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Recovery of antioxidants from olive mill wastewaters: A viable solution that promotes their overall sustainable management

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Abstract

Olive mill wastewaters (OMW) are rich in water-soluble polyphenolic compounds that show remarkable antioxidant properties. In this work, the recovery yield of compounds, such as hydroxytyrosol and tyrosol, as well as total phenols (TPh) from real OMW was investigated. Antioxidants were recovered by means of liquid-liquid solvent extraction. For this purpose, a laboratory-scale pilot unit was established and the effect of various organic solvents, namely ethyl acetate, diethyl ether and a mixture of chloroform/isopropyl alcohol, on process efficiency was investigated. It was found that the performance of the three extraction systems decreased in the order: ethyl acetate > chloroform/isopropanol > diethyl ether, in terms of their antioxidant recovery yield. It was estimated that treatment of 1 m^3 OMW with ethyl acetate could provide 0.247 kg hydroxytyrosol, 0.062 kg tyrosol and 3.44 kg of TPh. Furthermore, the environmental footprint of the whole liquid-liquid extraction system was estimated by means of the life cycle assessment (LCA) methodology to provide the best available and most sustainable extraction technique. From an environmental perspective, it was found that ethyl acetate and diethyl ether had similar environmental impacts. Specifically, for the production of 1 g hydroxytyrosol, tyrosol or TPh, 13.3, 53.1 or 0.949 kg CO_2 equivalent would be released to the atmosphere, respectively. On the other

hand, the chloroform/isopropyl alcohol mixture had detrimental effects onto ecosystems, human health and fossil fuels resources. In total, ethyl acetate yields low environmental impacts and high antioxidant recovery yield and thus it can be considered as the best solution, both from the environmental and technical point of view. Three alternative scenarios to improve the recovery performance and boost the sustainability of the ethyl acetate extraction system were also investigated and their total environmental impacts were estimated. It was found that with small process modifications the environmental impacts could be reduced by 29%, thus achieving a more sustainable antioxidants recovery process.

Keywords: OMW; hydroxytyrosol; tyrosol; LCA; solvent extraction; polyphenols

1. Introduction

Nowadays, there are growing concerns about a variety of environmental issues that accompany OMW and its effective treatment. OMW is a by-product of olive oil production and is characterized by seasonal large volumes and high organic loads (Chatzisymeon et al., 2009a; Gotsi et al., 2005). However, if properly managed, it is an inexpensive and convenient source of natural antioxidants, mainly due to its high polyphenolic content (Niaounakis and Halvadakis, 2006). Polyphenols are water soluble organic compounds and, therefore, they are found in abundance in OMW (Obied et al., 2005). So far, more than forty phenols have been identified in OMW with hydroxytyrosol being the main natural polyphenolic compound due to its high bio-antioxidant capacity (Tsimidou et al., 1992). In general, all polyphenolic compounds exhibit potential antioxidant properties; the way they act is through elimination of free radicals in cells, thus providing protection against oxidative stress in biomolecules like proteins, lipids and DNA (Boskou, 2006). Moreover, as natural substances their high potential antioxidant properties are reflected in their high market price, and their great demand in the cosmetic, pharmaceutical and food industry. Among them, hydroxytyrosol is in abundance in OMW and it is considered to be the most active antioxidant (Gordon et al., 2001). On the other hand, if polyphenols are left without any further treatment into the OMW, they are gradually oxidized and/or polymerized rendering OMW highly toxic and recalcitrant (Chatzisymeon et al., 2009b; Celano et al., 2008; Martirani et al., 1996). Hence, the recovery of the polyphenolic content of OMW not only provides economic benefits but it also makes OMW less toxic and easier to treat, thus promoting the overall sustainability of the OMW management (Federici et al., 2009).

Until now, several methods for polyphenols recovery have been suggested, including solvent extraction (Bertin et al., 2011; De Leonardis et al., 2007; Grizis et al., 2003; Lesage-Meessen et al.,

2001), adsorption onto resins (Scoma et al., 2011; Agalias et al., 2007), supercritical fluid extraction (Lafka et al., 2011), selective concentration by ultrafiltration (Galanakis et al., 2010; Lin and Juang, 2009) and integrated membrane systems (El-Abbassi et al., 2011; Garcia-Castello et al., 2010; El-Abbassi et al., 2009). Moreover, interesting alternative processes include the use of microparticles (Puoci et al., 2012) and biofilters (Ena et al., 2012), cloud point extraction (Gortzi et al., 2008) and ultrasound-assisted extraction (Klen and Vodopivec, 2011). Among them, liquid-liquid solvent extraction is an easy to operate technique thus, it could be applied even in small, family-owned olive oil mills that exist in most Mediterranean countries, including Greece. The concept has been very recently demonstrated by Sannino et al. (2013) who proposed liquid-liquid extraction using ethyl acetate followed by chromatographic fractionation for the production, at small-scale, of high grade, purified hydroxytyrosol. Furthermore, many researchers found that the most promising solvents for liquid-liquid extraction were diethyl ether, ethyl acetate and a mixture of chloroform with isopropyl alcohol (De Leonardis, 2007; Grizis et al., 2003; Lesage-Meessen et al., 2001). Due to the different polar properties of the target phenolic compounds typically found in OMW, such as hydroxytyrosol which is the main constituent in OMW exhibiting amphiphilic properties, different solvent systems were chosen to assess their selectivity to extract the target compounds.

At this point, it is of great significance to mention that the sustainability of the aforementioned polyphenols recovery techniques has never been studied before. Besides, the environmental footprint of any process can reveal important information regarding the scaling-up of the process and enhance its overall sustainability by introducing alternative "green" scenarios.

Life cycle assessment (LCA) is the concept of analyzing and quantifying the environmental impacts of any given product, process, service or activity throughout its lifespan (Tsoutsos et al., 2010). It is also known as cradle-to-grave assessment due to the fact that one has to take into account all the stages of a product's or service life, which start from the extraction of resource inputs and ends to the eventual disposal of the product or its waste (ISO 14040, 2006). The main benefit of LCA is its capability to link the environmental loads of any product, process, service or activity with its mass and energy flows (Kniel et al., 1996).

The aim of this work was to investigate the recovery yield of various polyphenolic compounds such as hydroxytyrosol and tyrosol, as well as TPh from real OMW by means of solvent extraction. This information was then employed to estimate the environmental footprint of the process by means of LCA and to provide the best available and sustainable extraction technique. For this purpose, a laboratory-scale pilot unit was setup and the type of the organic solvent was investigated.

2. Materials and methods

2.1 Materials

Ethyl acetate ($C_4H_8O_2$), diethyl ether ($C_4H_{10}O$), chloroform ($CHCl_3$) and isopropyl alcohol (C_3H_8O) were purchased from Merck (analytical grade) and used as extraction solvents. Chloroform and isopropyl alcohol were mixed at a 7:3 (v/v) ratio according to the work of Grizis et al. (2003).

Hydroxytyrosol ($C_8H_{10}O_3$, ≥90%), tyrosol ($C_8H_{10}O_2$, ≥95%) and oleuropein ($C_{25}H_{32}O_{13}$, ≥98%) were purchased from Extrasynthese Chemicals Company. Caffeic (≥98%) and gallic (≥98%) acids were purchased from Sigma Aldrich Company.

A model solution containing 250 mg/L oleuropein, 1000 mg/L tyrosol, 250 mg/L gallic acid, 250 mg/L caffeic acid and 270 mg/L hydroxytyrosol in ultrapure water was prepared to evaluate the ability of liquid-liquid extraction to recover them. It should be noted that the mixture of phenolic compounds was stirred vigorously for one day to ensure complete dissolution. The pH of the synthetic solution was fixed to 5 adding NaOH to match that of the actual OMW. The degree of recovery of the phenolic compounds from the model solution was calculated measuring their concentration at the initial solution and immediately after the liquid-liquid extraction had been completed. The discrepancy between these two values divided by the initial concentration results in the recovery yield of each compound.

The actual effluent was provided by a three-phase, olive oil mill located in Chania, Western Crete, Greece. Its main physicochemical characteristics were as follows: pH=5, initial soluble COD=98.6 g/L, total solids=70.5 g/L, conductivity=9.25 mS/cm and TPh=6 g/L, while its color was black-brown.

2.2 Sample pretreatment

Due to the effluent's high content of solids, the samples were first filtered through mess gauge filters and then centrifuged at 3000 rpm for 20 min to remove most of the solid particles. Afterwards, the supernatant fats and the solid precipitates were removed, and the remaining aqueous phase was subjected to further filtration by means of a 0.45 μm filter. Following this pretreatment, its main properties were: pH=4.8, initial COD=90 g/L, total solids=0.5 g/L, conductivity=9.5 mS/cm and TPh=5 g/L.

It should be noted here that filtration is unlikely to be employed as a preconditioning stage for solids removal in actual applications; on the contrary, coagulation-flocculation using inexpensive materials (i.e. ferrous ions) would be a reasonable pretreatment method prior to extraction as has been demonstrated in a recent study of our group (Papaphilippou et al., 2013). Moreover, sample pretreatment is not a trivial procedure since it can influence the efficiency of extraction.

2.3 Recovery procedure

Sample pretreatment was followed by liquid-liquid extraction to efficiently separate the target polyphenols from the OMW. A laboratory-scale pilot unit was setup in order to perform experiments in batch mode operation. The solvent to OMW ratio was always constant at 2:1 v/v (100:50 in mL). Moreover, it is important to note that all runs were carried out at ambient temperature (ca 27°C) in order to avoid enzymatic degradation and oxidation of polyphenols.

At first, the OMW and the appropriate solvent were loaded in an Erlenmeyer flask with 250 mL headspace, which was then tightly sealed and continuously stirred magnetically for 30 min at 120 rpm. During this step, the polyphenolic compounds were extracted from the aqueous phase (i.e. OMW) to the organic liquid phase (i.e. solvent). Afterwards, this OMW-organic solvent mixture was left to settle in a separate funnel for 30 min. After settling, the two liquid phases were separated and the organic phase was recovered. Recovery was followed by evaporation of the organic liquid phase to dryness. Evaporation took place at $40^{\circ}C$ in a bathwater under vacuum, in order to condense the organic solvent and obtain the crude organic extract (i.e. polyphenolic content). This organic deposit was then reconstituted in 25 mL methanol and analyzed. The methanolic extract is two-fold concentrated and the recovery is expressed in total mg of target phenolic compounds extracted from one liter OMW.

Process efficiency was estimated measuring the TPh recovery yield, as well as monitoring the concentration of tyrosol and hydroxytyrosol after the end of the liquid-liquid extraction technique. It should also be noted that all experiments were performed in duplicate and mean values are quoted as results with standard deviation never exceeding 5% for the range of concentrations in question.

2.4 High performance liquid chromatography (HPLC)

HPLC (Alliance 2690, Waters) was employed to monitor the concentrations of tyrosol, hydroxytyrosol, caffeic acid, gallic acid and oleuropein. Separation was achieved on an ACE C18-R reverse phase column (stainless steel, 250x4.6 mm, id 5 μm), while detection was achieved through a UV-Vis detector set at 280 nm. More details can be found elsewhere (Papaphilippou et al., 2013). The polyphenolic compounds were tentatively identified matching their elution times from the column to those of standard stock solutions. Calibration curves were also prepared at various concentrations and used for quantitation purposes.

2.5 Total phenols

The TPh content was determined colorimetrically at 765 nm on a Shimadzu UV 1240 spectrophotometer using the Folin-Ciocalteau reagent according to the procedures described in

171   detail elsewhere (Singleton et al., 1999). Gallic acid monohydrate was used as standard to quantify

172   the concentration of phenols in OMW.

173

174   **2.6 LCA**

175   LCA methodology was utilized in order to identify and, therefore, further reduce the environmental

176   footprint of the liquid-liquid extraction process and provide alternatives to decision makers and

177   developers (Foteinis et al., 2011). The software package SimaPro 7.3.3, a widely used tool, both by

178   professionals and researchers, was used in this work. Moreover, two impact assessment methods

179   were used and these are IPCC 2007 and ReCiPe. The first one compares processes based on CO_2

180   emissions equivalents. CO_2 emissions are a worldwide known unit, namely global warming

181   potential (GWP), and an acceptable way to express the sustainability of a system for a timeframe of

182   100 years. The ReCiPe framework is the most recent impact assessment method that exhibits

183   certain advantages comparing to other approaches, such as Eco-Indicator 99. In particular, ReCiPe

184   includes the broadest set of midpoint impact categories and the possibility of using impact

185   mechanisms that have global scope (Goedkoop et al., 2009). Analytically, the ReCiPe method can

186   transform the life cycle inventory results into a limited number of indicator scores that are

187   expressed per environmental impact category and also as an aggregated single score. These

188   indicator scores express the relative severity on an environmental impact category at two levels, the

189   midpoint indicators (impact categories) and the endpoint indicators (damage categories), as shown

190   in Table 1. Furthermore, in the case of the ReCiPe method, the perspective that was used was the

191   Egalitarian. This takes into account the long term, precautionary environmental impacts if compared

192   with the other available perspectives.

193

194   Table 1.

195

196   **3. Results and discussion**

197   **3.1 Polyphenols recovery from model solution**

198   Given the complexity of the actual OMW, preliminary tests were performed with the synthetic

199   solution.

200   Table 2.

201

202   As seen in Table 2, the extraction efficiency expectedly depends on the solvent/polyphenolic

203   compound pair; caffeic acid and tyrosol exhibited the highest recoveries irrespective of the solvent

204   system employed, followed by hydroxytyrosol, oleuropein and gallic acid. These preliminary

separations confirm that the selected solvent systems can, to a certain degree, extract all of the compounds from the mixture; the only exception was oleuropein, which could not be recovered by diethyl ether. Judging the relative extraction efficiency of the various solvents for the set of polyphenols rather than on an individual basis, ethyl acetate appears to be superior to the rest. This has also been confirmed by Papaphilippou et al. (2013) who tested ethyl acetate, dichloromethane, diethylether and a 7:3 (v/v) mixture of chloroform:isopropyl alcohol for the extraction of model, single-component aqueous solutions of tyrosol, oleuropein, caffeic and gallic acids.

Allouche et al. (2004) assessed the ability of several solvents to extract batchwise the polyphenolic fraction of OMW and reported that the extraction power decreased in the order: ethyl acetate > methyl isobutyl ketone > methyl ethyl ketone > diethyl ether. The superiority of ethyl acetate over diethyl ether to extract OMW phenols was also verified by Klen and Vodopivec (2011) who found that the former was nearly twice as effective as the latter. El-Abbassi et al. (2012) reported that ethyl acetate was capable of extracting hydroxytyrosol and tyrosol, as well as gallic, caffeic and p-coumaric acids from OMW.

3.2 Polyphenols recovery from OMW

Figure 1 shows a typical chromatograph representing the qualitative and quantitative composition of the OMW extract when ethyl acetate solvent is used. (It should be noted that the OMW was not subjected to chromatographic characterization prior to the extraction.) It is observed that hydroxytyrosol and tyrosol are among the most abundant target polyphenolic compounds, sharing 7.2% and 1.8% of the OMW polyphenolic content, respectively (these are computed from the extract concentrations, as well as the respective TPh value shown in Table 3). On the other hand, oleuropein, caffeic and gallic acids were also tested but found only in traces in the ethyl acetate extract. Therefore, only the concentrations of tyrosol and hydroxytyrosol were followed for the rest of the experimental work.

Figure 1.

The results, shown in Table 3, confirm that ethyl acetate is the most effective solvent in terms of hydroxytyrosol, tyrosol and TPh extraction with the respective concentrations being 247.2, 61.6 and 3440 mg/L, respectively. Specifically, ethyl acetate achieved higher extraction yields than the chloroform/isopropyl alcohol system by 49.7% and 30.5% for hydroxytyrosol and tyrosol, respectively. It should be noted that although the chloroform/isopropanol mixture shows a similar

TPh extraction yield to ethyl acetate, its selectivity towards tyrosol and hydroxytyrosol (i.e. target polyphenolic compounds with significant antioxidant capacity) is substantially lower than that of ethyl acetate. Moreover, diethyl ether exhibits even lower extraction yields in terms of all the extracted target antioxidants. Hence, the performance of the three extraction systems decreased in the order: ethyl acetate > chloroform/isopropanol > diethyl ether.

De Leonardis et al. (2007) reported that extraction with ethyl acetate could yield up to 0.34 kg hydroxytyrosol and 0.083 kg tyrosol per m^3 of OMW treated; these values are in good agreement with the results reported in this work (i.e. 0.25 kg hydroxytyrosol and 0.062 kg tyrosol per m^3 of OMW). Allouche et al. (2004) reported that a three-stage, continuous, counter-current process using ethyl acetate as the solvent could extract 1.23 kg of hydroxytyrosol per m^3 of OMW. In other studies, Agalias et al. (2007) reported a hydroxytyrosol recovery of up to 0.58 kg using various adsorbent resins. Visioli et al. (1999) used a benchtop mill to process olives and the resulting OMW was subjected to ethyl acetate extraction yielding 1.2 g hydroxytyrosol and 0.45 g tyrosol per 100 g of dry matter; these values increased to 9.79 g and 4.72 g, respectively when the extract was further subjected to chromatographic fractionation. Besides three-phase OMW, efforts have been made to isolate hydroxytyrosol from two-phase olive mill wastes using acid- or alkali-catalyzed hydrothermal treatment; about 5 kg of hydroxytyrosol could be obtained from 1 tn of solid waste containing 70% humidity (Fernandez-Bolanos et al., 2002).

Table 3.

3.2 Life cycle assessment of solvent extraction

3.2.1 General considerations

LCA was used to assess the environmental footprint of the antioxidants extraction when three different types of solvents were employed. The LCA study was based on the experimental data that are shown in Table 3.

First of all, the system boundaries were set as follows: the OMW production, its transportation and its final treatment/disposal, as well as the stirrer and the vacuum evaporator are not included inside the boundaries, since this work deals with the extraction of the high value natural compounds from OMW and not with its treatment.

Moreover the vials (flask, separation funnel) were not considered in the system flow (outside of the system boundaries) since their contribution to the total environmental impact is quite low. Preliminary LCA simulation runs were performed (data not shown) and the results showed that the material (i.e. glass) of the laboratory-scale vials does not pose serious environmental impacts.

Therefore, the main LCA system flows were (i) the energy inputs; (ii) the organic solvents and their

273 releases to the environment; (iii) the solvent recovery yield during the evaporation step of the
274 extraction technique.

275 All the related data (i.e. the important inputs/outputs) were identified, quantified and archived in
276 order to create an inventory of the flows (e.g. energy, raw materials, and releases to air, land and
277 water) of the LCA system from and to nature (Table 4). The main energy inputs that were identified
278 were the energy consumed by (i) the magnetic stirrer (45 kJ), and (ii) the vacuum evaporator that
279 was estimated at 435.6 kJ, 226.8 kJ and 680.4 kJ for ethyl acetate, diethyl ether and the
280 chloroform/isopropyl alcohol mixture, respectively. The outputs were the released solvent amounts
281 both to the aqueous phase and to the atmosphere. The solvent was diffused into the aqueous phase
282 during the separation of the two liquid phases and it was estimated measuring the volume of the
283 organic solvent before and after the end of this separation step. Analytically, the diffusion of ethyl
284 acetate, diethyl ether and chloroform/isopropyl alcohol into the aqueous phase was 5.1%, 4.4% and
285 4.5%, respectively. Moreover, the organic solvent was emitted into the atmosphere during its
286 evaporation which was inevitable in order to obtain the crude organic extract (i.e., polyphenolic
287 content). Solvent emissions to the atmosphere were monitored by measuring the volume of the
288 organic solvent after its condensation during the evaporation step. Solvent emissions were estimated
289 at 7%, 17% and 8% for ethyl acetate, diethyl ether and chloroform/ isopropyl alcohol, respectively.
290 Afterwards, three functional units were set, and these are the extraction yield (mg/L) of
291 hydroxytyrosol, tyrosol and TPh.

292

293   Table 4.

294

3.2.2 Global warming potential

296 The aforementioned inventory was used as an input in the software SimaPro 7.3.3 in order to
297 simulate the environmental loads/impacts of the two-phase extraction process and compare the three
298 different organic solvents by estimating their environmental footprint. Hence, the results are quoted
299 as global warming potential in CO_2 equivalents for a timeframe of 100 years for the extraction of 1
300 g of tyrosol, hydroxytyrosol and TPh and they are shown in Table 5.

301

302   Table 5.

303

304 These revealed that diethyl ether was the most environmentally friendly solvent, closely followed
305 by ethyl acetate for the extracted target antioxidants. Besides, chloroform/isopropyl alcohol was
306 found to pose serious threats to the environment in terms of its global warming potential.

307   Specifically, as far as tyrosol extraction yield is concerned, diethyl ether was found to be the most

308   environmentally friendly solvent releasing only 32.1 $kgCO_2eq$, while ethyl acetate and

309   chloroform/isopropyl alcohol released 53.1 and 206 $kgCO_2eq$, respectively. Undoubtedly, the

310   chloroform/isopropyl alcohol extraction system exhibits an extremely high GWP if compared with

311   the other two systems. The same trend, regarding GWP, stands for the extraction of hydroxytyrosol

312   and TPh.

313   GWP is strongly related to energy consumption as this is the main reason for increased CO_2

314   emissions worldwide (Forster et al., 2007). Hence, the better performance of diethyl ether, which is

315   followed by ethyl acetate and chloroform/isopropyl alcohol is primarily attributed to the fact that

316   the energy requirements for evaporation are lower for diethyl ether (226.8 kJ) than for ethyl acetate

317   (435.6 kJ) and chloroform/isopropyl alcohol (680.4 kJ). Moreover, the high GWP of the

318   chloroform/isopropyl alcohol solvent system can be also attributed to the fact that isopropyl alcohol

319   is a well-known green house gas, exhibiting a considerably higher amount of CO_2 equivalent

320   (Salazar and Nanny, 2010).

321

322   **3.2.3 Severity of the environmental impacts onto human health, ecosystem and resources**

323   **depletion**

324   The aggregated environmental impacts during the extraction of hydroxytyrosol, tyrosol and TPh

325   from OMW, based on the ReCiPe method, are presented in Figures 2, 3 and 4, respectively. Figure

326   2a shows that the most severe impact indicator during the extraction of hydroxytyrosol is the fact

327   that the overall process can cause toxic effects on humans for all the extraction solvent systems.

328   Besides, the human toxicity impact of the chloroform/isopropyl alcohol exceeds by far the impacts

329   of the other two solvent systems, i.e. it can cause about twice higher toxic effects than the use of

330   ethyl acetate or diethyl ether. Furthermore, the chloroform/isopropyl alcohol solvent system yields

331   higher environmental impact on fossil fuel depletion and on human health through its interference

332   with climate change phenomena than the other systems. All the other impact indicator scores are

333   practically not affected by the liquid-liquid extraction technique to recover antioxidants from

334   OMW. These findings are presented in a different way in Figure 2b, where one can see the general

335   environmental damage categories of the extraction process on human health, ecosystem and the

336   depletion of resources. Chloroform/isopropyl alcohol is by far the most toxic and hazardous

337   material for human health and ecosystems and yields the higher requirements in terms of fossil

338   fuels. On the other hand, ethyl acetate is the most environmentally friendly reagent to recover

339   hydroxytyrosol from OMW, although it requires higher amounts of fossil fuels than diethyl ether.

340

341   Figure 2.

342

343 Like hydroxytyrosol, the most significant environmental damage category for tyrosol extraction is

344 human health (Figure 3b). Firstly, this is threatened (Figure 3a) through the human toxicity, which

345 is caused by the toxic nature of the organic reagents in combination with the solvent amount that is

346 released to the atmosphere during the evaporation step of the extraction process. Secondly, human

347 health is affected through (i) the fact that some of the solvents, such as isopropyl alcohol are

348 greenhouse gases, and (ii) the potential formation of organic by-products, during the extraction

349 process, that contribute to the greenhouse effect. The accumulation of greenhouse gases can

350 interfere with the global climate causing serious impacts on human health through its change.

351 Furthermore, it was observed that chloroform/isopropyl alcohol poses detrimental effects primarily

352 on human health and secondarily to fossil fuels depletion sources and to ecosystem. Diethyl ether

353 was found to be the most environmentally friendly solvent reagent to perform the recovery of

354 tyrosol from OMW.

355

356 Figure 3.

357

358 Finally, Figure 4 clearly shows that diethyl ether is the most environmentally friendly solvent to

359 recover TPh from OMW, while ethyl acetate follows with its total environmental impacts being

360 twice as much. Chloroform/isopropyl alcohol poses even more environmental impacts that are

361 about four times higher than diethyl ether.

362 When a more holistic and precautionary principle thinking method like ReCiPe is used then the

363 results are slightly different from those obtained during the GWP estimation. This is evident in the

364 case of hydroxytyrosol recovery where it was found that diethyl ether is the most environmentally

365 friendly option regarding its GWP with 12.9 kg CO_2eq/g, followed by ethyl acetate with 13.3 kg

366 CO_2eq/g. However, considering the ReCiPe assessment (Figure 2), ethyl acetate shows a somewhat

367 (about 20%) more environmentally friendly footprint than diethyl ether.

368 This difference can be explained by considering the two applied impact assessment methods. To

369 begin with, IPCC 2007 estimates CO_2 emissions equivalent and it compares processes based on this

370 unit. On the other hand, the ReCiPe method transforms the life cycle inventory results into a

371 broader number of impact categories (Table 1) including the GWP mainly into its fossil depletion

372 impact category. Moreover, the key advantage of the ReCiPe method lies within the fact that it

373 takes into account the severity of each impact category to assess the sustainability of the process.

374 Hence, in the case of hydroxytyrosol recovery, the IPCC results declare that diethyl ether solvent

375 system is more environmentally friendly in terms of its GWP. This is consistent with the results

376 shown in Figure 2a, where fossil depletion impact is lower for the diethyl ether system. However,
377 the ReCiPe method indicates in Figure 2 that the impact of human toxicity is the most severe for the
378 overall extraction system and thus its total environmental footprint will be assessed according to
379 this impact category, mostly, where it is clearly shown that ethyl acetate is less toxic to humans than
380 the other solvent systems.

381 Accordingly, taking into account that ethyl acetate exhibits (i) the best efficiency to recover
382 hydroxytyrosol, tyrosol and TPh from OMW (Table 3), and (ii) a very good environmental footprint
383 during the recovery of the target antioxidant compounds, it is considered as the most viable and
384 sustainable option to recover target polyphenolic compounds from OMW.

385

386   Figure 4.

387

388 **3.3 Process modifications and alternative scenarios**

389 In order to reduce further the environmental impacts of the use of ethyl acetate, the following
390 diversifications, in terms of its operating parameters, can be applied to the liquid-liquid extraction.

391 The first suggestion is to minimize the partial diffusion of the organic solvent into the aqueous
392 phase during the liquid-liquid separation step; this is crucial because the presence of solvent in the
393 extracted effluent presumably increases its organic load and, consequently, the need for additional
394 treatment. This can be achieved adding salt into the OMW prior to the extraction step, thus
395 exploiting the salting-out effect (Grizis et al., 2003). Increasing the salt concentration of the
396 aqueous phase some of the water molecules are attracted by the salt ions, thus decreasing the
397 number of water molecules available to interact with the organic solvent. Therefore, the weak
398 intermolecular forces (e.g. hydrogen bonds) between organic molecules and water are easily
399 disrupted by the addition of salt. For this purpose, an experiment was performed to test the
400 efficiency of the extraction process in the presence of 8% (w/v) Na_2SO_4 in OMW and ethyl acetate
401 as the solvent (referred to as scenario 1). It was observed that solvent diffusion from the organic
402 into the aqueous phase decreased by 75%, while the respective environmental benefits are shown in
403 Figures 5 and 6 for hydroxytyrosol; Figure 5 shows that the total environmental impacts were
404 reduced by about 12.5%, while the GWP also decreased from 13.3 to 11 $kgCO_2eq/g$ hydroxytyrosol
405 (Figure 6). It should be noted here that following the recovery of precious antioxidants, the
406 remaining OMW will still contain several grams per liter of various organics and, therefore, must
407 undergo subsequent chemical and/or biological treatment prior to its final discharge. A point of
408 concern regarding scenario 1 is that increased salinity may have negative ramifications in the
409 treatability of the extracted effluent and this will depend on the concentration and type of added

410 salt, as well as the type of post-extraction treatment. Processes like phytoremediation or
411 electrochemical oxidation are amongst those that have been successfully employed for OMW
412 treatment (Di Bene et al., 2013; Goncalves et al., 2012; Bodini et al., 2011), while they can tolerate
413 increased salinity (e.g. using halophytes in phytoremediation (Manousaki and Kalogerakis, 2011))
414 or even require it (e.g. to enhance conductivity in electrochemical treatment (Chatzisymeon et al.,
415 2009c)).

416 Another option would be the use of more environmentally friendly raw materials, like bioethanol,
417 for the production of solvents and this would expectedly reduce the environmental impacts of the
418 extraction. Ethyl acetate production from bioethanol can have positive environmental impacts as
419 discussed in detail elsewhere (Nguyen et al., 2011; Ashley, 2006). Based on this assumption and
420 simulating the extraction procedure regarding its total environmental impacts, these were reduced
421 by 6% (scenario 2 in Figure 5). Moreover, the GWP decreased by 19% (scenario 2 in Figure 6).

422 Finally, the combination of the two aforementioned scenarios was also investigated and the results
423 are shown as scenario 3 in Figures 5 and 6; this scenario can lead to 17% and 29% reduction in
424 terms of its total environmental impacts and GWP, respectively. Hence, the results are very
425 promising since almost one fourth of the total environmental impacts can be reduced by applying
426 the proposed alternative scenarios.

427 Furthermore, it is worth noticing that although scenario 1 yields higher GWP than scenario 2, it is a
428 more environmentally friendly option regarding its damage categories and their impacts. This can
429 be explained by the fact that the use of bioethanol (i.e. an alternative and environmentally friendly
430 biofuel), for ethyl acetate production, reduces significantly the CO_2 emissions to atmosphere.
431 Conversely, scenario 1 achieves lower diffusion rates of the liquid organic phase into the aqueous
432 phase, thus reducing the amount of the hazardous organic solvent which is finally discharged into
433 aquatic bodies. Considering the fact that the severity of human toxicity is exceptionally higher than
434 the resources depletion impact, scenario 1 seems to present a more sustainable option, according to
435 the ReCiPe methodology.

436 Similar results (shown in Figures S1-S4 of supplementary material) were obtained for tyrosol and
437 TPh recovery based on scenarios 1-3.

438

439   Figure 5.

440

441   Figure 6.

442

443 **4. Conclusions and final remarks**

1

The aim of this work was to investigate the recovery yield of various polyphenolic compounds with known antioxidant properties such as hydroxytyrosol and tyrosol from real OMW, by means of liquid-liquid solvent extraction. Moreover, the environmental footprint of the whole liquid-liquid extraction system was estimated by means of the LCA methodology to provide the best available and sustainable extraction technique. The main conclusions drawn from this work are summarized below:

- The efficiency of the three extraction solvent systems decreases in the order: ethyl acetate > chloroform/isopropanol > diethyl ether, in terms of their antioxidant recovery yield.
- The use of ethyl acetate or diethyl ether, as extraction solvents, leaves similar environmental footprint regarding their environmental impacts on human health, ecosystem, fossil fuels resources and their contribution to global warming effect. However, ethyl acetate yields low environmental impacts and high antioxidant recovery performance and, therefore, it is assumed as the best option, both from an environmental and technical point of view.
- Chloroform/isopropyl alcohol mixture was found to pose detrimental effects onto the ecosystem, human health and fossil resources. Therefore, its use for antioxidants extraction from OMW is strictly not recommended.
- Alternative extraction scenarios were investigated to decrease further the overall environmental impacts, thus achieving a more sustainable recovery technique from agro-industrial effluents.

It should be emphasized that this work only dealt with a certain stage of effluent valorization, i.e. the extractive recovery of certain OMW constituents both in terms of experimentation and, mainly, assessment of its environmental footprint. Although there are indications that liquid-liquid extraction is a feasible methodology that can be applied by small-medium enterprises (like olive oil mills) for the recovery of the high added-value by-products of OMW, safe conclusions regarding the viability of such project cannot be reached unless:

(i)   the whole process is taken into account including the purification of crude extract by chromatographic fractionation, adsorption, distillation etc, evaluation of the antioxidant properties, and certainly the management of the post-valorization, residual effluent. The latter is of great importance since the recovery of polyphenols will only marginally reduce the organic loading of OMW;

(ii)   process scaling-up and optimization in terms of economic cost and technical feasibility (i.e. minimization of the use of costly solvents through lowering the solvent to substrate

478   volumetric ratio and/or their losses) are undertaken;

479   (iii)   a comparison between liquid-liquid extraction and other separation technologies takes
480   place using LCA and other decision-making tools and criteria;

481   (iv)   antioxidants recovery (irrespective of the chosen separation process) is regarded as part
482   of the "olive tree biorefinery" context described in detail by Federici et al. (2009).

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484   **Acknowledgements**

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LIST OF TABLES

Table 1.

Impact categories	Damage categories	Unit
Climate change human health		DALY (Disability Adjusted Life Years). Disadvantages / constraints caused by diseases
Ozone depletion		
Human toxicity	Human Health	
Photochemical oxidant formation		
Particulate matter formation		
Ionizing radiation		
Climate change ecosystems		
Terrestrial acidification		
Freshwater eutrophication		
Freshwater ecotoxicity		
Terrestrial ecotoxicity	Ecosystems	Depletion of species per year
Marine ecotoxicity		
Agricultural land occupation		
Urban land occupation		
Natural land transformation		
Metal depletion	Resources	US dollar ($)
Fossil depletion		

Table 2.

Solvent	Hydroxytyrosol, (%)	Tyrosol, (%)	Gallic acid, (%)	Caffeic acid, (%)	Oleuropein, (%)
Ethyl acetate	51.8	63.8	48.6	66.2	48.3
Diethyl ether	31.2	63.7	32.6	78.6	NR
Chloroform:isopropyl alcohol 7:3 (v/v)	40.2	73.6	15.1	74.1	72.7

Table 3.

Solvent	Hydroxytyrosol, (mg/L)	Tyrosol, (mg/L)	TPh, (mg/L)
Ethyl acetate	247.2	61.6	3440 (57%)
Diethyl ether	102.6	41.7	2800 (47%)
Chloroform:isopropyl alcohol 7:3 (v/v)	165.7	47.2	3380 (56%)

653   Table 4.

Equipment/ resource	Processes that are included in this study	Source
Energy from the Greek grid	- Lignite (54%) - Oil (11%) - Natural gas (17%) - Renewable sources (18%)	Greek Public Power Corporation S.A. (PPC) – Constructed from Ecoinvent v.2.2 system processes
Solvents	- ethyl acetate ($C_4H_8O_2$) - diethyl ether ($C_4H_{10}O$) - isopropyl alcohol (C_3H_8O) - chloroform ($CHCl_3$) - methanol	Ecoinvent v.2.2 system processes
Experimental equipment	- magnetic stirrer - vacuum evaporator	Manuals and own estimates - Constructed from Ecoinvent v.2.2 system processes

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658   Table 5.

Solvent	Hydroxytyrosol, (kg CO_2eq/g)	Tyrosol, (kg CO_2eq/g)	TPh, (10^2 kg CO_2eq/g)
Ethyl acetate	13.3	53.1	94.9
Diethyl ether	12.9	32.1	39.4
Chloroform/isopropyl alcohol	58.8	206.0	349.0

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Figure 1.

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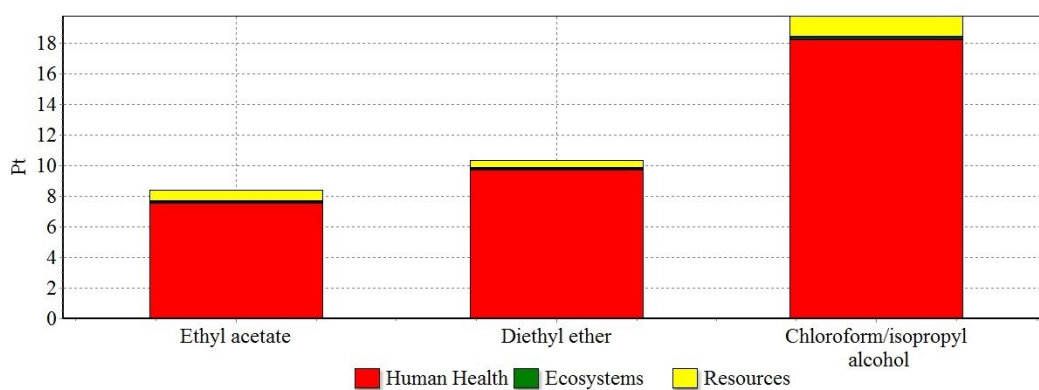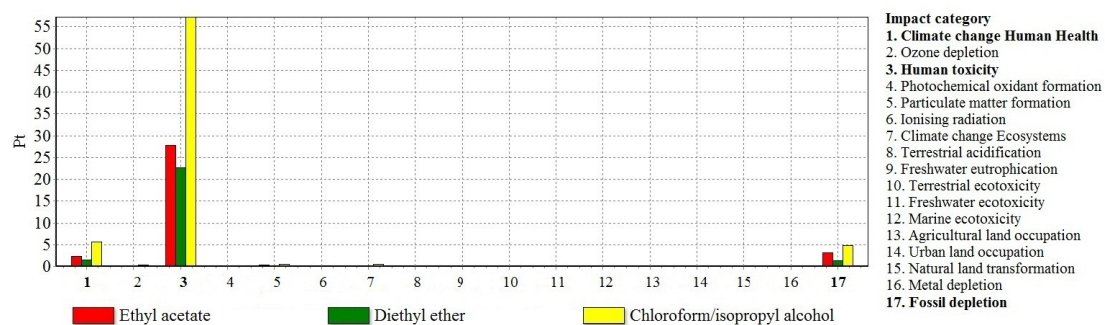689   Figure 2.

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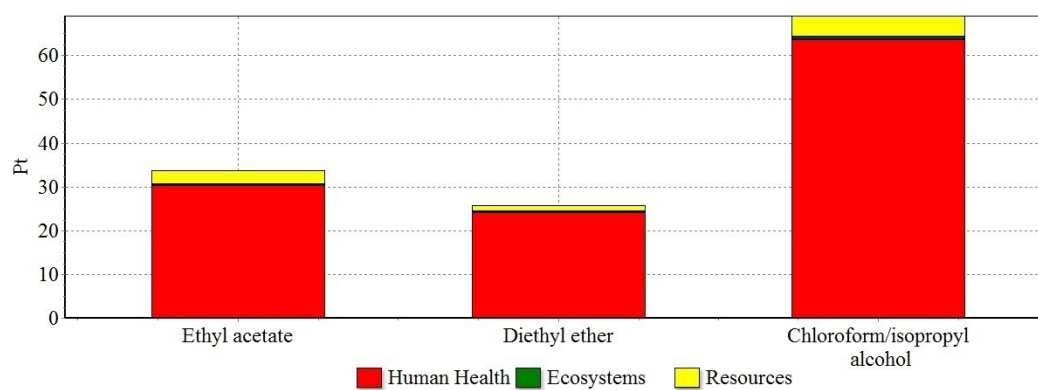692

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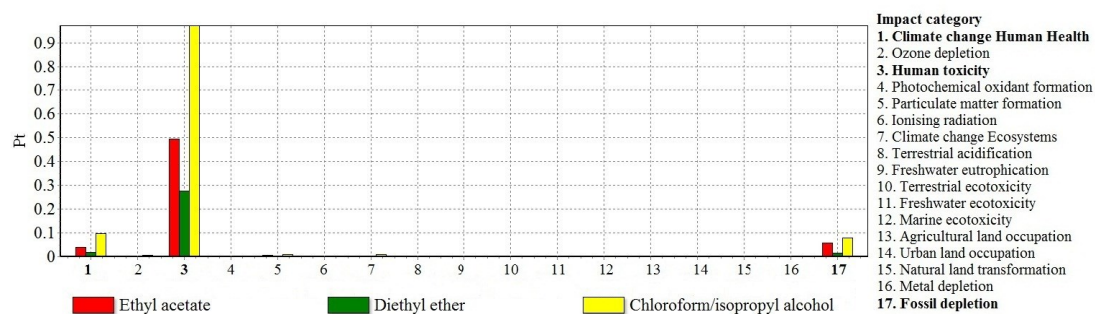

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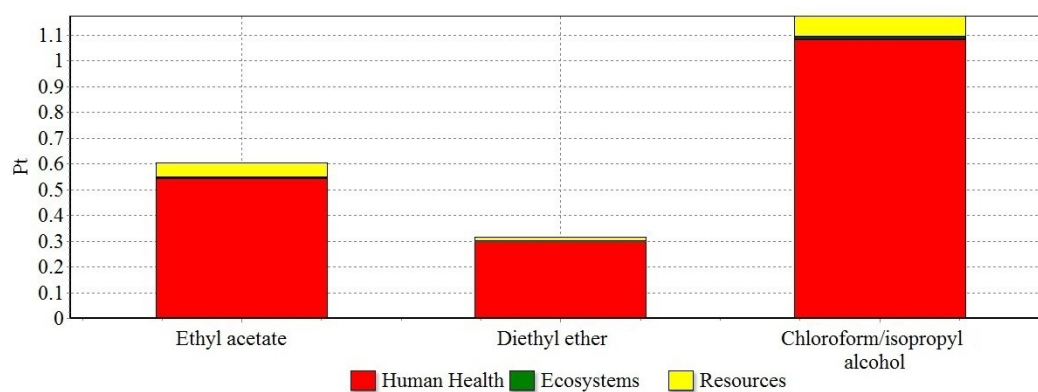
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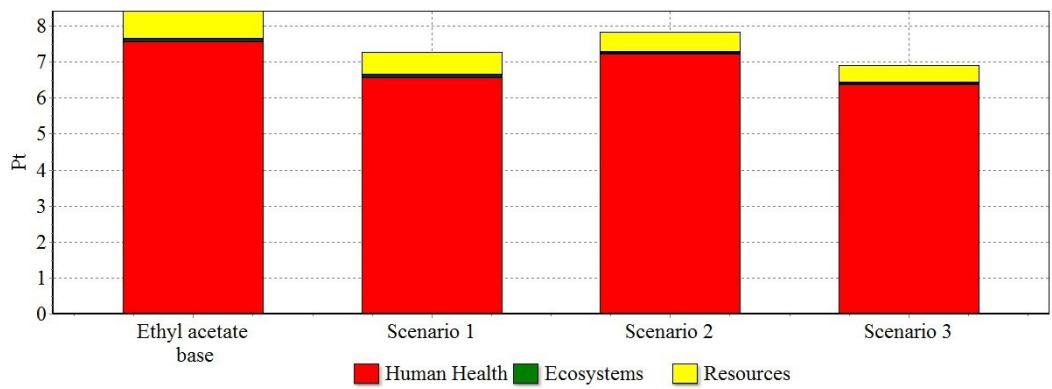
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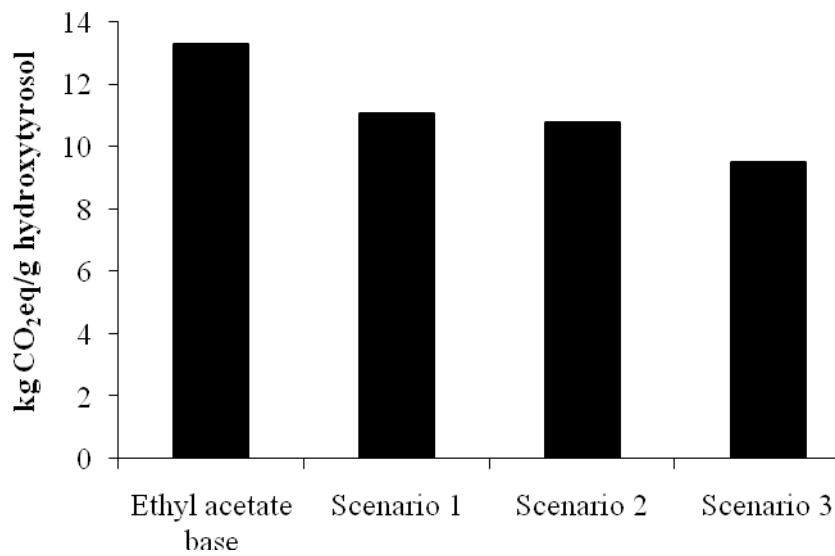707   Figure 4.

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Figure 5.

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Figure 6.

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