Valorization of olive tree leaves: Extraction of oleanolic acid using aqueous solutions of surface-active ionic liquids

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ABSTRACT

The global olive oil industry annually generates approximately 750,000–1,500,000 tons of *Olea europaea* leaves as waste that are typically burned for energy production. Yet, this agricultural by-product is a rich source of oleanolic acid, a high value triterpenic acid with outstanding pharmaceutical and nutraceutical activities. The present study focuses on the extraction of oleanolic acid from dried *O. europaea* leaves using aqueous solutions of surface-active ionic liquids as alternative solvents. A number of imidazolium-based ionic liquids with variable chain length, different anions and optional side-chain functionalization was synthesized and employed in the extraction of oleanolic acid. Ionic liquids with long alkyl chains remarkably enhance the solubility of oleanolic acid in water, thus being able to compete with the solubilities afforded by molecular organic solvents, such as chloroform. Consequently, they are suitable alternatives for the solid-liquid extraction of triterpenic acids from natural matrices and provide improved extraction yields of up to 2.5 wt% oleanolic acid extracted from olive tree leaves.

1. Introduction

Biomass has emerged as key source of a wide variety of fine chemicals [1]. This perspective is in straight line with the emerging biorefinery concept, which postulates the integrated exploitation of agroforest biomass as a source of chemicals, materials, fuels and energy [2]. In this context, the development of environmentally friendly strategies for the recovery of high-value compounds from biomass by-products prior to their further valorization, typically carried out by burning, is of crucial importance.

Triterpenic acids are a typical example of such high-value compounds that can be extracted from agricultural by-products prior to burning, while adding substantial economic value to biorefinery-based processes [3]. Triterpenic acids, such as oleanolic, betulinic and ursolic acids, are secondary plant metabolites typically found in barks, leaves or peels, with potential pharmaceutical and nutraceutical applications [4]. A number of studies demonstrated that triterpenic acids have potent antimicrobial, antitumor, hepatoprotective, anti-inflammatory, cytotoxic, anti-allergic and anti-HIV activities [4–6]. Oleanolic acid (Fig. 1), in particular, is a key component of olive pomace, and a major

contributor to the health promoting effect of the human Mediterranean diet [7]. Consequently, an increasing number of studies addressed the molecular mechanisms of action of plant triterpenes, but also their occurrence and improved isolation from various plant materials [3,8]. Due to their beneficial properties and interest for several industries there is considerable interest in identifying novel sources of triterpenic acids to guarantee the increasing world-wide demand [9]; it should be remarked that the commercial value of oleanolic acid, depending on its purity, can reach 1200 €/g [10].

While the leaves, fruits or bark of many plants are reported as potential sources of this compound, oleanolic acid can be also found in agroforestry waste streams, such as in the leaves of *O. europeae*, the common European olive tree [11]. These by-products are abundantly produced as a result of the activities of olive oil industries, which generate large amounts of wastes currently burned for energy production [3]. Taking the total world production of olive oil in 2012 into account, 750,000–1500,000 tons of leaves are discarded on an annual base. Assuming a maximum content of up to 3.1 wt% of oleanolic acid, the main triterpenic acid present in the leaves of *O. europeae*, this corresponds to large amounts of this high-value compound that could
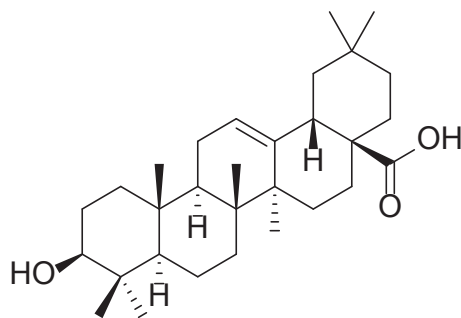

Fig. 1. Chemical structure of oleanolic acid (1).

be potentially extracted [12,13] contributing to the integrated valorization of the olive oil value chain.

Still considering the biorefinery concept, and in addition to the use of agroforestry by-products, one of the main challenges for the efficient exploitation of high-value compounds involves the replacement of conventional extraction and purification systems, usually carried out with volatile and often hazardous organic solvents in multistep procedures, by more efficient and environmentally friendly alternatives [14]. Among new prospective extractive solvents for added-value products, ionic liquids (ILs) have gained considerable attention [15]. Unlike molecular solvents, they present outstanding properties due to their ionic nature, namely negligible vapor pressure and high solvation ability. Since the physicochemical properties of ionic liquids are strongly dependent on their ionic nature and composition, the possibility of tailoring them is an important advantage for manipulating their extraction abilities and selectivities [16,17]. As a result, the ionic liquid-assisted targeted extraction of high-value natural products from biomass became a rapidly growing field of research in the past years, and numerous studies demonstrated improved extraction yield and selectivity compared to conventional solvents [18–20]. More recently, the possibility of using aqueous solutions of ionic liquids instead of their pure forms, prompted an increased interest due to substantial improvement in extraction efficiency and cost reduction. Moreover, advanced technologies for natural product extraction such as subcritical water extraction can be further boosted by the addition of ionic liquids, as recently demonstrated on the extraction of phenolics or carrageenan from brown or red seaweed [21,22]. Ionic liquid aqueous solutions promote extraction through the formation of hydrotropes, enhancing the solubility of more hydrophilic compounds [23–26], or as surface-active compounds, increasing the solubility/dispersion of hydrophobic substances in aqueous media [27–29]. Most recently, Faria et al. reported remarkably increased solubility of ursolic acid in solutions of surface-active ionic liquids in water and applied these solvents for the extraction of triterpenic acids from apple peels [30].

Here, we investigate the extraction of oleanolic acid from *O. europeae* leaves, an industrial waste stream from olive oil industry, relying on aqueous solutions of surface-active ionic liquids, and discuss the impact of ionic liquid chemical structure, extraction method and operational conditions such as microwave irradiation on the extraction yield.

2. Materials and methods

2.1. General

Commercially available reagents and solvents were used as received from Sigma Aldrich, unless otherwise specified. Doubly-distilled deionized water was obtained from a Millipore Milli-Q water purification system (Millipore, USA). All ionic liquids were dried for at least 24–48 h at 50 °C and 0.01 mbar before use and were stored under argon.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 400 at 400 and 100 MHz, respectively, using the solvent peak as reference. *J* values

are given in Hz. ^{13}C NMR spectra were run in proton-decoupled mode.

For the quantification of oleanolic acid, HPLC analysis was performed relying on reversed phase C18-column set-up via the following methods:

Method A: A GILSON HPLC unit coupled to an oven with manual injector and equipped with an analytical C18 reversed-phase column (250 × 4.60 mm, Kinetex 5 μm C18 100 A) from Phenomenex was used. The column temperature was set to 30 °C. The mobile phase consisted of 87 (v/v) % of methanol, 13 (v/v) % of water + 0.1 (v/v) % of tri-fluoroacetic acid (TFA). Separations were conducted in isocratic mode, at a flow rate of 1 mL/min. Detection was done at a wavelength of 210 nm. Data acquisition and evaluation were performed using the Jasco-Borwin 1.21 software and based on a previously established calibration curve ($R^2 > 0.9998$).

Method B: A Jasco HPLC unit equipped with a Maisch Reprosil 5 μm C18 100 column (250 × 4.60 mm) and security guard pre-column was used. The column temperature was set to 30 °C. The mobile phase consisted of 87 (v/v) % of methanol, 13 (v/v) % of water + 0.1 (v/v) % of trifluoroacetic acid (TFA) at a flow of 1 mL/min. Detection was done at a wavelength of 210 nm. Data evaluation was based on a previously established calibration curve ($R^2 > 0.9993$) prepared using 1-methyl-cyclohexene in MeOH as internal standard.

Microwave-assisted extractions were performed on a BIOTAGE Initiator[TM] sixty microwave unit. The reported times are hold times.

SEM pictures were taken with a FEI Inspect F50 at 15 kV. All samples were coated with a 3.5 nm thick gold-layer using a Leica Cool Sputter Coater EM SCD005.

Olive tree leaves were collected in Aveiro, Portugal and pre-dried at 25 °C for 5 d. The dried leaves were milled to a particle size > 1 mm using a Retsch ZM 100 cryo mill.

2.2. Synthesis of ionic liquids

The 1-alkyl-3-methylimidazolium halide ionic liquids [C$_6$mim]Cl, [C$_8$mim]Cl, [C$_{10}$mim]Cl, [C$_{12}$mim]Cl, [C$_{12}$mim]Br, [C$_{12}$mim]I, [C$_{14}$mim]Cl, [C$_{16}$mim]Cl and [C$_{18}$mim]Cl were prepared from freshly distilled *N*-methylimidazol and the corresponding alkyl halide, according to literature [31,32]. Solid surface-active ionic liquids, [C$_n$mim]X with n ≥ 12, were repeatedly crystallized from tetrahydrofuran or ethyl acetate until colorless crystals were obtained. Sulfonate- and phosphonate-based surface-active ionic liquids were synthesized through a two steps procedure involving the alkylation of 1-dodecylimidazole, which was previously synthesized following a procedure reported in literature and distilled before use [33]. 1-Dodecylimidazole was reacted with the corresponding methyl ester to yield 1-dodecyl-3-methylimidazolium mesylate ([C$_{12}$mim]OMs), 1-dodecyl-3-methylimidazolium tosylate ([C$_{12}$mim]OTs), and 1-dodecyl-3-methylimidazolium dimethylphosphate ([C$_{12}$mim]Me$_2$PO$_4$). Analytical data were in accordance with literature and details can be found in previous work [31,32,34,35]. In case of 1-dodecyl-3-methylimidazolium acetate ([C$_{12}$mim]OAc), the procedure given by Ferguson et al. [36] was adapted, which includes the neutralization of the corresponding hydroxide ionic liquid with acetic acid to obtain the acetate counterion. Analytical data were in accordance with literature and details can be found in our previous work [27]. The synthesis of ionic liquids with ester-functionalization, namely [C$_{12}$bet]Cl and [C$_{12}$COmim]Cl, relied on the pre-formation of dodecyl 2-chloroacetate that was further reacted with trimethyl amine or methylimidazole according to literature protocols [37,38].

2.2.1. 3-(2-(Dodecyloxy)-2-oxoethyl)-1-methylimidazolium chloride ([C$_{12}$COmim]Cl)

1-Methylimidazol (1.19 g, 14.49 mmol) and dodecyl 2-chloroacetate (3.81 g, 14.49 mmol) were stirred at ambient temperature for 24 h. The obtained solid was crystallized from THF, collected via filtration and washed with anhydrous THF and diethyl ether. After drying in vacuum,
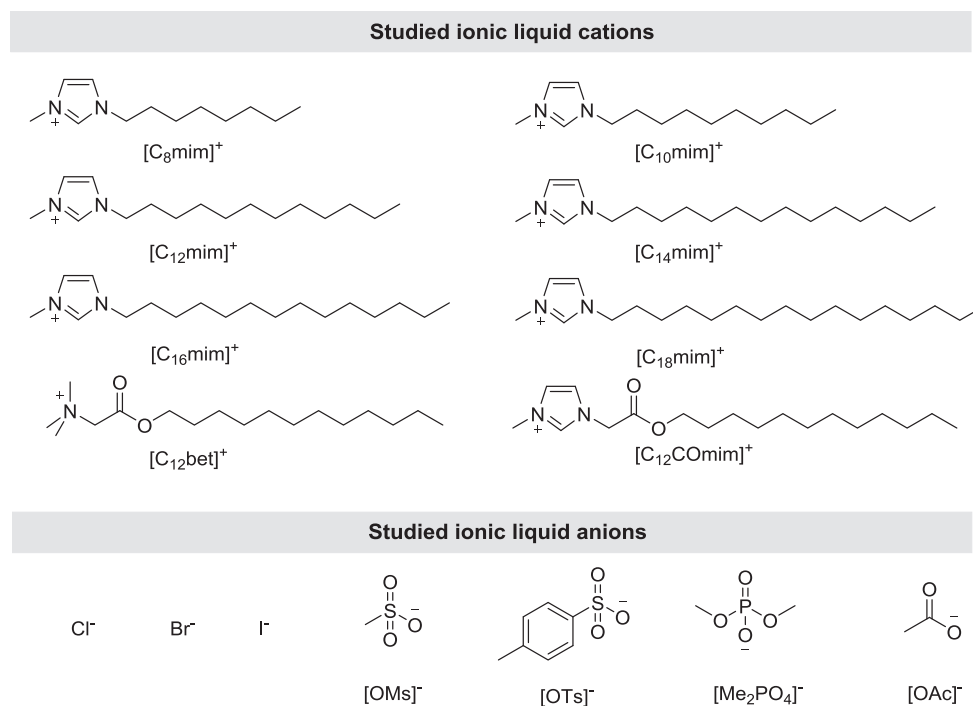
Fig. 2. Chemical structure and abbreviation of the ILs cations and anions used in this study.

a colorless solid was yielded in 81% (4.1 g). ^{1}H NMR (200 MHz, CDCl$_3$): δ_H = 0.86 (t, J = 6.36, 3 H, -CH$_2$-CH_3), 1.18–1.36 (m, 18 H, -O-CH$_2$-CH$_2$-(CH_2)$_9$-CH$_3$), 1.64 (quint, J = 6.55, 2 H, -O-CH$_2$-CH_2-(CH$_2$)$_9$-CH$_3$), 4.06 (s, 3 H, N-CH$_3$), 4.16 (t, J = 6.84, 2 H, -O-CH_2-CH$_2$-(CH$_2$)$_9$-CH$_3$), 5.47 (s, 2 H, N-CH$_2$-COO-), 7.40 (s, 1H, H-4), 7.51 (s, 1H, H-5), 10.58 (s, 1 H, H-2).

2.2.2. 2-(Dodecyloxy)-N,N,N-trimethyl-2-oxoethanaminium chloride ([C$_{12}$bet]Cl)

Dodecyl 2-chloroacetate (5.39 g, 20.51 mmol) was dissolved in 15 mL anhydrous THF. A solution of trimethylamine in THF (102.5 mmol) was added dropwise at room temperature. After stirring overnight, the precipitate was collected via filtration and washed with anhydrous THF and diethyl ether. After drying in vacuo (2×10^{-2} mbar) overnight, [C$_{12}$bet]Cl was obtained as colorless crystals in 88% yield. ^{1}H NMR (CDCl$_3$): δ = 0.81 (3H, t, J = 6.95, -C$_{11}$H$_{22}$-CH_3), 1.19 (18H, m, -C$_2$H$_4$-C$_9$H$_{18}$-CH$_3$), 1.58 (2H, t, J = 6.74, -CH$_2$-CH_2-C$_{10}$H$_{21}$), 3.60 (9H, s, N-(CH$_3$)$_3$), 4.10 (2H, t, J = 6.95, -CH_2-C$_{11}$H$_{23}$), 5.01 (2H, s, Cl-CH_2-CO).

2.3. Solubility studies

Oleanolic acid was added in excess amount to [C$_{12}$mim]Cl aqueous solutions (100, 500, 1000 mM) and to pure water. The mixture was then stirred under constant agitation (1000 rpm), at 80 °C $\pm$ 0.5 °C for 2 h (preliminary tests on the time required to achieve the equilibrium were carried out). After saturation of the aqueous solutions, and always assuring the presence of a solid phase and thus of oleanolic acid in excess, an aliquot of 200 µL was taken, mixed with 800 µL of methanol, filtered over a 0.2 µm syringe filter, and measured immediately via HPLC (*Method A*) using a previously established calibration curve. Results given are based on three independent experiments.

2.4. Extraction experiments

2.4.1. General extraction process using surface-active ionic liquids

A 8 mL screw-cap vial was charged with olive tree leaves (200 mg) and aqueous ionic liquid solution (1800 mg) in a concentration range varying from 100 to 1000 mM. The extractions were carried out with magnetic stirring (1000 rpm) and at different temperatures within $\pm$ 0.5 °C and times (25 °C/24 h or 80 °C/2 h).

After the extractions, the suspensions were centrifuged, and the supernatant was filtered using a 0.20 µm syringe filter. An aliquot of 200 µL was taken, mixed with 800 µL of internal standard solution (0.0464 mg 1-methylcyclohexene/mL of methanol) filtered over a 0.2 µm syringe filter and measured immediately via HPLC (*Method B*). Results given are based on three independent experiments.

2.4.2. General extraction procedure using ionic liquids under microwave irradiation

A 5 mL microwave vial was charged with olive tree leaves (200 mg) and aqueous ionic liquid solution (1800 mg), sealed with a Teflon septum and heated for 30 min at 80 °C under microwave irradiation (high absorption level) with magnetic stirring.

An aliquot of 200 µL was taken, mixed with 800 µL of internal standard solution (0.0464 mg 1-methylcyclohexene/mL of methanol), filtered over a 0.20 µm syringe filter, and measured immediately via HPLC (*Method B*). Results given are based on three independent experiments.

3. Results and discussion

Based on previous successful results on the extraction of hydrophobic substances from biomass using aqueous solutions of surface-active ionic liquids [27], herein we focused on the use of 1-alkyl-3-methylimidazolium-based ionic liquids combined with several anions, [C$_n$mim]X. Several studies already addressed the aggregation behavior in terms of critical micelle concentration [31,32], size and shape of aggregates over a broad concentration range [39,40] so that the physico-chemistry nature of 1-alkyl-3-methylimidazolium-based ionic liquids in water is relatively well explored and known. Therefore, we selected a set of N-methylimidazolium-based surface-active ionic liquids with the chloride anion and variable chain length, ranging from n = 8 to 16, for the extraction of oleanolic acid. In case of 1-dodecyl-3-methylimidazolium, the anion was optionally modified to study the impact of different anions, including other halides, sulfates,

Table 1
Solubility of oleanolic acid in aqueous solutions of the ionic liquid [C$_{12}$mim]Cl at variable concentrations.

Entry[a]	Concentration [C$_{12}$mim]Cl (mM)	Solubility (mg/mL)
1	0	n.d.[b]
2	100	2.828 ± 0.016
3	500	11.530 ± 0.039
4	1000	21.097 ± 0.234

[a] Performed with an excess amount of oleanolic acid added in [C$_{12}$mim]Cl aqueous solutions and in pure water at 80 °C ± 0.5 °C. Results are expressed as average ± standard deviation (STD), n = 3.

[b] Below the analytical equipment detection limit.

phosphonates or carboxylates on the extraction of oleanolic acid (Fig. 2). As the inherent toxicity and biodegradability of long-chain imidazolium salts might restrict their application, we also included in this study a surface-active betaine-derived ionic liquid [C$_{12}$bet]X with improved biodegradability [41].

Before addressing the extraction of oleanolic acid from native plant material, and in particular to infer the magnitude of the saturation of the target compound in aqueous solutions of ionic liquids, preliminary studies on the solubility of oleanolic acid in [C$_{12}$mim]Cl aqueous solutions were made. Pure oleanolic acid was added in excess to pure water and to aqueous ionic liquid solutions at different concentrations and stirred under fixed conditions. Due to the increasing viscosity of ionic liquid solutions at higher concentrations, we decided to work at a fixed temperature of 80 °C. The solubility of oleanolic acid in pure water could not be determined as it is below the detection limit of the used analytical equipment. This is in accordance with literature data, were extremely low values for the solubility of rather hydrophobic triterpenic acids in aqueous media are reported [42,43]. While no value is given for pure water, Jin et al. reported an oleanolic acid solubility of 1.4 ± 1.3·10^{-3} mg/mL in a 1 N aqueous solution of NaOH at 25 °C [42]. Similarly, Jäger et al. reported extremely low solubilities of oleanolic and betulinic acids in water (up to 2·10^{-5} mg/mL) [43]. Despite this low solubility in water, the solubility of oleanolic acid in a 100 mM aqueous solution of [C$_{12}$mim]Cl in water increased up to 2.83 mg/mL (Table 1, entry 3). A further increase on the ionic liquid concentration to 500 and 1000 mM drastically improves the solubility of oleanolic acid to a value of 21.10 mg/mL, indicating that aqueous solutions of surface-active ionic liquids lead to a remarkable increase (up to 10^6-fold) on the solubility of oleanolic acid, being thus able to compete with the solubilities afforded by molecular organic solvents [12]. A comparable increase in solubility was reported by Faria et al., who studied the solubility of ursolic acid in solutions of surface-active ionic liquids in water in a remarkable enhancement of solubility by 8
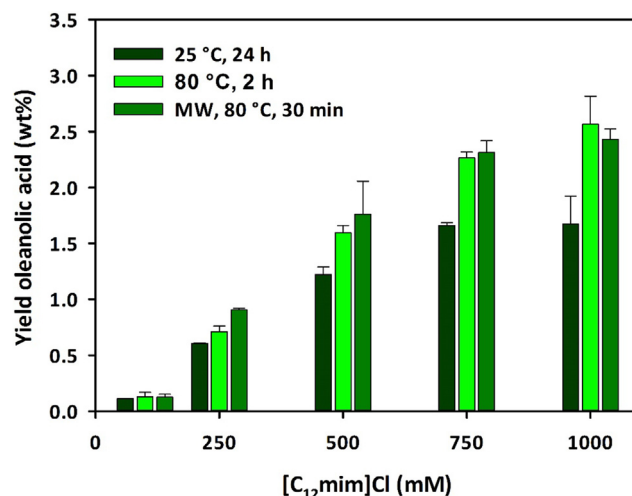

Fig. 4. Oleanolic acid extraction yield from olive tree leaves with aqueous solutions of [C$_{12}$mim]Cl and at different conditions (constant condition: ratio S/L = 1:10; d < 1 mm). Results are expressed as average extraction yield ± STD, n = 3. MW: microwave heating.

orders of magnitude [30].

Encouraged by these initial results of oleanolic acid solubility, we addressed the extraction of oleanolic acid as the main triterpenic acid occurring in the leaves of *O. Europaea* using aqueous solutions of ionic liquids, either via conventional extraction or assisted by microwave heating. After drying and milling of olive tree leaves to a particle size < 1 mm, we established an extraction procedure as outlined in Fig. 3.

The pre-processed olive tree leaves were suspended in the IL aqueous solutions at a fixed solid/liquid ratio of 1:10 (w:w). After extraction, the biomass was separated via centrifugation at 750 rpm. After filtration of the supernatant using a 0.2 μm syringe filter, the extract was diluted with methanol and directly analyzed via HPLC. Based on our previous experience in the analysis of pentacyclic triterpenes in the presence of ionic liquids, we relied on a reversed phase C-18 set-up for the quantification of oleanolic acid [44,45]. To prolong the column life time, sample obtained from biomass extractions were strictly measured on a reversed phase column equipped with protecting guard pre-column. The high polarity of alkylmethylimidazolium-based ionic liquids compared to triterpenes found in olive tree leaves allowed for a directly quantification of oleanolic acid in the crude extract as the ionic liquid was eluted at very short retention times of 2 min. Oleanolic acid was typically eluted at considerable higher retention times of approx.
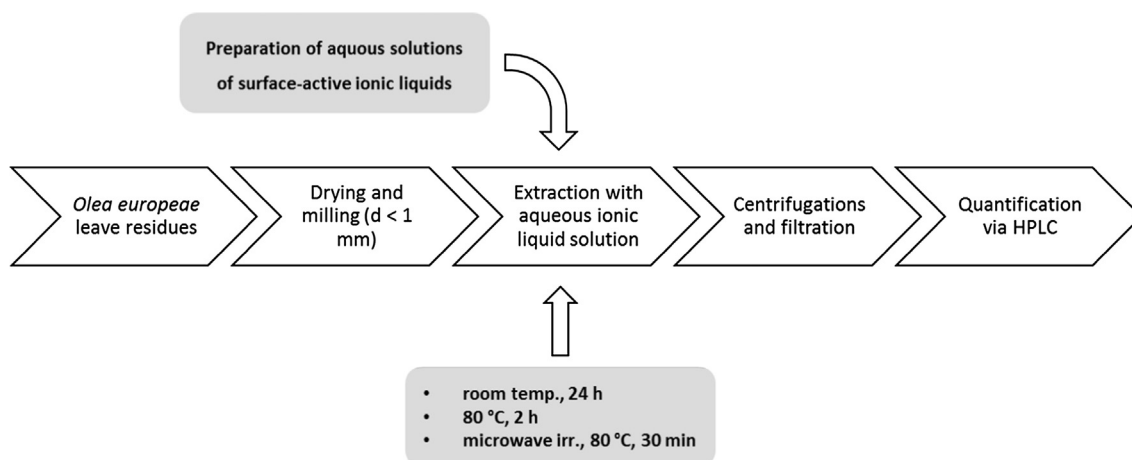

Fig. 3. Flow-scheme for the ionic liquid assisted extraction of oleanolic acid from *O. europeae* leaves using aqueous solutions.
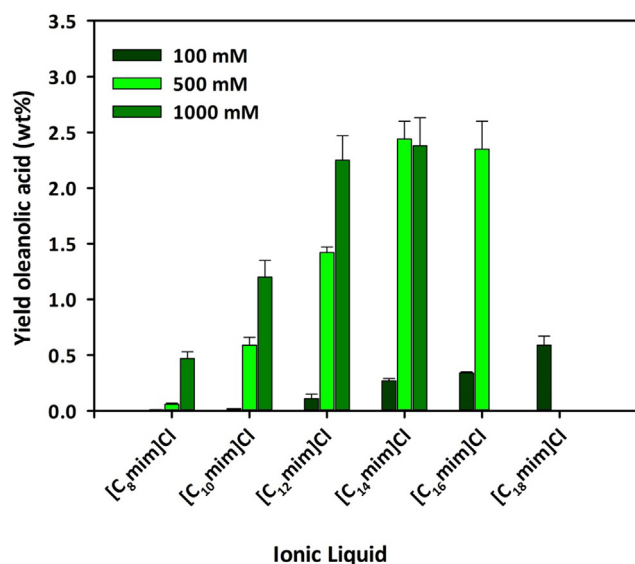
Fig. 5. Oleanolic acid extraction yield from olive tree leaves with different ILs, namely [C$_n$mim]Cl (n = 8, 10, 12, 14, 16, 18), at different concentrations and fixed conditions (T = 80 °C, t = 2 h, ratio S/L = 1:10, d < 1 mm). Results are expressed as average extraction yield ± STD, n = 3.
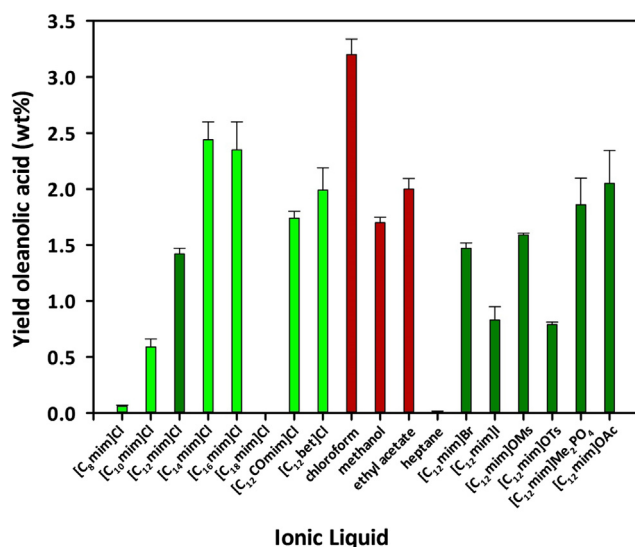

Fig. 6. Oleanolic acid extraction yield from olive tree leaves with several solvents at fixed conditions (T = 80 °C, [IL] = 500 mM, t = 2 h, ratio S/L = 1:10, d < 1 mm). Results are expressed as average extraction yield ± STD, n = 3.

15.5 min, and peak assignment was verified via standard addition of an authentic sample (see supplementary information).

The oleanolic acid extraction yield is expressed as weight percent (wt%) of extracted oleanolic acid per weight of pre-dried biomass. Initial studies were again performed with aqueous solutions of the surface-active ionic liquid [C$_{12}$mim]Cl over a concentration range of 100–1000 mM, which is well above the critical micelle concentration (CM) of the respective ionic liquid. As for the solubility studies, a strong influence of ionic liquid concentration was observed, with an increased ionic liquid concentration resulting in strongly improved extraction yields (Fig. 4). This is in good accordance with the data obtained in solubility studies, and points to an aggregate-mediated extraction mechanism.

These results show that significantly high amounts of oleanolic acid could be extracted with conventional heating at 80 °C, with an extraction time to 2 h. These strong improvements in extraction yield can be

explained by an increased solubility of oleanolic acid in the solvent, but also by the decrease of the viscosity of the aqueous solution at higher temperatures. As microwave-assisted extraction was already suggested as an useful method for the extraction of oleanolic acid and ursolic acid from plant materials, such as traditional Chinese herbs [46] we further investigated the influence of microwave irradiation on the extraction yield. Microwave-assisted extraction at 80 °C could further reduce the required extraction time, and up to 2 wt% of oleanolic acid could be obtained after 30 min. Independent of the chosen extraction parameters the concentration dependency was maintained, and the highest extraction yields were obtained with a 1000 mM of [C$_{12}$mim]Cl in water.

Since oleanolic acid can suffer speciation as a function of the pH (pKa = 4.74) [47], we further investigated the effect of pH over the extraction yields obtained, mainly to address if the target compound could be better extracted in its neutral or charged forms. A change of the pH value of the aqueous solution of ionic liquid from 3.13 to 9.6 did not influence the extraction yield (cf. the results shown in Fig. S1 in the supplementary information), meaning that electrostatic interactions between the charged oleanolic acid and ionic liquids do not play a major role on the extraction mechanism. We did not observe any trends when varying the pH value at a fixed ionic liquid concentration of 500 mM [C$_{12}$mim]Cl, and similar yields were obtained.

After optimizing extraction time and temperature, we addressed the impact of the ionic liquid structure. Fig. 5 shows the effect of the ionic liquid cation alkyl side chain length on oleanolic acid extraction yield. In general, there is an increase on the oleanolic acid extraction yield with the increase of the ionic liquid cation alkyl side chain length. However, from [C$_{12}$mim]Cl to [C$_{16}$mim]Cl, particularly at higher concentrations, no major differences on the extraction yields are observed. Furthermore, an increase of the ionic liquid concentration enhances the oleanolic acid extraction yield in the studied cases. However, some viscosity problems were found with long alkyl side chain ionic liquids at higher concentrations. In case of 1-alkyl-3-methylimidazolium-based ionic liquids, [C$_n$mim]Cl with n = 16 and 18, solutions at a concentration of 500 mM and/or 1000 mM became unstirrable after the addition of biomass at the given conditions; consequently, these results are missing in Fig. 5. Best yields were obtained with the surface-active ionic liquid 1-methyl-3-tetradecylimidazolium chloride, and up to 2.5 wt% of oleanolic acid could be extracted using a 500 mM aqueous solution.

The impact of the ionic liquid anion and of surface-active ionic liquids with improved biodegradability was further investigated at a fixed concentration of 500 mM (Fig. 6). The ionic liquid anion also influences the oleanolic acid extraction. In particular, acetate- and phosphate-based ionic liquids appear as promising candidates, whereas lower yields were found for surface-active ionic liquids with less hydrated anions, such as iodide or tosylate. This effect might be based on a different association of oleanolic acid to the cationic head groups in surface-active ionic liquids with different degrees of counterion binding [48], but also on strong hydrogen-bonding interactions established between oleanolic acid and anions with high hydrogen-bond basicity [49] although more investigations would be required to prove this.

For a better comparison with current state of art the results obtained with aqueous solutions of ionic liquids were compared with those obtained with organic solvents frequently used for the extraction of triterpenic acids, namely chloroform, ethyl acetate and methanol, under the same experimental conditions [50,51] as depicted in Fig. 6. Although extraction yields for oleanolic acid obtained with toxic chloroform are still slightly higher, it should be pointed out that aqueous solutions of ionic liquids are certainly a more environmentally-friendly option and can outperform the usually employed organic solvents, namely methanol, ethyl acetate and n-hexane while requiring considerably lower amount of ionic liquid as it would be the case with pure ionic liquids as extractants. It is also worthwhile to notice that the side-chain functionalized ionic liquids, and in particular the betaine derivative [C$_{12}$bet]Cl, are able to outperform methanol, ethyl acetate and n-
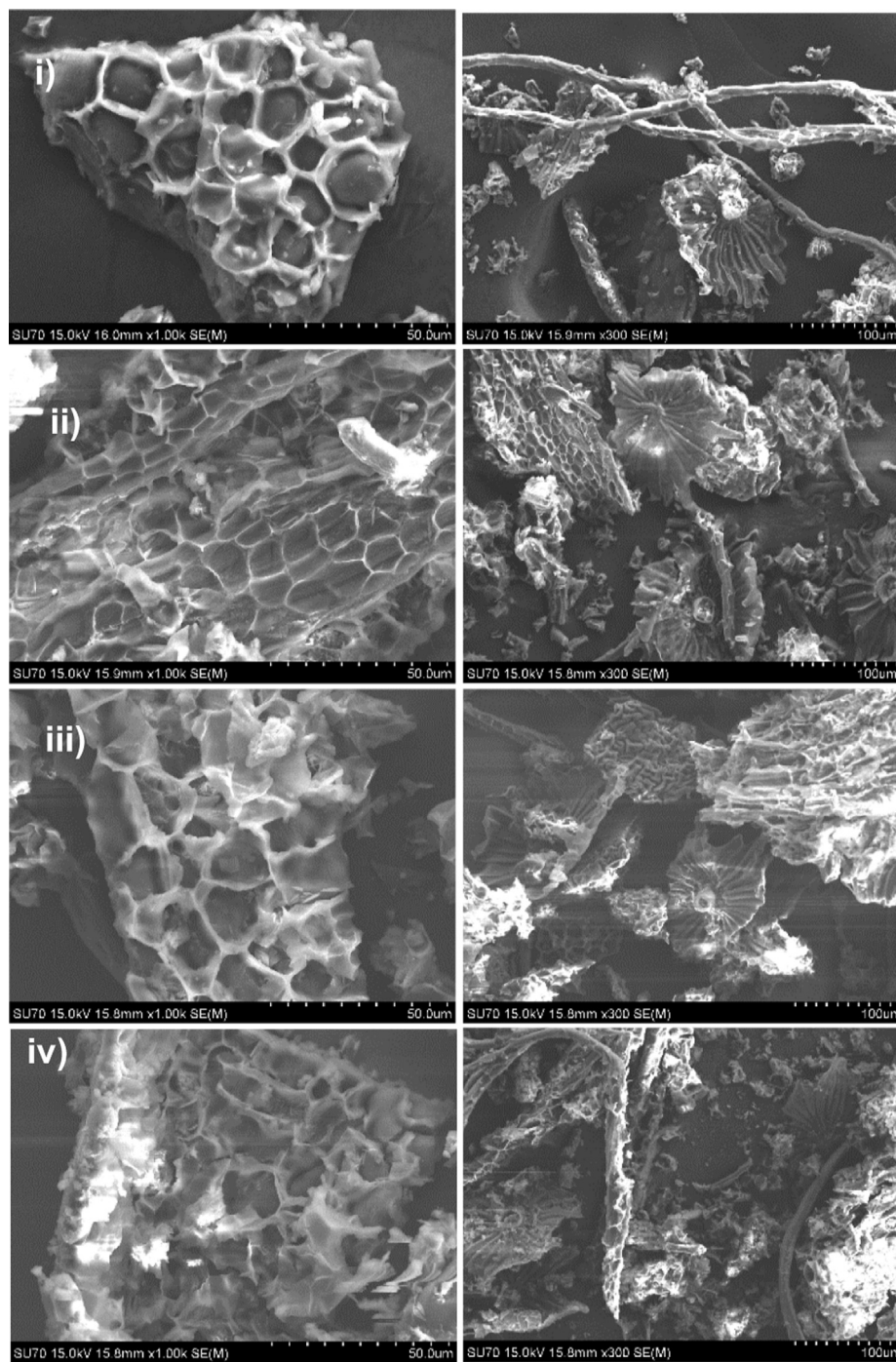
Fig. 7. SEM images of the olive leaves samples (i) without extraction; after extraction (T = 80 °C, t = 2 h, ratio S/L = 1:10, d < 1 mm) with (ii) water, (iii) an aqueous solution of [C$_{12}$mim]Cl at 500 mM and (iv) chloroform.

hexane and can compete with unfunctionalized imidazolium-based ionic liquids, thereby providing an attractive alternative and solution to the toxicity and biodegradability problems that are associated with long-chain imidazolium salts.

Finally, we investigated the residual biomass (after the extraction step) via scanning electron microscopy (see Fig. 7). Pictures were taken before and after extraction with water, ionic liquid solution or the conventional solvent chloroform, and compared. In general, small influence or modifications were found in the morphology of the biopolymeric matrix, independently of the extraction being performed with pure water, chloroform or ionic liquid solutions at 500 mM. This is in contrast to previous experiments, were a complete or partial

deconstruction and dissolution of biomass was observed, particularly when pure ionic liquids were used [52,53]. It seems that the use of aqueous solutions of ionic liquids can avoid the lignocellulosic matrix dissolution while still allowing the extraction of target compounds, which from the perspective of the recovery of the target compounds, in this case oleanolic acid, is an advantage given the lower complexity of the resulting extract.

4. Conclusions

In this work, we investigated the extraction of the high-value oleanolic acid from olive tree leaves, an agricultural by-product from

olive oil production, using aqueous solutions of surface-active ionic liquids. Initial studies on the solubility of oleanolic acid showed that the extremely low solubility of triterpenic acids in water can be drastically increased (up to 10^6 orders of magnitude) by the addition of surface-active ionic liquids, here confirmed with [C$_{12}$mim]Cl, pointing to an aggregation-mediated solubilization phenomenon.

After the solubility tests, aqueous solutions of various ionic liquids with surfactant activity were investigated for the extraction of oleanolic acid from olive tree leaves. The results obtained clearly confirm that aqueous solutions of ionic liquids are suitable alternatives for solid-liquid extractions of triterpenic compounds from biomass by-products. An increase in the ionic liquid concentration from 500 to 1000 mM and an increase in the side chain length of the surface-active ionic liquid to chain length, $\geq C_{12}$, resulted in higher extraction yields, although both effects lead to an increased viscosity of the aqueous solution not feasible to act as an adequate solvent. Variation of extraction parameters and ionic liquid structure allowed identifying optional extraction conditions. Under optimized conditions, namely 80 °C for 2 h, and with MW irradiation for 30 min up to 2.5 wt% oleanolic acid could be extracted using a 500 mM solution of [C$_{14}$mim]Cl at 80 °C. Eventually, this protocol provides an excellent strategy for the valorization of waste olive tree leaves prior to the burning for energy production, demonstrating that aqueous solutions of surface-active ionic liquids can be used as alternatives over conventional organic solvents.

Acknowledgements

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.seppur.2018.04.042.

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