Extraction and recovery processes for cynaropicrin from *Cynara cardunculus* L. using aqueous solutions of surface-active ionic liquids

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Abstract

Due to the wide range of relevant biological activities and high commercial value of cynaropicrin, and aiming at developing cost-effective processes, aqueous solutions of ionic liquids (ILs) were investigated for the extraction and recovery of cynaropicrin from the leaves of *Cynara cardunculus* L. Both cationic (1-alkyl-3-methylimidazolium chloride) and anionic (cholinium carboxylate) surface-active ILs were investigated, as well as a wide range of conventional surfactants and molecular organic solvents, allowing us to conclude that aqueous solutions of cationic surface-active ILs display a better performance for the extraction of cynaropicrin. Operational conditions were optimized, leading to a cynaropicrin extraction yield of 3.73 wt%. The recycling of both the biomass and the solvent were further investigated to appraise the extraction media saturation and to achieve a higher cynaropicrin extraction yield (6.47 wt%). Finally, it was demonstrated that 65 wt% of the extracted cynaropicrin can be efficiently recovered by precipitation from the IL aqueous extract through the addition of water as anti-solvent, allowing us to put forward both the extraction and recovery processes of the target value-added compound from biomass followed by solvent recycling. This approach opens the door to the development of more sustainable processes using aqueous solutions of ILs instead of the volatile organic solvents commonly used in biomass processing.

Keywords Cynaropicrin · Biomass · Extraction · Recovery · Ionic liquids · Aqueous solutions

Introduction

Cynara cardunculus L. (Asteraceae) is a Mediterranean plant species comprising three varieties, namely var. *syvestris* (L.) Fiori (wild cardoon), var. *scolymus* (L.) Fiori (globe artichoke), and var. *altilis* (DC) (cultivated cardoon) (Wiklund 1992). Of these, cultivated cardoon has been largely explored due to its fleshy stems and leaf petioles. This variety is used in regional dishes in European

countries (Gatto et al. 2013), as a source of aspartic proteinases for milk clotting during ewe cheese manufacturing (Verissimo et al. 1995), and used in traditional medicine (Koubaa et al. 1999). In addition to these more conventional uses, several industrial applications involving cardoon have also been considered, namely in pulp and paper production (Gominho et al. 2009), power generation and heating (Fernandez et al. 2006), among others.

C. cardunculus species are known for the bitter taste of their leaves, which is mainly due to the presence of guaianolide-type sesquiterpenes lactones, among which cynaropicrin (Fig. 1) is the most abundant, accounting for ~80% of the characteristic bitterness of the plant (Ramos et al. 2013). *C. cardunculus* extracts are of commercial interest due their hepatoprotective effect, and anti-inflammatory, antispasmodic, proapoptotic and antitrypanosomal properties (Cho et al. 2000, 2004; Emendörfer et al. 2005; Zimmermann et al. 2010). Therefore, more fundamental investigations, particularly regarding the extraction and recovery of bioactive compounds (mainly cynaropicrin) from cardoon, have been explored, envisaging their use in nutraceutical

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Fig. 1 Chemical structure of cynaropicrin

formulations (Kumar et al. 2017; Ramos et al. 2013). Considering the biological activity of cynaropicrin, together with its abundance in *C. cardunculus* leaves (up to 87 g/kg) (Ramos et al. 2013) and its high current commercial value, it is of utmost interest to develop more effective, environmentally friendly and economically viable extraction and recovery methods. Currently, cynaropicrin is mainly extracted from different types of biomass using volatile and often toxic organic solvents, such as chloroform (Bhattacharyya et al. 1995) and dichloromethane (Ramos et al. 2013), leading to several human risks and safety issues and to a poor environmental performance. Other methods used include the extraction of cynaropicrin with water at elevated temperatures (Bernhard 1982), and with supercritical CO₂ (Sovová et al. 2008), or related mixtures making use of co-solvents (Kaminskii et al. 2011), to improve the extraction yields. These methods have some disadvantages since they require several hours of extraction, are energy-consuming, may require the use of more sophisticated equipment and result in low cynaropicrin yields (Bernhard 1982; Bhattacharyya et al. 1995; Kaminskii et al. 2011; Sovová et al. 2008). Therefore, in the past decade, much interest has been devoted to the development of more cost-efficient and sustainable solvents and methods for the extraction of value-added compounds from biomass, which, apart from supercritical CO₂, comprises mainly ionic liquids (ILs) and, more recently, deep eutectic solvents (DES) and their natural counterparts (NADES) (Paiva et al. 2014; Passos et al. 2014; da Ponte 2017).

Within alternative solvents, ILs are among the most studied, particularly for the extraction of bioactive compounds from biomass, as summarized in recent reviews and books on the subject (Bogdanov 2014; Bogel-Lukasik 2016; Passos et al. 2014; Ventura et al. 2017). For instance, ILs have been used in the extraction of shikimic acid from *Illicium verum* (Ressmann et al. 2011) or from *Ginkgo biloba* leaves (Usuki et al. 2011), in the isolation of tannins from *Acacia catechu* (catechu) and *Terminalia chebula* (myrobolan) (Chowdhury et al. 2010), in the dissolution of suberin from *Quercus suber* (cork) (Garcia et al. 2010), and in the extraction of artemisinin from *Artemisia annua* (Bioniquis 2006, 2008). In addition to the extraction

and dissolution studies, some attempts at product recovery from the IL solution have also been carried out, by means of back-extraction steps using organic solvents, precipitation with anti-solvents, and use of resins, among others (Passos et al. 2014). A recent study focused on the use of more benign ILs that could be used together with the biomass-rich extracts, avoiding the need of the recovery step (Ferreira et al. 2017). In summary, given the proven efficiency of ILs as powerful solvents for the extraction of value-added compounds from several biomass sources (Passos et al. 2014; Ventura et al. 2017), and since their unique properties can be tailored by adequate cation–anion combinations, ILs are excellent alternatives to dissolve and/or extract target value-added compounds from natural resources (Passos et al. 2014).

In addition to pure ILs, recent studies have demonstrated that their aqueous solutions, or hydrated ILs, are more efficient for the extraction of bioactive components from biomass, by applying ILs with surface-active (Ressmann et al. 2013) or hydrotropic behavior (Cláudio et al. 2013). The high efficiency achieved using hydrated ILs has been related to the enhanced solubility of biomolecules in aqueous solutions of ILs when compared with their solubility in the respective pure solvents (Passos et al. 2014; Ventura et al. 2017). The concept of hydrated IL systems has been exploited to improve the biomolecule stability and biological activity of, e.g., enzymes (Fujita et al. 2007; Ohno et al. 2015), and as extraction media for target compounds from biomass, such as in the extraction of caffeine from guaraná seeds (Cláudio et al. 2013), of piperine from white pepper (Cao et al. 2009), and of 7-hydroxymatairesinol from Norway spruce knots (Ferreira et al. 2017), among others (Passos et al. 2014). More recently, it has been demonstrated that aqueous solutions of ILs can provide an impressive improvement in the solubility and further extraction of triterpenic acids from apple peel (Faria et al. 2017). With aqueous solutions, there is a decrease on the ILs consumption (which are replaced by water, the greenest solvent overall) and on the solvent viscosity, thus enhancing the mass transfer and reducing the energy consumption, resulting in more low-cost and environmentally friendly processes (Passos et al. 2014).

Previous investigations have demonstrated that some ILs can form aggregates in aqueous solution (Miskolczy et al. 2004), being known as surface-active ILs. This is the case of long-chain 1-alkyl-3-methyl-imidazolium chloride salts, [C_nmim]Cl with *n* = 8–18 (Łuczak et al. 2008). However, and despite their potential, only a few studies have explored the extraction of bioactive compounds from biomass using surface-active ILs, either pure or in aqueous media (Ferreira et al. 2012, 2013; Huang et al. 2014; Lin et al. 2012; Ressmann et al. 2013; Vieira et al. 2017; Wu et al. 2009; Yu et al. 2015; Zhou et al. 2015). Based on this,

in the present study we have chosen to investigate aqueous solutions of surface-active ILs for the extraction of cynaropicrin from the leaves of cultivated cardoon.

Different families of ILs were evaluated, namely surface-active 1-alkyl-3-methyl-imidazolium chloride and cholinium carboxylate salts, allowing us to explore the potential of both cationic and anionic surfactants. Organic solvents and aqueous solutions of conventional surfactants were also investigated for comparative purposes. After identifying the most promising IL, the process variables for the solid–liquid extraction were optimized, namely the solid–liquid ratio (S/L ratio, weight of dried biomass per weight of solvent), the temperature (T), the time of extraction (t) and the concentration of the IL. Finally, based on the cynaropicrin solubility dependence on the IL concentration, its recovery was accomplished using water as an anti-solvent, allowing us to put forward both the extraction and recovery processes.

Experimental

Chemicals and materials

Two families of surface-active ILs have been investigated, namely 1-alkyl-3-methylimidazolium chloride (acting as cationic surfactants) and cholinium carboxylate (acting as anionic surfactants) salts, i.e. 1-octyl-3-methylimidazolium chloride, $[C_8mim]Cl$, 1-decyl-3-methylimidazolium chloride, $[C_{10}mim]Cl$, 1-dodecyl-3-methylimidazolium chloride, $[C_{12}mim]Cl$, 1-tetradecyl-3-methylimidazolium chloride, $[C_{14}mim]Cl$, cholinium octanoate, $[Ch][C_8CO_2]$, cholinium decanoate, $[Ch][C_{10}CO_2]$, and cholinium dodecanoate $[Ch][C_{12}CO_2]$. In addition to these, 1-ethyl-3-methylimidazolium chloride, $[C_2mim]Cl$, 1-butyl-3-methylimidazolium chloride, $[C_4mim]Cl$, and 1-butyl-3-methylimidazolium dicyanamide, $[C_4mim][N(CN)_2]$, were investigated to confirm the relevance of surface-active ILs and to address the possible potential of hydrotropic ILs (Cláudio et al. 2013) to extract the target compound. All imidazolium-based ILs were purchased from Iolitec (99% purity), while the cholinium-based ILs were synthesized by us according to well-known protocols (Sintra et al. 2015). The three cholinium-based carboxylates investigated were synthesized by the neutralization of $[Ch]OH$ with octanoic, decanoic and dodecanoic acids. The reaction mixtures were stirred overnight at room temperature, under nitrogen atmosphere, and protected from light, resulting in the cholinium carboxylate with water as by-product. Unreacted acids were eliminated by washing steps with ethyl acetate, which, together with the water, was then removed under reduced pressure at 60 °C. Finally, the obtained ILs were dried under high vacuum for at least 48 h at

50 °C. After this purification step, the purity of all ILs was confirmed by 1H and ^{13}C NMR and shown to be ≥ 98 wt%.

In addition to ILs, aqueous solutions of conventional cationic, anionic and nonionic surfactants were used for comparison purposes, namely cetyltrimethylammonium bromide (CTAB), sodium dodecylsulfate (SDS), cetyltrimethylammonium chloride (CTAC), cetylpyridinium chloride (CPC), sodium dodecylbenzenesulfonate (SDBS), and Genapol C-100. All conventional surfactants were acquired from Fluka with a purity higher than 99.0 wt%. The chemical structures of the ILs and conventional surfactants investigated are shown in Fig. 2.

Organic solvents, namely acetone and dichloromethane, with a purity of ≥ 99.99 wt%, and n -hexane with a purity of 95 wt%, all acquired from Sigma-Aldrich, were also tested as extraction media.

Dried *C. cardunculus* L. leaves, packed under vacuum, with a granulometry between 40 and 60 mesh, were supplied by the Experimental Center of Agriculture School of the Polytechnic Institute of Beja, Southern Portugal. The cynaropicrin standard was purchased from Extrasynthesis, with a purity ≥ 97.5 wt%.

Determination of critical micellar concentration (CMC)

Aiming at better understanding the role played by surface-active ILs in the extraction process, the critical micellar concentration (CMC) of the studied ILs, when unavailable in the literature (Bai et al. 2008; Łuczak et al. 2008, 2009; Rengstl et al. 2014; El Seoud et al. 2007; Smirnova and Safonova 2012), was determined by electric conductivity (Inoue et al. 2007). The conductivity of several aqueous solutions of different concentrations of IL was determined by continuous dilution of an IL concentrated solution in water using a Russel RL105 Conductivity Meter at 25 °C. Each conductivity value was recorded when its fluctuation was less than 1% within 2 min.

Quantification, extraction and recovery of cynaropicrin

S/L mixtures were prepared with *C. cardunculus* L. leaves and aqueous solutions of ILs at different concentrations (100, 250, 500, 750, and 1000 mM) in closed glass vials. The mixtures were kept under controlled stirring and temperature (± 0.5 °C). To optimize the extraction yield of cynaropicrin, different temperatures (25, 35 and 45 °C), extraction times (30, 40, 50, 60, 120, 180, 300 and 1440 min) and S/L ratios (1:10, 1:20, 1:30 and 1:40, weight of dried biomass per weight of solvent, considering a fixed biomass weight of 0.25 mg) were investigated, while keeping the stirring speed constant (1000 rpm). A similar procedure was applied in the extraction using

Fig. 2 Chemical structures of ionic liquids and conventional surfactants tested in the extraction of cynaropicrin

conventional organic solvents and conventional surfactants, in which the IL aqueous solutions were replaced by these solvents. A sample of *C. cardunculus* L. leaves (5 g, dried weight) was also Soxhlet-extracted with dichloromethane (150 mL) for 7 h for comparison purposes.

After the extraction step, the mixtures were centrifuged, and the supernatant filtered using a 0.20- μ m syringe filter. Before HPLC injection, a 200- μ L aliquot was taken from the filtered supernatant, diluted with 800 μ L of methanol, and filtered again. The identification and quantification of the extracted cynaropicrin was performed by HPLC-DAD (Shimadzu, model PROMINENCE) using an analytical C18 reversed-phase column (250 $\times$ 4.60 mm), kinetex 5- μ m C18 100 A from Phenomenex. The mobile phase consisted of 75% (v:v) of water and 25% (v:v) of acetonitrile. The separation was conducted in isocratic mode, at a flow rate of 0.5 mL $\cdot$ min $^{-1}$, using an injection volume of 10 μ L. DAD was set at 198 nm. The column oven and the autosampler were operated at a constant temperature (30 $^{\circ}$ C). The quantification of cynaropicrin was based on a calibration curve previously established under the same conditions, with a cynaropicrin concentration ranging between 4.98×10^{-3} and 4.98 mg/mL, $R^2 = 0.9993$. The cynaropicrin extraction yield is expressed as the percentage ratio between the total weight of extracted

cynaropicrin and the total weight of the dried biomass. The quantitative results presented correspond to the average of at least three independent experiments.

To infer the maximum amount of cynaropicrin in the *C. cardunculus* L. leaves and on solvent saturation effects, six successive extraction cycles with the same biomass and new aqueous solutions of IL, and four successive cycles of extraction using the same solvent and fresh biomass, were carried out. After the four extraction cycles with the reuse of the same aqueous solution of IL, leading to the solvent saturation, the recovery of cynaropicrin by induced precipitation was evaluated using water as an anti-solvent. To this end, different amounts of water were added in order to decrease its solubility/saturation; thus, 0.25 mL of the IL-water solution used in the extraction experiments were diluted in 2.5 and 5 mL of distilled water. After the addition of water, the solution was centrifuged at 6000 rpm for 30 min, and vacuum-filtered with a 0.45- μ m microporous membrane. The solid residue was dried in an air oven at 50 $^{\circ}$ C for 2 days. A sample of the precipitated cynaropicrin was dissolved in methanol for identification and quantification by HPLC-DAD. The quantification of the residual cynaropicrin present in the IL aqueous solution after the dilution step was additionally determined.

Results and discussion

Effect of the ILs structural features and operational conditions

The remarkable potential of ILs as solvents in biomass processing is mainly due to their tunable properties, enabling them to dissolve a wide variety of compounds of different hydrophobicity, as well as to swell and dissolve raw biomass which may lead to an improved access to the valuable ingredients embedded in the biopolymer matrices (Bogdanov 2014; Passos et al. 2014). In addition to the use of pure ILs, the use of aqueous solutions of ILs can avoid the cellulosic matrix dissolution while still allowing the extraction of target compounds (Ressmann et al. 2011; Wang et al. 2012), e.g., cynaropicrin. Moreover, a higher selectivity is usually obtained with aqueous solutions of ILs, as well as improvements in the mass transfer phenomenon and energetic consumption due to a decrease on the overall solvent viscosity (Passos et al. 2014).

Knowing that cynaropicrin has a hydrophobic character ($\log K_{ow} = 1.08$) and is thus poorly water-soluble (774.6 mg/L at 25 °C) (Chemspider 2017), it is expected to achieve a good extraction performance using aqueous solutions of surface-active ILs. To confirm this hypothesis, we investigated the cynaropicrin extraction with aqueous solutions of both cationic, with $[C_n\text{mim}]\text{Cl}$ ILs ($n = 8–14$), and anionic surface-active ILs, with $[\text{Ch}][C_n\text{CO}_2]$ ($n = 8–12$). In the same line, i.e. to prove that surface-active ILs are really among the most relevant options and that aqueous solutions of ILs that act as hydrotropes ($\text{Cláudio et al. 2013}$) are not the most adequate solvents for cynaropicrin extraction; aqueous solutions of $[C_2\text{mim}]\text{Cl}$, $[C_4\text{mim}]\text{Cl}$ and $[C_4\text{mim}][\text{N}(\text{CN})_2]$ were also investigated.

An initial screening with $[C_4\text{mim}]\text{Cl}$ and $[C_{14}\text{mim}]\text{Cl}$ at different concentrations (100, 500 and 1000 mM) was carried out in order to address the impact of using surface-active versus hydrotropic ILs and the most relevant concentrations which lead to high extraction yields of cynaropicrin; the results are shown in Fig. 3. An increase in the concentration of $[C_4\text{mim}]\text{Cl}$, from 100 to 1000 mM, leads to an increase in the cynaropicrin extraction yield, from 0.83 to 1.19 wt%. On the other hand, with the surface-active $[C_{14}\text{mim}]\text{Cl}$, the extraction yield goes through a maximum (3.18 wt%), occurring at 500 mM of IL. However, a decrease in the yield above 500 mM of IL was observed, which might be due to an increased viscosity of the solution at higher IL concentrations, which is particularly relevant for ILs with longer alkyl side chains, thus hindering the mass transfer phenomena.

These results allowed us to select the 500 mM concentration to screen the potential of different ILs in the extraction of cynaropicrin. The operational conditions used in the ILs screening were: S/L ratio of 1:10, extraction time of 60 min,

Fig. 3 Weight fraction percentage of cynaropicrin extracted from the leaves of *C. cardunculus* L. with several ILs at different concentrations: 100 mM (orange), 500 mM (blue), 1000 mM (red) ($R_{S:L} = 1:10$, $T = 25$ °C and $t = 60$ min). The results correspond to the average of three independent experiments with standard deviations <5%

and temperature of 25 °C (Fig. 3). The detailed results are given in the Electronic Supplementary Material (Table S1). As shown in Fig. 3, higher extraction yields are obtained with aqueous solutions of surface-active ILs (mainly $[C_n\text{mim}]\text{Cl}$ ILs, with $n = 8–14$) over those obtained with aqueous solutions of hydrotropic ILs ($[C_2\text{mim}]\text{Cl}$, $[C_4\text{mim}]\text{Cl}$ and $[C_4\text{mim}][\text{N}(\text{CN})_2]$). Although aqueous solutions of hydrotropic ILs have high potential to extract less hydrophobic compounds, such as alkaloids and phenolic acids ($\text{Cláudio et al. 2013, 2015}$), when dealing with more hydrophobic compounds, such as cynaropicrin, aqueous solutions of surface-active ILs are known to exhibit a better extraction performance (Faria et al. 2017).

With surface-active cationic ILs ($[C_n\text{mim}]\text{Cl}$ ILs, $n = 8–14$), there is an increase in the cynaropicrin extraction yield with the decrease of the ILs CMC (Ressmann et al. 2013). Cationic ILs with longer alkyl side chains and lower CMC values are more effective solvents for the extraction of the target compound. On the other hand, the results obtained also show that anionic surface-active ILs ($[\text{Ch}][C_n\text{CO}_2]$), even with CMC values of the same order of magnitude as those of $[C_n\text{mim}]\text{Cl}$ ILs (ranging from 4 to 383 mM), display the lowest cynaropicrin extraction performance (yield <0.12 wt%). The CMC values of the surface-active ILs investigated were taken from the literature (Bai et al. 2008; Łuczak et al. 2008, 2009; Rengstl et al. 2014; El Seoud et al. 2007; Smirnova and Safonova 2012) and determined in this work when not available (see the Electronic Supplementary Material with detailed data in Table S2).

In summary, these results clearly demonstrate the higher capability of cationic surface-active ILs, and in particular of $[C_{14}mim]Cl$ at 500 mM, to extract cynaropicrin, in which a maximum extraction yield of 3.18 wt% was obtained. Based on these results, we then studied the influence of different concentrations of $[C_{14}mim]Cl$ in aqueous solution, ranging from 0 to 1000 mM, on the cynaropicrin extraction yield. The results obtained are shown in Fig. 4a, whereas the detailed results are provided in the Electronic Supplementary Material (Table S3).

At low concentrations (5 mM) of $[C_{14}mim]Cl$ and close to the CMC (4 mM) (Bai et al. 2008) of this IL, the cynaropicrin extraction yield is 0.69 wt%, which is similar to the value obtained with pure water (0.67 wt%) under the same conditions. However, as the CMC of $[C_{14}mim]Cl$ is overwhelmed, a significant increase in the cynaropicrin extraction yield is observed. The efficiency continuously increases with the IL concentration, but seems to reach a maximum around 500 mM, above which the extraction yield of cynaropicrin starts to decrease. This maximum in the extraction yield along the IL concentration is most probably due to the solution viscosity, as mentioned above and as reported in the literature (Passos et al. 2014).

After optimizing the concentration of the $[C_{14}mim]Cl$ aqueous solution, we proceeded to the optimization of the extraction temperature (Fig. 4b), performing extractions at

25, 35 and 45 °C, keeping the remaining conditions constant, namely a S/L ratio of 1:10, a concentration of 500 mM, and an extraction time of 60 min. The detailed results are given in the Electronic Supplementary Material (Table S4). As shown in Fig. 4b, an increase in temperature is not favorable for cynaropicrin extraction. Although an increase in cynaropicrin extraction with temperature would obviously be expected, the observed reduction in the recovery of this compound is most probably due to the dissolution of polysaccharides at higher temperatures, as previously reported with this type of biomass using other solvents (Fernandes et al. 2015), and given the extremely viscous solutions obtained after the extraction process at higher temperatures. However, this aspect can be seen as an advantage since good extraction yields of cynaropicrin are obtained at temperatures close to room temperature, without requiring additional energetic inputs.

The extraction time was also varied between 30 and 1440 min, keeping the remaining variables constant (S/L ratio of 1:10, a concentration of IL of 500 mM, and a temperature of 25 °C). The influence of this variable towards the extraction yield of cynaropicrin is illustrated in Fig. 4c (detailed data are provided in the Electronic Supplementary Material, Table S5). An increase in the extraction time shows a positive effect on the cynaropicrin extraction efficiency, up to 60 min with a maximum yield of cynaropicrin of 3.13 wt%. Although a small decrease in the extraction yield is observed between

Fig. 4 Optimization of the operational conditions on the yields of cynaropicrin extracted from *C. cardunculus* leaves using aqueous solutions of $[C_{14}mim]Cl$ at several conditions, namely: **a** concentration of IL in aqueous solution (IL); **b** temperature; **c** extraction time, and **d** solid–liquid ratio. The results correspond to the average of three independent experiments with standard deviations <5%

60 and 120 min, there is a dramatic decrease in the yield from 120 min onwards. This may again be associated with the dissolution of biomass polysaccharides in the IL aqueous solution, as discussed above for the temperature increase effect, becoming more relevant for longer extraction periods. In fact, aqueous solutions of high viscosity have been obtained for extraction times longer than 120 min. Similar effects with an increase in the extraction time have been observed in the extraction of several value-added compounds from biomass using different solvents (Tang et al. 2014; Trendafilova et al. 2010).

If the saturation of the solvent is reached, the extraction yield usually increases with the decrease of the S/L ratio. However, an increase in the volume of the solvent used further results in more expensive and less sustainable processes, in which higher amounts of solvents need to be recycled or disposed (Qi et al. 2015). To address this issue, the S/L ratio was finally optimized and the data obtained are shown in Fig. 4d, whereas the detailed results are provided in the Electronic Supplementary Material (Table S6). S/L ratios of 1:10, 1:20 and 1:30, keeping the remaining conditions constant, namely an IL concentration of 500 mM, a temperature of 25 °C, and an extraction time of 60 min, were tested. The results obtained (Fig. 4d) show a positive effect on the cynaropicrin yield when increasing the amount of the solvent (liquid), reaching a maximum extraction yield of 4.09 wt%. However, this increment is more relevant between 1:10 and 1:20, while after that and up to a 1:40 S/L ratio, the increase in the extraction yield is not significant enough to justify the use of larger volumes of solvent (taking into account the economic and environmental impact of the process). In this perspective, and considering the amounts of solvent used in the 1:20 and 1:40 ratios, and the slight differences in the extraction yields obtained (3.73 and 4.09 wt%, respectively), the whole process becomes more feasible with a S/L ratio of 1:20. Among the studied variables in the optimization of the extraction process, the S/L ratio was found to be the variable with the weakest influence on the extraction yield of cynaropicrin.

The results showed here clearly confirm the remarkable ability of aqueous solutions of surface-active ILs, namely $[C_{14}mim]Cl$, to extract cynaropicrin from the leaves of *C. cardunculus* L. To maximize the extraction yield of cynaropicrin under more sustainable conditions, a concentration of IL of 500 mM, a temperature of 25 °C, an extraction time of 60 min, and a S/L ratio of 1:20, should be adopted. To demonstrate the high potential of ILs aqueous solutions as alternative solvents, and particularly the relevance of the surface-active $[C_{14}mim]Cl$ IL, we further carried out the extraction of cynaropicrin from *C. cardunculus* L. leaves using several conventional surfactants, namely cationic (CPC, CTAC and CTAB), anionic (SDS and SDBS) and nonionic

(Genapol C-100) ones, with or without aromatic rings, in concentrations ranging between 10 and 500 mM, under the previously optimized operational conditions. The results obtained are shown in Fig. 5 and in the Electronic Supplementary Material (Table S7).

The best results were obtained with $[C_{14}mim]Cl$ followed by CPC aqueous solutions. In general, a relevant influence of the cationic/anionic nature of the surfactant and of the presence of aromatic rings towards the extraction of cynaropicrin from *C. cardunculus* L. leaves was found. This is in agreement with the results obtained with the two classes of ILs investigated and discussed above. However, the most relevant observation is that the surface-active $[C_{14}mim]Cl$ leads to the highest cynaropicrin extraction yield, notably 36% higher than that obtained with the best conventional surfactant (CPC). These results thus demonstrate the remarkable potential of surface-active ILs on the cynaropicrin extraction from *C. cardunculus* L. leaves.

Finally, the extraction of cynaropicrin from *C. cardunculus* leaves using water, acetone, *n*-hexane and dichloromethane under the same conditions, as well as by Soxhlet extraction with dichloromethane, was performed for comparison purposes (detailed data are provided in the Electronic Supplementary Material, Table S8). The maximum yield of cynaropicrin achieved was 8.65 wt% by Soxhlet extraction, in agreement with previously published values (Ramos et al. 2013). The higher yields obtained with this technique, when compared to solid–liquid extractions with the same solvent (4.53 wt%) can be explained by the specific conditions enabled by the Soxhlet technique (high temperature, time and number of extraction cycles with the use of fresh solvent) that

Fig. 5 Cynaropicrin extraction yields obtained with aqueous solutions of conventional surfactants and $[C_{14}mim]Cl$ at fixed conditions: S/L ratio = 1:20, $t = 60$ min, $T = 25$ °C. SDS, Genapol and CTAB were not applied at 500 mM due to their lower water solubility. The results correspond to the average of three independent experiments with standard deviations <5%

allows reaching a maximum extraction capacity. Regarding the use of pure water, *n*-hexane and acetone under the same conditions used with the IL aqueous solutions, these lead to substantially lower extraction yields (0.68, 0.04 and 0.35 wt%, respectively, vs. 3.73 wt% obtained with the IL aqueous solution). Although dichloromethane stands out as the best evaluated solvent for the extraction of cynaropicrin (4.53 wt%), the competitive results obtained with $[C_{14}mim]Cl$ aqueous solutions (3.73 wt%) support their high potential as alternative solvents to extract this compound from biomass without requiring the employment of volatile organic solvents. Moreover, low concentrations of ILs and moderate temperatures and time are used, with no significant energetic requirements.

Recovery process for cynaropicrin from biomass

After the identification of the optimum extraction conditions, we turned our attention towards the development of scaled and more sustainable extraction/recovery processes. To this end, we addressed the use of the same biomass in consecutive extractions with “fresh” solvent samples (for six cycles) to appraise the maximum amount of cynaropicrin that can be extracted, and the use of the same $[C_{14}mim]Cl$ aqueous solution using new biomass samples (for four cycles) to infer the solvent saturation. The detailed data are provided in the Electronic Supplementary Material (Table S9; Fig. S2).

The first test allowed the determination of the maximum amount of cynaropicrin that could be extracted from the same biomass; according to the results, a total extraction yield of 6.47 wt% after six extraction cycles was obtained using fresh IL–water samples. The sum of the first three recycles of biomass led to a higher cynaropicrin extraction yield than that obtained with dichloromethane, namely 6.33 versus 4.53 wt%, under the same extraction conditions, yet lower than that obtained by Soxhlet extraction.

Tests with the same aqueous solution of $[C_{14}mim]Cl$ for cynaropicrin extraction from new biomass samples were also performed. Detailed results are given in the Electronic

Supplementary Material (Table S10, Fig. S3). At the end of each extraction, the mixture was immediately filtrated in order to remove the biomass residue and to reuse the recovered IL–water solution. This process was repeated four times. The results obtained show that there is an increase in the extraction yield in each new cycle, from 3.73 to 4.16 wt%. However, this increment is of low significance, meaning that the solvent almost reaches saturation in the first step of extraction under the optimized operational conditions.

Taking advantage of the solvent saturation and of the effect of the IL concentration on the solubility/extraction of cynaropicrin, it is possible to envision a recovery process based simply on the addition of water, which will act as an anti-solvent. To confirm this hypothesis, at the end of the extraction process, the saturated solution was filtrated to remove solid biomass residues. The supernatant was then diluted with water in two different ratios (1:10 and 1:20 v:v), leading to the formation of a solid residue that was recovered by filtration. Detailed data are given in the Electronic Supplementary Material. The HPLC analysis of the remaining aqueous solution, while taking into account the dilutions carried out, allowed the confirmation of the precipitation of 65% of the extracted cynaropicrin. Cynaropicrin thus recovered by filtration and the surface-active IL can be used again after an evaporation step to remove the excess water, falling within an integrated biorefinery approach.

In summary, cynaropicrin can be recovered from the IL aqueous solutions by the addition of water as an anti-solvent, inducing the precipitation/recovery of 65 wt% of the target compound, and further allowing solvent recycling. An example of a DAD-HPLC chromatogram of the recovered extract is shown in the Electronic Supplementary Material. Although pure cynaropicrin was not obtained, a fact that only occurs if totally selective solvents could be identified, the obtained extracts may contain other valuable components, as reported in previous studies (Ramos et al. 2013, 2014). These cynaropicrin-rich extracts can be directly used in nutraceutical and cosmetic applications, as shown in the literature (Ferreira et al. 2017), without the need to obtain pure cynaropicrin.

Fig. 6 Schematic representation of the extraction and recovery process for cynaropicrin from biomass using aqueous solutions of surface-active ILs

Figure 6 depicts the developed process for the extraction and recovery of cynaropicrin from biomass. Although ILs have been described as greener replacements for harmful volatile organic solvents, numerous studies have demonstrated that this group of solvents, depending on their chemical structure, can be less, equally or even more toxic than commonly used organic solvents (Bubalo et al. 2017). Nevertheless, ILs display negligible vapor pressures, and at least losses to atmosphere will not occur as happens with conventional organic solvents. Moreover, aqueous solution of ILs are being proposed as remarkable solvents over pure ILs, being water the greenest solvent overall, thus contributing towards the sustainability of the proposed process and opening new perspectives for the development of biorefinery approaches based on the use of aqueous solutions of ILs as alternative and more efficient solvents.

Conclusions

Aqueous solutions of surface-active ILs were shown to be efficient extraction media for cynaropicrin from *C. cardunculus* L. leaves and can effectively replace commonly used organic solvents. Based on the cynaropicrin extraction profile, it was demonstrated that the extraction yield depends on the IL concentration and respective CMC, and that cationic surface-active ILs have a better performance as extraction solvents.

Several operational conditions were optimized to improve the extraction of cynaropicrin: the best conditions were obtained with a concentration of 500 mM of $[C_{14}mim]Cl$, a temperature of 25 °C, an extraction time of 60 min and a S/L ratio of 1:20, allowing to obtain a cynaropicrin extraction yield of 3.73 wt%, a significantly higher value than that obtained with pure water, aqueous solutions of conventional surfactants or organic solvents under the same conditions. The recovery of the target compound by the addition of water as an anti-solvent has been shown, as well as the recycling of the biomass and of the solvent, thus leading to the development of more sustainable extraction processes.

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Compliance with ethical standards

Conflict of interest Emanuelle L. P. de Faria declares that she has no conflict of interest. Melissa V. Gomes declares that she has no conflict of interest. Ana Filipa M. Cláudio declares that she has no conflict of interest. Carmen S. R. Freire declares that she has no conflict of interest. Armando J. D. Silvestre declares that he has no conflict of interest. Mara G. Freire declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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