Perspective Article

Selective extraction of high-added value carboxylic acids from aqueous fermentative effluents with new hydrophobic eutectic solvents (HES)

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ABSTRACT

Summary: Recovering these carboxylic acids from the fermentative streams in a sustainable, green, and economical way is a significant challenge. This work assessed hydrophobic eutectic solvents (HES) – water-immiscible – for the selective recovery of carboxylic acids via liquid–liquid extraction. Different trioctylphosphine oxide (TOPO) mixtures with menthol and thymol were studied and deeply characterized by ^1H and ^{31}P NMR to yield stable eutectic solvents, including a novel experiment of ^{31}P NMR at variable temperatures for the first time. Those stable eutectic solvents were tested in the liquid extraction of complex aqueous mixtures containing C_2–C_6 carboxylic acids and simple sugars (glucose and xylose). The back-extraction of the carboxylic acids for the recovery of the HES was optimized, being necessary in three stages for the complete cleaning of the eutectic solvent using NaOH 0.1 M. The eutectic mixture of TOPO and thymol in a molar ratio of 1:2 exhibited an overall recovery of C_5 and C_6 carboxylic acids over 70 %, allowing its selective extraction from the rest of the compounds in the complex mixture. Likewise, this HES (after back extraction) was successfully reused in a second extraction cycle, keeping the performance of the fresh one. Therefore, this study demonstrated that HES can have a high extraction selectivity for carboxylic acids of $\geq C_5$. Moreover, these solvents were stable and allowed reusability, reducing the environmental impact and process costs.

1. Introduction

Waste accumulation is one of the major environmental problems facing the world today, contributing dramatically to global warming. Sustainable and affordably managing solid wastes (SW) is one of the challenges of the 21st century and one of the critical responsibilities of a city government [1]. In this context, transitioning to a circular economy where wastes are valorized and returned to the production cycle as raw materials and/or products is imperative. Consequently, it is essential to change the mentality and consider waste as a complementary raw material from which a wide range of industrial products can be manufactured, replacing their analogs synthesized from fossil feedstocks. Wastes of biological origin (biowastes) are of great interest since they represent a high percentage of the overall waste generation. Currently, a significant fraction of these biowastes is landfilled without any valorization and, in the best scenario, are composted or anaerobically digested. These alternatives are considered of low value and need to take advantage of the high potential of these resources. Future approaches will prioritize the valorization of organic wastes into biomaterials rather than compost and energy in the framework of the biorefinery concept [2].

Biorefineries combine innovative technologies to produce biofuels, high-added-value products, and bioenergy. The carboxylate platform is gaining significant interest compared to thermochemical (pyrolysis, gasification, and hydrothermal treatments) and sugar (enzymatic fermentation towards alcohol products) platforms. In the carboxylate approach, an anaerobic fermentation process (either dark fermentation or acidogenic fermentation) can produce carboxylic acids as building blocks to generate further high added-value products. The carboxylate platform comprises organic acids with at least one carboxyl group and up to 20 carbon atoms. The carboxylate platform has the advantages of being resilient and environmentally sound, having higher product yields [3], and being industrially applicable and scalable [4,5]. Carboxylic acids with carbon atoms equal to or greater than 5 ($\geq C_5$) have been found as one of the most interesting chemical products since can be replacing its source from fossil-C to waste by-products and could be used for producing a wide range of high-added value products [5,6]. While carboxylic acids of fewer carbon atoms (<C5), which can be distilled at atmospheric pressure, are common carboxylic acids, mainly acetic (C_2), propionic (C_3), butyric (C_4), and their isomers [7]. Carboxylic acids of higher carbon atoms ($\geq C_5$) are suitable for a wide range of applications, including the production of bioplastics [5,8–10], bioenergy [5,10,11], and even hydrogen [5,10,11]. Once the carboxylic acids have been produced through fermentative processes, a key challenge is the selective extraction and purification of the ($\geq C_5$) carboxylic acids since they

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have a higher added value in comparison with ($<C_5$) carboxylic acids [12]. These aqueous fermentation effluents also contain a mixture of sugars, oligomers, and other compounds, making this separation more difficult [13,14]. Even if these acids are produced by non-contaminant methods [15,16], their extraction and purification are imperative for the correct assembly of the new waves of biorefineries [17].

Various separation and purification techniques have been traditionally used, such as crystallization [18], membrane filtration [19] or pervaporation [20]. However, Liquid-Liquid Extraction (LLE) might be an interesting alternative for separating carboxylic acids from aqueous fermentative effluents using suitable and more effective extraction results[21]. It works under mild conditions without clogging problems and hence has lower energy requirements than the purification mentioned above techniques.

Despite this, the conventional method of using a single solvent to extract organic acids has certain limitations. Sub-optimal extraction efficiency, phase separation problems and environmental pollution result from the overuse of solvents such as ethyl acetate and methyl *tert*-butyl ether. Although alternative solvents such as tri-n-butyl phosphate (TBP) and tri-n-octylamine (TOA) are often used for organic acid separation due to their hydrophobicity and high extraction efficiencies, these solvents are typically toxic and flammable [22,23].

One of the most studied solvents in LLE has been ionic liquids (ILs); their performance has been dramatically improved compared to the above-mentioned methods and solvents [24]. They have chemical stability, negligible vapor pressure, and low flammability from strong electrostatic forces between ions [25]. However, ionic liquids are expensive to produce, the synthesis of most ionic liquids is not "green", and they are unavailable in industrial quantities [26]. Other kinds of solvents, denoted as "green solvents", are deep eutectic solvents (DES), which have been reported to be a more sustainable option for several applications [27]. Unlike ILs, they are easy to prepare from readily available materials at high purities and are inexpensive compared to ILs [28]; they also have low volatility, wide liquid range, and non-flammability [28].

Water-immiscible hydrophobic eutectic solvents (HESs) are a new type of solvents introduced in the literature for the first time in 2015 [29]. Among the five types of eutectic solvents reported [30], the type V (hydrophobic) is the only one that shows immiscibility in water, a valuable property to be used as an extracting agent in aqueous effluents, thus simplifying their purification. HESs are formed by hydrogen bonds between a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA). Derived from these interactions, they exhibit a highly structured liquid phase that plays a crucial role in interacting with some metals [31] and carboxylic acids [32]. The strong molecular interactions between these components depress the mixture's melting point, leading to a liquid phase at room temperature [30]. HESs are typically easy to synthesize and store and cost low; DESs are extensively applied in electrochemistry, organic synthesis, catalysis, and extraction [22,32]. They are comparable to conventional organic solvents in terms of, the economic feasibility of their use [27] and physical properties, chemical stability and negligible volatility [33]. The effectiveness of HESs for extracting carboxylic acids has been demonstrated by other authors [13,26,29,34,35], but the selective extraction in a complex mixture has not been already reported, so it is necessary to be optimized.

This study aims to develop novel hydrophobic eutectic solvents (HESs) tailored specifically for the selective extraction of carboxylic acids ($\geq$C5) from aqueous solutions under mild conditions. The research focuses on optimizing the composition of HESs, with a particular emphasis on incorporating natural-source terpenes as hydrogen bond acceptors (HBAs), thereby creating a solvent with a partially natural origin. Natural compounds such as terpenoids and terpenes are being explored as potential candidates for this purpose due to their inherent hydrophobic properties. They are abundant and diverse plant-based compounds, readily sourced from various botanical materials including fruits and aromatic plants. Among these, notable examples

include thymol, menthol, and geraniol. Despite their renewable characteristics, studies of their use as eutectic solvent compounds are scarce, and most of the cases focus on the extraction of lipids from microalgae [13] or even biomedical applications [33]. These components possess desirable characteristics such as biodegradability, relatively low cost, and less solubility in water [22]. In this work, thymol and menthol are employed as HBA agents [13,36] and trioctylphosphine oxide (TOPO) as HBD [37].

Liquid-liquid extraction with pure TOPO has already been studied for the extraction of carboxylic acids [38,39], but this molecule is in a solid state at room temperature that causes it to work at high temperatures to make it liquid; note that its melting point is 50–52 °C. Selective extraction of ($\geq$C$_5$) carboxylic acids from an aqueous solution has been proven in the literature using the DES formed by TOPO as HBD but using as HBA other petroleum-based compounds as kerosene [40,41], oil lamp [40] or Aliquat 336 [42], being all toxic and with hazardous properties. So, using terpenes as menthol and thymol combined with TOPO allows it to work at mild conditions, enhancing the selectivity towards the high-value carboxylic acids of the mixture treated.

Particular focus will be placed on the HES characterization to understand their formation and interaction with carboxylic acids through NMR techniques, including a novel experiment of ^{31}P NMR at variable temperatures. A model-fermentation aqueous solution containing a mixture of sugars and carboxylic acids has been evaluated to prove the concept. The reusability of the optimized HES has also been studied in two successive extraction cycles. This work constitutes a precedent of a non-toxic and environmentally friendly method to extract selectively carboxylic acids with a high added value from fermentative aqueous effluents.

2. Materials and experimental methodology

2.1. Materials

All chemicals were used as received without further purification from the supplier Sigma Aldrich. Trioctylphosphine oxide (TOPO) $\geq$ 99.0 %, Thymol $\geq$ 98.5 %, and Menthol 99 % were used to synthesize the HES. Acetic acid 99.0 %, Propionic acid $\geq$ 99.0 %, Butyric acid $\geq$ 99.0 %, Valeric acid $\geq$ 98.0 %, Hexanoic acid $\geq$ 99.0 %, Fumaric acid $\geq$ 99.0 %, Glucose $\geq$ 99.0 % and Xylose $\geq$ 99.0 % were used to prepare synthetic mixtures of carboxylic acids.

2.2. Synthesis and characterization of HES

In this study, six mixtures were synthesized with a different molar ratio of TOPO to menthol and thymol: 1TOPO:1Thymol (1T:1T), 1TOPO:2Thymol (1T:2T), 2TOPO:1Thymol (2T:1T), 1TOPO:1Menthol (1T:1M), 1TOPO:2Menthol (1T:2T) and 2TOPO:1Menthol (2T:1M). Thymol and menthol were selected because of their availability, natural source, and good performance in extracting single components [33]. The following synthesis method was a thermal procedure as described in literature [25]. The temperature selected was 40 °C, and if the mixture did not reach the liquid state, the temperature was raised to a maximum of 100 °C, and the temperature was kept for 1 h. Afterward, the resultant mixtures were allowed to cool naturally at room temperature and kept isolated from the light to prevent degradation for 24 h. Those mixtures that remain in a liquid state after cooling and storing for 24 h were selected for the extraction assays. Otherwise, they were discarded. Because of the aqueous nature of the extraction feed phase, it is essential to ensure that HES is hydrophobic.

To demonstrate the hydrophobic nature of the synthesized hydrophobic eutectic solvents (HESs), they were subjected to a one-hour mixing with water, followed by decantation. Subsequently, the water content in each HES was measured using the Karl-Fisher titration method. The results showed values of $<$1 g/L of water, indicating the immiscibility of HESs in water.

Furthermore, analysis of the extracted aqueous samples using HPLC, in both the extraction and the back-extraction, revealed no signals of TOPO, menthol, or thymol. This observation further proves the absence of HES degradation into the aqueous phase during extraction.

The formation of the HES was monitored using nuclear magnetic resonance (^{31}P and ^{1}H NMR). NMR has also been used to explore the interactions of HES molecules with the different compounds to extract. The experiments were conducted using a Bruker Advance NEO 9.4T spectrometer (400 MHz 1H resonance frequency) with a 5 mm probe. The ^{31}P resonance frequency was 161.99 MHz and a single pulse sequence with ^{1}H decoupling. The Press delay (D1) was 30 s for all the experiments. In the case of the experiments at variable temperatures, variations of 10 °C were used. ^{31}P reference was triphenyl phosphate in acetone-d_6 (TPP) at −17.59 ppm. ^{31}P NMR experiments at variable temperatures were conducted under two modes: (1) an experiment increasing the temperature from 10 °C to the temperature formation established when the synthesis was optimized, i.e., 40 °C (in intervals of 10 °C), and final increase of the temperature up to 60 °C to check the stability of the molecule's interaction; and (2) decreasing the temperature from 60 °C to 20 °C (in intervals of 10 ° C) to prove the stability of the molecule's interaction with the temperature variation again. In addition, a control experiment of TOPO was also carried out to ensure the signal offset was not due to the temperature variation, as is usually the case in NMR techniques at variable temperatures [43].

2.3. Liquid-Liquid extraction

Aqueous solutions of acetic, propionic, butyric, valeric, hexanoic, pyruvic, and fumaric acids were prepared individually and all together with a concentration of 1 g/L each. Additionally, glucose and xylose were added at 1 g/L for some experiments to make the mixture more representative of the composition of a real fermentation effluent. In real fermentation effluents, the concentration of each acid can vary significantly based on various operating conditions, including the substrate type, inoculum, pH, temperature, and retention time [44–46]. To maintain consistency and avoid potential saturation of components in the HES, an average concentration of 1 g/L was established for all the compounds in the mixture. Furthermore, it's essential to note that the primary focus of this study is to understand and determine the key factors influencing the interaction of individual components or their collective presence.

As Fig. 1 shows, HES and aqueous solution were introduced in a 20 mL Ace pressure glass and stirred at 300 rpm at 30 °C for different times, with a feed/HES volume ratio of 1:1. Both phases were separated by pouring in a settling funnel and allowing a decantation time until a clear separation between phases was observed. The concentration of different compounds in the aqueous phase (raffinate) was analyzed by High-Performance Liquid Chromatography (HPLC) in an Agilent 1260 Infinity apparatus equipped with a refractive index (RID)-G13662A as detector and a Hi-Plex H$^+$ column (300 x 7.7 mm) from Phenomenex. The extraction yield (Y_E) and distribution coefficient (K_i) were calculated as shown in Eqs. (1) and (2).

$$Y_E(\%) = \frac{C_i^F - C_i^R}{C_i^F} \times 100 \tag{1}$$

where C_i^F refers to the initial concentration of the compounds in the aqueous phase (feed) and C_i^R is the component concentration in the aqueous phase (raffinate) after the extraction. The distribution coefficient was defined as the concentration of solute in the organic solvent divided by the concentration of solute in the aqueous phase [47], when the equilibrium is reached.

$$K_i = \frac{C_i^E}{C_i^R} \tag{2}$$

where C_i^E, refers to the concentration of the compounds in the HES phase after the extraction (extract), and C_i^R is the compound concentration in the aqueous phase (raffinate) after the extraction and under equilibrium conditions.

On the other hand, Fourier-Transform Infrared Spectroscopy (FTIR) analyses of the HES samples after extraction were carried out to determine whether the structure of the solvents is maintained after extraction and whether water is being absorbed by the HES. Fourier-Transform Infrared Spectroscopy (FTIR) analysis was carried out of the HES after use. A Nicolet 5700 spectrometer apparatus equipped with an iS50 ATR
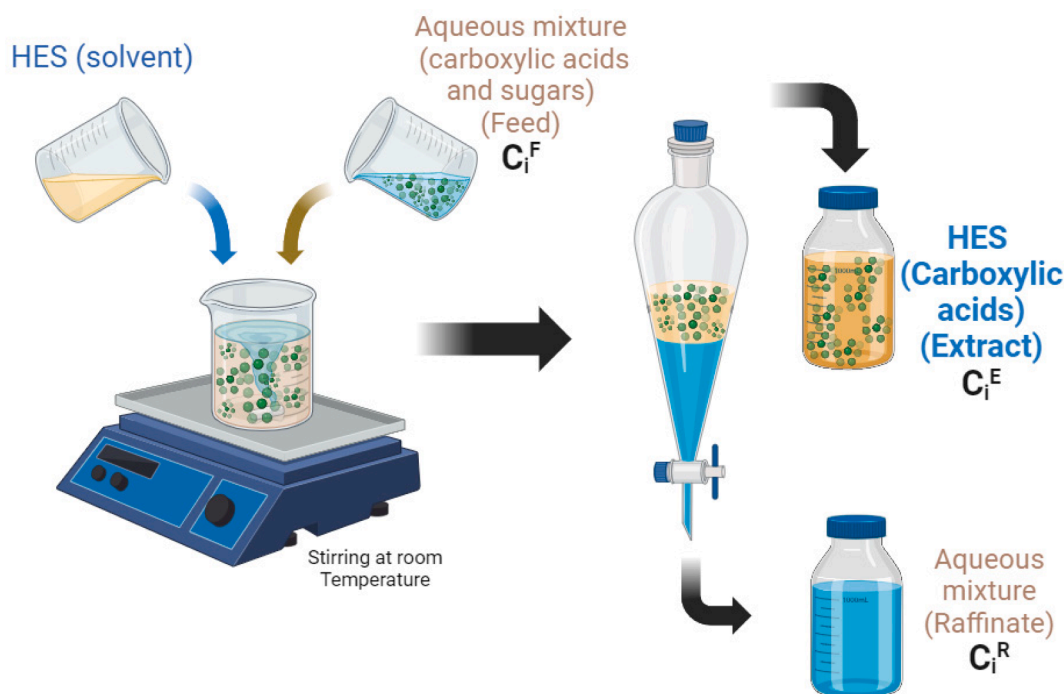

Fig. 1. Scheme of extraction experiments.

device was used for measurements in the range of 500–4000 cm^{-1}.

2.4. Recovery of carboxylic acids from the HES by liquid phase extraction (Back-extraction)

The previously extracted acids were recovered through a new extraction stage by putting the HES enriched in the acids of interest in contact with an alkaline solution, which was enriched with the acids, leaving the HES "clean" for its subsequent reuse. This second extraction stage was called "back-extraction" (Fig. 2). The back-extraction yields (Y_{BE}) were calculated as shown in Equation (3).

$$Y_{BE}(\%) = \frac{C_i^E}{C_i^F} \times 100 \tag{3}$$

where C_i^E, refers to the compound's concentration in the alkali phase after the back- extraction (extract') and C_i^F, refers to the compound's concentration in the HES before the back-extraction (feed') which is the same as the C_i^E (Fig. 1).

Different alkali solutions (NH$_4$, NaOH, and Na$_2$CO$_3$) were studied for the back extraction step since basic agents have been used successfully in the extraction of carboxylic acids in both DES [13] and ILs [24]. Since we were interested in obtaining an HES that is as "clean" as possible to be suitable for reusing, this "back-extraction" step must be optimized to ensure that practically no carboxylic acid remains. For this purpose, we optimized the following extraction variables: the type of alkaline agent, the concentration of the alkaline agent, the extraction time, the alkali/HES volume ratio, and the number of required steps.

Finally, the overall extraction yield (Y) was calculated considering the initial loading of compounds in the raw feed (C_i^F) and that obtained in the recovered aqueous solution after the back extraction ($C_i^{E'}$), as shown in equation (4) (Fig. 3).

$$Y(\%) = \frac{C_i^{E'}}{C_i^F} \bullet 100 \tag{4}$$

3. Results and discussion

3.1. Screening and characterization of HES

Table 1 summarizes the synthesis conditions (components, molar ratio, and temperature) of the mixtures of TOPO, thymol, and menthol studied in this work and the matter state after 24 h at room temperature. Different phases appear depending on the components' molar ratio, such as solid, semi-solid, or liquid. As can be seen in Fig. 4, only three mixtures were stable liquids after 24 h. It is worth highlighting that these samples were kept unaltered after over a month. According to these results, the mixtures referred to A (1TOPO:2Thymol), B (1TOPO:1Thymol), and D (1TOPO:2Menthol) HES were selected as potential extracting agents for recovery of carboxylic acids from fermentative effluents.

Fig. 5 illustrates the ^{1}H NMR spectra of (a) 1T:1T, (b) 1T:2T, and (c) 1T:2M eutectic solvents overlapped with the spectrum corresponding to pure thymol or menthol. From Fig. 5 a and b, it was possible to detect a signal associated with the –OH group that was shifted to a higher displacement (for example, from 4.70 to 8.61 ppm for the mixture 1T:1T) due to the interaction between protons of the –OH moiety and oxygen from P=O of TOPO. This displacement was not observed in the mixtures formed by menthol, even in the pure compound. However, considering that the interaction also influences the protons closer to the –OH group and analyzing the ^{1}H NMR of 1T:2M (Fig. 5c), it was easy to detect a displacement (from 3.35 to 3.41 ppm) of the signal belonging to the protons attached to the carbon where the –OH group was bonded (H in orange in the picture), thus confirming the interaction between the two molecules. It is worth highlighting that when two molecules were interacting with TOPO, the displacement of the ^{1}H was less affected, probably because the electron cloud, responsible for the H—O—H interactions, was shared with two molecules of thymol or menthol and hence being lower its influence. Additionally, when the liquid state was not stable, the eutectic solvent was not formed, e.g., 1T:1M, and no signal was changed in the spectrum.

To clear up whether the hydrogen bond was caused by the interaction of P = O moiety from TOPO and –OH groups from menthol/thymol or an intramolecular interaction between H donor molecules, ^{31}P NMR at fixed and variable temperatures was performed (Fig. 6). ^{31}P NMR spectra at room temperature evidenced that the signal changed drastically in the mixtures when a stable liquid state was achieved. ^{31}P NMR spectrum showed a change from 50.61 ppm corresponding to the TOPO compound to higher values for stable liquid mixtures (1T:1T (50.73 ppm); 1T:2T (53.06 ppm) and 1T:2M (55.23 ppm)). The shift to lower fields suggests that the protons of the –OH group, in menthol or thymol, could form hydrogen bonds with TOPO. The unshielding observed in the P atom is caused by the proximity of the O coming from the –OH. This electronegative atom removes electron density from P when forming the

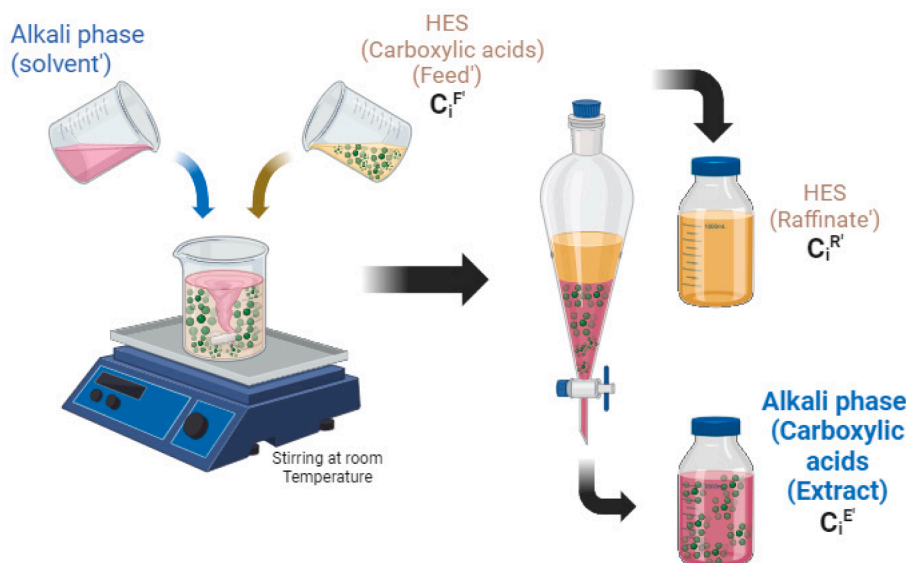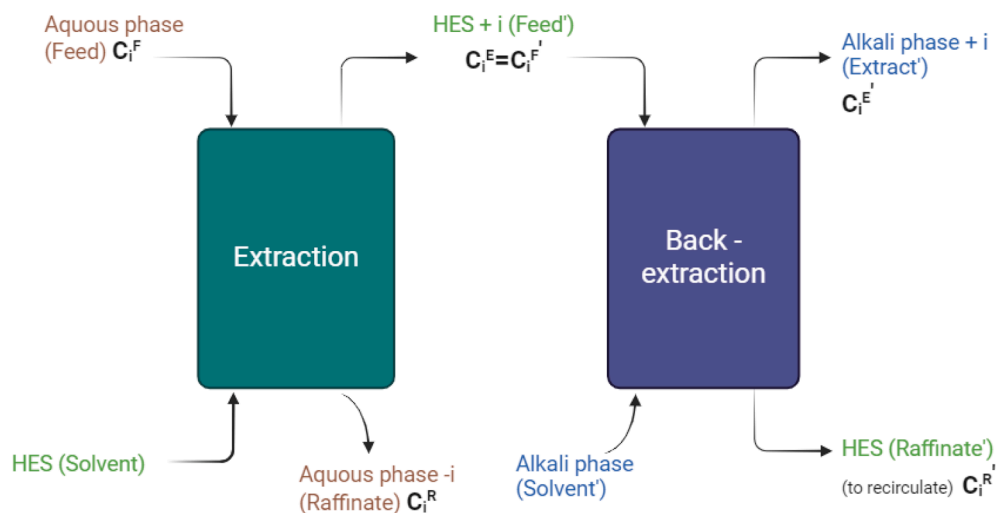**Fig. 2.** Scheme of back-extraction experiments.

Fig. 3. Complete extraction system for the recovery of carboxylic acids.

Table 1
Synthesis conditions of HES.

Mixture	Component 1	Component 2	Molar ratio	T (°C)	State [a]
1T:1T	TOPO	Thymol	1:1	40	Liquid
2T:1T	TOPO	Thymol	2:1	80	Solid
1T:2T	TOPO	Thymol	1:2	40	Liquid
1T:1M	TOPO	Menthol	1:1	40	Solid
2T:1M	TOPO	Menthol	2:1	60	Solid
1T:2M	TOPO	Menthol	1:2	40	Liquid

[a] Matter state after 24h at room temperature

hydrogen bond. However, in the mixtures that do not lead to a stable liquid, the interaction does not occur (1T:1M and 2T:1M). A small change was observed in the 31P NMR, evidencing the absence or low stability of the hydrogen bonds. A strange behavior was observed for the 2T:1T mixture because although the interaction occurred, this was not stable at room temperature, and after 15–30 min, the liquid mixture became solid, evidencing the low stability of the hydrogen bond. These results confirm that the strong interaction of P=O and OH groups by hydrogen bonds is the key to forming stable liquid mixtures.

^{31}P NMR at variable temperatures was carried out to gain more insights into the stability and formation of these intramolecular interactions. Fig. S1 and Fig. 7 show the ^{31}P NMR spectrum of the 1T:2T HES (one of the stable mixtures) under variable temperatures (increasing from 10 °C to 60 °C). It is possible to see a clear and progressive shift of the signal to a lower field, which means that an

interaction was happening and evidencing an interaction of both components of the mixture. At low temperatures, the interaction between molecules was not strong enough to cause a high signal shift. However, as temperature increases, the strength of the interaction is enhanced, thus provoking the displacement of the signal. At the HES formation temperature, 60 °C, the atoms in the molecule may stabilize over time. So this is the reason why, at the beginning of the experiment, the signal was observed at a very low field, but when the molecules and the H-bonds were stabilized, the signal changed to a higher field, remaining there for the rest of the ^{31}P NMR analysis. As the temperature is increased to 60 °C and remains there for one hour, it was possible to observe the stabilization of the signal moving in a range from 51.8 to 52.5 ppm, different and higher than the ^{31}P signal of isolated TOPO (50.6 ppm). The signal recorded was in the field of the signal observed when a ^{31}P NMR of the HES formed was acquired at room temperature. When the ^{31}P NMR analysis was performed, decreasing the temperature, small changes in the signal were observed (the signal is kept at the interval of 53.8–52.6 ppm). This fact evidences again a high stability of the interaction of that hydrogen bond (Fig. S2).

Finally, a control analysis with only TOPO evidenced that the ^{31}P NMR signal was shifted to values of 49.5–51.8 ppm (Fig. S3). This fact confirms that the differences in the signal with the temperature increase observed in Fig. S1 and Fig. S2 are caused by the formation of the hydrogen bond between P from TOPO and –OH from menthol or thymol, being this δ = 52.6 ppm.
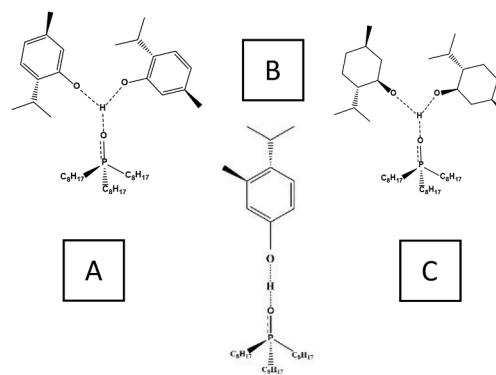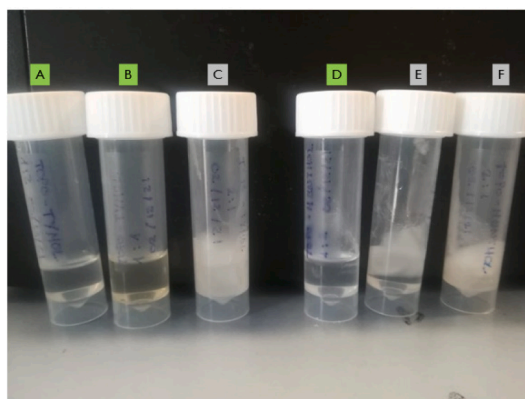

Fig. 4. HES samples. **A**: 1TOPO:2Thymol (1T:2T), **B**: 1TOPO:1Thymol (1T:1T), **C**: 2TOPO:1Thymol (2T:1T), **D**: 1TOPO:2Menthol (1T:2M), **E**: 1TOPO:1Menthol (1T:1M), **F**: 2TOPO:1Menthol (2T:1M). Structures of stable HES A, B, and D.
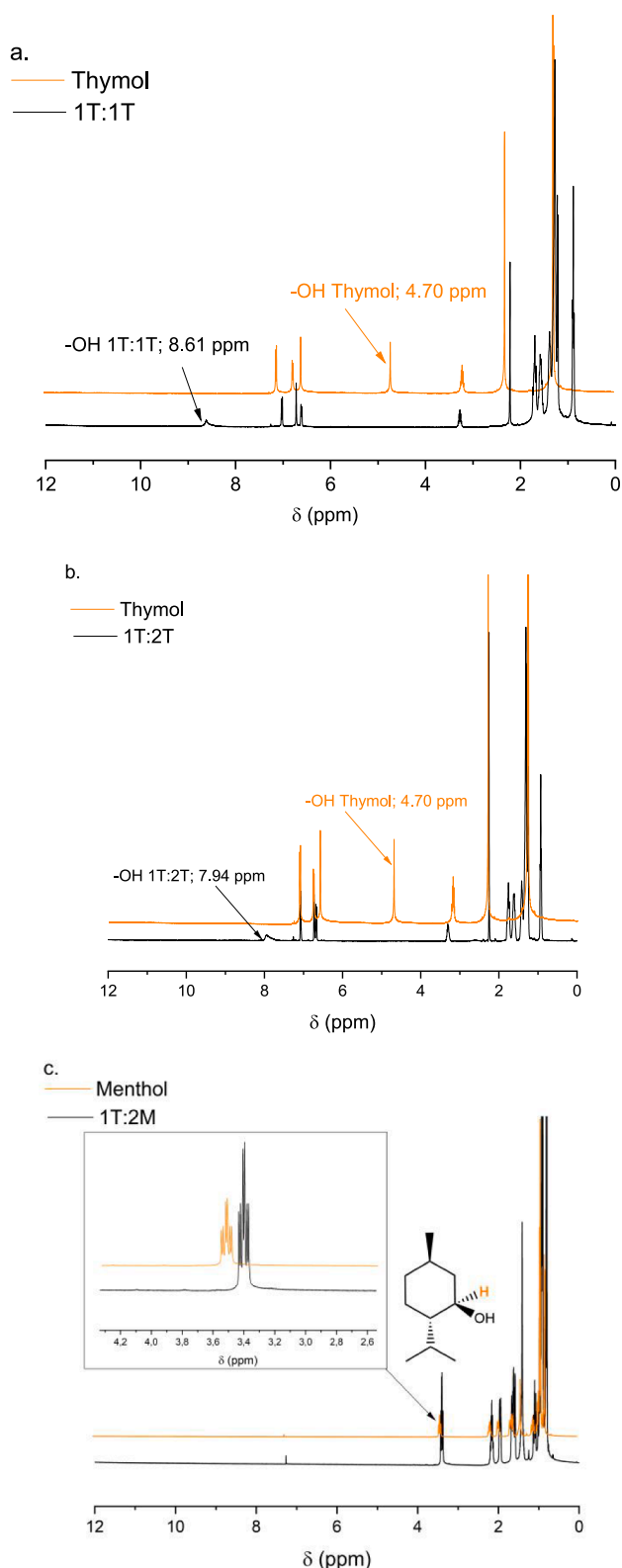
Fig. 5. Overlapped ^{1}H NMR of (a) 1T:1T and thymol; (b) 1T:2T and thymol; (c) 1T:2M and menthol.

3.2. Carboxylic acid extraction with HES from aqueous solutions

3.2.1. Extraction of carboxylic acids as single compounds

Extraction of single carboxylic acids was performed to calculate their distribution coefficients under equilibrium conditions (Table 2) and to elucidate how the length of the carbon chain and the type of carboxylic acids (mono or di-carboxylic) affect the interaction with HES. Fig. 8 shows the extraction yield of each single carboxylic acid at 10, 30, and 60 min for the three stable HES previously selected. The three solvents evidenced a dominant preference for hexanoic acid with an extraction yield of over 90 % in only 10 min. However, as the size of the hydrocarbon chain decreases, the three solvents display more different extraction yields. 1T:2T showed lower extraction yields for low carbon chain carboxylic acids (C < 5) in comparison with 1T:2M and 1T:1T, making it a better candidate for selective separation of high carbon carboxylic acids (C > 4).

The increase of the extraction yield with the carboxylic acid carbon chain length correlates with the acids' hydrophobicity. Hydrophobicity was related to the octanol/water partition coefficients (K_{OW}) of the different carboxylic acids. Thus, the order of the extraction yield was the same as the log K_{ow} values of acetic, propionic, butyric, valeric, and hexanoic acids, which are −0.31, 0.25, 0.79, 1.39, and 1.88, respectively [48]. Table 2. Distribution coefficient Ki of carboxylic acids with HES depicted in Table 2 confirms this trend, the longer the carbon chain, the better the extraction yield. It is important to highlight the K_i values for the case of 1T:2M mixture; Ki for valeric acid was more than 10-fold compared to 1T:1T and 1T:2T mixtures and over 20-fold for the hexanoic acid. Regarding the results with the dicarboxylic fumaric acid (C_4), the Ki values were in the range of the mono-carboxylic butyric acid (C4), and the presence of two carboxylic acids did not significantly change the extraction selectivity of the tested HES.

To deeply understand the interaction of HES with the different acids, ^{31}P NMR of the mixture of the acids and more selective HES (1T:2T) was performed. Fig. S4 shows the overlapping 31P NMR spectrum of hexanoic, butyric, acetic acid, and acetic acid in the presence of 1T:2T solvent and the acid-free spectrum of 1T:2T. A clear displacement of the P signal from the TOPO compound was observed when acids were presented, more notable as the hydrocarbon chain length increased. The ^{31}P NMR signal was associated directly with the interaction of carboxylic acids with the HES. The signal was shifted to a lower field as the interaction was more robust. ^{31}P NMR indicates a close correlation between the carbon length and the interaction by H-bond, which translates into a better affinity to the high-carbon carboxylic acids.

The experimental results were compared with previous studies in literature in terms of extraction yield of acetic, propionic, and butyric acid; no references were found regarding the extraction of carboxylic acids with carbon lengths higher than 4 [13,34,35]. The extraction yields of 1T:1T solvent were mainly like those found in the literature. However, in the case of 1T:2T solvent, the extraction yields were much lower than those reported, but there was a rise in the extraction yield of valeric and hexanoic acid, 77 % and 96 %, respectively. Concerning the performance of 1T:2M solvent, the extraction yields of (≥C_5) carboxylic acids were the highest found in the literature to date, but it was less selective to (≥C5) carboxylic acids than the 1T:2T solvent. Therefore, the most selective HES to extract (≥C_5) carboxylic acids was the 1T:2T solvent. Due to no previous studies in the literature on the extraction of hexanoic or major carbon chain acids, it was impossible to compare with other solvents. In the case of valeric acid, only one reference is described in the literature, with less selective extraction than that obtained in our studies [32].

3.2.2. Extraction of carboxylic acids in a multicomponent mixture

Fig. 9 shows the extraction of the carboxylic acids studied in a multicomponent mixture for the three stable HES. Clear differences were observed in the selectivity of the extraction towards (<C_5) carboxylic acids, with the HES formed by thymol being the most selective solvents for (≥C_5) carboxylic acids. In particular, the 1T:2T solvent was quite selective for (≥C_5) carboxylic acids, with an extraction performance at 60 min of 58.6 % and 86.6 % for valeric and hexanoic acid, respectively (Fig. 9a). In the case of 1T:1T, although it was more selective than the 1T:2M solvent, it was not able to separate selectively (≥C_5) carboxylic
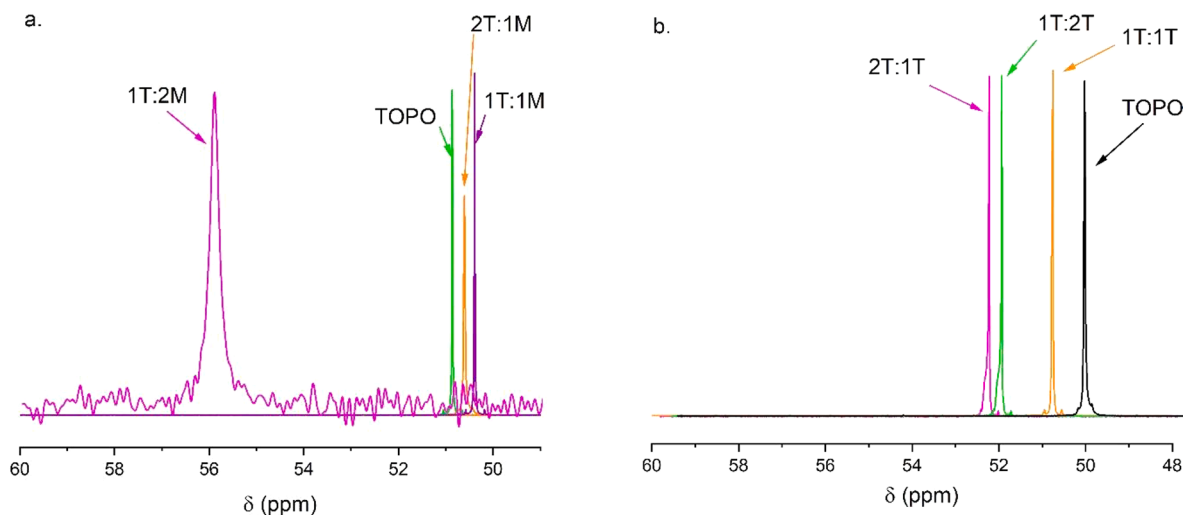
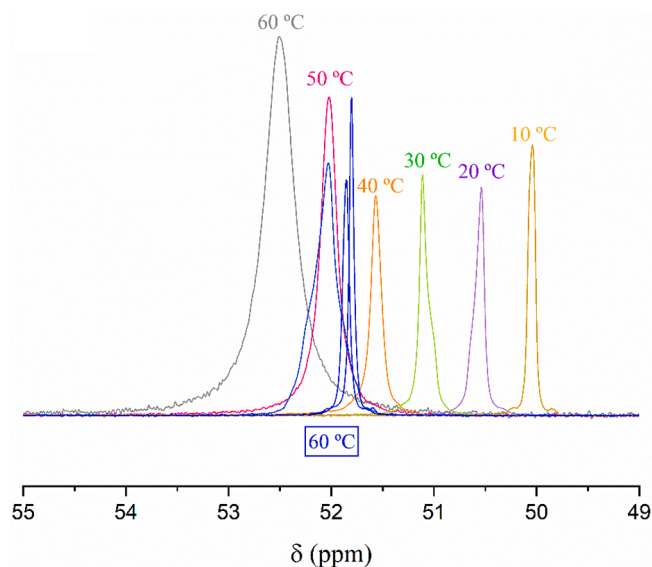**Fig. 6.** Overlapped ^{31}P NMR of TOPO with different molar ratios of (a) menthol and (b) thymol.

Fig. 7. ^{31}P NMR at an increased temperature of 1T:2T mixture.

Table 2
Distribution coefficient K_i of carboxylic acids with HES.

HES\ACIDS	Acetic	Propionic	Fumaric	Butyric	Valeric	Hexanoic
1T:1T	0.79	2.06	1.67	3.75	4.89	13.26
1T:2T	0.14	0.48	0.11	2.46	2.99	12.12
1T:2M	1.42	0.97	5.44	5.68	40.57	247.87

Data calculated under equilibrium conditions being different for each HES.

acids from ($<C_5$) carboxylic acids. Regarding the 1T: 2 M solvent, although the extraction yields were higher, all the acids were extracted without a clear preference by the ($\geq C_5$) carboxylic acids (Fig. 9c). Moreover, based on the results at different extraction times, 30 min was considered the optimal time for selective recovery of ($\geq C_5$) carboxylic acids.

As shown in S1, the three HES used in this study were more selective than the DES used in the literature regarding VFA extraction yield. The extraction yields using 1T:1T were similar to those in the literature. However, most notably, there were no references to the use of terpenoids to form an HES, which selectively extracts ($\geq C5$) carboxylic acids but rather extracts them in their entirety.

In Table 3, the comparison of the extraction performance of the tested eutectic solvent for single and the multi-component mixture of carboxylic acids after 30 min, evidences the high affinity of the thymol-based solvents to the extraction of hexanoic and valeric acid in the multicomponent mixture. This fact must be attributed to a saturation of acids with a longer chain (C6 and C5 mainly), preventing the smaller acids from being extracted. The study of the multi-component mixture confirms the outstanding selectivity of 1T:2T HES towards the high carbon carboxylic acids, which even have better affinity than single extraction experiments. On the other hand, extraction data for the 1T:2M solvent confirms its low selectivity, as previously evidenced with single acid compounds. Therefore, the HES with the best performance was the 1T:2T solvent due to its high selectivity for ($\geq C_5$) carboxylic acids and low extraction for short-chain acids.

To complete the study of the multi-component mixtures, two monomeric sugars (glucose and xylose) were added to the acid mixture at a concentration of 1 g/L each (Table 3). These sugars are frequently presented in aqueous fermentative effluents. The eutectic solvent also highly prefers carboxylic acids with carbon numbers over 4, even in the presence of sugars in the aqueous effluent. Likewise, the affinity of this HES with the sugars was negligible as it arises from their null extraction yield.

3.2.3. Back–extraction conditions for recovery of the 1T:2T solvent

To achieve the recovery of carboxylic acids and further reuse of the 1T:2T solvent, several operational variables of a back-extraction step were studied: the back-extractor alkaline agent, alkaline concentration, volume ratio of the alkaline aqueous solution to the 1T:2T solvent, and the number of back extraction steps. Fig. 10 shows the overall extraction yield for the mentioned variables. The maximum overall yields were also included, calculated as the product of extraction and back-extraction yields, assuming a 100 % performance in the back-extraction step. As feed for the back-extraction step, the 1T–2T solvent was used after extracting the multicomponent mixture of carboxylic acids (extraction conditions: 30 °C, a feed/HES volume ratio of 1:1 and 60 min). Fig. 10a shows the yield in the presence of NH_4 (0.075 M), NaOH (0.1 M), and Na_2CO_3 (0.1 M) as typical back-extraction agents. Ammonia shows a negligible recovery of the carboxylic acids from the 1T:2T solvent. This is probably due to the weak basicity of this alkaline agent. Sodium carbonate was an interesting alternative for safety reasons compared with the strong NaOH base, but the recovery yield was below 10 % for most acids except butyric acid. The NaOH solution shows the best performance but is far from the complete recovery of the acids, being the recovery agent typically used with terpene-formed HES [22]. Thus, the
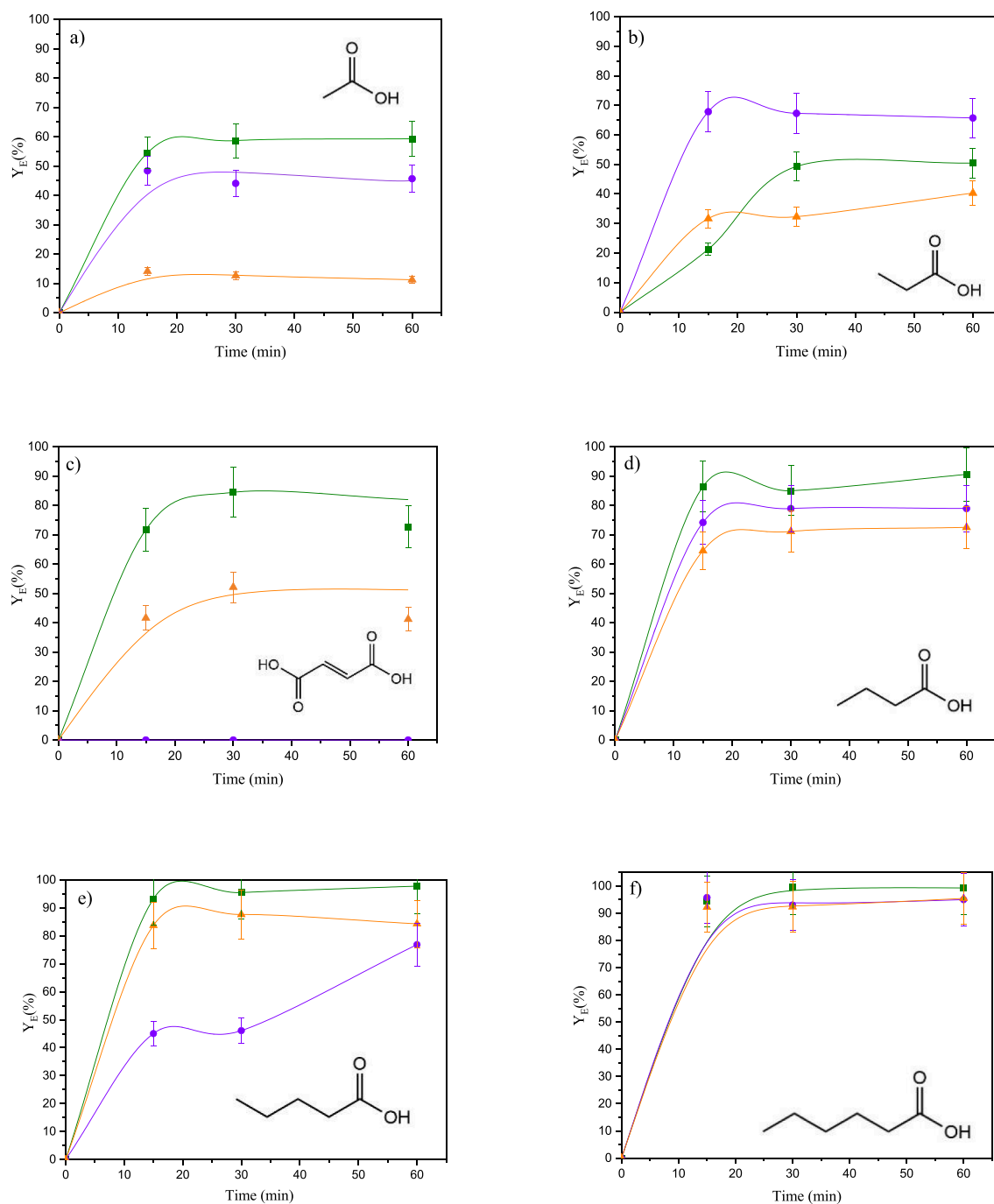
Fig. 8. Extraction yields of individual compounds: (a) Acetic acid, (b) Propionic acid, (c) Fumaric Acid, (d) Butyric acid, (e) Valeric acid (f) and Hexanoic acid using as extracting agents: 1T:2M (■), 1T:1T (●) and 1T:2T (▲). Operation conditions: T = 30 °C and feed/HES volume ratio of 1:1.

NaOH concentration ranging from 0.05 to 0.2 M was also tested (Fig. 10b). A lower and higher concentration of 0.1 M did not improve the recovery of the acids from the 1T:2T solvent. According to these results, the NaOH solution was considered the best back-extraction agent, and 0.1 M was the optimal concentration to increase the back-extraction yield. To improve the recovery of the acids, a volume ratio of the 1T:2T solvent to the alkali agent of 1:3 was also evaluated (Fig. 10c). Although the overall yield was enhanced using the threefold alkaline agent volume, the yield still needs to reach complete recovery (in all cases, the yield is below the potential maximum value). The slight differences did not justify increasing the amount of alkaline solution; hence, a volume ratio of 1:1 was selected as more convenient from an economical point of view. Finally, the number of 2 and 3 alkaline back-

extraction steps using fresh alkaline aqueous solution for each step was evaluated (Fig. 10d). It can be observed that three stages of back-extraction could maximize the recovery of the acids extracted from 1T:2T solvent. Under this scenario, the back-extraction yield reached an almost complete recovery of hexanoic and valeric acids as more abundant, fumaric, and propionic acids (that means that the overall extraction yield almost equals the maximum value). Regarding butyric and acetic acids, the recovery decreased to ca. 80 and 50 %, respectively. Likewise, the developed back-extraction conditions using the alkaline NaOH solution provided an effective method on alkaline for the recovery of the employed HES (1T:2T) for further reusing in the extraction of carboxylic acids.
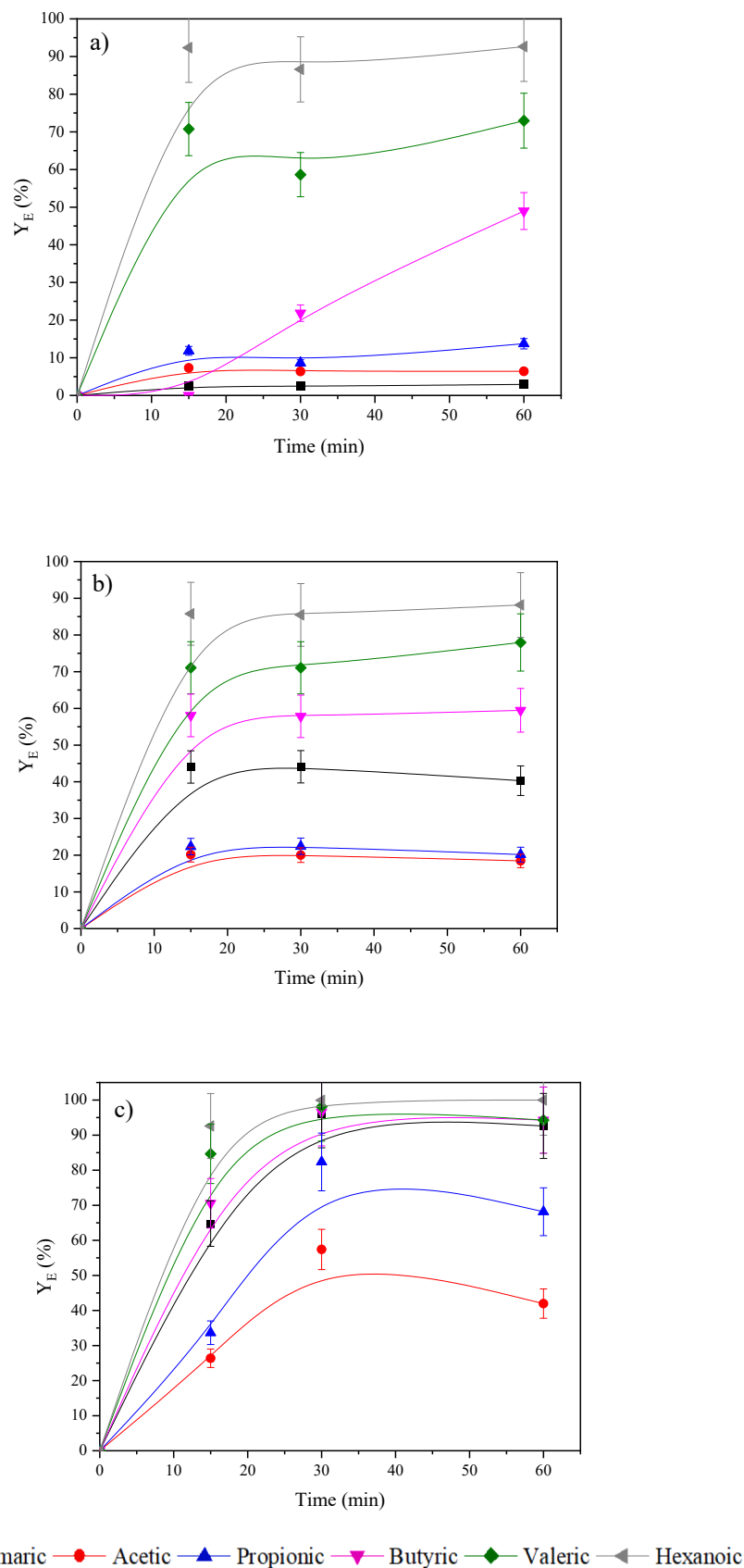
Fig. 9. Extraction yields of a multi-component mixture of carboxylic acids with (a) 1T:2T, (b) 1T:1T, and (c) 1T:2M. Operation conditions: T = 30 °C and feed/HES volume ratio of 1:1.

Table 3
Extraction yields (%) of single and multi-component mixtures of carboxylic acids at 30 min.

Solvent	Extraction Yield (%)					
	Single component			Multicomponent mixture		
	1T:1T	1T:2T	1T:2M	1T:1T	1T:2T	1T:2M
Glucose	–	–	–	0	0	10.2
Xylose	–	–	–	0	0	14.4
Fumaric	0	41.2	72.8	40.3	2.9	92.6
Acetic	44.1	12.6	58.7	20.0	6.4	57.4
Propionic	67.3	32.3	49.3	22.4	8.6	82.3
Butyric	78.9	71.1	85.0	57.8	21.9	96.7
Valeric	46.1	87.6	95.5	71.1	58.6	98.1
Hexanoic	92.9	92.4	99.6	85.5	86.6	99.9

Extraction time = 30 min, T = 30 °C and feed/HES volume ratio of 1:1.

3.2.4. Reuse of the recovered HES

After optimizing a first cycle of extraction (1 stage) and back-extraction (3 stages), respectively, a second cycle with the recovered eutectic 1T:2T solvent was performed to assess the recyclability of the HES. This issue is critical for the design of an environmentally friendly industrial process. The HES was reused under the same extraction and back extraction conditions optimized in the first cycle. Fig. S5 shows a schematic view of the process with the two cycles and all stages based on the above-obtained results, and Fig. 11 shows the overall extraction yields for the two cycles. It was confirmed that the reused 1T:2T solvent achieved a similar performance for extraction of ($\geq C_5$) carboxylic acids than the fresh one. In some cases, a higher overall extraction performance can be observed after the second cycle since the reused HES was not entirely "cleaned" in the first cycle.

FTIR analyses of the eutectic 1T:2T solvent before and after the extraction and the alkali back-extraction steps (Fig. S6) were performed to confirm the structural stability of the HES after use in one complete cycle. In the case of TOPO, the band at 2960 cm^{-1} was associated with the asymmetric stretching vibration of CH$_3$, and the bands at 2920 and 2854 cm^{-1} were the dissymmetric and symmetric stretching vibrations of CH$_2$, respectively. 1460 cm^{-1} was a scissoring band of CH2, and 722 cm^{-1} was in phase rocking vibration of C–H in long-chain alkyl (CH$_2$)$_n$ (n > 4). The stretching vibration band of P=O in TOPO appears around 1149 cm^{-1} [49]. The FTIR spectrum of thymol has characteristic peaks in the 1250–1750 cm^{-1} region of the spectrum, which are assigned to C=C stretching, –OH bending, and C–O stretching of phenolic groups of thymol [50]. Analyzing the spectra, it was possible to observe that bands associated with TOPO and thymol are kept unaltered after each step of the first cycle of extraction and back-extraction steps of the solvent. Likewise, it could also be observed that the band corresponding to the water did not appear (wide band between 3000 and 3700 cm^{-1} or the band at 1700 cm^{-1}) [51], confirming the hydrophobic character of the synthesized HES.
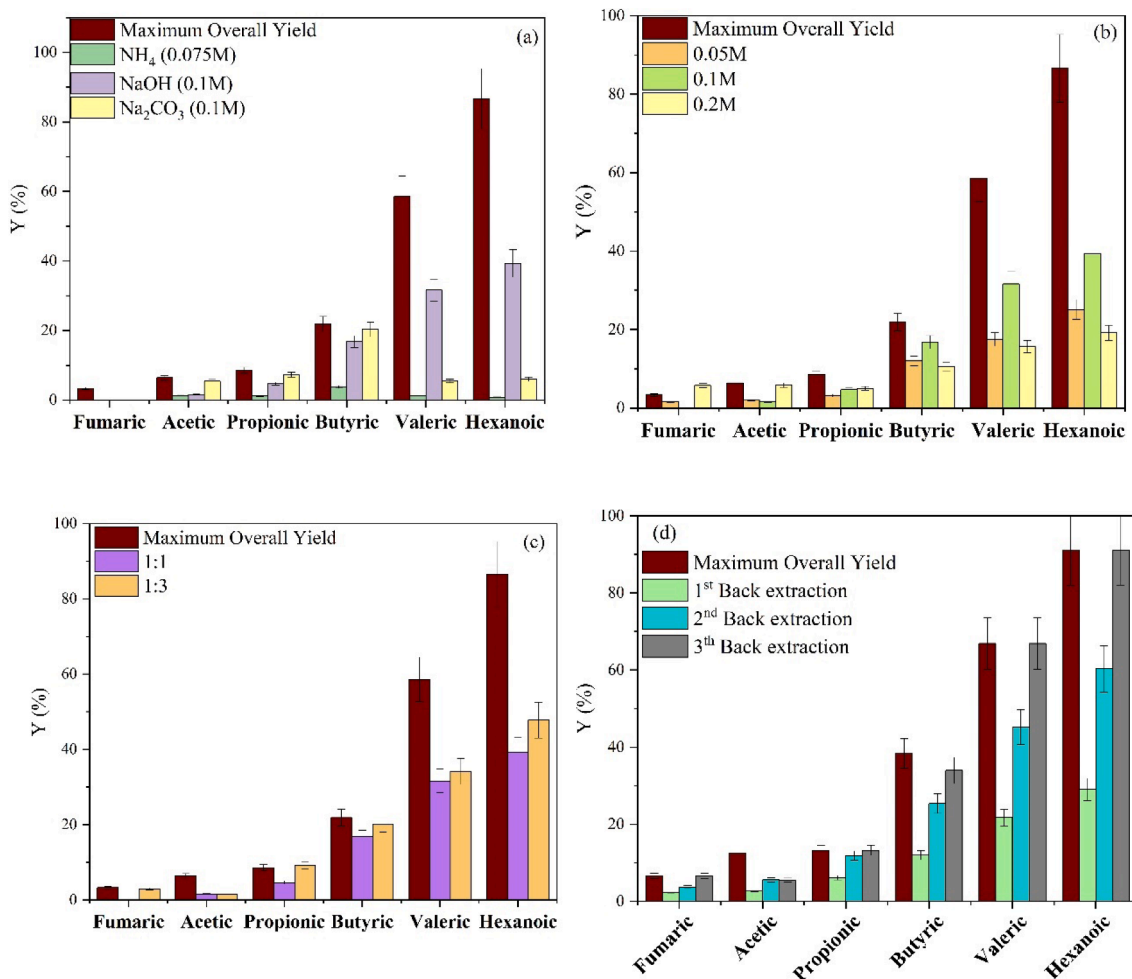

Fig. 10. Overall process yield (%) using (a) different alkaline agents, (b) NaOH with different concentrations, (c) different volume ratios of HES to the alkaline agent, and (d) several back extraction steps with fresh alkaline solution. Back-extraction conditions: T = 30 °C, t = 30 min, alkali/1T:2T eutectic solvent volume ratio of 1:1 (except in Figure (c)) and one back-extraction step (except in Figure (d)). Maximum overall yield assuming a 100 % back-extraction yield respect.
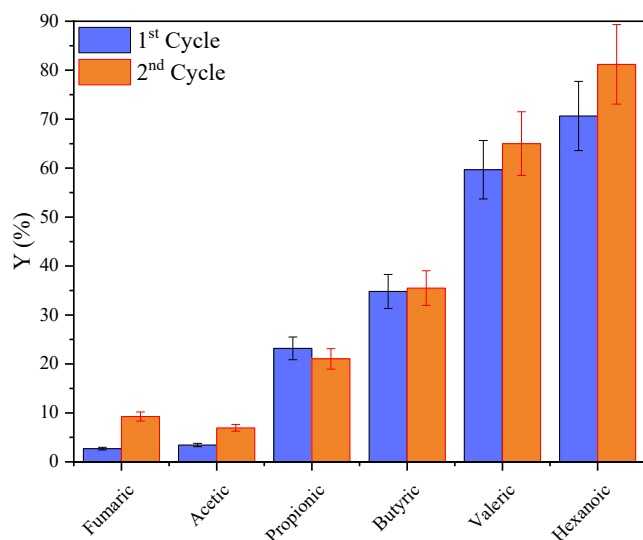
Fig. 11. Overall extraction yields for carboxylic acids of the reuses. Extractions conditions: T = 30 °C, t = 60 min and feed/HES volume ratio of 1:1. Back-extraction conditions: T = 30 °C, t = 30 min, with NaOH (0.1 M) as an alkaline agent, alkali/HES volume ratio of 1:1 and three back-extraction steps.

4. Conclusions

Deep hydrophobic eutectic solvents (HES) were synthesized in adequate ratios by combining natural compounds such as menthol or thymol with trioctylphosphine oxide (TOPO). ^{31}P-NMR spectra evidence the formation of a stable supramolecular structure mainly due to hydrogen bonds between the molecules. The HESs synthesized effectively extracted a multi-component mixture of typical carboxylic acids present in fermentative effluents. The HES, with a composition of 1TOPO:2Thymol, provided a remarkable selectivity for extracting ($\geq$C$_5$) carboxylic acids in a single extraction stage up to 92 %. The backstage recovery of the HES for further reuse and separation of the extracted carboxylic acids was successfully achieved employing an alkaline NaOH solution (0.1 M), HES/alkali agent volume ratio 1:1, and three consecutive backstage extractions. The recovered HES showed similar results of performance extraction than the fresh one, proving a feasible process for recovery of ($\geq$C$_5$) carboxylic acids from fermentation effluents and being interesting for a prospective industrial scaling up and having recovery yields of 65 % for valeric acid and 81 % for hexanoic acid in the total process.

CRediT authorship contribution statement

N. Vidal: Writing – original draft, Methodology, Investigation, Conceptualization. **M. Ventura:** Writing – review & editing, Methodology, Investigation, Conceptualization. **F. Martínez:** Writing – review & editing, Visualization, Supervision. **J.A. Melero:** Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2024.127540.

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