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ORIGINAL PAPER

Green and Non-conventional Extraction of Bioactive Compounds from Olive Leaves: Screening of Novel Natural Deep Eutectic Solvents and Investigation of Process Parameters

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Abstract

Olive leaf as an agricultural waste contains valuable bioactive compounds that are mainly used for pharmaceutical and cosmetic industries. Lately the major component, oleuropein, has gained extra attention due to the anti-viral activity against SARS-CoV-2 that causes Coronavirus disease (Covid-19). In this study, extraction of the bioactive compounds from olive leaves was conducted using a non-conventional and green method. New generation green solvents, natural deep eutectic solvents (NADES) were used in combination with ultrasound assisted extraction. Screening of NADES type, temperature, and particle size were investigated using one-pot-at-a-time method while, NADES amount and liquid-to-solid ratio were optimized using experimental design. The results were evaluated in terms of total polyphenol yield (Y_{TP}), total flavonoid yield (Y_{TF}) and antiradical activity (A_{AR}). At the optimized conditions, the highest total polyphenol yield and the highest total flavonoid yield were achieved with choline chloride–fructose–water (CFW) (5:2:5) as 187.31 ± 10.3 mg gallic acid equivalent g^{-1} dw and 12.75 ± 0.6 mg apigenin equivalent g^{-1} dw, respectively. The extracts were also analyzed for oleuropein, caffeic acid and luteolin contents. The highest amount of oleuropein and caffeic acid were extracted by glucose–fructose–water (GFW) (1:1:11) as 1630.80 mg kg^{-1} dw and 112.77 mg kg^{-1} dw, respectively.

Graphic Abstract

Keywords Olive leaf · Natural deep eutectic solvents · Ultrasound assisted extraction · Green extraction, experimental design, response surface methodology

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Statement of Novelty

This study presents the first time use of novel natural deep eutectic solvents for the extraction from olive leaves using ultrasound assisted extraction and contributes for improving and broadening the use of natural deep eutectic solvents for the extraction of bioactive compounds from different sources within 'green extraction' domain.

Introduction

Olive leaf (*Olea europaea*) extracts have been regarded as valuable items since ancient times. It is known that pharaohs were mummified using olive leaf extracts by Egyptians [1]. In the next years, olive leaf extracts were used for health purposes such as healing fevers, and afterwards healing tropical diseases such as malaria [1]. In 1854, the treatment of these diseases could be executed formally by the olive leaf extracts [2]. Later on, research activities on olive leaves revealed promising significant effects such as antioxidant capacity [3–5], antifungal activity [6], antibacterial activity [7], anti-HIV property [8], vasodilator effect [9], and hypoglycemic effect [10] both in vivo and in vitro. Regarding these properties, olive leaf components have been under research for their potential anti-viral effect against SARS-CoV-2 that causes Coronavirus disease (Covid-19). Lately, remarkable results were presented on the blockage of the SARS-CoV-2 spike protein-ACE-2 interface by oleuropein dimer and dihydro oleuropein [11]. Additionally, demethyloleuropein was reported to block SARS-CoV-2 main protease [11]. On the other hand, oleuropein, quercetin, luteolin-7-glucoside, apigenin-7-glucoside, catechin and epicatechin-gallate were reported to be investigated as the potential inhibitor of Covid-19 main protease [12].

The valuable content of olive leaves is comprised of mainly phenolics and several flavonoids. Principally five groups of phenolic compounds present in the olive leaves, as oleuropeosides (oleuropein and verbascoside), flavones (luteolin-7-glucoside, apigenin-7-glucoside, diosmetin-7-glucoside, luteolin, and diosmetin); flavonols (rutin); flavan-3-ols (catechin), and substituted phenols (tyrosol, hydroxytyrosol, vanillin, vanillic acid, and caffeic acid). One of the major compounds in olive leaves is oleuropein, which is followed by hydroxytyrosol [13].

There are approximately 890 million olive trees in the world and 172 million of them are in Turkey, covering around 1.8 million ha of area [14]. Olive cultivation is a significant issue for Turkey both in economic and cultural aspects. During the harvesting of olives together with pruning stages, considerable amount of by-product mainly consisting of olive leaves are accumulated, that is almost 25 kg per tree [13]. This biomass is generally used to feed animals or burned out to remove. Considering the curing, healing and nutritional properties of the leaves, valorization of these by-products have a great importance.

Researchers working on this subject reported successful conventional extraction methodologies from olive leaves such as the use of dimethyl sulfoxide [4], hexane [15], ethanol [16] and methanol [17] as solvents. However, the requirement of long extraction times was the bottleneck of the conventional methods. It was later reported that the extraction rate could be enhanced by the change of the type of the solvent or by increasing the agitation rate or using high temperatures. However, considering the reduction of both the phenolic content and antioxidant capacity at high temperatures, researchers studied on alternative procedures named as non-conventional extraction processes. Non-conventional procedures aim to enhance extraction yield, decrease the cost and enhance the selectivity of the extraction. These procedures include the use of ultrasound, microwave, supercritical fluid extraction, pressurized liquid extraction, pulsed electric fields and high voltage electrical discharges [18–23]. Among these non-conventional methods, ultrasound assisted extraction is considered to be one of the most interesting techniques because it is simple, efficient and also cheap [24]. Ultrasound is reported to enhance mass transfer mainly by inducing cavitation. Gas bubbles lead to high localized pressures and micro-streaming that disrupt the plant tissue; therefore, enhancing the intracellular substances into the solvent [25]. Beyond these, ultrasound creates interfacial instabilities and efficient compressions and expansions influencing external and internal mass transfers. Additionally, this principal non-conventional method is regarded as a green extraction method due to the reduction in energy consumption and ensuring safety as well as sustainability [23, 26, 27]. Ultrasound-assisted extraction is known to improve the efficiency of the green solvents, enhance both the extraction yield and rate, additionally known to be safe for the heat sensitive components [27]. Successful green extractions using ultrasound from olive leaves were previously reported in the literature [28–35].

In terms of the nature of the extraction solvent, the use of non-toxic, natural and renewable substances has gained great importance in the last two decades due to ecological aspects. To promote sustainable extraction processes and the utilization of green chemistry, non-petroleum derived solvents have been encouraged in many fields of research [36]. From this point of view, deep eutectic solvents (DESs) are good candidates as extraction solvents. They are new generation green solvents, non-toxic, recyclable, non-flammable and they have low vapor pressure [37–39]. They are mostly composed of natural substances and

numerous types of DESs can be formed easily in the laboratory. DESs that are prepared using natural substances are called natural deep eutectic solvents, NADESs [40]. NADESs are reported to probably occur in living cells and involve in many processes in the cell such as biosynthesis, solubilization and also storage of different poorly water-soluble metabolites and unstable compounds in cells [40]. Therefore, they are very attractive solvents to be used in a broad research areas such as drug delivery systems, bone-therapy scaffolds, and other food, pharmaceutical and cosmetics related applications such as extractions [41].

In the literature, many studies have been performed using NADESs such as; extraction from olive oil [42], grape and olive pomaces [43, 44], *Firus carica* L. [45], Greek medicinal plants [46], almond, sesame, cinnamon and olive oil [47] and agro-food waste [48]. There is also an increasing interest of the use of DESs on the extraction from olive leaves especially in the last 2 years [49–52].

However, the combination of the use of NADES and ultrasound assisted extraction was only reported by Dedousi et al. [53] and Mouratoglou et al. [48], who investigated sodium potassium tartarate–glycerol–water (7:1:2), and choline chloride–glycerol (1:3) together with sodium acetate–glycerol (1:3), respectively.

In this study, the aim is to (i) screen novel NADESs for the ultrasound assisted extraction of bioactive compounds from olive leaves for the first time, (ii) optimize the extraction process, (iii) present an advantageous green procedure that encourage the scale-up for industrial applications.

Materials and Methods

Chemicals and Reagents

Olive leaves were harvested from Burhaniye, Balıkesir/Turkey in 2018. Olive leaves were washed with distilled water and dried overnight at 45 °C. Dried leaves were grounded using a domestic blender (Profilo Mambo, 500 W) and separated to three different sizes using molecular sieves as < 106 µm, 106–425 µm and 425–1400 µm (Endecotts, Octagon 200, England). Dried and grounded olive leaves were stored at − 20 °C until further use. Choline chloride (C1879), lactic acid (27714), glycerol (G5516), apigenin (10,798), gallic acid (G7384), 1,1-diphenyl-2-picrylhydrazyl (D9132) (DPPH), Folin–Ciocalteu reagent (9252) were purchased from Sigma. Ethylene glycol (1.009.49), D-fructose (104.007), malonic acid (800,387) were obtained from Merck whereas D-glucose (A3666) and D-sucrose (A2211) were from Applichem. All other chemicals were of reagent grade.

Preparation of NADESs

NADESs were prepared by mixing required amount of the components in a screw-capped bottle and heating till a clear liquid was formed. Choline chloride was dried under vacuum over silica gel in a desiccator prior to use. The NADESs prepared and used in this study are as follows: glucose–fructose–sucrose–water (1:1:1:11) (GFSW), glucose–fructose–water (1:1:11) (GFW), glucose–sucrose–water (1:1:11) (GSW), fructose–sucrose–water (1:1:11) (FSW) [54], choline chloride (ChCl)–glucose–water (5:2:5) (CGW), ChCl–fructose–water (5:2:5) (CFW), ChCl–sucrose–water (4:1:4) (CSW) [55], ChCl–lactic acid (1:2) (CLa) [44], ChCl–malonic acid (1:1) (CMa) [55], ChCl–ethylene glycol (1:2) (CEG) [38] and ChCl–glycerol (1:2) (CGly) [56].

Ultrasound Assisted Extraction

Certain amount of grounded leaves (< 106 µm, 106–425 µm and 425–1400 µm) were placed in a screw cap-tube with a certain amount of (8.61–90%) NADES. After mixing thoroughly, the tubes were placed in a temperature controlled sonication bath (Elma S30H, Singer, Germany) at a certain temperature (55–75 °C) for 60 min, at a sonication power of 140 W, a frequency of 37 kHz, and an acoustic energy density (AED) of 35 W L^{-1}. The extract was filtered and the clear supernatant was used for the analyses. 50% (v/v) aqueous methanol was also used for comparison (30 mL g^{-1}, 75 °C).

The effects of different NADES type, temperature (55–75 °C) and particle size were investigated using one-pot-at-a-time method on the total Y_{TP}, Y_{TF} and A_{AR}. On the other hand, optimization was performed to investigate the amount of selected NADES (%) and liquid-to-solid ratio ($R_{L/S}$) on the total Y_{TP} and Y_{TF}.

Design of Experiments and Response Surface Methodology

To consider the influence of the two of the critical parameters, an experimental design was performed. The design included the amount of NADES (%) and liquid-to-solid ratio ($R_{L/S}$) as independent variables and Y_{TP} and $Y_{TF,}$ as responses. A circumscribed central composite design (CCD) was used to determine and optimize the parameters to accomplish maximum extraction efficiency of phenolic substances and flavonoids. The range of the factors were obtained from preliminary experiments.

Design-Expert® 9.0 (Stat-Ease, Inc., USA) was used for the experimental design and statistical analysis. Circumscribed central composite design included axial points beyond the factorial points. Fourteen runs were conducted which included the replication of five runs at the central

point. The replications were used to estimate the experimental uncertainty variance. The runs were performed randomly to prevent systematic bias. In order to derive an equation expressing the relation between the independent variables and response, stepwise regression analysis was performed for the data collected from experimental runs. To confirm the results, the experiments were run again at optimum level of independent variables. Adequacy of the model was evaluated through analysis of variance (ANOVA). 'Backward elimination' was performed to remove the insignificant terms ($p > 0.05$) which leaded the improvement of the significance of the model. The visualization of the model was performed by using Response Surface plots. For statistical calculations, the relation between the coded values and actual values are described by the following equation:

$$x_i = \frac{X_i - X_{cp}}{\Delta X_i}, \quad i = 1, 2, 3, \ldots, k. \tag{1}$$

Here x_i, describes the dimensionless value of an independent variable; X_i, real value of an independent variable; X_{cp}, real value of an independent variable at the center point; and ΔX_i, step change of real value of the variable i corresponding to a variation of a unit for the dimensionless value of the variable i.

Most of the relationship of the independent variables and the responses were calculated by the second order polynomial. The quadratic model is expressed as;

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} {x_i}^2 + \sum \beta_{ij} x_i x_j, \tag{2}$$

where Y is the predicted response, x_i and x_j represent the variables or parameters, β_0 is the offset term, β_i is the linear effect, β_{ij} is the first order interaction effect and β_{ii} is the squared effect. The coded and actual factors for the experimental design are listed in Table 1.

Determination of Total Polyphenol Yield

Total polyphenol yield was determined as reported by Blidi et al. [57] Olive leaf extract (0.02 mL) was mixed with water (0.78 mL) and Folin–Ciocalteu reagent (0.05 mL) was added to the mixture and left for 1 min at room temperature. Then, sodium carbonate [20% (v/v)] was added and the mixture was incubated at room temperature in the dark for 1 h. The absorbance was read at 750 nm and the total polyphenol concentration (C_{TP}) was calculated from the calibration curve prepared using gallic acid. Total polyphenol yield was expressed in mg gallic acid equivalents (GAE) g^{-1} of dry weight (dw) from Eq. (3)

$$Y_{TP}\left(\text{mg GAE g}^{-1}\text{ dw}\right) = \frac{C_{TP} \times V}{m}. \tag{3}$$

Here, V is the volume of the extraction medium (L) and m is the dry weight of the material (g).

Determination of Total Flavonoid Yield

Total flavonoid yield was determined as reported by Lee et al. [58]. Diethylene glycol (10 mL), olive leaf extract (1 mL) and 1 N NaOH solution (1 mL) were mixed in a test tube and incubated at room temperature for 30 min. The absorbance was measured at 420 nm. The total flavonoid content was calculated as mg apigenin equivalents (ApE) per g of dry weight and was calculated using Eq. (4).

$$Y_{TF}\left(\text{mg ApE g}^{-1}\text{ dw}\right) = \frac{C_{TF} \times V}{m}. \tag{4}$$

Determination of the Antiradical Activity

Antiradical activity assay was performed using the method reported by Shehata et al. [59]. An aliquot of extract (0.025 mL) sample was mixed with 100 µM DPPH solution in methanol (0.975 mL). The absorbance at 515 nm was read immediately after mixing ($A_{515(i)}$) and after exactly 30 min ($A_{515(f)}$). A_{AR} was calculated using Eq. (5).

$$A_{AR}\left(\text{µmol DPPH g}^{-1}\text{ dw}\right) = \frac{C_{DPPH}}{C_{TP}} \times \left(1 - \frac{A_{515(f)}}{A_{515(i)}}\right) \times Y_{TP}. \tag{5}$$

Here C_{DPPH} is the initial concentration of DPPH, in mol L^{-1}; C_{TP} is the total polyphenol concentration of the extract, in mg GAE L^{-1}.

HPLC–ESI–QTOF-MS Analysis

Analyses were performed using an Agilent 1200 Liquid Chromatography system (Agilent Technologies, Palo Alto, CA, USA) equipped with a standard autosampler. The HPLC column was Inertsil Diol C18 (3 µm, 4.6 × 100 mm), with a

Table 1 Independent variables and their coded and actual values used for optimization

Independent variable	Unit	Symbol	Code levels				
			− α	−1	0	1	+ α
NADES	%	*A*	8.61	20	47.5	75	86.39
$R_{L/S}$	mL g^{-1}	*B*	9.64	20	45.0	70	80.36

flow rate of 0.4 mL min^{-1} at 25 °C. The mobile phase consisted of 20 mM ammonium format in water (A) (60%) and acetonitrile (B) (40%), and analyses was performed using isocratic mode. The injection volume was 5 µL. The HPLC system was coupled to a Q-TOF mass spectrometer equipped with a Jet Stream ionization source (Agilent 6530, Agilent Technologies, Palo Alto, CA, USA) operating in negative ion mode. JSI-QTOF-MS parameters were; drying gas temperature, 300 °C; drying gas flow, 8 L min^{-1} and nebulizing gas pressure, 40 psi. Detection was carried out within a mass range of 60–1000 m/z. The MS/MS analyses were acquired by automatic fragmentation where the three most intense mass peaks where fragmented. Nitrogen was used as drying, nebulizing and collision gas.

Statistics

All extractions were carried out in duplicate. All determinations were carried out at least in triplicate and values were averaged. Statistics was performed with ANOVA with Design-Expert® 9.0 (Stat-Ease, Inc., USA).

Results and Discussion

Effect of NADES Type

Eleven green solvents were utilized to investigate the effect of different types of NADESs on the total polyphenol yield, total flavonoid yield and antiradical activity, using one-pot-at-a-time method. The solvents used were grouped in four classes, as sugar based NADESs, choline chloride-sugar based NADESs, acid based NADESs and polyalcohol based NADESs and the properties are presented in Table 2.

*Measured in our laboratory

**The properties given in the table are at 25 °C unless otherwise stated

N/A not available

Figure 1 shows the effect of NADES type on the total polyphenol yield and total flavonoid yield of the extracts. Among NADESs used, GFW provided the highest polyphenol yield as 20.49 ± 0.50 mg GAE g^{-1} dw, followed by CEG and CLa as 18.65 ± 0.85 and 17.53 ± 0.43 mg GAE g^{-1} dw, respectively. On the other hand, the highest Y_{TF} was obtained with CLa as 8.44 ± 0.30 mg ApE g^{-1} dw, followed by CEG and GFW as 7.23 ± 0.20 and 6.10 ± 0.30 mg GAE g^{-1} dw, respectively.

Antiradical activities of the extracts are presented in Fig. 2. The highest value was obtained using GFW as 394.49 ± 10.58 µmol DPPH g^{-1} dw, followed by CMa and CFW as, 357.94 ± 15.37 µmol DPPH g^{-1} dw and 318.70 ± 12.05 µmol DPPH g^{-1} dw, respectively. Consequently, GFW, CEG and CLa were the NADESs that showed up for the polyphenol and flavonoid yields. These three pioneering NADESs belong to the subclasses of sugar based, polyalcohol based and acid based NADESs. On the other hand, despite providing lower polyphenol and flavonoid yield, CFW was the best among ChCl-sugar based NADESs. Considering the properties of NADESs given in Table 1, GFW has the lowest pH among sugar based NADESs as 4.48 and the handling was easy with GFW due to its low viscosity, especially when compared to GFSW. Similarly, CFW has the lowest pH among ChCl–sugar based NADESs as 1.96. Despite the close pH values of CEG and CGly, CEG was found to provide higher polyphenol and flavanoid yields. Another advantage was the lower viscosity of CEG than CG (Table 1).

Mourtzinos et al. [65] reported that CEG provided the highest extraction efficiency among the polyalcohol based DESs used in the extraction of olive leaves. This result is

Table 2 The properties of natural deep eutectic solvents

Group	NADES type	Density (g cm^{-3})	Viscosity (mPa s)	pH	Conductivity (µS cm^{-1})	Water activity, a_w
Sugar based NADESs	GFSW (1:1:1:11)	1.3657 [56] (40 °C)	983.304 [56] (40 °C)	4.76*	0.09*	0.689*
	GFW (1:1:11)	1.3006 [54]	N/A	4.48*	0.83*	0.798*
	GSW (1:1:11)	1.3511 [54]	N/A	5.83*	0.24*	0.754*
	FSW (1:1:11)	1.3597 [54]	N/A	5.24*	0.26*	0.776*
ChCl-sugar based NADESs	CFW (5:2:5)	1.2095 [60] (30 °C)	598 [60] (30 °C)	1.96 [55]	1399 [60] (30 °C)	0.16*
	CGW (5:2:5)	1.2094 [60] (30 °C)	584 [60] (30 °C)	2.32 [55]	2820 [60] (30 °C)	0.159*
	CSW (4:1:4)	1.2164*	853.3 [61] (30 °C)	4.03 [55] 5.29*	471*	0.158*
Acid based NADESs	CLa (1:2)	1.134 [44] (40 °C)	29.5 [44] (40 °C)	0.46*	1764*	0.208*
	CMa (1:1)	1.2112 [60] (30 °C)	616 [60] (30 °C)	0 [55]	732 [60] (30 °C)	0.089*
Polyalcohol based NADESs	CEG (1:2)	1.120 [62]	37 [62]	3.96*	7960* (24 °C), 7610 (20 °C) [62]	< 0.03*
	CGly (1:2)	1.181*, 1.180 [63]	351*, 259 [64]	3.91*	1189*, 1300 [39]	< 0.03*

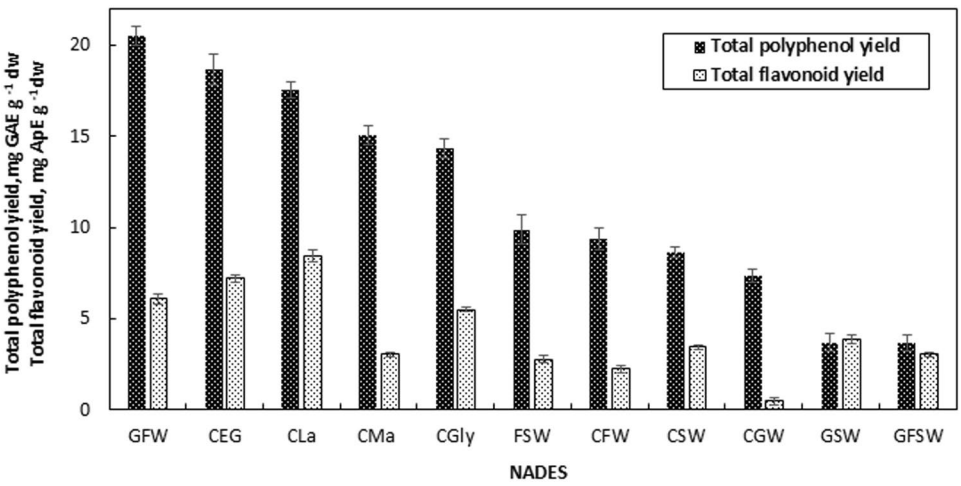

Fig. 1 The effect of NADES type on total polyphenol yield and total flavonoid yield of the extracts [liquid-to-solid ratio 50 mL g^{-1}, 65 °C, 90% (v/v) NADES, particle size 425–1400 µm]

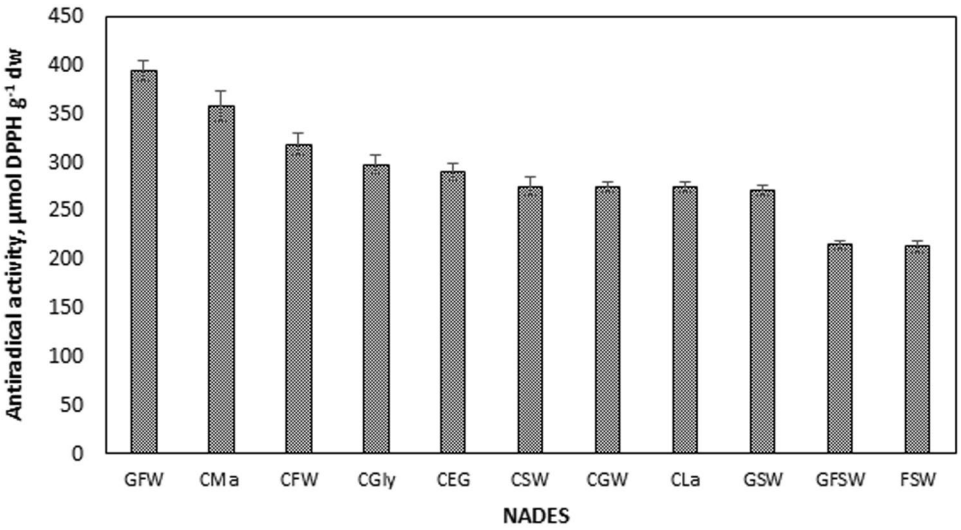

Fig. 2 The effect of NADES type on total antiradical activities (A_{AR}) of the extracts [liquid-to-solid ratio 50 mL g^{-1}, 65 °C, 90% (v/v) NADES, particle size 425–1400 µm]

compatible with the findings of this study. Due to the polar nature of EG, it is likely to show dipole-type and H-bond interactions with phenolic compounds. On the other hand, the lower extraction efficiency with glycerol based DESs compared to EG may arise from the branched structure of glycerol creating a steric hindrance [47].

When acid based NADESs were compared within, the use of CLa resulted in higher Y_{TP}, Y_{TF} and A_{AR} than CMa. This may be due to the higher viscosity of CMa (Table 1) that led to lower mass transfer during the extraction process. CLa was also reported to provide higher extraction yield than other organic acid based DESs tested by Alañón et al. [50]. However, they reported higher extraction ability for polyalcohol based DESs than acid base DESs [50]. On the other hand, Şahin et al. [52] declared that carboxylic acid based DESs resulted in more efficient extracts in terms of oleuropein when compared to other DESs they tested.

Consequently, considering the effect of NADES type on total polyphenol yield, total flavonoid yield and total antiradical activities of the extracts; GFW, CFW, CLa and CEG were the four NADESs selected to be used in further experiments.

Effect of the Amount of NADES

The effect of NADES type was tested at a high amount, as 90% (v/v) in this study. The aim was to use as much NADES amount as possible to take the advantage of the solubility capacity of the green solvents to extract bioactive compounds. Successful extractions with 90% were also reported in the literature [66]. However, the viscosity of the green solvents change significantly with the addition of water, affecting the mass transfer rate and therefore, the extraction capacity [56]. Additionally, water as a polar solvent, increases the polarity of the NADESs, facilitating the extraction of polar compounds [41]. Also, many studies reported the enhancement of the extraction performance with the addition of water to DESs at a specific range [49,

50, 53, 56]. Therefore, lower NADES amounts such as 50, 70 and 90% (v/v) were tested and the results are presented in Fig. 3 and Table 3. For all four types of NADESs used, Y_{TP} and Y_{TF} were found to be at their highest values with 50% (v/v) amount (Fig. 3a, b). When NADES content was decreased to 50%, approximately 3.2-fold

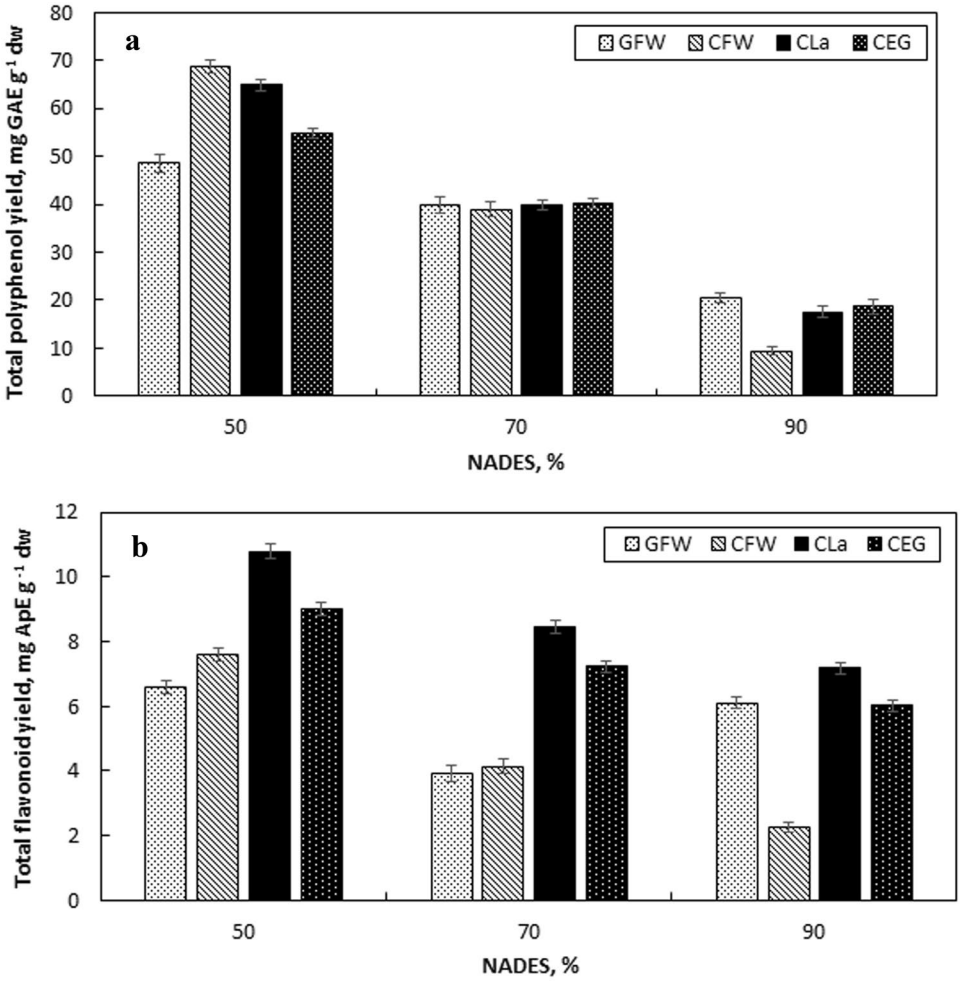

Fig. 3 The effect of the amount of NADES on total polyphenol yield (**a**) and total flavonoid yield (**b**) of the extracts (liquid-to-solid ratio 50 mL g^{-1}, 65 °C, particle size 425–1400 µm)

Table 3 Effects of NADES amount, temperature, liquid-to-solid ratio and particle size on the antiradical activity of the extracts

Antiradical activity of the extracts				
	GFW	CFW	CLa	CEG
Amount of NADES [% (v/v), liquid-to-solid ratio = 50 mL g^{-1}, T = 65 °C, particle size = 425–1400 µm]				
50	463.99 ± 13.1	463.77 ± 9.8	374.77 ± 7.6	458.63 ± 12.0
70	455.94 ± 11.6	453.71 ± 10.5	373.43 ± 9.2	457.51 ± 8.7
90	394.50 ± 9.5	318.70 ± 11.2	274.38 ± 8.8	290.37 ± 13.2
Temperature, T (°C, liquid-to-solid ratio = 50 mL g^{-1}, NADES = 50%, particle size = 425–1400 µm)				
55	461.76 ± 11.9	468.69 ± 10.7	424.42 ± 9.9	463.33 ± 12.5
65	463.99 ± 13.1	463.77 ± 9.8	374.77 ± 7.6	458.63 ± 12.0
75	461.98 ± 10.6	461.98 ± 9.8	416.14 ± 12.4	460.86 ± 11.7
Effect of particle size [µm, liquid-to-solid ratio = 50 mL g^{-1}, NADES = 50% (v/v), T = 75 °C]				
< 160	433.50 ± 13.3	437.50 ± 12.6	342.50 ± 12.1	457.50 ± 8.4
160–425	457.50 ± 11.1	462.50 ± 10.7	394.00 ± 12.4	461.00 ± 9.8
425–1400	461.98 ± 10.6	461.98 ± 9.8	416.14 ± 12.4	460.86 ± 11.7

increase was detected at Y_{TP}, while 1.4-fold increase was detected at Y_{TF} in comparison with 90% (v/v). The highest Y_{TP} was obtained using 50% CFW as 68.66 ± 1.4 mg GAE g^{-1} dw and the highest Y_{TF} was obtained using 50% CLa as 10.78 ± 0.22 mg GAE g^{-1} dw.

Similarly, the highest antiradical activities were found using 50% of NADESs as 463.99 ± 13.1 µmol DPPH g^{-1} dw with GFW, 463.77 ± 9.8 µmol DPPH g^{-1} dw with CFW, 374.77 ± 7.6 µmol DPPH g^{-1} dw with CLa and 458.63 ± 12.0 µmol DPPH g^{-1} dw with CEG (Table 3). In the literature, a similar optimum water content as 43.3% (v/v) was reported for CEG (1:2) [50] while much lower optimum water content 20% (w/v) was reported for glycerol–glycine–water (7:1:3) [49]. Therefore, the optimum water content varies depending on the nature and viscosity of the DES.

According to the results, NADES amount was determined to be very significant parameter on the extraction yields. Therefore, it was selected as a factor to be optimized using experimental design.

Effect of Temperature

The effect of temperature was investigated at 55, 65 and 75 °C. Higher values were not tested due to possible negative effect of high temperature on the phenolic content and antioxidant capacity [65]. According to the results, 75 °C was found to provide the highest Y_{TP} and Y_{TF} (Fig. 4a, b). CFW was found to provide the highest Y_{TP} as 76.62 ± 0.99 mg GAE g^{-1} dw while CLa provided the highest Y_{TF} as 11.29 ± 0.35. In terms of A_{AR}, similar values were obtained at tested temperature range (Table 2). The viscosity of NADES decrease with the increase in the temperature and therefore facilitates the penetration of the solvent to the plant. This destructs the intermolecular interaction in the plant and leads to increased extraction at high temperatures [67].

Our results were found to be compatible with, Alañón et al. [50] who reported 79.6 °C as the optimum temperature for the extraction of phenolics from olive leaf. On the

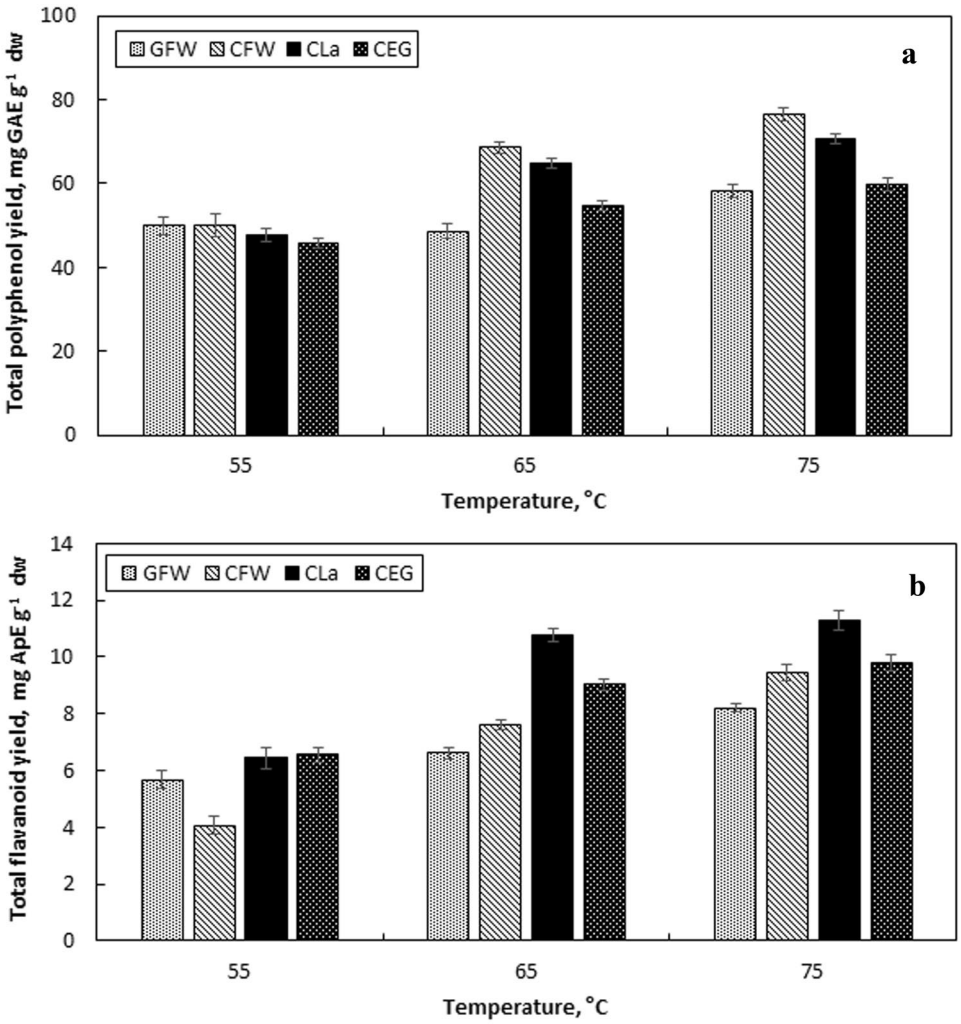

Fig. 4 The effect of temperature on total polyphenol yield (**a**) and total flavonoid yield (**b**) of the extracts [liquid-to-solid ratio 50 mL g^{-1}, 50% (v/v) NADES, particle size 425–1400 µm]

other hand Athanadiadis et al. reported an enhancement in the extraction kinetics from olive leaf at 80 °C [49].

Effect of Particle Size

To investigate the effect of the particle size, grounded olive leaves were fractionated into three different particle sizes. The leaf particles that were < 106 µm and between 106 and 425 µm provided similar values, whereas 425–1400 µm provided higher Y_{TP} and Y_{TF} for all NADESs used (Fig. 5). CFW and CLa were the NADESs that let the highest Y_{TP} and Y_{TF} as 76.62 ± 1.5 mg GAE g^{-1} dw and 11.29 ± 0.35, respectively. In terms of A_{AR}, the particles between 160 and 425 µm and 425–1400 µm provided very close results except for CLa. CLa provided the highest A_{AR} for 425–1400 µm size. Therefore, the optimum particle size was detected as 425–1400 µm. This results is consistent with the recommended average particle size that is reported to be 0.4–0.8 mm [41].

Process Optimization by Response Surface Methodology

The effects of the temperature, particle size and the type and the amount of NADESs were investigated using one-pot-at-a-time method. The optimum values were identified as; 75 °C, 425–1400 µm and 50% (v/v) NADES (GFW, CFW, CLa and CEG). The results showed that a fine tuning of the amount of NADES (%) would provide higher extractions efficiency. Beyond these, another significant parameter on the extraction is the solid-to-liquid ratio ($R_{L/S}$). Therefore, the effect of solid-to-liquid ratio ($R_{L/S}$) and the amount of

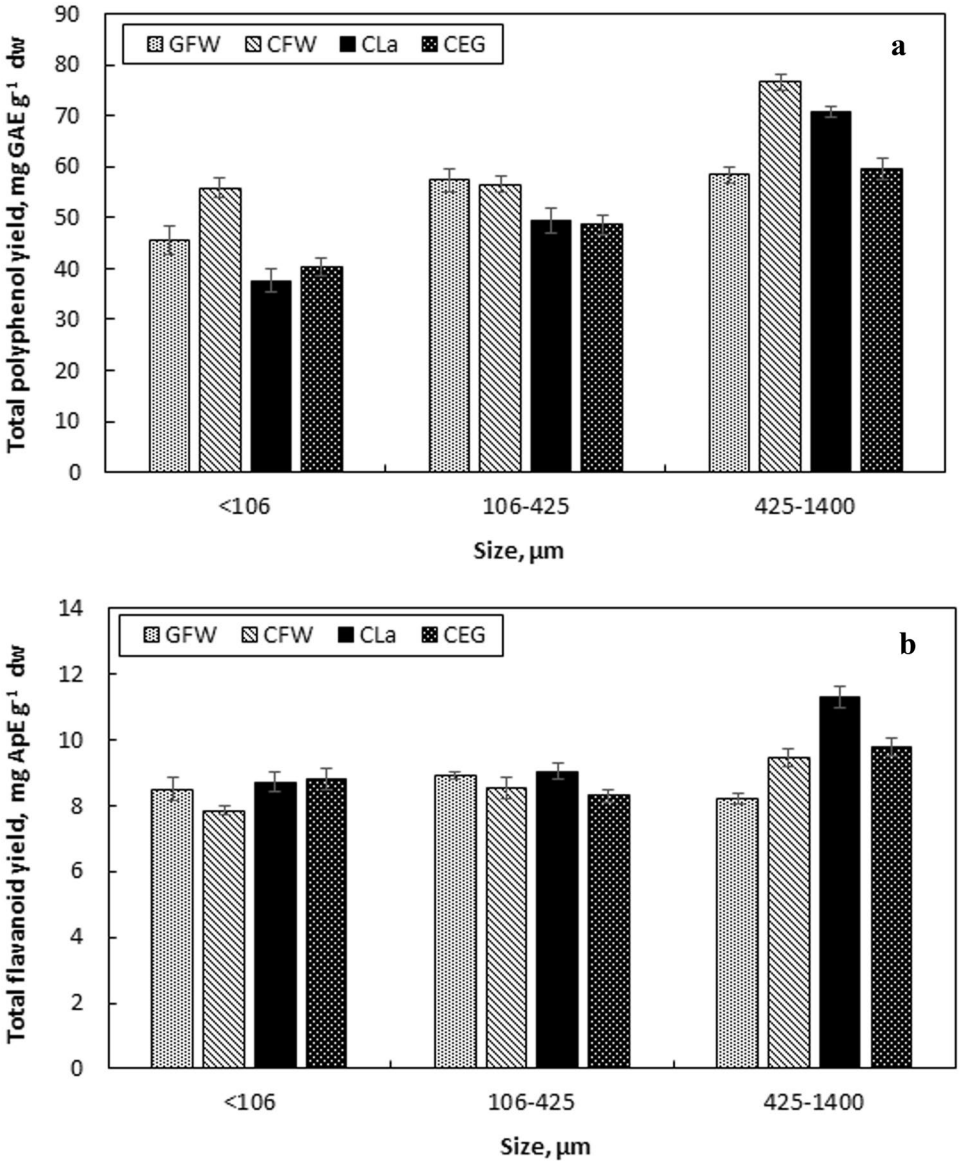

Fig. 5 The effect of particle size on total polyphenol yield (**a**) and total flavonoid yield (**b**) of the extracts [liquid-to-solid ratio 50 mL g^{-1}, 50% (v/v) NADES, 75 °C]

NADES (%) on Y_{TP} and Y_{TF} were investigated in detail, using an experimental design.

Experimental design was performed by using CCD and the responses were revealed by RSM. This let to find out the joint effects of the two factors; NADES amount and $R_{L/S}$ on the responses; total polyphenol yield and total flavonoid yield. Four pioneering NADESs from each group, as GFW, CFW, CLa, and CEG, were used in the experimental design and each of them were optimized separately. The experimental design, levels of the two independent variables and responses are tabulated in Table 4.

Table 5 shows the ANOVA results for both of the responses; total polyphenol yield (mg GAE g^{-1} dw) and total flavonoid yield (mg ApE g^{-1} dw) obtained with four different NADESs. On the other hand, the mathematical models representing the responses in the experimental region and also the R^2 values are presented in Table 6. ANOVA results, p values and R^2 in accordance implied the reliability of the models to predict the responses.

First of all, total polyphenol yield values obtained with four different NADESs are discussed. The model F-value of 8.34 for GFW indicated a statistically significant reduced cubic model. The significant model terms of this equation, that was modified using 'backward elimination', were identified as B, B^2 and A^2B (Table 6). Y_{TP} values obtained with GFW were in the range of 116.24–11.81 mg GAE g^{-1} dw. When response surface is analysed (Fig. 6a), a unique 3D plot was observed representing the reduced cubic model. High values of Y_{TP} was achieved around 45–70 mL g^{-1} of $R_{L/S}$ and 33.75–61.25% of GFW. At low values of $R_{L/S}$, especially around 20–32.5 mL g^{-1}, Y_{TP} was low regardless of the amount of GFW, as indicated in green color.

In the case of CFW, quadratic model was found to express the responses thoroughly for the working space (Table 6). The maximum and minimum values were obtained as, 195.00 and 3.87 mg GAE g^{-1} dw. All of the model terms were found to be significant. High values of Y_{TP} could be obtained at average values of the working space. At the high levels of the both of the independent variables, Y_{TP} reached its lowest values as indicated with blue colour (Fig. 6b). Similar to the effect of the high levels of the independent variables, low levels also let to a decrease in Y_{TP} (green area), but not as dramatic as the blue area.

Y_{TP} values were in the range of 133.36 and 3.32 mg GAE g^{-1} dw when CLa was used for the extractions (Fig. 6c). The ANOVA results indicated that the response could be expressed by a quadratic model (Table 6). Additionally, all of the model terms were identified to be statistically significant. The quadratic surface showed that high values of the response could be obtained at medium values of $R_{L/S}$ together with relatively low amount of CLa. On the other hand, low values were obtained at highest levels of the independent variables. Y_{TP} was found to increase with decreasing amount of NADES at constant $R_{L/S}$, while it showed an increasing and decreasing trend with increasing $R_{L/S}$ at constant amount of NADES.

The design of the experiments conducted using CEG resulted in a quadratic model for Y_{TP}, indicating A, B, AB, A^2 and B^2 as significant terms (Table 6). The quantity changed between 98.24 and 2.14 mg GAE g^{-1} dw. High values of Y_{TP} could be achieved at both lower $R_{L/S}$ and NADES values (Fig. 6d) as indicated in red. Y_{TP} decreased dramatically with the decrease in the CEG amount at $R_{L/S}$=70 mL g^{-1}. A similar decrease was also observed with increasing $R_{L/S}$ at constant NADES amount. The lowest level of the response surface showed up for high levels of both of the variables.

When the four selected NADESs are compared in terms of the Y_{TP} values obtained, CFW was found to provide the highest values.

The response surface plots of Y_{TF} are presented in Fig. 6e–h. In the case of GFW, the response values fitted best in a reduced cubic model (Table 6). The reduction was performed in order to eliminate the insignificant factors by 'backward elimination'. The significant model terms were identified as A, B, A^2, B^2 and A^2B. The highest and lowest values obtained were 8.33 and 3.80 mg ApE g^{-1} dw, respectively. The 3D plot showing the response surface indicated that the highest values of Y_{TF} were achieved at lower values of NADES amount and at medium values of $R_{L/S}$. On the other hand, the lowest Y_{TF} values were obtained at low $R_{L/S}$ (20–30 mL g^{-1}) and high values of NADES amount (47.5–75%) (Fig. 6e). However, decreasing amount of GFW had a positive effect on Y_{TF} around 20–30 mL g^{-1} of $R_{L/S}$. Additionally, Y_{TF} showed an increasing and decreasing trend at constant amount of NADES.

Quadratic model was obtained as the equation to describe the total flavonoid yield for CFW extractions. The values were in the range of 13.27–7.23 mg ApE g^{-1} dw. A, B, A^2 and B^2 were the significant model terms (Table 6). The 3D plot indicated a clear increase of Y_{TF} at low amount of CFW together with high level of $R_{L/S}$ (Fig. 6f). On the other hand, a slight shift of the working space to higher values of $R_{L/S}$ would provide a better view of the entire reddish area.

The ANOVA results of the design for CLa showed that *predicted* R^2 (0.717) was in reasonable agreement with *adjusted* R^2 (0.909). All of the model terms were found to be significant (Table 6). Total flavonoid yield values were in the range of 10.16–1.26 mg ApE g^{-1} dw. Response surface plot indicated that highest Y_{TF} values were achieved at medium to low values of $R_{L/S}$ but medium to high lower values of CLa (Fig. 6g). NADES amount did not have a dramatic effect on Y_{TF} at constant low values of $R_{L/S}$.

CEG also resulted in a quadratic model to describe the response surface for Y_{TF}, with the significant terms as A, B, AB, A^2 and B^2 (Table 6). The quantities changed

Table 4 Central composite design matrix for NADESs (experimental variables and responses)

Run	Variables				Responses							
	NADES (%)		$R_{L/S}$ (mL g^{-1})		GFW		CFW		CLa		CEG	
	Level	*Actualvalue*	*Level*	*Actualvalue*	Total polyphenol yield (mg GAE g^{-1} dw)	Total flavonoid yield (mg ApE g^{-1} dw)	Total polyphenol yield (mg GAE g^{-1} dw)	Total flavonoid yield (mg ApE g^{-1} dw)	Total polyphenol yield (mg GAE g^{-1} dw)	Total flavonoid yield (mg ApE g^{-1} dw)	Total polyphenol yield (mg GAE g^{-1} dw)	Total flavonoid yield (mg ApE g^{-1} dw)
	A		B									
1	0	47.50	0	45.00	101.79	7.78	166.95	12.43	133.36	10.16	92.24	9.11
2	0	47.50	0	45.00	89.79	7.50	195.00	11.52	111.58	9.78	98.24	8.65
3	− 1	20.00	− 1	20.00	82.38	6.84	90.36	9.76	74.98	7.53	81.94	7.56
4	+ 1	75.00	+ 1	70.00	83.11	6.14	3.87	8.39	3.32	1.26	2.14	6.89
5	+ 1	75.00	− 1	20.00	72.01	5.19	120.03	7.66	81.05	9.32	73.35	3.90
6	0	47.50	0	45.00	110.50	7.69	157.59	12.60	114.00	9.00	95.00	8.11
7	− 1	20.00	+ 1	70.00	77.79	6.57	144.39	13.27	85.00	8.47	83.5	7.20
8	0	47.50	0	45.00	98.68	7.15	180.69	11.83	108.55	8.3	87.8	7.53
9	0	47.50	0	45.00	116.24	8.33	154.02	11.20	118.25	9.58	76.68	8.30
10	0	47.50	− α	9.64	11.81	3.8	112.05	9.48	91.18	9.03	80.32	4.20
11	0	47.50	0	45.00	93.44	7.33	175.11	11.90	105.00	10.00	80.00	7.82
12	0	47.50	+ α	80.36	84.61	6.46	7.50	10.91	9.37	1.52	3.77	6.90
13	+ α	86.39	0	45.00	75.57	5.46	96.03	7.27	80.90	3.54	51.79	5.90
14	− α	8.61	0	45.00	83.12	7.20	119.37	9.89	94.66	7.75	72.24	7.30

Table 5 ANOVA results for the responses obtained with NADESs

NADES	Model	Source	Sum of squares	df	Mean square	F	P	Inference
Total polyphenol yield (mg GAE g^{-1} dw)								
GFW	Reduced cubic model	Model	6899.26	6	1149.88	8.34	0.0066	S
		A-NADES %	30.92	1	30.92	0.22	0.6502	
		B-R	2649.92	1	2649.92	19.23	0.0032	
		AB	61.54	1	61.54	0.45	0.5254	
		A2	408.35	1	408.35	2.96	0.1289	
		B2	3907.74	1	3907.74	28.35	0.0011	
		A2B	1162.70	1	1162.70	8.44	0.0228	
		Residual	964.71	7	137.82			
		Lack of fit	456.66	2	228.33	2.25	0.2013	*NS*
		Pure error	508.05	5	101.61			
		Cor total	7863.97	13				
CFW	Quadratic model	Model	42,326.51	5	8465.30	23.23	0.0001	S
		A-NADES %	*2586.88*	*1*	*2586.88*	*7.10*	*0.0286*	
		B-R	*5511.77*	*1*	*5511.77*	*15.12*	*0.0046*	
		AB	*7241.16*	*1*	*7241.16*	*19.87*	*0.0021*	
		A2	*6846.47*	*1*	*6846.47*	*18.79*	*0.0025*	
		B2	*21,862.78*	*1*	*21,862.78*	*59.99*	*< 0.0001*	
		Residual	2915.71	8	364.46			
		Lack of fit	*1746.26*	*3*	*582.09*	*2.49*	*0.1750*	*NS*
		Pure error	*1169.46*	*5*	*233.89*			
		Cor total	45,242.22	13				
CLa	Quadratic model	Model	17,260.19	5	3452.04	21.08	0.0002	S
		A-NADES %	*1129.78*	*1*	*1129.78*	*6.90*	*0.0303*	
		B-R	*4204.76*	*1*	*4204.76*	*25.68*	*0.0010*	
		AB	*1925.02*	*1*	*1925.02*	*11.76*	*0.0090*	
		A2	*1810.20*	*1*	*1810.20*	*11.06*	*0.0105*	
		B2	*8743.32*	*1*	*8743.32*	*53.40*	*< 0.0001*	
		Residual	1309.90	8	163.74			
		Lack of fit	*808.04*	*3*	*269.35*	*2.68*	*0.1575*	*NS*
		Pure error	*501.86*	*5*	*100.37*			
		Cor total	18,570.09	13				
CEG	Quadratic model	Model	10,999.23	5	2199.85	15.31	0.0006	S
		A-NADES %	*1766.28*	*1*	*1766.28*	*12.29*	*0.0080*	
		B-R	*3956.41*	*1*	*3956.41*	*27.53*	*0.0008*	
		AB	*1323.87*	*1*	*1323.87*	*9.21*	*0.0162*	
		A2	*910.71*	*1*	*910.71*	*6.34*	*0.0360*	
		B2	*3284.65*	*1*	*3284.65*	*22.85*	*0.0014*	
		Residual	1149.83	8	143.73			
		Lack of fit	*786.46*	*3*	*262.15*	*3.61*	*0.1006*	*NS*
		Pure error	*363.38*	*5*	*72.68*			
		Cor total	12,149.06	13				

between 9.11 and 3.90 mg ApE g^{-1} dw. All of the terms of the equation representing the responses were shown to be significant. On the other hand, *predicted* R^2 (0.717) was found to be in reasonable agreement with *adjusted* R^2 (0.865). It was observed that high amount of NADES together with low amount $R_{L/S}$ resulted in very low values of Y_{TF}. On the other hand, when NADES amount decreased Y_{TF} increased at constant $R_{L/S}$ (Fig. 6h). The high extraction yield for flavonoid were obtained at lower NADES amount and medium values of $R_{L/S}$.

When the four selected NADESs are compared in terms of the Y_{TF} values CFW was found to provide the highest responses while GFW provided the lowest responses.

Table 5 (continued)

NADES	Model	Source	Sum of squares	df	Mean square	F	P	Inference
Total flavonoid yield (mg ApE g^{-1} dw)								
GFW	Reduced cubic model	Model	17.63	6	2.94	16.00	0.0009	S
		A-NADES %	*2.58*	*1*	*2.58*	*14.03*	*0.0072*	
		B-R	*3.54*	*1*	*3.54*	*19.26*	*0.0032*	
		AB	*0.37*	*1*	*0.37*	*2.03*	*0.1976*	
		A2	*2.12*	*1*	*2.12*	*11.56*	*0.0114*	
		B2	*9.53*	*1*	*9.53*	*51.91*	*0.0002*	
		A2B	*1.19*	*1*	*1.19*	*6.46*	*0.0385*	
		Residual	1.29	7	0.18			
		Lack of fit	*0.43*	*2*	*0.22*	*1.27*	*0.3590*	*NS*
		Pure error	*0.85*	*5*	*0.17*			
		Cor total	18.92	13				
CFW	Quadratic model	Model	42.48	5	8.50	18.62	0.0003	S
		A-NADES %	*14.33*	*1*	*14.33*	*31.42*	*0.0005*	
		B-R	*4.90*	*1*	*4.90*	*10.74*	*0.0112*	
		AB	*1.94*	*1*	*1.94*	*4.25*	*0.0731*	
		A2	*18.22*	*1*	*18.22*	*39.93*	*0.0002*	
		B2	*4.33*	*1*	*4.33*	*9.49*	*0.0151*	
		Residual	3.65	8	0.46			
		Lack of fit	*2.24*	*3*	*0.75*	*2.65*	*0.1605*	*NS*
		Pure error	*1.41*	*5*	*0.28*			
		Cor total	46.13	13				
CLa	Quadratic model	Model	115.94	5	23.19	27.16	< 0.0001	S
		A-NADES %	*16.17*	*1*	*16.17*	*18.94*	*0.0024*	
		B-R	*39.34*	*1*	*39.34*	*46.07*	*0.0001*	
		AB	*20.25*	*1*	*20.25*	*23.72*	*0.0012*	
		A2	*19.29*	*1*	*19.29*	*22.59*	*0.0014*	
		B2	*23.96*	*1*	*23.96*	*28.06*	*0.0007*	
		Residual	6.83	8	0.85			
		Lack of fit	*4.38*	*3*	*1.46*	*2.97*	*0.1358*	*NS*
		Pure error	*2.45*	*5*	*0.49*			
		Cor total	122.77	13				
CEG	Quadratic model	Model	27.55	5	5.51	17.69	0.0004	S
		A-NADES %	*4.43*	*1*	*4.43*	*14.21*	*0.0055*	
		B-R	*5.20*	*1*	*5.20*	*16.69*	*0.0035*	
		AB	*2.81*	*1*	*2.81*	*9.01*	*0.0170*	
		A2	*4.14*	*1*	*4.14*	*13.28*	*0.0065*	
		B2	*11.98*	*1*	*11.98*	*38.45*	*0.0003*	
		Residual	2.49	8	0.31			
		Lack of fit	*0.87*	*3*	*0.29*	*0.89*	*0.5072*	*NS*
		Pure error	*1.62*	*5*	*0.32*			
		Cor total	30.04	13				

S significant, *NS* not significant

The optimization of the responses was also performed using Design Expert. The experimental conditions providing the highest values of the responses Y_{TP} and Y_{TF} were predicted separately using Design Expert. The predictions of the program for Y_{TF} indicated that flavonoid yield would not change significantly between its own optimum conditions or the other response's (Y_{TP}) optimum conditions. Moreover, the desirability function that was used to optimize both of the responses predicted lower Y_{TP} than a single optimization. Considering the choices, the predicted optimized conditions

Table 6 Mathematical equations expressing the responses in coded factors

NADES	Models (Coded Factors)	R^2	Adj R^2	CV (%)
Total polyphenol yield (mg GAE g^{-1} dw)				
GFW	$101.74 - 1.9 *A + 25.74 * B + 3.92 * A * B - 7.44 * A^2 - 23.00 * B^2 - 24.11 * A^2 * B$	0.877	0.772	13.92
CFW	$171.56 - 17.98 * A - 26.25 * B - 42.55 * A * B - 30.45 * A^2 - 54.41 * B^2$	0.936	0.895	15.51
CLa	$115.12 - 11.88 * A - 22.93 * B - 21.94 *A * B - 15.66 * A^2 - 34.41* B^2$	0.930	0.885	14.79
CEG	$88.33 - 14.86 * A - 22.24 * B - 18.19 * A * B - 11.11 * A^2 - 21.09 * B^2$	0.905	0.846	17.14
Total flavonoid yield (mg ApE g^{-1} dw)				
GFW	$7.63 - 0.57 * A + 0.94 * B + 0.31 * A * B - 0.54 * A^2 - 1.14 * B2 - 0.77 * A^2 * B$	0.932	0.874	6.42
CFW	$11.91 - 1.34 * A + 0.78 * B - 0.70 *A * B -1.57 * A^2 - 0.77 * B^2$	0.921	0.871	6.38
CLa	$9.47 - 1.42 * A - 2.22 * B - 2.25 * A* B - 1.62 * A^2 - 1.80 * B^2$	0.944	0.909	12.29
CEG	$8.25 - 0.74 * A + 0.81 * B + 0.84 * A * B -0.75 * A^2 - 1.27 * B^2$	0.917	0.865	7.86

for one of the responses, Y_{TP} were used in the experiments and the results are given at Table 7. Additionally, antiradical activity assays were also performed and presented at the optimum conditions. According the results, the difference between predicted and experimental results were found to be lower than 6%, which showed the convenience of the experimental design. The highest Y_{TP} ,Y_{TF} and A_{AR} were obtained with CFW as 187.31 ± 10.3 mg GAE g^{-1} dw, 12.75 ± 0.6 mg ApE g^{-1} dw and 480 ± 26 µmol DPPH g^{-1} dw, respectively.

The Principle Bioactive Compounds Detected in the Olive Leaf Extracts

The extracts obtained at the optimum conditions were subjected to LC–MS analysis for the detection of oleuropein, luteolin and caffeic acid and compared with MeOH extract which was obtained at non-optimized conditions. According to the results (Table 8), NADESs were found to extract comparable amounts with MeOH. The highest oleuropein content was achieved with GFW as 1630.80 mg kg^{-1} dw, followed by CEG as 1031.57 mg kg^{-1} dw; whereas MeOH provided 1221.17 mg kg^{-1} dw. GFW was also found to provide highest caffeic acid as 112.77 mg kg^{-1} dw, followed by MeOH as 41.54 mg kg^{-1} dw. GFW could extract higher amount of oleuropein and caffeic acid than MeOH, showing the comparable extraction performance of the NADES with the organic solvent. On the other hand, MeOH extract provided the highest amount of luteolin as 2.59 mg kg^{-1} dw followed by GFW and CFW extracts, as 1.34 mg kg^{-1} dw and 0.49 mg kg^{-1} dw, respectively.

According to the results, oleuropein was found to be the most abundant compound among the three phenolics analyzed. This was inevitable since it is also the most abundant phenolic compound in the olive leaf [13]. Despite the highest Y_{TP} and Y_{TF} values were obtained with CFW, higher amount of oleuropein, caffeic acid and luteolin could be detected in GFW extracts. This may be due to the modification of the extracted substances into their derivatives that could not be analyzed. Moreover, other phenolic substances may be present in CFW extract. The results showed that NADESs can be good candidates to be used as an alternative of conventional solvents.

Conclusions

Green, simple and cheap extraction procedure from an agricultural waste, olive leaf, was presented as an alternative to conventional extraction methods, using the potential of green and low cost natural deep eutectic solvents. This extraction method meets many principles of green extraction, such as simple and inexpensive preparation of the solvents, minimum amount of solvent, sustainable production, decreased waste and also the use of safer solvents. The novelty of this study is the first time use of NADESs that are not previously used for the extraction from olive leaves using ultrasound assisted extraction. Moreover glucose–fructose–water—as a firstly presented NADES in the ultrasound assisted extraction from olive leaves, was found to extract higher amount of oleuropein and caffeic acid than MeOH, showing an encouraging possible shift of organic solvents with NADESs for an environmentally-friendly process. The sustainable utilization of the resources, can only be managed by avoiding existing chemical-based methods and by using green and non-conventional methods such as ultrasound assisted-extraction, as presented in this study. In addition to the shift of the extraction method with an environmentally-friendly method, the substitution of the hazardous solvents (especially chlorinated solvents) with the green solvents

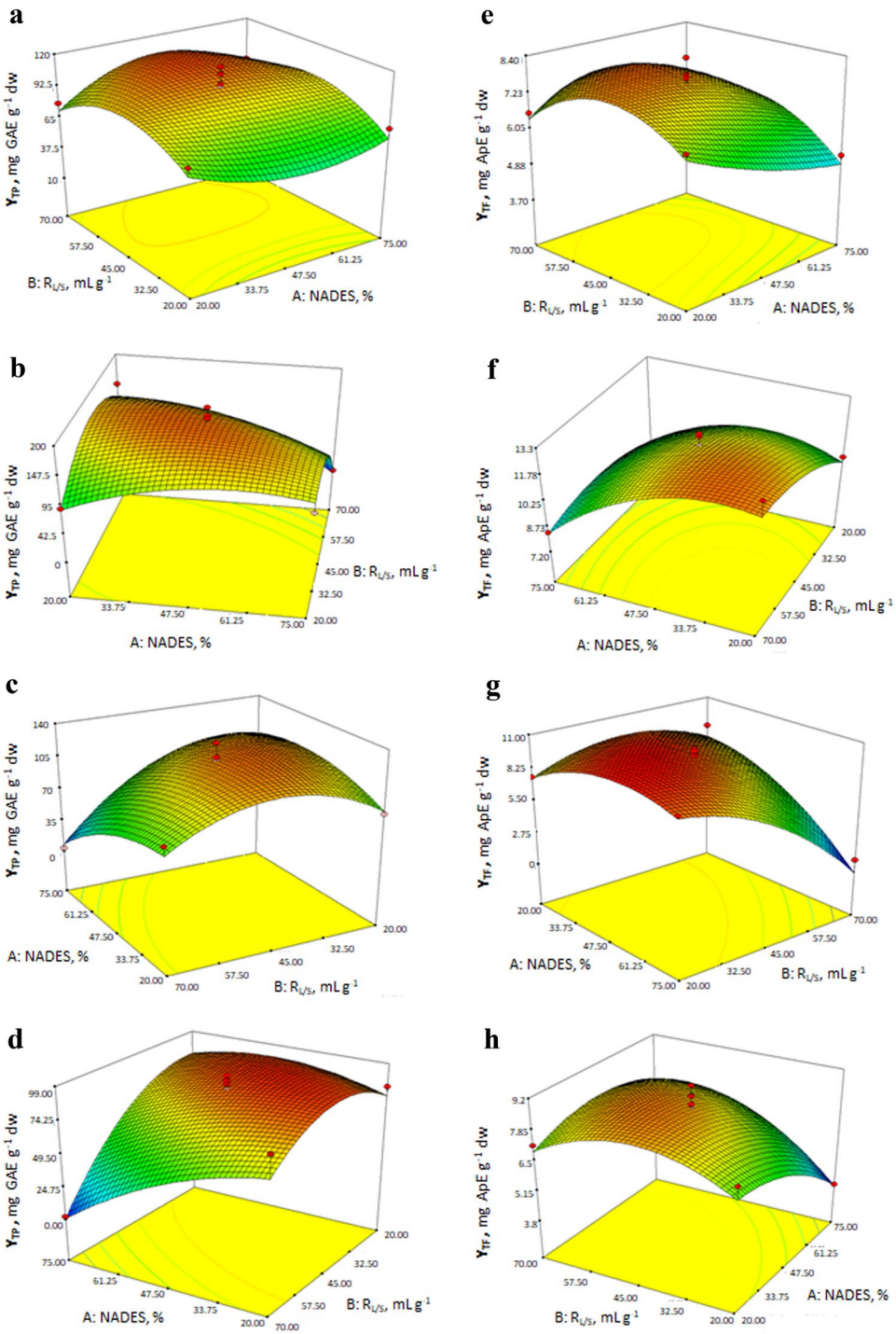

Fig. 6 3D plots of the response surfaces of the response Y_{TP} for GFW (**a**), CFW (**b**), CLa (**c**), CEG (**d**) and Y_{TF} for GFW (**e**), CFW (**f**), CLa (**g**), and CEG (**h**)

will result in satisfactory clean processes. Similar changes in the industrial processes—even a partial change will probably mark a new epoch for a clean and healthy earth. The presented procedure is a promising route for the green extraction from olive leaves that will contribute to the elimination of the hazardous processes. On the other hand, the optimum green solvent content that was found to be around 50%, offers a clear away of the disadvantage of the difficulty of pumping and stirring of high viscosity deep eutectic solvents in the industrial scale. Additionally, the extract has the potential to be used without further purification steps, due to the non-toxic and natural structure of natural deep eutectic solvents.

Table 7 Optimized conditions for the ultrasound assisted extraction with NADESs

NADES	Optimum conditions	Y_{TP}, mg GAE g^{-1} dw Predicted	Y_{TP}, mg GAE g^{-1} dw Experimental	Y_{TF}, mg ApE g^{-1} dw Predicted	Y_{TF}, mg ApE g^{-1} dw Experimental	A_{AR}µmol DPPH g^{-1} dw
GFW	47.09%, 63.27 mL g^{-1}	116.64 ± 4.6	122.47 ± 4.7	8.06 ± 0.4	8.46 ± 0.5	470 ± 27.2
CFW	42.69%, 40.66 mL g^{-1}	175.38 ± 8.2	187.31 ± 10.3	11.91 ± 0.7	12.75 ± 0.6	480 ± 26
CLa	40.00%, 38.84 mL g^{-1}	119.28 ± 6.2	124.05 ± 4.7	10.02 ± 0.5	10.42 ± 0.5	435 ± 28.2
CEG	50.00%, 30.85 mL g^{-1}	93.65 ± 6.1	99.45 ± 6.6	7.88 ± 0.48	8.12 ± 0.4	472 ± 22.1

*Extraction conditions: T = 75 ℃, t = 1 h

Table 8 Bioactive compounds extracted from olive leaves

NADES/Solvent	Oleuropein (mg kg^{-1} dw)	Caffeic acid (mg kg^{-1} dw)	Luteolin (mg kg^{-1} dw)
GFW	1630.80	112.77	1.34
CFW	853.46	0.00	0.49
CLa	290.07	0.09	0.01
CEG	1031.57	0.08	0.25
MeOH	1221.17	41.54	2.59

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