Application of natural deep eutectic solvents for extraction of polyphenolics from olive oil by-products using microwaves

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Abstract

Natural deep eutectic solvents (NADESs) considered as "green" solvents offer an efficient, safe, sustainable, and cost effective method alternative to conventional extraction while microwave assisted extraction (MAE) is a convenient, rapid, environmental friendly and efficient technique. The combination of NADESs with MAE was applied in this study for the recovery of phenolic compounds from olive kernel and olive leaves. Various NADESs were used consisting of choline chloride (ChCl) with maltose, glycerol, citric and lactic acid. The choline chloride and lactic acid NADES system achieved good extractability results of phenolic compounds in short extraction time from the two substrates expressed in mg GA /g d.w. of substrate; 29.57 ± 0.13 for olive kernel and 36.71 ± 0.07 for olive leaves respectively.

Keywords: olive kernel, olive leaves, microwave-assisted extraction, natural deep eutectic solvents

1. Introduction

Among the diverse ways of green technology, the developing green solvents are one of the most important subjects, thus a new type of solvent named deep eutectic solvents (DESs) has emerged. A DES is composed of a mixture of two components: a hydrogen acceptor (usually choline chloride) and a hydrogen bond donor such as amino acids, carboxylic acids, sugars etc. In the case that certain natural components are combined, the DES mixture is called natural deep eutectic solvent (NADES). The resulting mixture after heating is eutectic with a lower melting point than either of the individual components, mainly due to the generation of intermolecular hydrogen bonds. DESs possesses a useful properties such as adjustable viscosity, non-flammability, non-toxicity, low volatility and solubility in water (García et al 2016; Dei et al 2013; Qi et al 2015; Weaver et al 2010). Therefore, they have the potential to act as effective solvents for the extraction of a wide range of non-polar and polar compounds and have been proposed as alternatives to several conventional and toxic organic solvents. There are an increasing number of studies on the extraction of bioactive plant compounds,

including flavonoids, catechin and phenolic acids by applying NADES as extraction solvents (Bi et al 2013; Garcia et al 2016; Qi et al 2015; Yao et al 2015; Wei et al 2015).

Microwave-assisted extraction (MAE) is a rapid and efficient extraction method that offers efficient extraction yields in shorter time, simplified manipulation, reduced solvent consumption and lower energy input (Li et al 2010). Compared to the traditional extraction methods, MAE is known as a more environmental-friendly process with economic advantages. Considering that NADESs can efficiently absorb microwave energy, it is a rather interesting approach to apply these green solvents in MAE in extraction processes.

Olive oil production is an important agricultural activity and one of the primary driving engines of the economy of Greece. A significant amount of solid residue, named as olive kernel, and olive leaves are the by-products of olive oil production process. These residues are rich in polyphenols; since only 2% of the phenolic compounds are transferred to the oil and as much as 98% retained in the residue (Chanioti et al. 2016; Lafka et al 2013). The direct disposal of olive kernel and leaves into environmental systems without any pretreatment constitute a major environmental problem due to their high organic and polyphenol content, the latter being toxic to water and soil ecosystems. However, polyphenols are one of the most important groups of natural antioxidants that confer an effective defence system against free radical attack. Recent studies demonstrated the presence of numerous bioactive components in the leaves and olive kernel, such as oleuropein, hydroxytyrosol, verbascoside, lutein and rutin, which have shown antiviral, antimicrobial, antioxidant, anti-inflammatory and anti-carcinogenic activities (Fernández-Bolaños et al 2006). Therefore, ways to valorize these by-products are seeking and investigating.

In this study, NADESs are proposed and applied to the microwave-assisted extraction of polyphenols from olive kernel and leaves. Four different NADES systems consisting of choline chloride combined with maltose, glycerol, citric and lactic acid and two different temperatures (40, 60°C) were studied. Conventional solvents such as ethanol: water 70% v/v and water were also applied to MAE using the same polyphenol sources. The antioxidant potential of extracts obtained from olive kernel and leaves in terms of their total phenolic content and their antioxidant radical scavenging was evaluated.

2. Materials and methods

2.1 Raw Materials

Olive kernel and olive leaves were used as raw materials for their phenolic compounds recovery. Olive kernel obtained from a local olive oil mill using a continuous three-phase centrifugation system consists of pulp fragments (21–33% w/w), pit (42–54% w/w), olive fruit skin (10–11% w/w) with initial moisture 45.0% w/w and olive leaves (initial moisture 49% w/w) were collected from the region of Thiva (Voiotia, Greece). Both raw materials were air dried at 35°C for 24 h (final moisture 5% w/w) and ground in 1 mm with a cutting mill (FRITSCH, cutting mill, pulverisette 15). The pretreated raw materials were kept at 4°C.

2.2 Chemicals and reagents

Folin–Ciocalteu's reagent, gallic acid, 1,1-diphenyl-2 picrylhydrazyl (DPPH), methanol (HPLC grade), ethanol, sodium carbonate, sodium acetate and maltose (>97.0%), were purchased from Sigma Aldrich Chemical Co. (St Louis, MO). Choline chloride (>98.0%) and lactic acid (>98.0%) were obtained from Acros Organics (Geel, Belgium), citric acid (>98.0%) was purchased from Univar (LaiwuTaihe Biochemistry Co. Ltd, China) and glycerol (>99.0%) was purchased from Lach-Ner (Neratovice, Czech Republic).

2.3 NADESs preparation

The NADESs mixtures were prepared by heating the two individual components at 80 °C under stirring until a homogeneous liquid formed. The mixture was allowed to cool at ambient temperature and then 20%v/v water was added. In the experiments the following types of NADESs: choline chloride–glycerol, choline chloride–maltose, choline chloride–citric acid and choline chloride–lactic acid were prepared.

2.4 Physicochemical properties of NADES

The following properties of the prepared NADESs were examined. The density was measured at 25 °C by a densitometer. The refractive index was determined at 40 °C using the refractometer (BOE 32400 ABBE, Boeco, Germany). The viscosity was measured at 40 °C by the Brookfield rheometer (Brookfield Engineering Laboratories Inc., Stoughton, MA) (strains S61 or S62 at 40 rpm). Air/NADESs surface tension was measured at 40 °C using a Wilhelmy plate tensiometer (Sigma702, Biolin Scientific, Sweden).

2.5 Microwave-Assisted Extraction (MAE) with different solvents

Olive kernel or leaves (2 g) was mixed with 25 mL of NADES in an extraction vessel and the mixture was subjected to microwave (MAE) at 200W using experimental equipment (Nanjing Xianou Instruments Manufacture Co., Ltd, China) at certain temperatures (40°C or 60°C) for 30 min duration (Table 1). After extraction, the mixture was centrifuged at 10000 rpm for 10 min and the extract was collected. Each experiment was repeated three times. MAE experiments were also carried out at 60°C for 30min with conventional solvents ethanol: water 70% v/v and water.

2.6 Total Phenolic Content (TPC)

Total phenolic content was determined by the Folin-Ciocalteu method, as described by Waterhouse (2002), using gallic acid as standard. In 0.1 mL of extract, 7.9 mL distillate water and 0.5 mL of Folin-Ciocalteu reagent were added and the mixtures were vortexed. Then, 1.5 mL of supersaturated Na_2CO_3 wer added, the mixture was revortexed and incubated for 2 h in darkness. The absorbance of

the solution was then measured using a spectrophotometer (Hitachi, U-2900 UV/Vis, 200 V) at 765 nm. The results were expressed as mg gallic acid equivalents (GAE) per g of dry weight of raw materials (mg GAE/g raw material (d.w.)).

2.7 Antioxidant Activity

Antioxidant activity was determined as described by Brand-Williams (1995), using the DPPH assay. 0.1 mL of the extract was added to 3.9 mL of DPPH radical solution (0.0025 g/100 mL methanol) and after 20 min remaining in darkness the absorbance of the mixture was measured at 515 nm. Results were expressed as IC50 (g d.w. /g DPPH). IC50 is the concentration of extract that decline 50% the initial concentration of DPPH radical. BHT (butylated hydroxytoluene) and a-tocopherol (vitamin E) were used as reference compounds for the comparative evaluation of antioxidant activity of the phenolic extracts.

2.8 Statistical Analysis

The experimental data were assessed by analysis of variance (ANOVA) using STATISTICA 7 (Statsoft Inc., Tulsa, USA), while significant differences of mean values were estimated at the probability level $P<0.05$.

3. Results and Discussion

3.1 Preparation and Physicochemical properties of NADES

NADES can be obtained as referred by combining ChCl with sugars, sugar alcohols, amino acids or organic acids. Four NADESs with components 1 and 2 at mole ratio 1:2 (Table 1) were used in the extraction experiments in order to evaluate the extractractability of phenolic compounds from olive kernel and olive leaves sources.

Table 1. Natural deep eutectic solvents (NADESs) used in extraction experiments

Components		Mole ratio	Code
Component 1	**Component 2**		
Choline chloride	Citric Acid	1:2	DES-CA
	Lactic Acid		DES-LA
	Maltose		DES-MA
	Glycerol		DES-GLY

As the physical or physicochemical properties of solvent themselves may generally affect the extractability of the target compounds from a solid matrix, thus, basic properties such as density, viscosity, refractive index and surface tension of used solvents were measured (Table 2). The density

of all tested NADES was higher than those of water and of ethanol: water 70% v/v. Viscosity is one of the largest obstacles for the application of NADES due to the low mass transport efficiency (Wei et al. 2015), while it is affected by water percentage and temperature. So, 20% (v/v) water in NADES solutions were used in these experiments. The viscosity of all types of NADESs examined was higher than that of water and of ethanol: water 70% v/v, while decreasing according to the following order: DES-MA > DES-CA > DES-GLY > DES-LA. Surface tension is also an important property, since the solid matrix can be percolated easily by a solvent of low surface tension which also decreases by increasing the temperature (Bi et al. 2013). The surface tension of the NADESs also differed from those of water and ethanol: water 70% v/v following the same order as viscosity values. These physicochemical properties were examined in order to correlate the resulting extraction yield with the type of NADESs.

Table 2. Physical/Physicochemical properties of natural deep eutectic solvents (NADESs)

Code	Water (%)	Density (40°C) (Kg/m^3)	Viscosity (40°C/50rpm) (cP)	Refractive index (25°C)	Surface tension (40°C) (mN/m)
DES-CA	20	1.311 ± 0.015	448.1 ± 0.95	1.468 ± 0.001	60.35 ± 0.27
DES-LA	20	1.134 ± 0.010	29.5 ± 0.87	1.442 ± 0.000	42.42 ± 0.12
DES-MA	20	1.431 ± 0.026	975.5 ± 1.02	1.487 ± 0.000	74.49 ± 0.90
DES-GLY	20	1.164 ± 0.017	47.5 ± 0.59	1.464 ± 0.001	56.12 ± 0.14
Water	100	0.992 ± 0.012	0.653 ± 0.010	1.333 ± 0.001	69.52 ± 0.12
Ethanol:water	30	0.886 ± 0.010	1.31 ± 0.05	1.365 ± 0.000	23.76 ± 0.08

3.2 Effect of NADES type and temperature - evaluation of the extracts

The effect of type of NADESs and extraction temperature by applying the microwave assisted extraction (MAE) of olive kernel and leaves on the phenolic compounds recovery is shown in Fig. 1. In both phenolic substrates, the type of NADES significantly influenced the total phenolic content (TPC) of the extracts ($P<0.05$). From the NADES examined, the DES-LA possessed the highest TPC for both olive kernel and leaves extracts (29.57 ± 0.13 and 36.71 ± 0.07 mg GA /g d.w., respectively). The TPC of extracts obtained by the various types of NADESs decreased in the order: DES-LA > DES-GLY > DES-CA > DES-MA for both olive oil by-products. It must be noted that viscosity and surface tension of NADESs may affect the extractability of phenolic compounds; for example the DES-MA showed high viscosity and surface tension value resulted in low TPC extract.

Moreover, the TPC of the extracts significantly increased ($P<0.05$) with the increase of temperature. Higher temperature (60 °C) may promote an increase in solubility of phenolic compounds and an increase of their diffusion rate into the solvent, thus increasing the mass transfer rate (Chanioti et al. 2016); additionally the dissolution of phenolics may be accelerated by the lower viscosity and higher diffusivity of NADESs at increased temperature. It must be notified that in all cases by increasing the extraction temperature from 40 to 60 °C, the TPC of olive kernel and leaves extracts increased respectively (Fig.1).

Figure 2 shows the antioxidant activity of olive kernel and leaves extracts obtained by MAE using different NADESs and temperature values. It can be observed that the antioxidant activity (IC50) of both extracts affected significantly ($P<0.05$) by the type of extraction solvent; the maximum antioxidant activity was obtained for olive kernel with DES-LA at 60 °C (17.51 ± 0.85 g d.w. /g DPPH) and for olive leaves with DES-GLY at 60 °C (4.76 ± 0.08 g d.w. /g DPPH), respectively. According to our previous work (Chanioti et al. 2016), the scavenging effect (IC50) obtained for the BHT and a-tocopherol standards was 0.41 and 0.29 g /g DPPH, respectively. Therefore, the best olive leaves extract proved 11 and 16 times less antioxidant activity than that of BHT and a-tocopherol, while the best olive kernel extract proved 42 and 60 times less antioxidant activity than that of BHT and a-tocopherol, respectively. Furthermore, by increasing the extraction temperature from 40 to 60 °C, the antioxidant activity values of olive kernel extracts increased significantly for all the NADESs used ($P<0.05$) (Fig 2A).

The antioxidant activity of extracts obtained by the different types of NADESs decreased in the following order: DES-LA > DES-CA > DES-GLY > DES-MA for olive kernel and DES-GLY > DES-MA > DES-LA > DES-CA for olive leaves extracts, respectively.

Comparison of the NADESs extracts with those obtained by conventional solvents

NADESs proved to be effective in phenolic compounds extraction in the case of olive kernel. Moreover, the olive kernel extracts achieved higher TPC by NADESs and better antioxidant activity than those of water and ethanol: water 70% v/v. However, in the case of olive leaves the ethanol: water 70% v/v was significantly efficient solvent proving higher TPC values and antioxidant activity. As far as the water is concerned, it resulted in almost equal TPC and antioxidant activity values to those of NADESs.

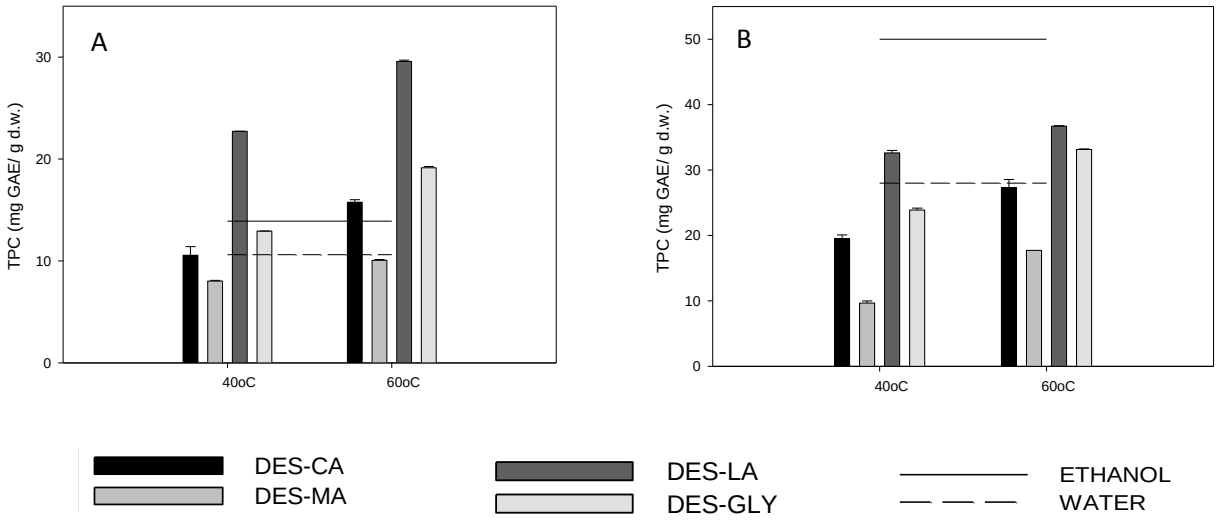

Figure 1. Effect of extraction temperature and NADESs' type on total phenolic content (TPC) of olive kernel (A) and leaves (B) extracts.

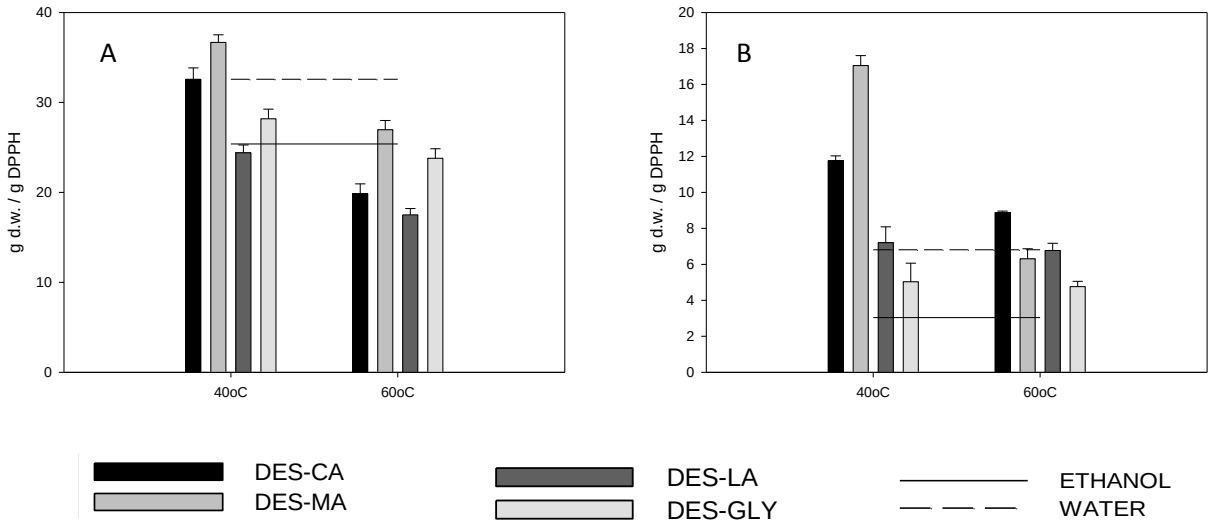

Figure 2. Effect of extraction temperature and NADESs' type on antioxidant activity of olive kernel (A) and leaves (B) extracts.

4. Conclusions

In this study the combination of promising green solvents NADESs with microwave-assisted extraction (MAE) proved to be efficient for the recovery of phenolic compounds from olive kernel and leaves. Choline chloride/lactic acid (DES-LA) possessed the highest total phenol content (TPC)

by applying MAE at 60°C for both olive kernel and leaves extracts. The TPC of extracts obtained by the different types of NADES decreased significantly in the following order: DES-LA > DES-GLY > DES-CA > DES-MA for both olive oil by-products. The excellent properties of NADESs, such as sustainability, biodegradability and high extractability of phenolics highlight their potential as green solvents for their extraction from olive oil by-products. The developed method based on the combination of MAE and NADES could be an alternative for the extraction of active components of plant materials providing higher extraction efficiency compared with conventional methods with/that commonly use solvents achieving significantly reduced extraction times. Due to its good extractability, the combination of the proposed green and effective microwave extraction method and the green NADESs may be promising for the extraction of natural bioactive compounds.

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