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Extraction of phenolic compounds from virgin olive oil by deep eutectic solvents (DESs)

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ABSTRACT

Deep eutectic solvents (DESs) are "green" solvents, applied in this study for the extraction of phenolic compounds from virgin olive oil (VOO). Different combinations of DES consisting of choline chloride (ChCl) in various mixing ratios with sugars, alcohols, organic acids, and urea, as well as a mixture of three sugars were used. The yields of the DES extractions were compared with those from conventional 80% (v/v) methanol/water. DES showed a good solubility of phenolic compounds with different polarities. The two most abundant secoiridoid derivatives in olive oil, oleacein and oleocanthal, extracted with ChCl/xylitol and ChCl/1,2-propanediol showed an increase of 20–33% and 67.9–68.3% with respect to conventional extraction, respectively. To our knowledge, this is the first time that phenolic compounds have been extracted from VOO oil using DES. Our results suggest that DES offers an efficient, safe, sustainable, and cost effective alternative to methanol for extraction of bioactive compounds from VOO.

1. Introduction

Deep eutectic solvents (DESs), environmentally friendly "green" solvents, have recently been applied extensively in several areas of chemistry, including the preparation of inorganic materials, organic synthesis, biochemistry, and analytical chemistry (Oliveira, Pereiro, & Luís, 2013; Zhang, De Oliveira-Vigier, Royer, & Jérôme, 2012). DES are a group of emerging solvents with excellent properties such as negligible volatility at room temperature, that are water miscible, non-flammable, and highly viscous. They have the potential to act as effective solvents for the complete dissolution and extraction of a wide range of non-polar and polar compounds and have been proposed as alternatives to several conventional and often toxic organic solvents. A DES is composed of a mixture of a hydrogen acceptor (usually choline chloride) and a hydrogen bond donor (generally natural plant-based organic ions, such as amino acids, carboxylic acids, sugars, etc.) in a solid state that associate by hydrogen bonding. The resulting mixture is eutectic, meaning it has a lower melting point than that of each individual component, and is liquid even at very low temperatures. H-bonding interactions between the DES component molecules are responsible for their high extractability and low melting points (Dai, van Spronsen, Witkamp, Verpoorte, & Choi, 2013). A certain combination of natural products in the solid state becomes liquid, so called natural deep eutectic solvents (NADES), are present in nature and play a role in all kinds of cellular processes of living organism (Choi et al., 2011).

DESs show some advantages as solvents, especially considering their easy and cheap preparation, biodegradability, and the precursors used are renewable, non-toxic, and natural compounds. Various DES have recently been used for drug dissolution (Dai, van Spronsen, et al., 2013; Morrison, Sun, & Neervannan, 2009), stabilisation of natural pigments (Dai, Verpoorte, & Choi, 2014) and the purification of crude oil (Gu et al., 2014; Pang et al., 2012) and biodiesel (Abbot, Cullis, Gibson, Harris, & Raven, 2007). There are an increasing number of studies on extraction and separation media for bioactive plant compounds, including flavonoids (Bi, Tian, & Row, 2013; Dai, Witkamp, Verpoorte, & Choi, 2013; Nam, Zhao, Lee, Jeong, & Lee, 2015), catechin (Zhang, Tang, & Row, 2014), phenolic acids (Park, Tang, & Row, 2014), terpenoids (Tang, Bi, Zhang, & Row, 2014), and saponin (Ribeiro, Coelho, & Marrucho, 2013). This study examined the efficiency of a wide range of solvents of different polarities for the extraction of phenolic compounds from olive oil and their comparison with a common extraction method of alcohol (methanol) extraction.

In recent decades, the phenolic compounds of olive fruit and especially of olive oil have received increased attention for their health promoting benefits (Ruiz-Canela, Estruch, Corella, Salas-Salvado, & Martinez-Gonzalez, 2014). Virgin olive oil (VOO) is

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one of the main components of the Mediterranean diet, consumers of which have a lower incidence of coronary heart disease and certain cancers (Cicerale, Lucas, & Keast, 2010). Four main classes of phenolic compounds with positive bioactive properties are represented in different proportions in olive oil: simple phenols (phenolic acids and phenolic alcohols, the latter from hydrolysed secoiridoids such as hydroxytyrosol (Hy) and tyrosol (Ty)), secoiridoid derivatives (the aglycone of oleuropein and ligstroside, elenolic acid linked to Hy and Ty (Hy-EA and Ty-EA) respectively, and their respective decarboxylated dialdehydic derivatives), lignans (pino- and acetoxypinoresinol), and flavones (luteolin and apigenin). The dialdehydic forms of decarboxymethyl oleuropein aglycone (Hy-EDA or oleacein) and ligstroside aglycone (Ty-EDA or Oleocanthal) are the two most abundant iridoid compounds in olive oil and have important biological properties. Oleocanthal has been demonstrated to exhibit anti-inflammatory activity with an ability to inhibit cyclooxygenase (COX-1 and COX-2) enzymes, similar to that seen with ibuprofen (Beauchamp et al., 2005). Also, the compound inhibits tumour necrosis factor (TNFα) and Interleukin (IL-1β), as well as nitric oxide (NO) production in macrophages, and thus has attracted attention as a potential therapeutic compound for the treatment of joint degenerative disease (Scotece et al., 2012). Furthermore, oleocanthal has been demonstrated to show a protective effect against Alzheimer's disease (Li et al., 2009) and possesses an antiproliferative effect in human breast and prostate cancer cell lines via inhibition of the proto-oncogene receptor tyrosine kinase c-Met (Elnagar, Sylvester, & El Sayed, 2011) or contributes to the prevention or treatment of colon cancer cells via the activation of AMP-activated protein kinase and COX-2 expression (Khanal et al., 2011). Oleacein exhibits antiproliferative and antioxidant properties (Vougogiannopoulou et al., 2014). The other phenolic compounds of olive oil also possess significant biological activities: Oleuropein aglycon (Hy-EA) was found to be an antiallergic active substance (Sato, Shinozaki, & Tamura, 2014), to directly regulate HER-2 in breast cancer cells (Menendez et al., 2007), and be protective against Alzheimer's disease (Abuznait, Qosa, Busnena, El Sayed, & Kaddoumi, 2013). Hydroxytyrosol has the capacity to inhibit proliferation and promote apoptosis in several tumour-cell lines (Granados-Principal, Quiles, Ramirez-Tortosa, Sanchez-Rovira, & Ramirez-Tortosa, 2010) and, in addition, has a broad range of physiological activities that are beneficial in terms of plasma lipoproteins, oxidative damage, platelet and cellular function, and bone health, due to its anti-inflammatory, antimicrobial, and antioxidant activities (Fernández-Bolaños, López, Fernández-Bolaños, & Rodríguez-Gutiérrez, 2008). Lignans are a group of phyto estrogens with a variety of estrogen-related functions, such as the prevention and treatment of cancer, arteriosclerosis, and osteoporosis (Fini et al., 2008).

Considering the biological importance of these classes of compounds, it would be very interesting to develop methods for their recovery from olive oil for bioassay tests or medical application. Traditionally, conventional organic solvents with liquid–liquid extraction or solid phase-extraction, were applied to extract phenolic compounds from olive oil. Nowadays, methanol and water are commonly and officially used for the extraction of polyphenols (International Olive Council Testing Method, 2009). However, from the viewpoint of green chemistry, methanol is a volatile, flammable, and toxic liquid. Furthermore, its production mainly relies on non-sustainable natural gas as a feedstock. DESs are gathering considerable interest as potential substitutes for conventional organic solvents. The natural and often biodegradable DES could be a potential extraction solvent for phenolic compounds with a broad range of polarities from olive oil although, prior to this study, this was yet to be tested. The aim of the present study was to compare the efficiency of the extraction of phenolic compounds from olive oil using a series of choline chloride-based DESs compared to extraction with the traditionally used organic solvent methanol. The separation and quantification of the principal phenolic compounds in the extracts isolated from VOO by DES, carried out by HPLC analysis coupled with DAD, allowed us to evaluate the extractability of these phenolic compounds.

2. Materials and methods

2.1. Samples, standards, and reagents

The virgin olive oil (VOO) used in this study was provided by Almazara Experimental at Instituto de la Grasa (Seville, Spain) in October 2014 and was preserved in PET bottles at 8 °C until its utilisation. The VOO was composed of 95% Manzanilla and 5% Lechin varieties.

The standard compounds vanillic acid, p-coumaric acid, luteolin, vainillin, apigenin, elenolic acid and Hy-Ac were obtained from Sigma Aldrich. Tyrosol was obtained from Fluka. Hydroxytyrosol was obtained by the method described by Fernández-Bolaños et al. (2013). Oleocanthal, oleacin, oleuropein aglycone, ligstroside aglycone, and 1-acetoxypinoresinol were obtained from VOO separated in an analytic C-18 column and eluted with MeOH:H_2O (García et al., 2001). Methanol and acetonitrile of HPLC grade were purchased from Panreac-Applichem. Deionised water was used acidified with TFA 6 N.

Glycerol, lactic acid, urea, sucrose, 1,4-butanediol, xylitol, 1,2-propanediol, malic acid, glucose, fructose, and choline chloride were purchased from Sigma Aldrich. Resin Amberlite XAD-16 was purchased from Vivaqua, Spain.

2.2. Preparation of the deep eutectic solvents (DESs)

DESs were fundamentally prepared by heating choline chloride with different hydrogen donors. These components were placed in a capped bottle and heated to 80 °C in a water bath with agitation until a homogeneous colourless liquid was formed. One DES was prepared by mixing three sugars (glucose, fructose, and sucrose) following the same procedure. Table 1 shows the different types of DES prepared and tested in this study, their abbreviated names, along with the molar ratios of their components.

2.3. Extraction of phenolic compounds from virgin olive oil by DES

14 g of the different DESs was added to 14 g of VOO sample in a glass tube and immersed in a water bath at 40 °C with agitation for 1 h. During the incubation period, the mixture was vortexed for 1 min every 15 min. The two phases were centrifuged at 1200*G* for 10 min and the upper oil phase was recovered. The procedure

Table 1
Composition of the deep eutectic solvents.

Abbreviation	Components			Mol ratio
	Component 1	Component 2	Component 3	
DES-1	Choline chloride	Glycerol		1:2
DES-2	Choline chloride	Lactic acid		1:2
DES-3	Choline chloride	Urea		1:2
DES-4	Choline chloride	Sucrose		1:1
DES-5	Choline chloride	Sucrose		4:1
DES-6	Choline chloride	1,4-Butanediol		1:5
DES-7	Choline chloride	Xylitol		2:1
DES-8	Choline chloride	1,2-Propanediol		1:1
DES-9	Choline chloride	Malonic acid		1:1
DES-10	Choline chloride	Urea	Glycerol	1:1:1
DES-11	D-(−)-Fructose	D-(+)-Glucose	Sucrose	1:1:1

was repeated once more. The combined polar extracts (lower phase) were washed with 60 mL of hexane to remove the residual oil and shaken vigorously until the two phases were observed by decantation. The polar extract was washed four times with hexane, and nitrogen was bubbled into the DES extract to eliminate the residual hexane.

2.4. Recovery of compounds from DES extracts

The obtained DES extracts were passed through a column of 3.0 cm inside diameter filled with pre-treated hydrated Amberlite XAD-16 to give a bed height of 15 cm. Following adsorption, the adsorbate-laden column was first washed with sufficient deionised water until all the DES was washed out (100 mL), then the phenolic compounds were eluted with 100 mL of MeOH. The methanolic sample was evaporated under vacuum at 35 °C and redissolved with 2 mL of MeOH for analysis by HPLC-DAD. The above experiments were performed in duplicate for each extract.

2.5. Extraction of phenolic compounds from virgin olive oil by methanol/water (80:20 v/v) (control sample)

The phenolic extract of virgin olive oil was obtained by following the procedure described by Montedoro, Servili, Baldioli, and Miniati (1992). Briefly, 14 g of virgin olive oil was extracted by using 14 mL of methanol/water (80:20 v/v). The mixture was stirred for 1 min in a vortex apparatus and centrifuged at 1200*G* for 10 min. The methanol layer was separated and the extraction repeated four times. The methanolic extracts were combined, and after removing the methanol, the residue was taken up to 15 mL with acetonitrile. Washes with hexane (3 × 20 mL) were performed, the resulting acetonitrile solution was evaporated under vacuum, and the residue was dissolved in 1 mL of MeOH. The extract was referred to as the control sample. Aliquots (20 μL) of the final solution were injected into the HPLC system.

2.6. Extraction of phenolic compounds from virgin olive oil by water (Sample W) and methanol/water mixture (Sample M) at 40 °C for 1 h

The phenolic compounds in VOO were also extracted by two different methods, one using water and the other using methanol/water (80:20 v/v) as solvents and following the same procedure as for DES extraction (Section 2.3), with extraction at 40 °C for 1 h on a mechanical shaker and the recovery of compounds using a macroporous resin (Section 2.4). The resulting extracts were named Sample W (for the water extracted sample) and Sample M (for the methanol/water extracted sample).

2.7. HPLC-DAD analysis

The phenolic compounds were quantified using a Hewlett–Packard 1100 liquid chromatography system with a C-18 column, Mediterranea SEA 18, (Teknokroma, Barcelona, Spain), 250 mm × 4.6 mm i.d. 5 μm. The system was equipped with a diode array detector (DAD; the wavelengths used for quantification were 280 and 340 nm) and Rheodyne injection valves (20 μL loop). Two different mobile phases were used to perform the separation. (1) *Methanol/water:* elution was achieved using a gradient of 90% water (pH adjusted to 2.5 with 6 N trichloroacetic acid,) and 10% methanol initially. The concentration of methanol was increased to 30% in 10 min and maintained for 10 min. Subsequently, the methanol percentage was raised to 40% in 10 min and maintained for another 5 min. Finally, the methanol percentage was increased to 60%, 70%, and 100% in 5-min periods. Initial conditions were reached in 15 min. A flow of 1 mL/min was used in all the experiments. The phenolic compounds were quantified by using the reference compounds obtained from commercial suppliers as previously described (see Section 2.1). (2) *Acetonitrile/water:* Separation of the phenolic compounds in urea-based DES extracts from olive oil was carried out at a flow rate of 1 mL/min using a mixture water (H_2O) acidified with 0.01% trichloroacetic acid (solvent A) and acetonitrile (CH_3CN) (solvent B) as mobile phase, utilising the following gradient over a total run time of 55 min: 95% A initially, 75% A at 30 min, 50% A at 45 min, 0% A at 47 min, 75% A at 50 min, 95% A at 52 min until the run was completed.

2.8. HPLC–MS analysis

The HPLC–ESI-MS system consisted of a Dionex Ultimate 3000RS U-HPLC (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation was performed using the same conditions as described above (Section 2.7) with water/methanol as the mobile phase. The injection volume was 20 μL and the flow rate was 1 mL/min. A stainless steel column (250 mm × 4.6 mm i.d.) packed with 5 μm C18 Mediterranea SEA 18 (Teknokroma, Barcelona, Spain) was used. A split post-column of 0.4 mL/min was introduced directly on the mass spectrometer electrospray ion source. Mass spectrometry was performed using a micrOTOF-QII High Resolution Time-of-Flight mass spectrometer (UHR-TOF) with qQ-TOF geometry (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionisation (ESI) interface. The instrument was operated in positive ion mode using a scan range from *m/z* 50 to 1200. Mass spectra were acquired in MS fullscan mode and data were used to perform multitarget-screening using TargetAnalysis™ 1.2 software (Bruker Daltonics, Bremen, Germany). MS–MS spectra were acquired in Auto-MS/MS mode (data-dependent acquisition) and were used for the structural confirmation of the compounds detected. Collision energy was estimated dynamically based on appropriate values for the mass and stepped across a ±10% magnitude range to ensure good quality fragmentation spectra. The instrument control was performed using Bruker Daltonics HyStar 3.2.

3. Results and discussion

3.1. Phenolic profiles of the DES extracts

Three alcohol-based DESs (DES-1, DES-6, and DES-8), four sugar-based DESs (DES-4, DES-5, DES-7 and DES-11), two organic acid-based DESs (DES-2 and DES-9), and two urea-based DESs (DES-3 and DES-10) were prepared, with differing mixing ratios of various alcohols, sugars, organic acids and urea to choline chloride (ChCl); DES-11 was a mixture of three sugars (fructose/sucrose/glucose) with no ChCl (Table 1), and the efficiency was tested for each of the 11 different solvents for the extraction of phenolic compounds from VOO. In certain cases, it was necessary to add water to decrease the viscosity, according to the proportions described by Dai, van Spronsen, et al. (2013).

In order to recover and concentrate the phenolic compounds from the DES extracts for analysis by HPLC-DAD, a macroporous adsorbent resin, XAD-16, was used. The separation of DES from the target compound using the resin was necessary as DES cannot be removed by conventional vacuum concentration due to its low vapour pressure. The resin adsorbs the phenolic compounds (Rubio-Senent, Rodríguez-Gutiérrez, Lama-Muñoz, & Fernández-Bolaños, 2012) while the polar moiety of DES can be eluted with water. Most phenolic compounds were recovered with methanol

after the elution of the polar compounds with water. The structure of the main phenolic compounds extracted are shown in Fig. 1S.

Fig. 1 presents the chromatogram of a characteristic DES extract obtained from VOO at 280 nm. Most of the peaks in DES chromatograms were similar to the control extract using the conventional extraction method (Montedoro et al., 1992) with 80% (v/v) methanol/water (Fig. 1). 13 representative compounds were detected and identified in almost all the DES extracts. The identification was carried out by the mass spectra (Fig. 1S) and comparison with reference compounds. Only in the case of urea-based DES (DES-3 and DES-10) extracts were the HPLC profiles (using methanol as mobile phase) significantly different to the control extract (Fig. 2a). However, after changing the mobile phase to acetonitrile, we confirmed that the chemical profile of DES-3 and DES-10 extracts were qualitatively similar to the control at 280 nm, although they remained a little different at 340 nm (Fig. 2b). The formation of adducts between methanol and urea during the chromatographic separation could be the reason for the differences in the profiles.

3.2. Extraction efficiency of DESs

To investigate the extraction efficiency of DESs, quantitative analysis of the ten most characteristic peaks, using the peak areas, was applied. The individual phenolic compounds were quantified using their corresponding external standard calibration curves. The extraction efficiency of DES was compared with the traditional solvent of 80% methanol:water (v/v) at room temperature, to avoid the degradation of certain compounds (control sample); with a simple extraction using water, a common general extraction solvent, but with the same conditions of temperature (40 °C) and time (1 h) as for the DES extraction (Sample W); and finally with 80% methanol:water (v/v) at the same conditions as DES (Sample M). In the case of the two latter solvents (Sample W and Sample M), the same protocol of recovery of the phenolic compounds from the XAD resin was used as the DES extracts, and eluted with methanol after first eluting the polar compounds with water.

The concentrations of simple phenols, secoiridoids, lignans, and flavonoids from extraction with the two organic acid-based DES

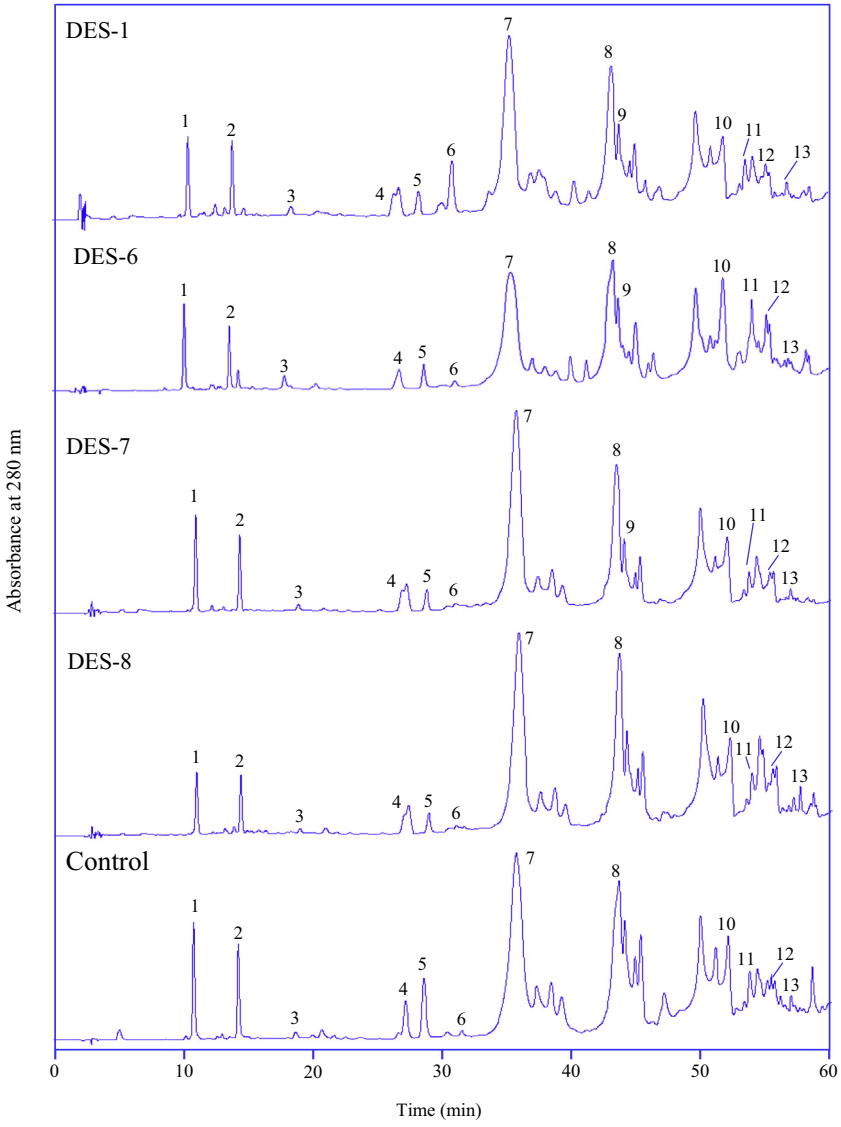

Fig. 1. HPLC chromatogram of phenolic compounds in virgin olive oil at 280 nm, extracted by means of different eutectic solvents DES 1, DES 6, DES 7, DES 8, and a control extract obtained with methanol:water (80:20). Peaks correspond to (1) hydroxytyrosol, (2) tyrosol, (3) vanillic acid, (4) 4-(acetoxyethyl)-1,2-dihydroxybenzene, (5) *p*-coumaric acid, (6) elenolic acid, (7) oleacin (dialdehydic form of elenolic acid linked to hydroxytyrosol), (8) oleocanthal (dialdehydic form of elenolic acid linked to tyrosol), (9) 1-acetoxypinoresinol, (10) oleuropein aglycon, (11) luteolin, (12) ligstroside aglycon, (13) apigenin.

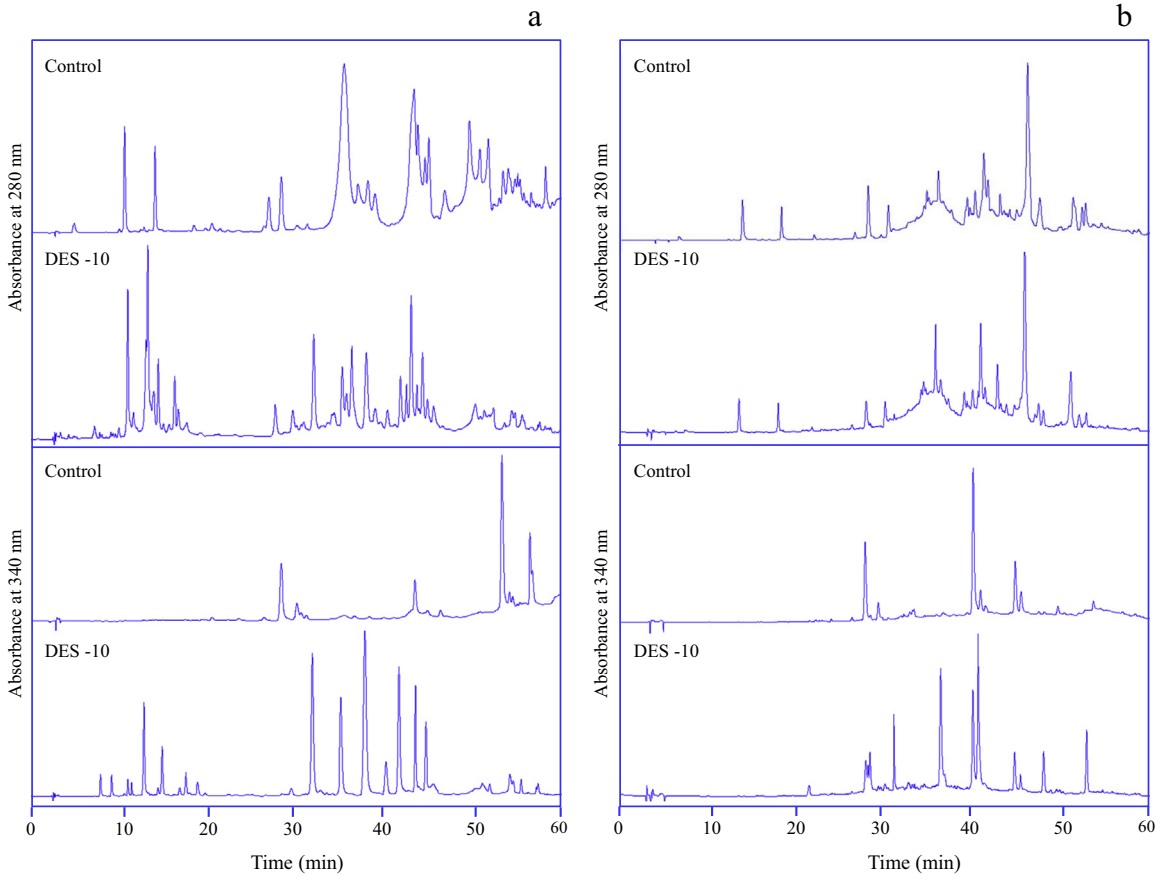

Fig. 2. HPLC chromatogram of phenolic compounds in virgin olive oil at 280 nm, and 340 nm extracted using eutectic solvent DES-10 or methanol:water (80:20) (control), (a) eluted with the mobile phase consisting of a methanol:water gradient, (b) eluted with the mobile phase consisting of a gradient acetonitrile:water.

(DES-2 and DES-9), the control sample, and Samples W and M are shown in Table 2. It should be noted that the simple phenols Hy and Ty constitute the main phenolic compounds identified in the organic acid-based DES extracts, while in the control sample present a typical comportment of VOO, in accordance with those reported in other VOO studies (García, Brenes, García, Romero, & Garrido, 2003), where the secoiridoid derivatives were the most abundant. Hy-EDA (oleacein) and Ty-EDA (oleocanthal), followed by oleuropein aglycon (Hy-EA) were the major compounds present in the olive oil studied. The observed increased levels of Hy and Ty could be related to the decrease in their derivatives, particularly Hy-EDA and Ty-EDA, respectively. This may reflect a chemical change during the extraction with these DESs due to the low pHs of the solvents (pH 2 for DES-2 and pH 0.5 for DES-9). However, curiously, other secoiridoid structures like oleuropein or ligstroside aglycones, and the flavonoids (luteolin and apigenin) showed a good recovery and were stable at these extreme pHs.

Also, three alcohol-based DES of ChCl mixed with glycerol, 1,4-butanediol, and 1,2-propanodiol (DES-1, DES-6, and DES-8, respectively) were used for extraction. The target compounds from the olive oil matrix are considered to be hydrogen bond donors and can interact competitively with the chloride anion. As shown in Table 2, the highest amounts of oleacein were extracted with DES-8, followed by DES-1 and the control sample, which had similar levels, then DES-6 and sample M, again with similar levels. In the case of oleocanthal the highest amounts were also extracted with DES-8, followed by DES-1 and Sample M, with approximately similar values, followed by the control and DES-6 samples. Extraction with 1,2-propanodiol (DES-8) yielded the highest amounts of both dialdehyde secoiridoids, being 19.6% and 68.3% higher than the control sample. DES-6 was found to be the best alcohol-based DES for the extraction of oleuropein aglycon (Hy-EA) which levels 48.4% higher than for the control sample while DES-8 was the best for ligstroside aglycon (Ty-EA) with a 156.6% respective increase. For extraction of the less polar compounds, the flavonoids (luteolin and apigenin) and lignan (acetoxipinoresinol), DES-8 was also the most efficient solvent, with results similar to the control sample and in agreement with those reported by Dai, van Spronsen, et al. (2013) for phenolic compounds extracted from safflower, *Carthamus tinctorius*.

In relation to the sugar-based DES (Table 2), choline chloride and sucrose was used to prepare two solvents (DES-4 and DES-5). In the case of DES-4, 25% of water was added to reduce the viscosity and improve the yield. An effectively higher extraction yield was achieved with DES-4 with respect to DES-5 for almost all of the phenolic compounds. In addition, the concentration of Hy-Ac and oleacein in the DES-4 extract was significantly higher than for the control, and W and M extracts, confirming that the solvent had an excellent efficiency. The oleocanthal concentration of DES-4 was similar to that of the control extract but slightly less than the extraction with methanol/water at 40 °C for 1 h (Sample M). Also, a xylitol-based DES (DES-7) with 25% of water added to reduce the viscosity, prepared according the proportion reported by Dai, van Spronsen, et al. (2013), showed a high capacity for extracting phenolic compounds from olive oil. The two secoiridoid derivatives most abundant in olive oil, oleacein and oleocanthal, were extracted in high proportions with increases of 33 and 68% with respect to the conventional solvent methanol/water (control sample), respectively. Also, the solvent exhibited a high efficiency for the lignan acetoxypinoresinol, whose concentration was

Table 2
Effect of acid-based DES (DES-2 and DES-9), alcohol-based DES (DES-1, DES-6 and DES-8) and sugar-based DESs (DES-4, DES-5, DES-7 and DES-11) on the amounts of phenolic compounds extracted (mg/kg) from olive oil and comparison with the traditional method using methanol:water 80% (v/v) at room temperature (control sample, SControl), and extraction with water (SW) or methanol:water 80% (v/v) (SM), in the same conditions of extraction as for DES.

Phenolic compounds	DES-2	DES-9	DES-1	DES-6	DES-8	DES-4	DES-5	DES-7	DES-11	SControl	SW	SM
Hydroxytyrosol (Hy)	31.86 ± 2.38	35.00 ± 3.05	3.54 ± 1.43	3.30 ± 0.92	3.53 ± 1.69	6.08 ± 4.38	4.81 ± 2.16	3.68 ± 0.64	3.15 ± 0.53	6.19 ± 0.06	2.70 ± 0.08	1.91 ± 0.18
Tyrosol (Ty)	23.09 ± 1.18	19.45 ± 2.00	3.65 ± 0.60	2.41 ± 0.27	3.11 ± 0.62	4.29 ± 0.97	3.05 ± 1.06	3.95 ± 0.24	3.28 ± 0.08	5.25 ± 0.06	2.36 ± 0.11	2.23 ± 0.20
Hy-Ac	nd	0.68 ± 0.05	1.62 ± 0.28	1.42 ± 0.04	1.39 ± 0.06	2.28 ± 0.14	0.98 ± 0.60	1.28 ± 0.29	1.64 ± 0.000	1.39 ± 0.13	0.43 ± 0.07	1.20 ± 0.07
Oleacein (Hy-EDA)	2.02 ± 0.5	5.10 ± 0.81	69.54 ± 5.10	54.56 ± 0.31	80.76 ± 2.22	77.36 ± 5.28	37.88 ± 3.14	89.82 ± 6.04	64.31 ± 8.38	67.54 ± 0.76	49.96 ± 2.33	58.19 ± 3.87
Oleocanthal (Ty-EDA)	7.25 ± 0.28	7.00 ± 1.01	53.65 ± 5.10	36.80 ± 1.35	69.32 ± 0.51	38.09 ± 1.22	2.12 ± 0.10	69.15 ± 4.74	14.59 ± 2.75	41.18 ± 1.00	7.28 ± 1.16	50.75 ± 0.17
1-Acetoxypinoresinol	nd	nd	4.00 ± 0.20	3.34 ± 0.14	4.50 ± 0.02	1.51 ± 0.04	1.84 ± 0.37	8.70 ± 0.60	0.42 ± 0.06	4.79 ± 0.71	0.46 ± 0.05	7.53 ± 0.10
Hy-EA	5.56 ± 0.02	11.71 ± 0.40	8.59 ± 1.62	14.93 ± 2.47	4.50 ± 0.02	7.63 ± 1.40	4.66 ± 1.93	9.10 ± 0.67	2.29 ± 0.38	10.06 ± 0.13	4.85 ± 0.87	7.88 ± 0.38
Luteolin	0.55 ± 0.003	0.60 ± 0.002	0.68 ± 0.02	0.68 ± 0.06	0.81 ± 0.04	0.54 ± 0.02	0.65 ± 0.18	0.74 ± 0.01	0.38 ± 0.01	0.98 ± 0.001	0.08 ± 0.004	0.68 ± 0.02
Ty-EA	2.31 ± 0.12	5.76 ± 0.30	2.38 ± 0.10	2.67 ± 0.30	5.22 ± 0.33	1.84 ± 0.19	2.46 ± 0.52	3.17 ± 0.13	0.39 ± 0.10	2.01 ± 0.22	1.64 ± 0.66	3.03 ± 0.10
Apigenin	0.091 ± 0.001	0.103 ± 0.002	0.12 ± 0.005	0.12 ± 0.003	0.15 ± 0.33	0.058 ± 0.005	0.09 ± 0.03	0.13 ± 0.01	0.03 ± 0.000	0.19 ± 0.003	0.003 ± 0.00	0.13 ± 0.007
Total	72.73 ± 4.5	85.40 ± 7.6	147.8 ± 14.4	120.2 ± 5.8	173.3 ± 5.8	139.7 ± 13.6	58.5 ± 10.1	189.7 ± 13.4	90.5 ± 12.3	139.6 ± 3.1	69.76 ± 5.23	133.5 ± 5.1

The data are expressed as mean ± SD based on duplicate values.

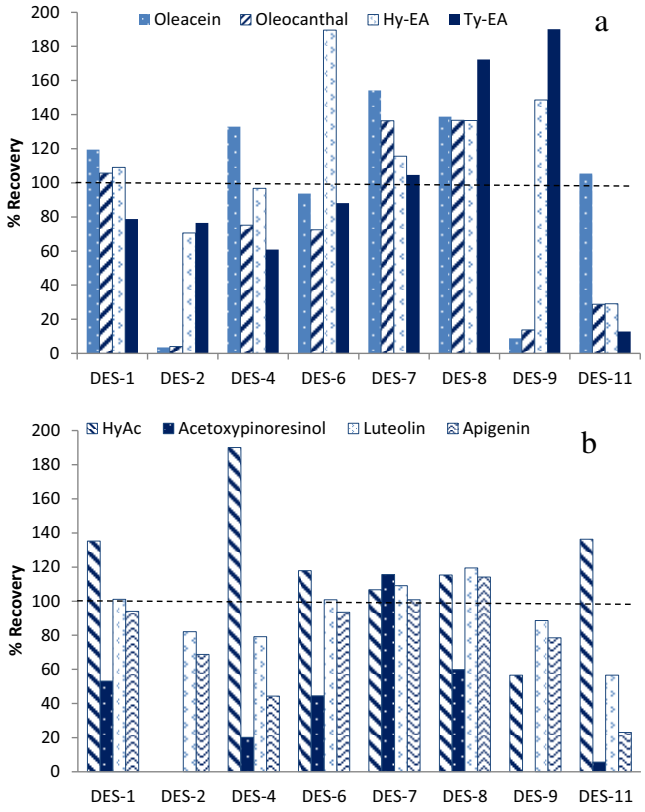

Fig. 3. Recovery yield (%) of (a) oleacein, oleocanthal, Hy-EA, and Ty-EA and (b) Hy-Ac, acetoxypinoresinol, luteolin, and apigenin from olive oil by different types of DES in relation to phenolic compounds obtained from a mixture methanol:water 80% (v/v) in the same conditions (Sample M).

81.8% higher with respect to the control, although there was little difference (15.6%) between the results when extracted with methanol/water in the same conditions as for the DES extraction (Sample M). Finally, a DES of fructose, glucose, and sucrose (DES-11) was prepared according to the proportions reported by Dai, van Spronsen, et al., 2013; Dai, Witkamp, et al., 2013. Although the solvent only showed a high efficiency for the extraction of Hy-Ac and oleacein, with levels slightly higher or similar to the control, respectively, and the yields were lower for the rest of the phenolic compounds, this DES remains interesting due to its composition of abundant non-toxic and environmentally friendly primary metabolites.

Taking into consideration all of the solvents (Tables 2), the order of efficiency for the extraction of phenolic compounds (the sum of total phenolic compounds) from olive oil was DES-7 > DES-8 > DES-1 > control sample ≈DES-4 > Sample M > DES-6 > DES-11 > DES-9 > DES-2 > H_2O > DES-5. Also, it is interesting to note that the concentrations of Ty-EDA, Ty-EA, and acetoxypinoresinol were higher when extracted with methanol in the same conditions as for DES (Sample M) than for the methanol extraction carried out at room temperature (control sample), yet this trend was the inverse for Hy-EDA and Hy-EA. Fig. 3a shows the extraction efficiency in relation to methanol in the same conditions as for DES for oleocanthal, oleacein, Hy-EA, and Ty-EA for all the different DES mixtures. Using DES-7 and DES-8, the amounts of the two major and pharmacologically relevant secoiridoids in olive oil, oleocanthal and oleacein, were higher than extractions with the other solvents. These results indicate that the polarity of DES-7 and DES-8 or/and their hydrogen bonding interactions with the target compounds appears to be most favourable for oleacein and oleocanthal. It is interesting to note that all the sugar-based

Table 3
Relative extraction yield of phenolic compounds as peak area from olive oil with the deep eutectic solvents (DES-1, DES-7, DES-8, and DES-10) and methanol:water 80% (v/v) (sample control, SControl).

	Hydroxytyrosol	Tyrosol	Oleacein	Oleocanthal	Hy-EA	Ty-EA
SControl	703[a] ± 15	611.5 ± 10	460.5 ± 6	940.8 ± 12	4249.5 ± 40	970.8 ± 18
DES-1	517.3 ± 10	543.5 ± 8	769.2 ± 10	824.4 ± 10	3237.9 ± 65	777.7 ± 10
DES-7	837.7 ± 20	674.5 ± 6	1181.9 ± 12	931.9 ± 12	3817.1 ± 19	1004.9 ± 10
DES-8	853.5 ± 15	683.6 ± 4	1151.8 ± 15	944.2 ± 13	3864.3 ± 22	975 ± 9
DES-10	458.5 ± 12	420.7 ± 10	1024.8 ± 20	1047.6 ± 21	3593 ± 20	1321.2 ± 11

[a] The data correspond to the area of each peak in the HPLC-DAD chromatography at 280 nm and mobile phase acetonitrile. The data are expressed as mean ± SD based on duplicate values.

DESs—DES-4, DES-7, and DES-11—showed a high capacity for extracting oleacein (Fig. 3a) and Hy-Ac (Fig. 3b), whereas DES-4 and DES-11, for example, showed little extraction capacity for the other phenolic compounds. DES-6 was the most efficient solvent for the extraction of Hy-EA (89.5% higher than methanol at 40 °C), and DES-8 and DES-9 were the most efficient for Ty-EA extraction (72% and 90% higher than methanol at 40 °C, respectively) (Fig. 3a). In the case of acetoxypinoresinol, DES-7 (Fig. 3b) showed the highest extraction yield (15.5% higher than methanol at 40 °C), and for the flavonoids, luteolin and apigenin (Fig. 3b), DES-7 and DES-8 were also the most efficient DES extraction solvents.

To investigate the urea-based DES-10, the mobile phase of HPLC was changed from methanol to acetonitrile (see Section 3.1), peaks were identified by HPLC–MS, and analysis of the compounds extracted was applied using the peak areas of different HPLC-DAD chromatograms at 280 nm (Fig. 2b). The DES-10 extract was compared to DES-1, DES-7, DES-8 and control extracts (Table 3). Hy and Ty area values of DES-10 with urea were the lowest, indicating that the bio-secoiridoid molecules are less degraded, and oleacein and oleocanthal with DES-10 to levels similar to those for DES-7 and DES-8.

4. Conclusions

We have demonstrated the successful capacity of deep eutectic solvents (DES) for the extraction of phenolic compounds from olive oil. Under the extraction conditions used in this paper, almost all the types of DES studied had an enhanced performance for the extraction of the phenolic compounds in olive oil compared with the classic method (methanol/water). DES proved to be an excellent extraction solvent for a wide range of compounds with a wide range of polarities, from those highly soluble in water (Hy and Ty), to those poorly soluble in water but methanol soluble (flavonoids). The two secoiridoid derivatives most abundant in olive oil and with major biological properties, oleacein and oleocanthal, were extracted with ChCl/xylitol and ChCl/1,2-propanediol in higher proportions than with methanol/water, with an increased yield of 20–33% and 67.9–68.3% respectively. Further studies are needed to determinate the optimal extraction conditions, including temperature, time, oil/solvent ratio, water/DES ratio, and recovery of compounds from the extract adsorption to an XAD resin. DESs, with their compositional flexibility and low cost and environmental impacts, have the potential to be utilised for possible industrial applications involving, as demonstrated here, the isolation and extraction of food constituents and bioactive compounds. Our results propose DESs as both a greener and more efficient alternative to methanol for the large-scale extraction of bioactive compounds from virgin olive oil.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.10.131.

References

Abbot, A. P., Cullis, P. M., Gibson, M. J., Harris, R. C., & Raven, E. (2007). Extraction of glycerol from biodiesel into a eutectic based liquid ionic. *Green Chemistry, 9*, 868–872.

Abuznait, A. H., Qosa, H., Busnena, B. A., El Sayed, K. A., & Kaddoumi, A. (2013). Olive-oil-derived oleocanthal enhances β-amyloid clearance as a potential neuroprotective mechanism against Alzheimer's disease: *In vitro* and *in vivo* studies. *ACS Chemical Neuroscience, 4*, 973–982.

Beauchamp, G. K., Keast, R. S. J., Morel, D., Lin, J., Pica, J., Han, Q., et al. (2005). Ibuprofen like activity in extra-virgin olive oil. *Nature, 437*, 45–46.

Bi, W., Tian, M., & Row, K. H. (2013). Evaluation of alcohol-based deep eutectic solvent in extraction and determination of flavonoids with response surface methodology optimization. *Journal of Chromatography A, 1285*, 22–30.

Choi, Y. H., van Spronsen, J., Dai, Y., Verbene, M., Hollmann, F., Arends, I. W. C. E., ... Verpoorte, R. (2011). Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology? *Plant Physiology, 156*, 1701–1705.

Cicerale, S., Lucas, L., & Keast, R. S. (2010). Biological activities of phenolic compounds present in virgin olive oil. *International Journal of Molecular Science, 11*, 458–479.

Dai, Y., van Spronsen, J., Witkamp, G.-J., Verpoorte, R., & Choi, Y. H. (2013a). Natural deep eutectic solvents as new potential media for green technology. *Analytica Chimica Acta, 766*, 61–68.

Dai, Y., Verpoorte, R., & Choi, Y. H. (2014). Natural deep eutectic solvents providing enhanced stability of natural colorants from safflower (*Carthamus tinctorius*). *Food Chemistry, 159*, 116–121.

Dai, Y., Witkamp, G.-J., Verpoorte, R., & Choi, Y. H. (2013b). Natural deep eutectic solvents as a new extraction media for phenolic metabolites in *Carthamus tinctorius* L.. *Analytical Chemistry, 85*, 6272–6278.

Elnagar, A. Y., Sylvester, P. W., & El Sayed, K. A. (2011). (−)-Oleocanthal as a c-Met inhibitor for the control of metastatic breast and prostate cancers. *Planta Medica, 77*, 1013–1019.

Fernández-Bolaños, J., Rodríguez-Gutiérrez, G., Lama-Muñoz, A., Rubio-Senent, F., Fernández-Bolaños, J.G., & Maya, I., et al. (2013). Method for obtaining hydroxytyrosol extract, mixture of hydroxytyrosol and 3,4-dihydroxyphenylglycol extract, and hydroxytyrosyl acetate extract, from by-products of the olive tree, and the purification thereof. PCT/ES2012/070491.

Fernández-Bolaños, J. G., López, O., Fernández-Bolaños, J., & Rodríguez-Gutiérrez, G. (2008). Hydroxytyrosol and derivatives: Isolation, synthesis, and biological properties. *Current Organic Chemistry, 12*, 442–463.

Fini, L., Hotchkiss, E., Fogliano, V., Graziani, G., Romano, M., De Vol, E. B., et al. (2008). Chemopreventive properties of pinoresinol-rich olive oil involve a selective activation of the ATM-p53 cascade in colon cancer lines. *Carcinogenesis, 29*, 139–146.

García, A., Brenes, M., García, P., Romero, C., & Garrido, A. (2003). Phenolic content of commercial olive oils. *European Food Research Technology, 216*, 520–525.

García, A., Brenes, M., Martínez, F., Alba, J., García, P., & Garrido, A. (2001). High-performance liquid chromatography evaluation of phenols in virgin olive oils during extraction at laboratory and industrial scale. *Journal of American Oil Chemists' Society, 78*, 625–629.

Granados-Principal, S., Quiles, J. L., Ramirez-Tortosa, C. L., Sanchez-Rovira, P., & Ramirez-Tortosa, M. C. (2010). Hydroxytyrosol: From laboratory investigations to future clinical trials. *Nutrition Review, 68*, 191–206.

Gu, T., Zhang, M., Tang, T., Chen, J., Li, Z., Zhang, Q., & Qiu, H. (2014). Deep eutectic solvents as novel extraction media for phenolic compound from model oil. *Chemical Communications, 50*, 11749–11752.

International Olive Council Testing Method (2009). Determination of biophenols in olive oils by HPLC. COI/T.20/DOC.29, Madrid, November. <http://www.internationaloliveoil.org/>.

Khanal, P., Oh, W. K., Yun, H. J., Namgoong, G. M., Ahn, S. G., Kwon, S. M., et al. (2011). *P*-HPEA-EDA, a phenolic compound of virgin olive oil, activates AMP-activated protein kinase to inhibit carcinogenesis. *Carcinogenesis, 32*, 545–553.

Li, W., Sperry, J. B., Crowe, A., Trojanowki, J. Q., Smith, A. B., & Lee, V. M. Y. (2009). Inhibition of tau fibrillization by oleocanthal via reaction with amino groups of tau. *Journal of Neurochemistry, 110*, 1339–1351.

Menendez, J. A., Vazquez-Martin, A., Colomer, R., Brunet, J., Carrasco-Pancorbo, A., Garcia-Villalba, R., et al. (2007). Olive oil's bitter principle reverses acquired autoresistance to trastuzumab (Herceptin™) in HER2-overexpressing breast cancer cells. *BMC Cancer, 7*, 80–96.

Montedoro, G. F., Servili, M., Baldioli, M., & Miniati, E. (1992). Simple and hydrolysable phenolic compounds in virgin olive oil. 1. Their extraction separation, and quantitative and semiquantitative evaluation by HPLC. *Journal of Agricultural and Food Chemistry, 40*, 1571–1576.

Morrison, H. G., Sun, C. C., & Neervannan, S. (2009). Characterization of thermal behavior of deep eutectic solvents and their potential as drug solubilization vehicles. *International Journal Pharmaceutics, 378*, 139.

Nam, M. W., Zhao, J., Lee, M. S., Jeong, J. H., & Lee, J. (2015). Enhanced extraction of bioactive natural products using tailor-made deep eutectic solvents: Application to flavonoid extraction from *Flos sophorae*. *Green Chemistry, 17*, 1718–1727.

Oliveira, F. S., Pereiro, A. B., & Luís, P. N. R. (2013). Deep eutectic solvents as extraction media for azeotropic mixtures. *Green Chemistry, 15*, 1326–1330.

Pang, K., Hou, Y., Wu, W., Gui, W., Peng, W., & Marsh, K. N. (2012). Efficient separation of phenols from oils via forming deep eutectic solvents. *Green Chemistry, 14*, 746–754.

Park, H. E., Tang, B., & Row, K. H. (2014). Application of deep eutectic solvents as additives in ultrasonic extraction of two phenolic acids from *Herba Artemisiae Scopariae*. *Analytical Letter, 47*, 1476–1484.

Ribeiro, B. D., Coelho, M. A. Z., & Marrucho, I. M. (2013). Extraction of saponins from sisal (*Agave sisalana*) and jua (*Ziziphus joazeiro*) with cholinium-based ionic liquid and deep eutectic solvents. *European Food Research and Technology, 237*, 965–975.

Rubio-Senent, F., Rodríguez-Gutiérrez, G., Lama-Muñoz, A., & Fernández-Bolaños, J. (2012). New phenolic compounds hydrothermally extracted from the olive oil by-product alperujo and their antioxidative activities. *Journal of Agricultural and Food Chemistry, 60*, 1175–1186.

Ruiz-Canela, M., Estruch, R., Corella, D., Salas-Salvado, J., & Martinez-Gonzalez, M. A. (2014). Association of Mediterranean diet with peripheral artery disease: The predimed randomized trial. *Journal of the American Medical Association*, 311–415.

Sato, A., Shinozaki, N., & Tamura, H. (2014). Secoiridoid type of antiallergic substances in olive waste materials of three Japanese varieties of *Olea europaea*. *Journal of Agricultural and Food Chemistry, 62*, 7787–7795.

Scotece, M., Gomez, R., Conde, J., Lopez, V., Gomez-Reino, J. J., Lago, F., et al. (2012). Further evidence for the anti-inflammatory activity of oleocanthal: Inhibition of MIP-1α and IL-6 in J774 macrophages and ATDC5 chondrocytes. *Life Sciences, 91*, 1229–1235.

Tang, B., Bi, W., Zhang, H., & Row, K. H. (2014). Deep eutectic solvent-based HS-SME coupled with GC for the analysis of bioactive terpenoids in *Chamaecyparis obtusa* leaves. *Chromatographia, 77*, 373–377.

Vougogiannopoulou, K., Lemus, C., Halabalaki, M., Pergola, C., Werz, O., Smith, A. B., et al. (2014). One-step semisynthesis of oleacein and the determination as a 5-lipoxygenase inhibitor. *Journal of Natural Products, 77*, 441–445.

Zhang, Q., De Oliveira-Vigier, K., Royer, S., & Jérôme, F. (2012). Deep eutectic solvents: Syntheses, properties and applications. *Chemical Society Reviews, 41*, 7108–7146.

Zhang, H., Tang, B., & Row, K. (2014). Extraction of catechin compounds from green tea with a new green solvent. *Chemical Research Chinese Universities, 30*, 37–41.