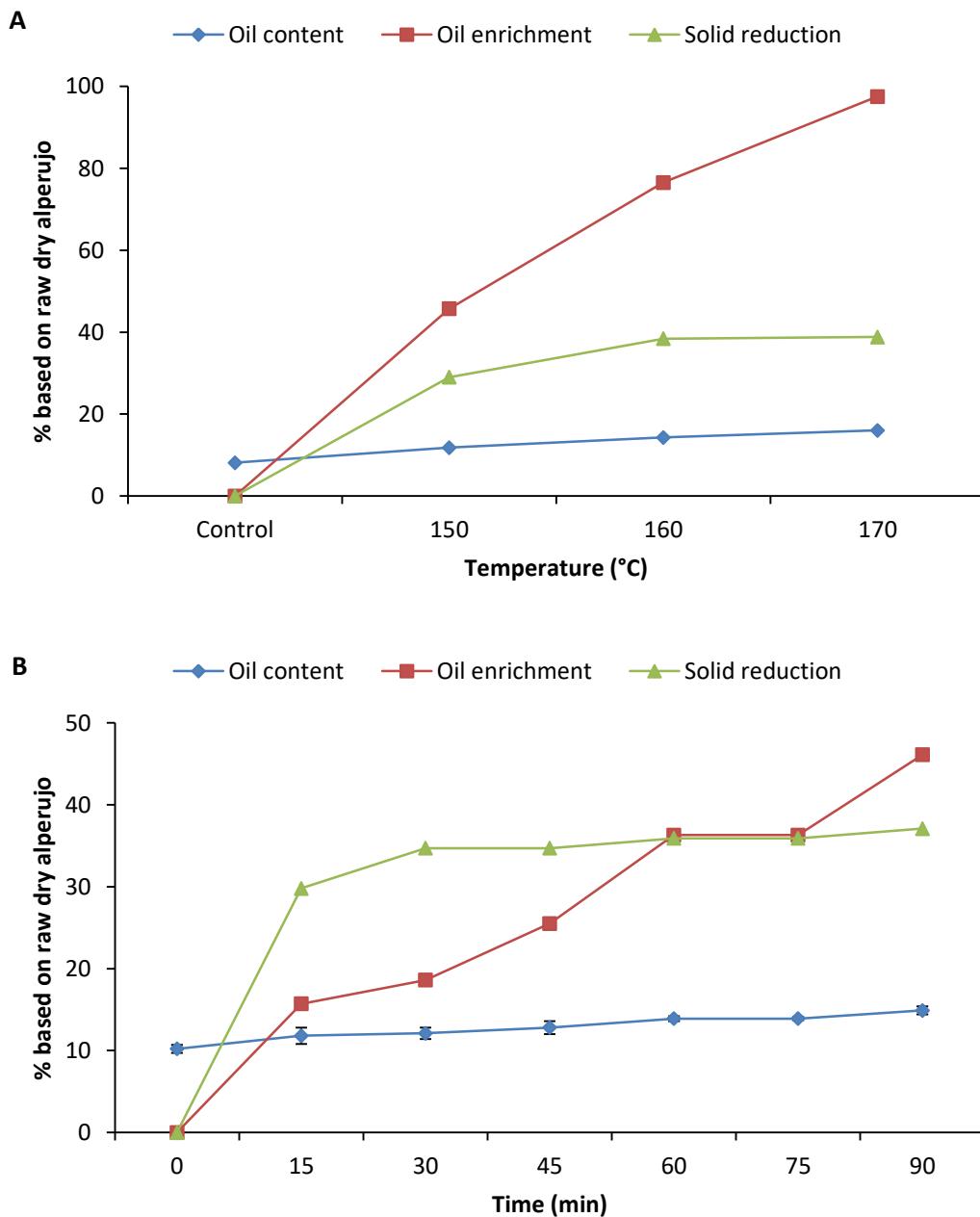


Figure 1.



**Figure 2.**



**Figure 3.**