

Aqueous Solutions of Surface-Active Ionic Liquids: Remarkable Alternative Solvents To Improve the Solubility of Triterpenic Acids and Their Extraction from Biomass

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Supporting Information

ABSTRACT: Triterpenic acids (TTAs) are well-known for their relevant biological properties and have been facing an increasing interest for nutraceutical and pharmaceutical applications. To overcome the concerns associated with the commonly used volatile organic solvents for their extraction from biomass, here we investigate the potential of aqueous solutions of ionic liquids (ILs) as alternative solvents. The solubility of ursolic acid (UA) was first determined in several aqueous solutions of ILs (hydrotropes or surface-active) at 30 °C for evaluation of the dissolution phenomenon. Conventional surfactants were also investigated for comparison purposes. The collected data reveal a remarkable enhancement in the solubility of UA (8 orders of magnitude) in surface-



active IL aqueous solutions when compared to that in pure water. Afterward, the potential of these IL aqueous solutions was confirmed by their use in the extraction of TTAs from apple peels. Total extraction yields of TTAs of 2.62 wt % were obtained using aqueous solutions of surface-active ILs at moderate conditions, overwhelming the extraction yields of 2.48 wt % obtained with chloroform and 1.37 wt % with acetone using similar conditions.

KEYWORDS: Biorefinery, Triterpenic acids, Solubility, Extraction, Surface-active ionic liquids, Apple peel

INTRODUCTION

It is well-known that there is a strong link between the consumption of fruits and vegetables and improved human health.^{1,2} Some compounds present in fruits and vegetables have a high potential to modulate many processes involved in the development of some diseases and degenerative disorders, including cancer,³ cardiovascular disorders,⁴ and diabetes.⁵ Among the vast plethora of bioactive natural compounds with the potential to improve human health are flavonoids, phenolic acids, carotenoids, tocopherols, alkaloids, lignans, tannins, and triterpenoids, among others.^{6,7}

Triterpenoids are a vast class of C-30 terpenic compounds, which can be classified into different groups depending on their carbon backbone, including, for instance, lupane, oleanane, and ursane-based compounds.⁸ Triterpenoids are widely distributed in medicinal and edible plants,⁹ and are part of the regular human diet because of their relevant health benefits.¹⁰ In the past few years, there has been a growing trend in the incorporation of triterpenoid-rich extracts in new functional foods, cosmetics, healthcare products, and drugs.^{9–11} To make

the application of these novel products possible it is required to have abundant natural sources of triterpenoids, as well as safe and cost-effective extraction techniques. Agro-food industry byproducts are an obvious resource for tackling this challenge, e.g., fruit peels which are rich in triterpenoids.¹² Apples are rich in triterpenoids, and particularly in triterpenic acids (TTAs), such as ursolic, oleanolic, and betulinic acids (Figure 1).¹³ In general, the occurrence of ursolic acid in apple peels is welldocumented;¹⁴ however, there is a growing list of other triterpenoids that are also present and still need to be fully characterized.^{10,15} According to the United Nations Food and Agriculture Organization (FAO) database, the global fruit production in 2013 was of 610 million tons, among which apples comprised 81 million tons, i.e., 13% of the total fruit production.¹⁶ Moreover, during the industrial processing of apples for the preparation of juices, jams, etc., large amounts of

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Figure 1. Chemical structures of some triterpenic acids present in apple peels: (a) ursolic, (b) oleanolic, and (c) betulinic acids.



Figure 2. Chemical structures of (a) cations and (b) anions comprising the ILs and (c) conventional surfactants used in this work.

residues are generated, such as apple peels, and thus, these are a promising raw material for the extraction of triterpenoid-rich extracts.

To obtain extracts rich in TTAs from apple peels, several extraction methods and solvents have been used, including extractions with ethanol (yields ranging between 0.01 and 1.3 wt %),¹⁷ with chloroform (yield of 0.7 wt %),¹⁸ and accelerated solvent extraction (ASE) with ethyl acetate (yields ranging between 0.2 and 2.1 wt %).⁹ All of these studies have been carried out with volatile organic solvents. Taking into account the envisaged application of TTA-rich extracts in nutraceutical and pharmaceutical products, there is a crucial demand to use safer and more biocompatible solvents, and to develop cost-effective extraction processes. Among the possible solvents, water appears as the greener and safer solvent overall; however, TTAs display negligible solubility in water.¹⁹ Therefore, aqueous solutions of ionic liquids (ILs) can be envisioned as

promising solvents if an increase in the solubility of TTAs and further extraction ability from biomass are verified.

ILs are organic salts with melting temperatures below 100 °C, typically composed of a large organic cation and an inorganic or organic anion.²⁰ Their chemical nature is responsible for a number of unique properties, such as negligible volatility and nonflammability at ambient conditions, and high thermal and chemical stabilities.^{21,22} Furthermore, physicochemical properties of ILs can be modulated by an adequate selection and combination of their ions, allowing the design of ILs with target properties.^{20,23} Because of these features, ILs display a good solvation capacity for a wide range of solutes and are well-known as potential substitutes of conventional organic solvents for the extraction of bioactive compounds from biomass.^{24,25} Despite the potential of pure ILs in the extraction of bioactive components from biomass, it was recently demonstrated that IL aqueous solutions display a

tremendous potential in this domain, in which they can act either as hydrotropes or as surface-active agents, promoting an increase in the solubility of bioactive compounds in aqueous media and favoring their extraction from raw materials.^{26–28} In addition, the use of IL aqueous solutions leads to foremost advantages since the IL consumption is reduced, and the viscosity of the extraction solvent is decreased, leading to enhancements in mass transfer phenomena and to a decrease in energy consumption.²⁵ Furthermore, IL aqueous solutions have also been shown to be advantageous since they are more selective to target compounds while avoiding the dissolution of the biomass lignocellulosic part.²⁵

TTAs (Figure 1) are aliphatic polycyclic structures with a low solubility in water.¹⁹ Therefore, in this work, we investigated the potential of IL aqueous solutions to increase the solubility of TTAs, using ursolic acid (UA) as a representative compound of this class, and further applied the most promising IL aqueous solutions in the extraction of TTAs (ursolic, oleanolic, and betulinic acids) from green apple peels. For comparative purposes, the solubility and extraction of TTAs using common surfactant aqueous solutions and organic solvents, respectively, were also addressed. To the best of our knowledge, no attempts have been previously reported in the literature on the use of IL aqueous solutions for the improvement of the solubility and extraction of TTAs from biomass.

EXPERIMENTAL SECTION

Materials. For inference of the molecular structure characteristics which enhance the solubility of TTAs in aqueous media, a large array of ILs was investigated (chemical structures shown in Figure 2): 1ethyl-3-methylimidazolium acetate, [C2C1im][CH3CO2]; 1-butyl-3methylimidazolium ethylsulfate, [C₄C₁im][C₂H₅SO₄]; 1-butyl-3-methylimidazolium octylsulfate, [C4C1im][C8H17SO4]; 1-butyl-3-methylimidazolium chloride, $[C_4C_1im]Cl;$ 1-butyl-3-methylimidazolium dicyanamide, $[C_4C_1im][N(CN)_2];$ 1-butyl-3-methylimidazolium thiocyanate, [C₄C₁im][SCN]; 1-butyl-3-methylimidazolium methylsulfate, [C₄C₁im][CH₃SO₄]; 1-butyl-3-methylimidazolium tosylate, $[C_4C_1 im][TOS];$ 1-butyl-1-methylpyrrolidinium chloride, $[C_4C_1 pyr]$ -Cl; 1-butyl-1-methylpyridinium dicyanamide [C₄C₁py][N(CN)₂], 1methyl-3-octylimidazolium chloride, [C8C1im]Cl; 1-decyl-3-methylimidazolium chloride, $[C_{10}C_1im]Cl;$ 1-dodecyl-3-methylimidazolium chloride, [C12C1im]Cl; 1-tetradecyl-3-methylimidazolium chloride, [C₁₄C₁im]Cl; 1-hexadecyl-3-methylimidazolium chloride, [C₁₆C₁im]-Cl; 1-octadecyl-3-methylimidazolium chloride, [C18C1im]Cl; and tributyltetradecylphosphonium chloride, [P444,14]Cl. The imidazolium-, pyridinium-, and pyrrolidinium-based ILs were purchased from Iolitec. The phosphonium-based IL was kindly offered by Cytec Industries Inc. All used ILs have a purity higher than 98 wt %, according to the information provided by suppliers.

In addition to ILs, conventional surfactants were also studied for comparison purposes (chemical structures depicted in Figure 2), namely, sodium dodecylsulfate (SDS) from Alfa Aesar, sodium dodecylbenzenesulfonate (SDBS) from Sigma-Aldrich, and hexadecyltrimethylammonium bromide (CTAB) from Fluka. All conventional surfactants have a purity higher than 99 wt %.

Ursolic acid (UA), oleanolic acid (OA), and betulinic acid (BA) standards, with a purity higher than 98 wt %, were acquired from Sigma. The solvents used for the extraction of TTAs, in addition to aqueous solutions of ILs and surfactants, included distilled water, and acetone and chloroform (purity \geq 99.99 wt %) from VWR Chemicals. The mobile phase used in the HPLC analysis was composed of methanol (purity \geq 99.99 wt %) from VWR Chemicals, and ultrapure water (purity \geq 99.99 wt %) from Merck, both HPLC grade.

Solubility of Ursolic Acid in Aqueous Solutions of ILs. While there is a large diversity of apple cultivars available for consumption and, consequently, a broad variation in their bioactive components composition,¹² ursolic acid (UA) is the most well-documented

triterpenic acid present in apple peels.¹⁴ Thus, we have chosen UA as a major representative of the TTAs class for carrying out screening studies of solubility in aqueous solutions of ILs, as well as in aqueous solutions of conventional surfactants for comparison purposes.

Pure UA (solid) was added in excess amounts to aqueous solutions of ILs and conventional surfactants of different concentrations (50, 250, 500, 750, and 1000 mM). Samples were kept under constant agitation with the use of the Eppendorf Thermomixer Comfort equipment at (30 ± 0.5) °C. Previously optimized equilibration conditions were established as follows: stirring velocity of 750 rpm and equilibration time of at least 72 h. At least two independent samples were prepared for determination of the average solubility value and respective standard deviation. After saturation of the aqueous solutions, always with the presence of a solid phase and thus of UA in excess, a 200 μ L aliquot was taken, diluted with 800 μ L of methanol, carefully filtered with the use of a 0.20 μ m syringe filter, and subsequently quantified with a GILSON HPLC unit coupled to an oven and with a manual injector, using a previously established calibration curve ($R^2 > 0.9990$). Data acquisition and evaluation were performed with Jasco-Borwin 1.21 software. An analytical C18 reversed-phase column (250 × 4.60 mm), Kinetex 5 μ m C18 100 A, from Phenomenex, was used. The mobile phase consisted of 87% (v/ v) methanol, 13% (v/v) water, and 0.1% (v/v) trifluoroacetic acid (TFA). Separations were conducted in isocratic mode, at a flow rate of 1 mL min⁻¹ and with an injection volume of 10 μ L. The wavelength was set at 210 nm. Each sample was analyzed at least two times. The column oven and the autosampler were operated at 30 °C. Illustrative HPLC chromatograms of standard TTAs and of an apple peel extract obtained using an aqueous solution of $[C_{14}C_1 \text{im}]Cl$ (500 mM) are provided in Figure S1 in the Supporting Information.

The pH of ILs and surfactants aqueous solutions was determined using a pH meter (Digimed, model DM21), previously calibrated with buffer solutions (pH 7.0 and pH 4.0, Reagent QM).

Because of the amphiphilic character of the studied ILs, and with the aim of better understanding the role played by surface-active ILs in solubility enhancement, the critical micellar concentration (CMC) of the studied ILs was determined by electric conductivity.²⁹ The conductivity of several aqueous solutions of different concentrations of IL was determined using a Russel RL105 conductivity meter at 25 °C, by continuous dilution of an IL concentrated solution in water. Each conductivity value was recorded when its fluctuation was less than 1% within 2 min.

Extraction of TTAs from Apple Peels Using Aqueous Solutions of ILs. Apple peels from Portuguese-origin golden apples were manually removed. The apple peels were dried at 25 $^{\circ}$ C for 2 days, and then they were ground with a commercial coffee grinder. The samples of ground apple peels were further divided and classified according to the particle size by means of stainless steel sieves. Samples with a diameter smaller than 1 mm were used.

Weighted amounts (with an uncertainty of 10^{-4} g) of ground apple peels were added to aqueous solutions of ILs with a concentration selected from the UA solubility studies, at a fixed solid–liquid ratio ($R_{s/\nu}$ weight of dried biomass per weight of the IL aqueous solution) of 0.1. The extractions were carried out at different temperatures (25, 50, 80, and 90 °C, within ±0.5 °C), with a fixed extraction time (60 min), and with constant stirring (1000 rpm), for determination of the effect of temperature on the extraction yield. A similar procedure was applied in the extractions with conventional organic solvents, in which the IL aqueous solution was replaced by organic solvents, however under reflux due to the lower boiling temperature of organic solvents. At least three independent extractions were carried out for each condition and solvent.

After the extraction step, the overall solution and extract were centrifuged, and the supernatant was filtered using a 0.20 μ m syringe filter. A 200 μ L aliquot was taken, mixed with 800 μ L of methanol, and filtered over a 0.2 μ m syringe filter, and the TTA content was determined by HPLC-DAD at 210 nm. Three major TTAs have been identified in the extracts, namely, oleanolic acid (OA), betulinic acid (BA), and ursolic acid (UA), according to the respective standards and retention-time values. Previous calibration curves have been

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established for each TTA ($R^2 > 0.9992$, 0.9995, and 0.9990, for oleanolic, betulinic, and ursolic acids, respectively). HPLC-DAD (Shimadzu, model Prominence) analyses were performed using the same column and conditions described previously in the solubility tests. The TTA extraction yield is expressed as the percentage ratio between the weight of TTAs and the total weight of the dried biomass.

RESULTS AND DISCUSSION

Solubility of Ursolic Acid in Aqueous Solutions of ILs. TTAs (Figure 1) are almost insoluble in water,¹⁹ and their extraction from biomass is usually carried out with volatile organic solvents.^{9,17,18} Aiming at improving the TTA solubility in aqueous-rich media and to favor their extraction from biomass, we first investigated the potential of IL aqueous solutions to increase their solubility, using ursolic acid (UA) as a representative compound of this class since it is the most well-documented tritterpenic acid present in apple peels.¹⁴ The solubility of ursolic acid was determined in several aqueous solutions of ILs, as well as aqueous solutions of conventional surfactants for comparison purposes, at concentrations of 50, 150, 250, 500, 750, and 1000 mM.

Although pure ILs have been described as potential solvents for the extraction of value-added compounds from biomass, e.g., betulin from birch bark,³⁰ aqueous solutions of ILs also display a high potential and additional advantages due to the use of lower amounts of IL as the solvent.²⁵ Aqueous solutions of ILs have additional benefits in terms of solvent toxicity and cost, and reduce the overall viscosity of the extraction media, thus enhancing the mass transfer phenomena and reducing energy consumption. In summary, whenever possible, IL aqueous solutions should be the preferred choice.²⁵ Upon consideration of compounds with a low solubility in water, such as TTAs, two classes of ILs can be selected for improvement of their solubility and extraction in aqueous media: ILs that act as hydrotropes²⁶ or as surfactants.²⁸ On the basis of this possibility, both classes of ILs were studied for an inference of the main IL structural characteristics which rule the solubility and extraction of TTAs. It should be remarked that it was not possible to determine the solubility of ursolic acid in pure water, as it was found to be below the detection limit of the used analytical method. Still, the water solubility of ursolic acid reported in the literature¹⁹ is 1.02×10^{-7} g L⁻¹, used as a reference in this work.

In a first approach, ILs that behave as hydrotropes^{26,31} were selected, namely, $[C_4C_1im][N(CN)_2]$, $[C_4C_1im][TOS]$, $[C_4C_1im][SCN], [C_4C_1im][C_2H_5SO_4], [C_4C_1py][N(CN)_2],$ and $[C_4C_1pyrr]Cl$, and tested in aqueous solutions in concentrations ranging from 50 to 1000 mM at 30 °C to dissolve ursolic acid. These ILs are composed of a butyl chain as the longest alkyl chain at the cation, and do not present a critical micellar concentration (CMC) nor are surface-active. With these ILs, and at different concentrations, the target TTA was not detected by HPLC analysis in any of the aqueous solutions, meaning that the solubility of UA is below the detection limit of the used analytical equipment and method (0.002 g L^{-1}) , as determined by us). Therefore, hydrotropy does not play a significant role in enhancing the solubility of TTAs in aqueous media. Aqueous solutions of hydrotrope ILs thus seem more valuable for enhancing the solubility of moderately hydrophobic compounds, such as phenolic acids, as previously reported.26

Subsequently, surface-active ILs, both cationic and anionic, were investigated. In particular, different ILs composed of long

alkyl side chains with known surface-active characteristics^{28,32} have been studied ($[C_nC_1\text{im}]X$ with n = 8, 10, 12, 14, 16, and 18 and X = Cl, $[C_8H_{17}SO_4]$, and $[P_{444,14}]$ Cl). Figure 3 shows



Figure 3. Solubility of UA in aqueous solutions of surface-active ionic liquids and conventional surfactants at different concentrations at 30 °C: (medium blue \bullet) [C₈C₁im]Cl, (brown \bullet) [P_{444,14}]Cl, (purple \bullet) [C₁₀C₁im]Cl, (yellow \bullet) [C₁₂C₁im]Cl, (green \bullet) [C₁₄C₁im]Cl, (red \bullet) [C₁₆C₁im]Cl, (pink \bullet) [C₁₈C₁im]Cl, (dark blue \bullet) [C₄C₁im]-[C₈H₁₇SO₄], (light blue ---) SDBS, (black ---) SDS, and (orange ---) CTAB.

the solubility data of UA at 30 $^{\circ}$ C in the different surface-active IL aqueous solutions in a 50–1000 mM concentration range; detailed data are provided in the Supporting Information (Tables S1 and S2). It should be remarked that some ILs were exploited up to lower concentrations because of the high viscosity obtained with more concentrated solutions and difficulties encountered in their handling for subsequent quantification. On the basis of the amphiphilic character of the studied ILs, we also determined their CMC values by conductivity, aiming at a better understanding of the role played by the IL regarding the dissolution mechanism which enhances the solubility of ursolic acid in aqueous media. These results are reported in the Supporting Information (Table S3).

In general, the addition of all the investigated surface-active ILs leads to an increase in the solubility of ursolic acid in aqueous solutions. The pH of almost all IL aqueous solutions is below the pK_a of UA ($pK_a = 4.90$),³³ meaning that UA is being solubilized in its protonated or neutral form. Thus, the gathered solubility data are a main result of the IL chemical structure and respective CMC and not of the solution pH. The pH detailed data combined with the solubility results are presented in the Supporting Information (Figure S2).

For most ILs, a monotonous increase was found in the solubility of the TTA along the IL concentration, whereas for $[C_{16}C_1\text{im}]Cl$ and $[C_4C_1\text{im}][C_8H_{17}SO_4]$, a maximum in the solubility was observed, occurring at 500 and 250 mM, respectively. This behavior is analogous to that observed with hydrotrope-based ILs, although for these the maximum solubility is observed at higher IL concentrations.²⁶ As also shown in Figure 3, an increase in the IL cation alkyl side chain leads to an increased capacity to solubilize UA in aqueous media. It is well-known that an increase in the IL alkyl side chain decreases the CMC and promotes the IL aggregation,³² as shown in the Supporting Information (Table S3) with the

CMC values determined in this work and respective comparisons with literature values, therefore supporting the higher capacity of ILs composed of longer alkyl side chains to solubilize UA. For instance, at 500 mM IL, the solubility of UA increases in the following order: $[C_{16}C_1 \text{im}]Cl (2.48 \text{ g L}^{-1}) >$ $[C_4C_1im][C_8H_{17}SO_4]$ (1.85 g L⁻¹) > $[C_{14}C_1im]Cl$ (1.66 g L⁻¹) > $[C_{12}C_1im]Cl (1.17 g L^{-1}) > [C_{10}C_1im]Cl (1.01 g L^{-1}) >$ $[P_{444,14}]Cl(0.37 \text{ g } \text{L}^{-1}) > [C_8C_1\text{im}]Cl(0.09 \text{ g } \text{L}^{-1}).$ This trend follows the CMC values of all the investigated ILs. However, an exception occurs with $[C_4C_1im][C_8H_{17}SO_4]$, since it presents a CMC (43.3 mM) between those displayed by $[C_{10}C_1 \text{im}]Cl$ (58.7 mM) and $[C_{12}C_1 \text{im}]Cl$ (15.2 mM), meaning that anionic surfactants may be promising options to enhance the solubility of UA in aqueous solutions. Although a maximum solubility of ursolic acid would be expected with $[C_{18}C_1 im]Cl$, as shown in Figure 3 for lower IL concentrations and according to its lower CMC, only a maximum concentration of 150 mM was used because of the high viscosity of $[C_{18}C_1 \text{ im}]Cl$ aqueous solutions and difficulties in handling such solutions for further quantification. Similar viscosity problems have been described by Ressmann et al.²⁸ with long-alkyl-chain ILs for the extraction of piperine from biomass.

Upon comparison of the solubility of UA achieved using an imidazolium-based ($[C_{14}C_1\text{im}]Cl$ (1.66 g L⁻¹)) IL with a phosphonium-based ($[P_{444,14}]Cl$ (0.37 g L⁻¹)) IL at 500 mM, it is clear that the presence of an aromatic ring in the structure of the IL cation is a relevant factor for an increase in the solubility of ursolic acid. According to these results, the role of the aromatic ring not only may be highly relevant to the increase in the solubility of UA in aqueous solutions, as demonstrated in Figure 3, but also will possibly influence the extraction process of TTAs.

Since a significant increase in the solubility of UA in surfaceactive IL aqueous solutions was observed, to truly confirm their potential, we further compared the obtained results with those gathered with aqueous solutions of conventional surfactants, such as sodium dodecylsulfate (SDS), sodium dodecylbenzenesulfonate (SDBS), and hexadecyltrimethylammonium bromide (CTAB). The obtained results are depicted in Figure 3. Although conventional surfactant aqueous solutions may lead to some competitive solubility data, these are limited by their lower solubility in water (if compared with ILs), and thus are more restricted in their ability to enhance the solubility of UA in aqueous media. A similar trend is expected with nonionic surfactants, and for this reason, this type of surfactants was not investigated in this work. Taking into account the surfactant molecular structures (Figure 1), the relevant role of anionic surfactants is evident, which is in agreement with the high performance discussed above with the IL $[C_4C_1im]$ - $[C_8H_{17}SO_4]$. Moreover, the presence of aromatic rings also appears as a relevant factor toward the enhancement of the solubility of UA in aqueous solutions. Overall, the obtained results emphasize the potential of IL aqueous solutions to solubilize poorly water-soluble compounds, such as TTAs.

Maximum solubility values of UA of 2.48 and 3.13 g L⁻¹ have been obtained with the two identified best ILs, namely, $[C_{16}C_{1}$ im]Cl and $[C_4C_1$ im] $[C_8H_{17}SO_4]$, respectively. If these values are compared with the solubility of the target compound in pure water $(1.02 \times 10^{-7} \text{ g L}^{-1})$,¹⁹ they represent an increase in the solubility of UA of 8 orders of magnitude. An increase in the solubility of UA up to 5 orders of magnitude has been obtained with volatile organic solvents, such as tetrahydrofuran, cyclohexane, and ethyl acetate,³⁴ and methanol, ethanol, and 2propanol.³⁵ This remarkable enhancement in solubility reveals the high potential of IL aqueous solutions as alternative solvents for the extraction of TTAs from biomass, as shown below.

This remarkable increment in the solubility of UA along the IL concentration (up to 8 orders of magnitude) can also be used as a way of recovering the target solutes from the ILwater solvent by a simple addition of water as an antisolvent. To test this hypothesis, we prepared aqueous solutions of $[C_4C_1im][C_8H_{17}SO_4]$ at 250 mM containing 2.5 mg mL⁻¹ of UA. At room temperature (ca. 25 °C) we added water under constant agitation to reach a concentration of IL down to 50 mM. During the addition of water, the precipitation of UA was macroscopically visible (cf. Figure S3 in the Supporting Information). This precipitate was recovered by filtration and washed several times with water at room temperature, with the aim of removing any traces of IL present. The precipitate was then dried up to constant weight at 50 °C, allowing the recovery of 89% of the initial UA added to the system. The IL can be further recovered by an evaporation step for removal of the excess water and thus recycled and reused. Despite the good performance of ILs to solubilize and extract value-added compounds from biomass, the isolation and purification of the target compounds from the IL-rich medium remains a challenge, mainly because of the inability to apply a simple solvent evaporation step due to the nonvolatile nature of the most studied aprotic ILs. With the aim of overcoming this drawback, some strategies have been proposed, including backextractions with organic solvents, precipitation with antisolvents, and the use of macroporous and ion-exchange resins.^{24,25} Herein, taking advantage of the remarkable solubility dependence of TTAs along the IL concentration, we can use water as an appropriate antisolvent, therefore making use of the overall greenest antisolvent.

Extraction of Triterpenic Acids from Green Apple Peels. After the previous screening on the ILs chemical features to enhance the solubility of TTAs in aqueous solutions, we selected $[C_{14}C_{1}\text{im}]Cl$, at 500 mM, to be used as an extraction solvent of TTAs from green apple peels. This IL and concentration were chosen since good solubility data for TTAs have been obtained (with values well above those that could be extracted from biomass taking into account the apple peel composition),¹³ and to work with IL aqueous solutions of lower viscosity. Moreover, it is generally accepted that the decrease in the extraction yields from biomass observed at higher IL concentrations mainly results from the increased solution viscosity which hinders an efficient solvent penetration into the plant tissues.^{25,28}

The extraction of TTAs from apple peels was carried out at 25, 50, 80, and 90 °C, while the other operational conditions were kept constant, namely, a biomass—solvent ratio of 1:10, an IL concentration of 500 mM, and 60 min of extraction time. Three TTAs have been identified by HPLC-DAD, namely, ursolic, oleanolic, and betulinic acids, according to a wide range of tested standards. The extraction yields of the three identified TTAs at the studied temperatures are shown in Figure 4.

Negligible yields have been obtained at 25 °C (below the analytical equipment detection limit), increasing from 50 to 80 °C, followed by a decrease at 90 °C. Therefore, temperature strongly influences the extraction yield of TTAs from biomass. Maximum extraction yields for the three identified TTAs have been obtained at 80 °C, namely, 0.079 wt % for UA, 0.90 wt % for BA, and 1.64 wt % for OA.



Figure 4. Extraction yields of TTAs [BA (green), OA (yellow), and UA (blue)] from green apple peels with $[C_{14}C_{1}\text{im}]Cl$ aqueous solutions at different temperatures and other fixed conditions ([IL] = 500 mM, t = 60 min, $R_{s/l} = 1:10$).

The increase in the extraction yield observed from 25 to 80 °C may result both from a solubility increment and from the solvent viscosity decrease at higher temperatures, thereby increasing both the target solute solvation and diffusion. The decrease in the extraction yields observed at higher temperatures may result from the codissolution of plant polysaccharides that turns the aqueous media into a gel, making the recovery of TTAs highly difficult, as discussed in other works.^{36,37} On the basis of the obtained results, and within the investigated temperature range, 80 °C is the best temperature for the extraction of TTAs from apple peels using [C₁₄C₁im]Cl aqueous solutions at 500 mM.

The TTA extraction yield using $[C_{14}C_{1}\text{im}]Cl$ aqueous solutions was then compared to those with common organic solvents well-described in the literature for the extraction of TTAs from biomass,^{18,38} such as acetone and chloroform, under reflux at 80 °C. The obtained results are shown in Figure 5.



Figure 5. Extraction yields of TTAs (BA, OA, and UA) from green apple peels with several solvents at fixed conditions (T = 80 °C, [IL] = 500 mM, t = 60 min, $R_{s/l} = 1:10$; organic solvents were used as pure and not as aqueous solutions).

 $[C_{14}C_{1}im]Cl$ aqueous solutions are particularly more efficient than the studied volatile and hazardous organic solvents for the extraction of OA. Upon comparison of the total amount of extracted TTAs while envisaging the preparation of TTA-rich extracts for incorporation in new functional foods, cosmetics, healthcare products, and drugs, $[C_{14}C_{1}im]Cl$ aqueous solutions appear as the most promising solvents, with a total extraction yield of TTAs of 2.62 wt %, when compared with 2.48 wt % obtained with chloroform and 1.37 wt % with acetone under similar conditions.

The interest in TTAs has increased over the past few years, as well as the number of extraction studies of these target compounds from a broad range of biomass sources (apple, grape,³⁶ tomato,³⁷ and olive³⁸), with several extraction methods (microwave,³⁹ maceration,⁴⁰ solid–liquid,³⁶ and supersonica-tion⁴¹) and different solvents (ethyl acetate,³⁶ *n*-hexane,¹⁵ and ethanol¹⁷). The results obtained in this work have been obtained with aqueous solutions of ILs, instead of the commonly used volatile organic solvents, under moderate temperatures and without using energy-intensive methods. Extraction processes based on aqueous solutions of ILs are thus potential platforms for enhancing the solubility and extraction of triterpenic acids and other value-added compounds present in biomass, performing even better than the pure ILs commonly investigated in the literature,²⁵ while avoiding the dissolution of the lignocellulosic fraction and allowing the recovery of richer value-added extracts. Moreover, on the basis of the solubility trends of TTAs along the IL concentration and the previously demonstrated induced-precipitation approach with the use of water as the antisolvent, the possibility of applying the same procedure is envisioned for the recovery of TTA-rich extracts from the IL-water solvent, and with which the IL can be recovered and reused after an evaporation step to remove the excess water.

CONCLUSIONS

In the past several years, there has been a growing trend in the incorporation of triterpenoid-rich extracts in new functional foods, cosmetics, healthcare products, and drugs. To make the application of these products possible, however, it is required to have abundant natural sources of triterpenoids, preferentially agro-food industry byproducts, as well as to use safer and more cost-effective extraction techniques. On the basis of these requirements, in this work, we investigated the potential of aqueous solutions of ILs as alternative solvents over the commonly used volatile organic solvents for the extraction of TTAs from biomass. Aiming at identifying the most promising IL aqueous solutions for the extraction of TTAs, we first addressed a comprehensive study based on the solubility of ursolic acid in aqueous solutions of ILs, allowing us to better understand the dissolution phenomenon and the IL chemical structure features which enhance the TTA solubility and further extraction yield. The collected data reveal that hydrotropy does not play a significant role in the improvement of the solubility of TTAs in aqueous media. However, surface-active ILs allow a remarkable increase in the solubility of ursolic acid in aqueous solutions, with an observed enhancement of 8 orders of magnitude upon comparison with its solubility in pure water. On the basis of the increase in the solubility of ursolic acid, a major representative of the TTA class, aqueous solutions of surface-active ILs were then tested in the extraction of TTAs from apple peels, allowing the simultaneous extraction of betulinic, oleanolic, and ursolic acids. A total extraction yield of

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TTAs of 2.62 wt % was obtained with the identified best conditions, overwhelming the total extraction yields of 2.48 wt % obtained with chloroform and 1.37 wt % with acetone (under the same conditions and determined in this work for comparison purposes). The obtained results clearly confirm that aqueous solutions of ILs are an improved alternative for the extraction of TTAs from biomass, representing a promising alternative over the commonly used volatile organic solvents.

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.7b01616.

Solubility values of ursolic acid, CMC values of the studied surface-active ILs, pH values of the IL aqueous solutions, extraction yields of TTAs from green apple peels, and chromatograms used for TTAs identification and quantification (PDF)

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Notes

The authors declare no competing financial interest.

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