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Recovery of avonoids using novel biodegradable choline amino acids ionic liquids based ATPS

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B S T R C T

In this work, different ionic liquids composed by choline as cation and amino acid as anion (CAAILs) have been synthetized and applied to establish aqueous two-phase systems (ATPS) with two phosphate salts. In order to understand the phase formation process of the ATPS, binodal curves, tie-lines and their respective tie-line lengths were determined at $T = 298.15$ K and atmospheric pressure. Besides, the tie-lines were fitted using Othmer-Tobias and Bancroft equations. Furthermore, with the aim to analyse the application of the studied ATPS in the recovery of avonoids, partition coefficients of rutin and quercetin were calculated at $T = 298.15$ K and atmospheric pressure. Finally, the obtained results were compared with those found in literature.

1 Introduction

Over the last years, the increasing concern about the environmental impact of industrial processes has led to the development of a new concept called “green engineering” [1], in which the design of more healthful and environmentally friendly processes has become a priority.

Nowadays, the development of new alternatives with low impact in the human health and in the environmental such as the use of solvents produced from natural sources, water, supercritical CO_2 or ionic liquids [2], as well as the economic and energy valorisation of wastes generated in the industry through obtaining high value-added compounds and/or, are being performed [3].

In this context, fruit processing industry generates more than half a billion tons of waste worldwide [3]. The wide availability of this raw material and its huge hitherto little exploited potential has given rise to numerous studies on the processing of high added value products present in these residues [4].

Such wastes are mainly composed by peels, seed and pomace of fruits that constitute a good raw material to products recovery like pectins, alkaloids, avonoids, essential oils, etc., denoted as bioactive compounds. The demand of this kind of compounds is increasing in a large variety of industries such as pharmaceutic, food, cosmetic or biorefineries [3–5]. Due to stricter regulations about the origin of these products, the use of these natural compounds instead of their synthetic counterparts is becoming more common [6].

One family of these interesting bioactive compounds are avonoids. Flavonoids are phenolic compounds present in many colourful fruits and vegetables such as oranges, grapes, strawberries, plums They are found in abundance not only in these food, but also in chocolate, nuts, red wine, soy and tea leaves [7]. These compounds present interesting properties, anti-in ammatory, anti-osteoporotic, antiviral and antioxidant, which are beneficial for the human health and interesting for the pharmaceutic and food industries [8,9]. One of their relevant properties is their antioxidant ability due to their high redox potential [10], which help to protect the plant against UV light, fungal parasites and oxidative cell injury [11]. Moreover, the regularly human consumption of avonoids has been associated with the reduction in the incidence of diseases [12,13]. Thus, there is currently great interest in the extraction of avonoids from natural sources due to the possibility to improve the health through diet, either with fruits and vegetables consumption or through food supplements. Within the widely variety of avonoids, we selected rutin and quercetin since they are two of the representative avonoids present in fruits and vegetables [14].

The conventional processes used in the extraction of avonoids include techniques as solid-liquid, liquid-liquid or supercritical

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uid extraction [15–17]. However, these methods present disadvantages such as high consumption of energy and solvents, risk of thermal degradation of the extracted compounds and long extraction times [3]. The use of solvents as hydrocarbons, alcohols, chloro-alkanes or acetone, which are toxic and harmful to the environment, can prevent the application of the extracted bioactive compounds for human consumption [18].

For these reasons, in this work, the extraction of avonoids using ionic liquids based on choline and amino acids (CAAILs), to achieve separation in a more healthful and environmentally friendly media is presented.

Ionic liquids (IL) have been widely studied as suitable alternative to replace the volatile organic solvents used in the extraction processes. However, the most used ILs are those based on imidazolium and pyridinium cations, which are toxic and poorly biodegradable [19]. Therefore, in the last years, a large number of studies are focused on the research of non-toxic and environmentally friendly ionic liquids. The ILs composed by choline as cation and amino acids as anions have emerged as promising alternatives because they are formed by no toxic natural compounds and consequently they respect the environment [20,21].

In this study, the application of three CAAILs in the extraction of two avonoids using aqueous two-phase systems (ATPS) as separation technique is presented, since it is recognized as a high efficient method for the separation and purification of biomolecules [22].

For this, the binodal curves and tie-lines for six ATPS {CAAIL (1) + salt (2) + water (3)} where the CAAILs are those composed by choline and three amino acids (L-alanine, glycine and L-serine) and the salts are tripotassium phosphate (K_3PO_4) and dipotassium hydrogen phosphate (K_2HPO_4), were experimentally determined. In order to confirm the absence of other possible salts formed by ion exchange, a series of ^{1}H NMR studies were carried out. Merchuck equation [23] was used to fit and predict the binodal curves and the reliability of the tie-lines was evaluated using Othmer-Tobias [24] and Bancroft [25] equations. With these systems, it is possible to assess the effect of the change of the amino acid in the CAAIL as well as the in uence of the salt on the behaviour of the studied ATPS. Finally, the experimental data were compared with those found in literature [26]. To our knowledge, only data for the ATPS {[Ch][Ala] and [Ch][Ser] (1) + K_3PO_4 (2) + water (3)} are available to date.

In order to check the capacity of separation of these systems, the partitioning of two avonoids (rutin and quercetin) was also carried out on the best previously determined ATPS {[Ch][Ala] (1) + K_3PO_4 or K_2HPO_4 (2) + water (3)}, which are those with the larger heterogeneous regions.

2 Experimental

2.1. Chemicals

Choline hydroxide, 45 wt (weight percent) aqueous solution, was purchased from ACROS Organics and it was used without any further purification. Amino acids, L-alanine, glycine and L-serine with mass fraction purity higher than 0.99, were acquired from Scharlau, Sigma-Aldrich and PanReac, respectively. Potassium phosphate tribasic (K_3PO_4) and di-potassium hydrogen phosphate (K_2HPO_4) were purchased from Sigma-Aldrich and Scharlau, respectively. All chemicals were used after drying with heating in order to reduce their water content. Solutions used in the experimental procedure were prepared with bidistilled water from Panreac.

Rutin hydrate ($C_{27}H_{30}O_{16}$ xH_2O) and quercetin ($C_{15}H_{10}O_7$) were purchased from Sigma-Aldrich with mass fraction purity higher than 0.94. These avonoids were used without further purification. Purities and suppliers of all chemicals used are listed in Table 1.

2.2. Apparatus and procedure

2.2.1. Synthesis of the CAAILs

The ionic liquids used in this work, namely cholinium alaninate [Ch][Ala], cholinium glycinate [Ch][Gly] and cholinium serinate [Ch][Ser], were synthesized in our laboratory according to methods already reported in bibliography [27,28]. The synthesis consists of a direct neutralization reaction between cholinium hydroxide and the corresponding amino acid. For this, an aqueous cholinium hydroxide 40 wt solution was mixed with an equimolar aqueous solution of the corresponding amino acid. The mixture was vigorously stirred at room temperature for 24 h. After neutralization, water was evaporated under vacuum at $T = 323.15$ K. The unreacted amino acid was precipitated by adding a solution of acetonitrile and methanol in 9:1 (v/v) ratio. After filtration, volatile compounds were evaporated under reduced pressure at $T = 313.15$ K. Finally, the purified CAAIL was dried in vacuum at $T = 323.15$ K for 48 h and was stored under moisture-free conditions before its use. Their water contents (180 ppm) were measured using a Mettler Toledo C20 Coulometric Karl Fisher Titrator with an uncertainty in the measurement of ±5 .

The chemical structures of the three synthesized CAAILs (Fig. 1) were confirmed by ^{1}H-RMN as indicated below. ^{1}H NMR spectra were recorded on a BRUKER ARX 4CO spectrometer at 400.1621 (^{1}H) MHz. D_2O (ACROS Organics, 99.8 + atom D) was employed as deuterated solvent as received from the supplier. Chemical shifts are quoted in parts per million (ppm) relative to the signals corresponding to the residual non-deuterated solvent (D_2O: $\delta_H = 4.79$ ppm).

(2-hydroxyethyl)trimethylammonium 2-amino-ethanoate [Ch][Gly]

^{1}H NMR (D_2O): δ 4.09 (m, 2H, Ch-2), 3.55 (m, 2H, Ch-1), 3.21 (s, 2H, Gly-2).

(2-hydroxyethyl)trimethylammonium 4-amino-5-hydroxypropanoate [Ch][Ser]

^{1}H NMR (D_2O): δ 4.07 (m, 2H, Ch-2), 3.76 (dd, 1H, $J_1 = 11.2$, $J_2 = 4.3$ Hz, Ser-3), 3.68 (dd, 1H, $J_1 = 11.2$, $J_2 = 5.8$ Hz, Ser-3), 3.53 (m, 2H, Ch-1), 3.34 (dd, 1H, $J_1 = 5.8$, $J_2 = 4.3$ Hz, Ser-2).

(2-hydroxyethyl)trimethylammonium 2-amino-ethanoate [Ch][Ala]

^{1}H NMR (D_2O): δ 4.10 (m, 2H, Ch-2), 3.55 (m, 2H, Ch-1), 3.36 (q, 1H, $J = 7.1$ Hz, Ala-2), 1.26 (d, 3H, $J = 7.1$ Hz, Ala-3).

The purity of the synthesized ionic liquids was determined by ^{1}H NMR and is given in Table 1.

2.2.2. Phase diagrams determination

For the binodal curves, aqueous solutions of salt (K_3PO_4 or K_2HPO_4) of known composition were placed into glass tubes and titrated with an aqueous solution of the corresponding CAAIL until a slight turbidity in the mixtures was observed. Then, the samples were weighed to calculate their composition. Drops of water were added until homogeneous mixtures were achieved, and the above procedure was repeated until enough data was obtained. Similarly, aqueous solutions of CAAIL of known composition were titrated with the corresponding salt solution, following the aforementioned procedure. These experimental data were merged to build a complete binodal curve. All binodal curves were fitted using the equation given by Merchuck et al. [23]:

Table 1
Purity and suppliers of pure compounds used in this work.

Chemicals	Supplier	Purity (wt)[a]
Choline hydroxide, 45 wt, aqueous solution	Acros Organics	
L-Alanine	Scharlau	≥99
Glycine	Sigma-Aldrich	≥99
L-Serine	PanReac	≥99
Potassium phosphate tribasic	Sigma-Aldrich	≥98
di-Potassium hydrogen phosphate anhydrous	Scharlau	≥98
Rutin hydrate	Sigma-Aldrich	≥94
Quercetin	Sigma-Aldrich	≥95
Cholinium alaninate	synthesized in our laboratory	≥93
Cholinium glycinate	synthesized in our laboratory	≥96
Cholinium serinate	synthesized in our laboratory	≥93

[a] wt = weight percent.

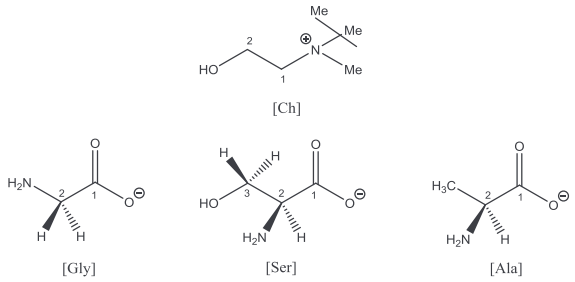


Fig 1 Structures of the cation ([Ch]) and anions ([Gly], [Ser], [Ala]) that form the synthesized ILs used in this work: [Ch][Gly], [Ch][Ser] and [Ch][Ala].

$$[\text{CAAIL}] = \text{A}\ \exp\left[\left(\text{B}\ [\text{salt}]^{0.5}\right) - \left(\text{C}\ [\text{salt}]^{3}\right)\right] \quad (1)$$

where [CAAIL] and [salt] are the ionic liquid and salt compositions in weight fraction, respectively, and A, B and C are the adjustable parameters obtained by nonlinear least squares regression.

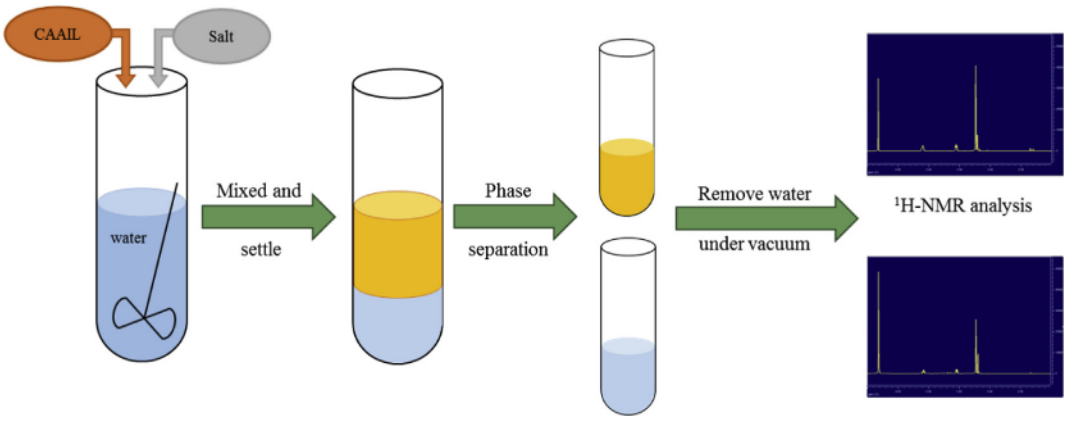
2.2.3. Tie-lines determination

To determine the tie-lines (TL), immiscible ternary mixtures {[Ch][Ala], [Ch][Gly] or [Ch][Ser] (1) + K_3PO_4 or K_2HPO_4 (2) + water (3)} of known composition were introduced into glass cells and sealed using a silicon cover. In order to ensure an intimate contact between phases, the mixtures were stirred using a magnetic stirrer for 4 h, and then they were left to settle down overnight in a thermostatic bath at $T = 298.15$ K to achieve a complete phase separation. Once equilibrium was reached, both phases were carefully separated and individually weighed.

With the aim of confirming the absence of other salts, such as K[Ser], K[Gly] or $[Ch]_3PO_4$, which could be formed in the immiscible ternary mixtures by ion exchange, a series of ^{1}H NMR studies on the composition of the two-phases formed, was carried out (Scheme 1).

Thus, a mixture of {[Ch][Gly] (1) + K_3PO_4 (2) + water (3)} on similar composition to the feed composition of the tie-lines determined in this work (33.28 wt for K_3PO_4 and 19.59 wt for [Ch][Gly]) was prepared and allowed to form the two phases using the same procedure as tie-lines determination. The ^{1}H NMR spectrum of the residue obtained after drying the upper phase showed the presence of [Ch] cation and [Gly] anion in 1:1 proportion, as indicated by the integration of the signals corresponding to protons Ch-1 (2H) or Ch-2 (2H) and Gly-2 (2H) (Fig. 2). It is important to point out that the complex multiplets observed for the protons located in positions 1 and 2 of [Ch] cation (Ch-1 and Ch-2), are due to the slow quadrupolar relaxation of the ^{14}N nucleus produced in some tetraalkylammonium salts, which makes possible the appearance of $J^{14}_{N\text{-}H}$ couplings [29]. On the other hand, the weight of solid obtained after drying the bottom phase, composed by K_3PO_4 contaminated with an insignificant rest of [Ch][Gly] (confirmed by ^{1}H NMR), was similar to the amount added to the initial mixture, indicating that no K^+ cation or PO_4^{3-} anions are exchanged to form other different salts.

The application of the same working protocol indicated in



Scheme 1 Working protocol applied to confirm the absence of the other salts on the immiscible ternary mixtures.

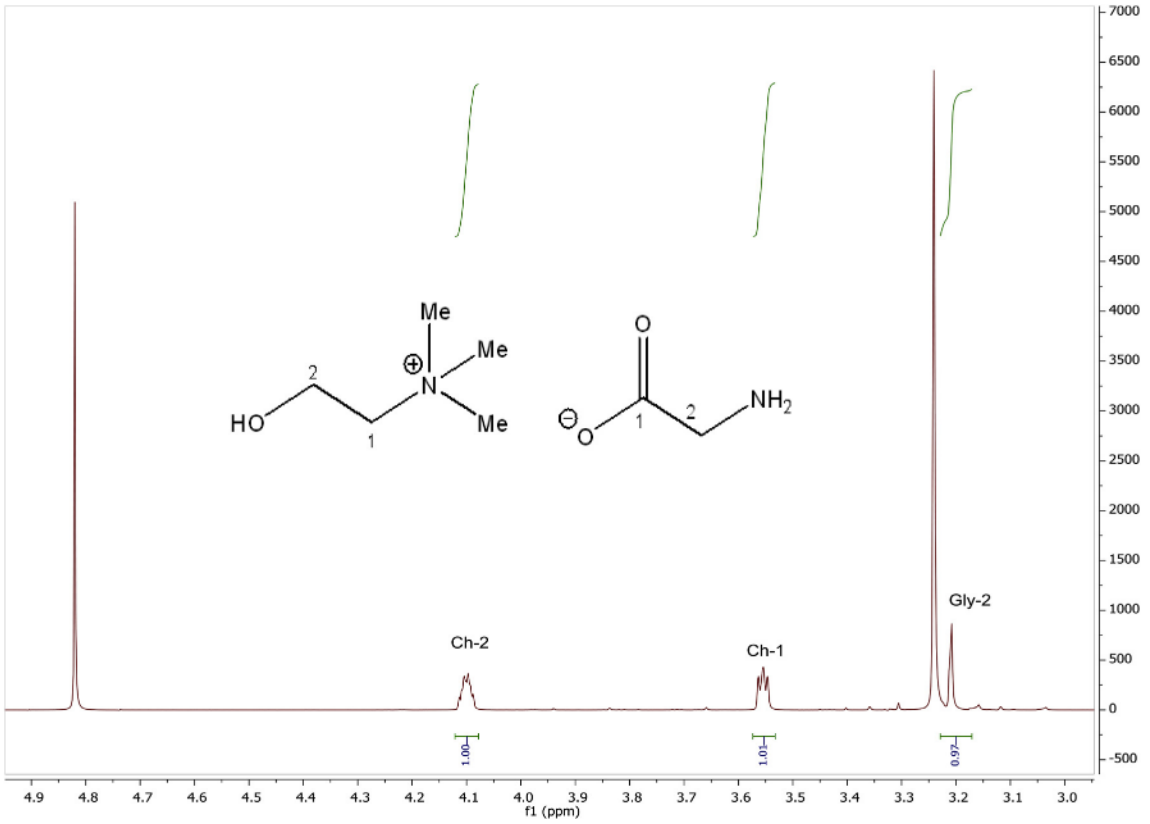


Fig 2 ^{1}H NMR spectrum of the residue obtained from the top phase formed from the ternary mixture {[Ch][Gly] (1) + K_3PO_4 (2) + water (3)}. Integration indicated in green and blue.

Scheme 1 to immiscible ternary mixtures containing [Ch][Ser] and [Ch][Ala] also confirmed that no ion exchange is produced. Thus, the ^{1}H NMR spectrum of the residue obtained after drying the upper phase formed from the mixture {[Ch][Ser] (1) + K_2HPO_4 (2) + water (3)} on similar composition to the feed composition of the tie-lines determined in this work (33.85 wt for K_2HPO_4 and 29.76 wt for [Ch][Ser]) showed the presence of [Ch] cation and [Ser] anion in 1:1 proportion, as indicated by the integration of the signals corresponding to protons Ch-1 (2H) or Ch-2 (2H) and Ser-2 (1H) (Fig. 3). Again, the weight of the solid (K_2HPO_4) obtained after drying the bottom phase was similar to the one added to prepare the initial ternary mixture. A similar result was obtained for the ternary mixture {[Ch][Ala] (1) + K_2HPO_4 (2) + water (3)}.

Compositions of the tie-lines (TL) were obtained using the following procedure: the ionic liquid concentration in both phases was measured using UV–Vis spectrophotometry (Thermo Scientific Varioskan ash) at 270 nm for the systems containing [Ch][Ala] and [Ch][Gly], and at 300 nm for the systems with [Ch][Ser]. The water content was determined gravimetrically after freeze drying and the salt amount was calculated by mass balance.

The ionic liquid concentration was determined using a calibration curve of the absorbance versus the ionic liquid concentration previously established; to minimize the possible interference from the salt, the calibration curve was determined using a dilution factor (DF) of 40; this DF eliminates the effect of salt on the absorbance value.

The tie-line lengths (TLL) were calculated by the following equation:

$$TLL = \sqrt{\left([CAAIL]_T - [CAAIL]_B\right)^2 + \left([salt]_T - [salt]_B\right)^2} \quad (2)$$

where [CAAIL] and [salt] are the CAAIL and salt compositions in weight fraction, respectively, and the subscripts T and B refer to top and bottom phases, respectively.

To fit of tie-line data, different equations have been proposed. In this work, Othmer-Tobias (eq. (3)) and Brancroft (eq. (4)) equations were chosen as they are the most widely used [24,25]:

$$log\left[\frac{1-[CAAIL]_T}{[CAAIL]_T}\right] = K_1 + n\ log\left[\frac{1-[salt]_B}{[salt]_B}\right] \quad (3)$$

$$\log\left[\frac{1-[water]_B}{[salt]_B}\right] = K_2 + r\ \log\left[\frac{1-[water]_T}{[CAAIL]_T}\right] \quad (4)$$

where $[water]_T$ and $[water]_B$ are the weight fraction of water in the top and bottom phases in the equilibrium, respectively and where $[CAAIL]_T$ and $[Salt]_B$ are the compositions in weight fraction for the CAAIL in the top and salt in the bottom, respectively.

2.2.4. Partitioning of avonoids

The partition of the two avonoids (rutin hydrate and quercetin) was determined experimentally in the lie-lines previously measured for the systems involving [Ch][Ala] as ionic liquid, that is to say, {[Ch][Ala] (1) + K_3PO_4 (2) + water (3)} and {[Ch][Ala] (1) + K_2HPO_4 (2) + water (3)} at $T = 298.15$ K and atmospheric pressure. These ATPS were adopted for the partitioning studies, as

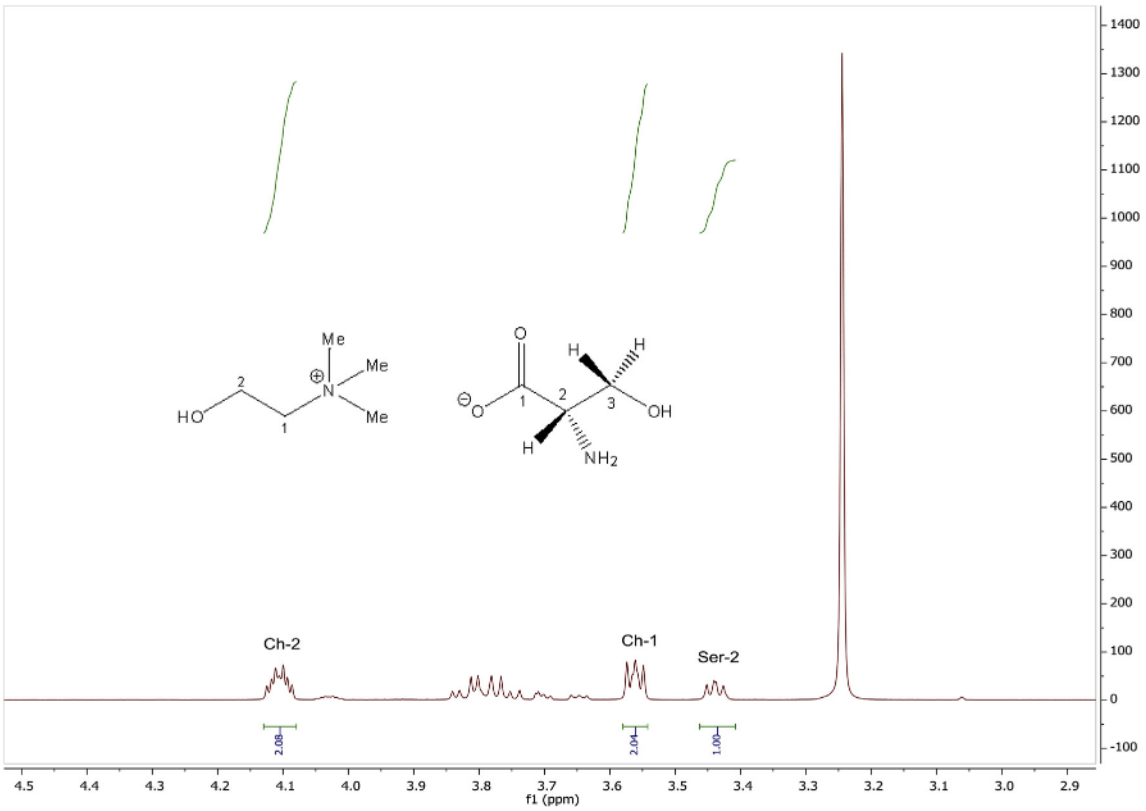


Fig 3 ^{1}H NMR spectrum of the residue obtained from the top phase formed from the ternary mixture {[Ch][Ser] (1) + K_2HPO_4 (2) + water (3)}. Integration indicated in green and blue.

they showed to have the larger biphasic regions from all systems previously studied.

Solutions of the two avonoids (0.020 wt and 0.007 wt for rutin and quercetin, respectively) were prepared dissolving a certain amount of each avonoid in aqueous solution of [Ch][Ala] (85 wt). Then, six replicates of the feed composition of each tie-line were prepared in Eppendorf tubes containing 0–1.5 mg of each avonoid in order to assess the effect of the change of concentration of avonoids in each tie-line. To keep the feed composition of each tie-line, water was added in the corresponding amount. An automatic pipette (Multipipette® XStream, Eppendorf) was used for the addition of all compounds. The tubes were thoroughly mixed on a vortex mixer for 2 min, and splitting of both phases was achieved using centrifugation (Minispin, Eppendorf) at 13.4×10^3 rpm for 15 min. In order to ensure that the equilibrium was reached, all mixtures were settled overnight at $T = 298.15$ K and atmospheric pressure. Then, samples of each phase were collected with a syringe and diluted conveniently.

The partition coefficients (K) for the avonoids were obtained from the measurement of the absorbance of each phase using a UV–Vis spectrophotometer (Thermo Scientific Varioskan Flash) at a wavelength of 368 and 472 nm for rutin and quercetin, respectively.

To minimize the possible interferences from other components (salt and ionic liquid) the top and the bottom phases were diluted with water, the dilution factor (DF) was 40 for the top phase and 10 for the bottom phase. In addition, the samples were analyzed against blanks which contained the same phase components but without amino acid. It is important to note that no interferences were found. [Ch][Ala] presents the maximum peak of absorbance at 270 nm and no peaks appears at 368 and 472 nm, wavelengths in which the absorbance of the avonoids studied in this work were measured.

These coefficients of partition were determined as the slope of the straight lines from the representation of the absorbance in the ionic liquid-rich phase (top phase) versus the absorbance in the salt-rich phase (bottom phase) and were corrected with their corresponding dilution factors, DF_{phase}, according to the following equation:

$$K = \frac{\text{Abs(top phase)}\ \ DF_{top}}{\text{Abs(bottom phase)}\ \ DF_{bottom}} \quad (5)$$

3 Results and discussion

3.1. Phase diagrams: binodal curves and tie-lines

In this work, binodal data for the aqueous two-phase systems involving the ionic liquids [Ch][Ala], [Ch][Gly] and [Ch][Ser] and the inorganic salts K_3PO_4 and K_2HPO_4 have been experimentally determined at $T = 298.15$ K and atmospheric pressure. The salts used to obtain the ATPS presented in this work, K_3PO_4 and K_2HPO_4, were selected due to their high-water solubility (500 g/L and 168 g/L at $T = 298.15$ K, respectively) and their salting-out ability owing to their kosmotropic nature [30].

The experimental data are depicted by weight percentage in Fig. 4 (a) and (b) and summarized in Table S1 of Supplementary Information. The adjustable parameters calculated following eq. (1) as well as the standard deviations for the six studied systems are

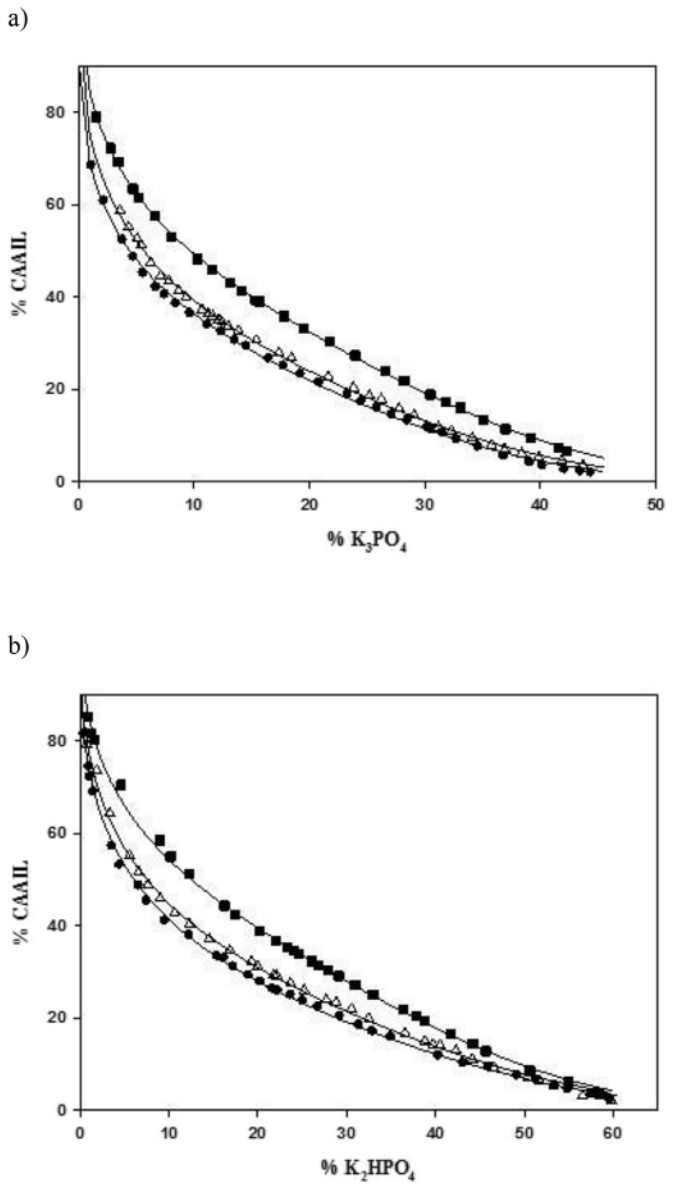


Fig 4 Binodal curve of the studied ternary systems at $T = 298.15$K and atmospheric pressure. (a) {ionic liquid (1) + K_3PO_4 (2) + water (3)} and (b) {ionic liquid (1) + K_2HPO_4 (2) + water (3)}. The symbols represent the experimental data and the line corresponds to the fit by eq (1). (●) [Ch][Ala]; ( ) [Ch][Gly] and (■) [Ch][Ser].

presented in Table 2. In this table, it can be observed that high correlation coefficients were achieved with equation (1) for all the 6 studied systems.

The comparison obtained with the values found in the bibliography [26] is presented in Fig. S1 in the Supporting Information.

Furthermore, the feed compositions, experimental tie-lines concentrations, along with their corresponding calculated length (TLL) and slope (STL) are shown in Table 3. Regarding the STLs, the variation is not highlighted as the results show that the obtained tie-lines are almost parallel. The steepest STLs among the investigated systems were obtained for the system {[Ch][Ala] (1) + K_3PO_4 (2) + water (3)}. An increase of the CAAIL composition in the feed leads to an increase in the concentration of salt and CAAIL in the respective top and bottom phases. As an example, the experimental tie-line data for the ternary systems involving [Ch][Ala] with K_3PO_4 and K_2HPO_4, respectively, are plotted in Fig. 5 a) and b). The experimental tie-lines for the other systems determined in this work are given in Supporting Information (Fig. S2 and Fig. S3). The empirical correlation equation given by Othmer-Tobias (equation (3)) and Brancroft (equation (4)) were satisfactorily used to fit the tie-line data. The values of the fitted parameters together with the corresponding R^2 values are showed in Table 4.

3.1.1. *Effect the amino acid on binodal curves*

By comparison of the phase diagrams containing the same salt, it is possible to analyse the effect of the change of amino acid (anion) in the ionic liquid on the phase diagrams. As it can be observed from Fig. 4 (a), in which the solubility curves for the ATPS with the salt tri-potassium phosphate are presented, the ability of phase separation of CAAIL follows the order: [Ch][Ala] > [Ch][Gly] > [Ch][Ser]. The closer the binodal curve comes to the origin of the phase diagram, the stronger the CAAIL ability to promote the phase splitting. The same behaviour is observed in the comparison of ATPS involving K_2HPO_4 as salt, which is drawn in Fig. 4 (b). These results may be explained based on the hydrophobicity of the studied amino acids. The hydrophobicity of the amino acids follows the order: alanine > glycine > serine [31]. This trend is the same regarding the capability of the phase separation of the studied CAAILs. Therefore, it can be inferred that the anions that present less affinity for water are more effective to promote the formation of ATPS. These conclusions are in agreement with previous results reported in literature [26,32]: the higher the hydrophobicity of the IL the more easily it is salted-out by K_3PO_4.

3.1.2. *Effect the salt on binodal curves*

In Fig. 6, a comparison between the experimental binodal curves for the two salts used in this work is presented. From this comparison, the ability of the phosphate salts (tri-potassium phosphate and di-potassium hydrogen phosphate) to promote phase segregation in aqueous solutions containing the different ionic liquids can be assessed. As expected, the salting-out trend observed followed the Hofmeister series, in other words, the systems composed by tri-potassium phosphate salt present larger biphasic region than all the other studied ternary systems and the result could be explained based on the salting-out effect produced from the

Table 2
Adjustable parameters (A, B and C) obtained by Merchuck equation (eq. (1)) and standard deviation for the experimental binodal curves data.

System	A	B	C	σ[a]
{[Ch][Ala] (1) + K_3PO_4 (2) + water (3)}	91.833	−0.287	$1.87\ 10^{-5}$	0.620
{[Ch][Gly] (1) + K_3PO_4 (2) + water (3)}	100.475	−0.291	$1.56\ 10^{-5}$	0.612
{[Ch][Ser] (1) + K_3PO_4 (2) + water (3)}	107.252	−0.240	$1.49\ 10^{-5}$	0.367
{[Ch][Ala] (1) + K_2HPO_4 (2) + water (3)}	96.408	−0.265	$6.04\ 10^{-6}$	0.652
{[Ch][Gly] (1) + K_2HPO_4 (2) + water (3)}	99.719	−0.250	$6.08\ 10^{-6}$	0.920
{[Ch][Ser] (1) + K_2HPO_4 (2) + water (3)}	104.120	−0.203	$7.51\ 10^{-6}$	0.669

[a] Standard deviation; $= \sum_{i}^{n_{dat}} ((z - z_{cal}))^2 / n_{dat}\}^{1/2}$ (z and z_{cal} are the values of the experimental and calculated property and n_{dat} is the number of experimental data points).

Table 3
Experimental phase equilibrium compositions (in weight percentage), together with their corresponding tie-line length (TLL) and slope (STL) for the systems {ionic liquid (1) + potassium phosphate tribasic (2) + water (3)} at $T = 298.15$ K and atmospheric pressure[a].

Tie-line (TL)	Feed		Top phase		Bottom phase		STL	TLL
	$[IL]_{feed}$	$[salt]_{feed}$	$[IL]_{top}$	$[salt]_{top}$	$[IL]_{bot}$	$[salt]_{bot}$		
{[Ch][Ala] (1) + K_3PO_4 (2) + water (3)}								
TL1	19.764	36.334	64.329	2.376	0.414	51.981	−1.288	80.906
TL2	25.021	29.529	61.271	2.964	1.065	48.270	−1.329	75.348
TL3	32.960	20.064	55.931	3.843	2.756	43.098	−1.355	66.095
TL4	40.200	10.870	49.203	5.570	7.305	35.109	−1.418	51.264
{[Ch][Gly] (1) + K_3PO_4 (2) + water (3)}								
TL1	15.003	37.768	47.960	7.054	1.105	52.421	−1.033	65.220
TL2	17.750	32.012	44.389	8.451	2.716	46.899	−1.084	56.699
TL3	21.071	28.177	43.157	8.925	3.222	45.733	−1.085	54.311
TL4	24.159	23.945	40.877	9.998	4.684	42.098	−1.128	48.377
TL5	27.927	19.965	39.779	10.530	5.476	40.719	−1.136	45.695
{[Ch][Ser] (1) + K_3PO_4 (2) + water (3)}								
TL1	19.125	38.125	61.927	6.293	2.745	51.769	−1.301	74.636
TL2	22.454	32.045	52.697	9.468	5.045	46.886	−1.273	60.587
TL3	23.987	30.003	49.374	10.976	6.114	44.867	−1.276	54.955
TL4	25.985	26.516	41.216	15.265	9.209	40.737	−1.257	40.906
{[Ch][Ala] (1) + K_2PHO_4 (2) + water (3)}								
TL1	29.860	34.197	61.969	3.862	3.487	61.596	−1.013	82.179
TL2	31.927	30.668	59.007	4.300	4.018	59.670	−0.993	78.036
TL3	36.098	23.006	54.085	5.917	5.283	55.757	−0.979	69.754
TL4	40.002	14.046	46.535	8.790	7.204	50.929	−0.933	57.642
{[Ch][Gly] (1) + K_2PHO_4 (2) + water (3)}								
TL1	31.369	39.990	66.992	3.717	1.683	72.883	−0.944	95.127
TL2	33.104	32.910	61.127	5.076	2.693	66.877	−0.946	85.053
TL3	36.111	24.979	53.912	7.374	4.038	61.550	−0.921	73.637
TL4	39.284	16.661	46.376	10.748	6.681	54.274	−0.912	58.908
{[Ch][Ser] (1) + K_2PHO_4 (2) + water (3)}								
TL1	35.085	40.403	76.288	3.453	1.911	73.758	−1.058	102.347
TL2	39.076	28.915	63.161	7.416	3.447	64.983	−1.037	82.944
TL3	40.756	22.998	54.695	11.233	5.468	59.622	−1.017	69.028
TL4	43.071	19.933	52.080	12.512	5.894	58.255	−1.010	65.004

[a] Standard uncertainties, u, are: $u(T) = 0.2$ K, $u(p) = 10$ kPa, $u(w,$ weight percentage$) = 10^{-3}$.

creation of water-ion complexes. Herein, K_3PO_4 acts as salt-out agent due to its preferential hydration over CAAIL.

According to the results obtained, it can be concluded that the most suitable ATPS is the one formed by ionic liquid [Ch][Ala] and salt K_3PO_4, as this system is the one that exhibits the larger biphasic region from all the studied systems. Moreover, the tie-line with the feed composition of 0.1976 and 0.3633 wt for [Ch][Ala] and K_3PO_4, respectively, present the highest ability to perform the extraction.

3.2. Partitioning of rutin and quercetin

The partition coefficients K) calculated using equation (5) for the two avonoids, rutin and quercetin, together with the feed composition and the tie-line length of the tie-lines for the best systems {[Ch][Ala] (1) + K_3PO_4 or K_2HPO_4 (2) + water (3)} are presented in Table 5. The high values of the partitioning coefficients demonstrate a high recovery in the CAAILs-rich phase. From this Table it can be observed that an increase in the value of the TLL leads to an increase on the partition coefficient value.

The logarithm of the partition coefficients for the avonoids as function of TLL are represented in Fig. 4. These results are in accordance with the commonly used expression:

$$\mathrm{Ln}K = \quad \mathrm{TLL} \tag{6}$$

where is a constant value which depends of the ATPS composition.

In Fig. 7 and in Table 5 it can be observed that the extraction of rutin on the two CAAILs-based ATPS is more favourable than the extraction of quercetin in the same studied systems. In addition, for the same avonoid, the ATPS that presents better capacity for the separation of the two avonoids is the ATPS containing the K_3PO_4 salt.

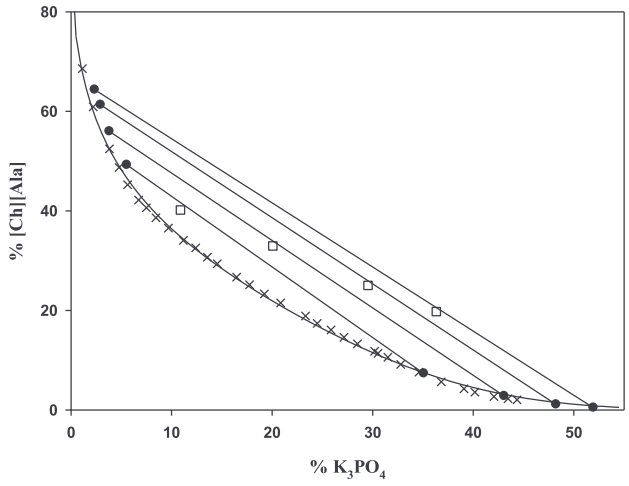
These results confirm the ability of the CAAIL-based ATPS in the selective extraction of the rutin and quercetin.

4 Conclusions

In this work, different ionic liquids composed by choline as cation and amino acids as anions (CAAILs) have been synthetized and their aqueous two-phase systems (ATPS) with two phosphate salts were studied. The CAAILs are those composed by the ions derived from choline and three amino acids (L-alanine, glycine and L-serine) to give [Ch][Gly], [Ch][Ser] and [Ch][Ala], while the salts are tripotassium phosphate (K_3PO_4) and dipotassium hydrogen phosphate (K_2HPO_4). The absence of other different salts than [Ch][Gly], [Ch][Ser] and [Ch][Ala], which could be formed by ion exchange, was confirmed by the ^{1}H NMR analysis of the top and bottom phases formed from the immiscible ternary mixtures {[Ch][Ala], [Ch][Gly] or [Ch][Ser] (1) + K_3PO_4 or K_2HPO_4 (2) + water (3)}.

In order to understand the phase formation process of the ATPS, binodal curves, tie-lines and their respective tie-line lengths were determined at $T = 298.15$ K and atmospheric pressure. Besides, the tie-lines were accurately fitted using Othmer-Tobias and Bancroft equations. Furthermore, with the aim to analyse the application of

a)



b)

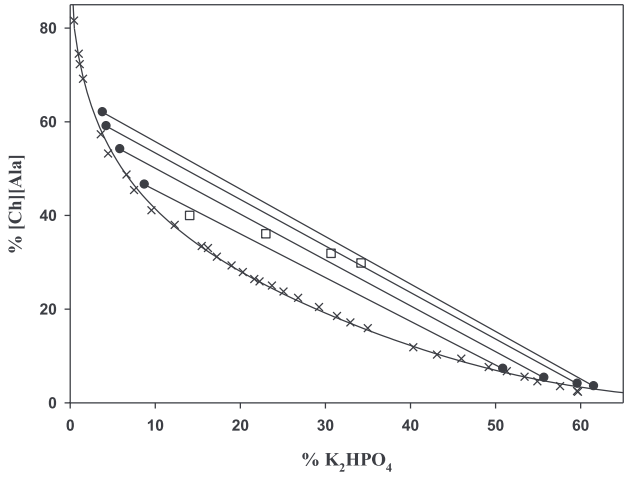


Fig 5 Binodal curves and tie-lines for the ternary systems composed of {ionic liquid (1) + salt (2) + water (3)} at $T = 298.15$K and atmospheric pressure. ( ) feed compositions, (X) binodal curve, (●) tie-line data and (–) fit of the binodal data. (a) [Ch][Ala] + K_3PO_4 + water; (b) [Ch][Ala] + K_2HPO_4 + water.

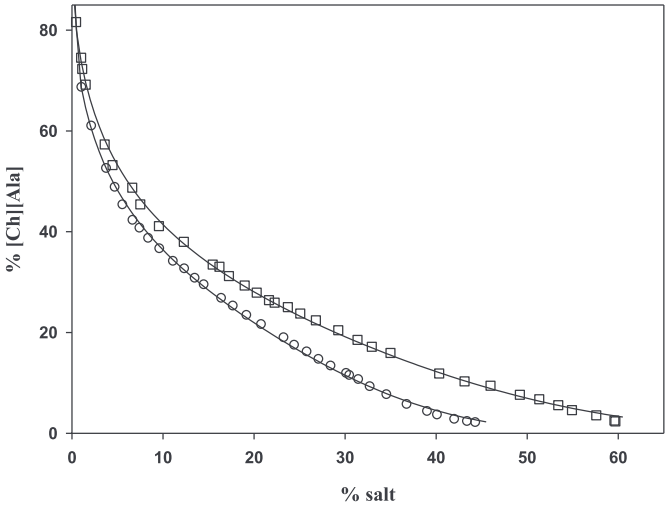


Fig 6 Binodal curve at $T = 298.15$K and atmospheric pressure. {[Ch][Ala] (1) + K_3PO_4 or K_2HPO_4 (2) + water (3)}. The symbols represent the experimental data and the line corresponds to the fit with eq (1). (○) K_3PO_4, ( ) K_2HPO_4.

Table 5
Partition coefficients obtained for rutin and quercetin for the systems {[Ch][Ala] (1) + K_3PO_4 or K_2HPO_4 (2) + water (3)} at $T = 298.15$ K and atmospheric pressure[a].

Number of TL	$[IL]_{feed}$	$[salt]_{feed}$	TLL	*K*	
				Rutin	Quercetin
{[Ch][Ala] (1) + K_3PO_4 + (2) + water (3)}					
TL 1	19.764	36.334	80.906	5717	324.1
TL 2	25.021	29.529	75.348	4025	195.2
TL 3	32.960	20.064	66.095	2310	127.9
TL 4	40.200	10.870	51.264	950.0	65.95
{[Ch][Ala] (1) + K_2HPO_4 + (2) + water (3)}					
TL 1	29.860	34.197	82.179	3506	234.3
TL 2	31.927	30.668	78.036	2900	169.3
TL 3	36.098	23.006	69.754	1800	107.5
TL 4	40.002	14.046	57.642	695.0	63.56

[a] Standard uncertainties, *u*, are: $u(T) = 0.2$ K, $u(p) = 10$ kPa, $u(K) = 10^{-2}$.

the studied ATPS in the recovery of avonoids, the partition coefficients of rutin and quercetin in the {[Ch][Ala] (1) + K_3PO_4 or K_2HPO_4+ (2) + water (3)} ATPS were determined at $T = 298.15$ K and atmospheric pressure.

The ability of phase splitting of CAAIL follows the order: [Ch][Ala] > [Ch][Gly] > [Ch][Ser].

The applicability of the these CAAILs-based ATPS in the extraction of two avonoids was evaluated through the determination of the distribution coefficients of rutin and quercetin on the {[Ch][Ala] (1) + K_3PO_4 or K_2HPO_4+ (2) + water (3)} ATPS as they showed to have the larger biphasic regions from all systems previously studied.

The high values of the obtained partition coefficients demonstrated that this kind of ATPS may be applied for the separation of the rutin and quercetin in the biotechnology industry.

Table 4
Parameters obtained using Othmer-Tobias equation (eq (3)) and Brancroft equation (eq (4)) with the correlation coefficients.

ATPS	Othmer-Tobias			Bancroft		
	n	K_1	R^2	*r*	K_2	R^2
{[Ch][Ala] (1) + K_3PO_4 + (2) + water (3)}	0.904	−0.223	0.997	0.998	0.249	0.998
{[Ch][Gly] (1) + K_3PO_4 + (2) + water (3)}	0.704	0.064	0.997	1.393	−0.012	0.996
{[Ch][Ser] (1) + K_3PO_4 + (2) + water (3)}	1.880	−0.153	0.999	0.468	0.080	0.999
{[Ch][Ala] (1) + K_2HPO_4 + (2) + water (3)}	1.422	0.080	0.998	0.681	−0.071	0.997
{[Ch][Gly] (1) + K_2HPO_4 + (2) + water (3)}	1.061	0.142	0.996	0.946	−0.111	0.996
{[Ch][Ser] (1) + K_2HPO_4 + (2) + water (3)}	1.539	0.182	0.999	0.667	−0.096	0.999

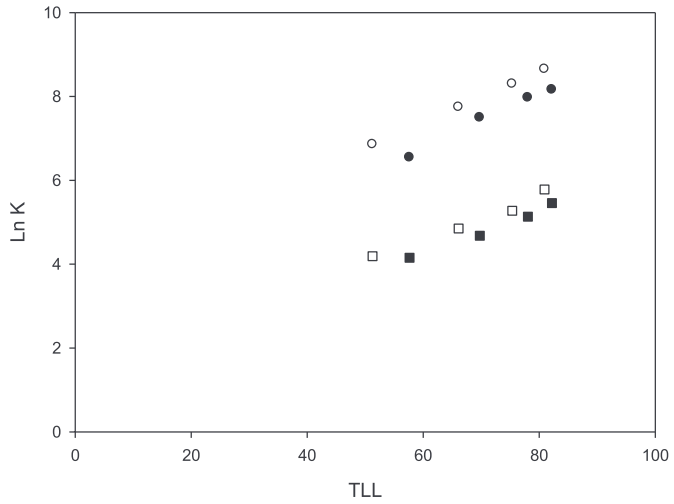


Fig 7 Logarithm of the partition coefficients of the rutin and quercetin versus tie-line length for the {[Ch][Ala] (1) + K_3PO_4 or K_2HPO_4 (2) + water (3)} systems at $T = 298.15$K and atmospheric pressure. (○) rutin for the {[Ch][Ala] (1) + K_3PO_4 (2) + water (3)}, (●) rutin for the {[Ch][Ala] (1) + K_2HPO_4 (2) + water (3)}, ( ) quercetin for the {[Ch][Ala] (1) + K_3PO_4 (2) + water (3)} and (■) quercetin for the {[Ch][Ala] (1) + K_2HPO_4 (2) + water (3)}.

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Appendix A Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j. uid.2019.03.024.

References

[1] P.T. Anastas, J.B. Zimmerman, Design through the 12 principles of green engineering, Environ. Sci. Technol. 37 (2003) 94–101.
[2] C. Capello, U. Fischer, K. Hungerbuhler, What is a green solvent? A comprehensive framework for the environmental assessment of solvents, Green Chem. 9 (2007) 927–934.
[3] J. Banerjee, R. Singh, R. Vijayaraghavan, D. MacFarlane, A.F. Patti, A. Arora, Bioactives from fruit processing wastes: green approaches to valuable chemicals, Food Chem. 225 (2017) 10–22.
[4] H. Passos, M.G. Freire, J.A.P. Coutinho, Ionic liquid solutions as extractive solvents for value-added compounds from biomass, Green Chem. 16 (2014) 4786–4815.
[5] A. Teixeira, N. Baenas, R. Dominguez-Perles, A. Barros, E. Rosa, D. Moreno, C. Garcia-Viguera, Natural bioactive compounds from winery by-products as health promoters: a review, Int. J. Mol. Sci. 15 (2014) 15638–15678.
[6] L. Zhang, D.M. Kujawinski, E. Federherr, T.C. Schmidt, M.A. Jochmann, Caffeine in your drink: natural or synthetic, Anal. Chem. 84 (2012) 2805–2810.
[7] A.N. Panche, A.D. Diwan, S.R. Chandra, Flavonoids: an overview, J. Nutr. Sci. 5 (2016) 1–15.
[8] R.J. Nijveldt, E. van Nood, D.E. van Hoorn, P.G. Boelens, K. van Norren, P.A. van Leeuwen, Flavonoids: a review of probable mechanisms of action and potential applications, Am. J. Clin. Nutr. 74 (2001) 418–425.
[9] C. Rice-Evans, N. Miller, G. Paganga, Antioxidant properties of phenolic compounds, Trends Plant Sci. 2 (1997) 152–159.
[10] R. Tsao, R. Yang, Optimization of a new mobile phase to know the complex and real polyphenolic composition: towards a total phenolic index using high-performance liquid chromatography, J. Chromatogr. A 1018 (2003) 29–40.
[11] N.C. Cook, S. Samman, Flavonoids–chemistry, metabolism, cardioprotective effects, and dietary sources, Nutr. Biochem. 7 (1996) 66–76.
[12] G.R. Beecher, Overview of dietary avonoids: nomenclature, occurrences and intake, J. Nutr. 133 (2003) 3248S–3254S.
[13] Q. Liu, W. Cai, X. Shao, Determination of seven polyphenols in water by high performance liquid chromatography combined with preconcentration, Talanta 77 (2008) 679–683.
[14] C. Faggio, A. Sureda, S. Morabito, A. Sanches-Silva, A. Mocan, S. Fazel Nabavi, S. Mohammad Nabavi, Flavonoids and platelet aggregation: a brief review, Eur. J. Pharmacol. 807 (2017) 91–101.
[15] I. Ignat, I. Volf, V.I. Popa, Critical review of methods for characterization of polyphenolic compounds in fruits and vegetables, Food Chem. 126 (2011) 1821–1835.
[16] M.A. Fernandez, M. Espino, F.J.V. Gomez, M.F. Silva, Novel approaches mediated by tailor-made green solvents for the extraction of phenolic compounds from agro-food industrial by-products, Food Chem. 239 (2018) 671–678.
[17] E. Revilla, J.-M. Ryan, G. Martín-Ortega, Comparison of several procedures used for the extraction of anthocyanins from red grapes, J. Agric. Food Chem. 46 (1998) 4592–4597.
[18] N.J.K. Simpson, Solid-Phase Extraction: Principles, Techniques, and Applications, Taylor Francis, 2000.
[19] M. Petkovic, K.R. Seddon, L.P.N. Rebelo, C. Silva Pereira, Ionic liquids: a pathway to environmental acceptability, Chem. Soc. Rev. 40 (2011) 1383–1403.
[20] B.L. Gadilohar, G.S. Shankarling, Choline based ionic liquids and their applications in organic transformation, J. Mol. Liq. 227 (2017) 234–261.
[21] T.P. Thuy Pham, C.-W. Cho, Y.-S. Yun, Environmental fate and toxicity of ionic liquids: a review, Water Res. 44 (2010) 352–372.
[22] M. Iqbal, Y. Tao, S. Xie, Y. Zhu, D. Chen, X. Wang, L. Huang, D. Peng, A. Sattar, M.A.B. Shabbir, H.I. Hussain, S. Ahmed, Z. Yuan, Aqueous two-phase system (ATPS): an overview and advances in its applications, Biol. Proced. Online 18 (2016) 1–18.
[23] J.C. Merchuk, B.A. Andrews, J.A. Asenjo, Aqueous two-phase systems for protein separation. Studies on phase inversion, J. Chromatogr. B Biomed. Sci. Appl. 711 (1998) 285–293.
[24] D.F. Othmer, P.E. Tobias, Liquid-liquid extraction data toluene and acetaldehyde systems, Ind. Eng. Chem. 34 (1942) 690–692.
[25] G. Tubio, L. Pellegrini, B.B. Nerli, G.A. Pico, Liquid–Liquid equilibria of aqueous two-phase systems containing poly(ethylene glycols) of different molecular weight and sodium citrate, J. Chem. Eng. Data 51 (2006) 209–212.
[26] R. Wang, Y. Chang, Z. Tan, F. Li, Applications of choline amino acid ionic liquid in extraction and separation of avonoids and pectin from ponkan peels, Separ. Sci. Technol. 51 (2016) 1093–1102.
[27] C.P. Song, R.N. Ramanan, R. Vijayaraghavan, D.R. MacFarlane, E.-S. Chan, C.-W. Ooi, Green, aqueous two-phase systems based on cholinium aminoate ionic liquids with tunable hydrophobicity and charge density, ACS Sustain. Chem. Eng. 3 (2015) 3291–3298.
[28] S. Hu, T. Jiang, Z. Zhang, A. Zhu, B. Han, J. Song, Y. Xie, W. Li, Functional ionic liquid from biorenewable materials: synthesis and application as a catalyst in direct aldol reactions, Tetrahedron Lett. 48 (2007) 5613–5617.
[29] M. Lorenzo, M. Vilas, P. Verdía, J. Salgado, M. Villanueva, E. Tojo, Long-Term thermal stabilities of ammonium ionic liquids designed as potential absorbents of ammonia, RSC Adv. 5 (2015) 41278–41284.
[30] W. Kunz, J. Henle, B.W. Ninham, 'Zur Lehre von der Wirkung der Salze (about the science of the effect of salts): franz Hofmeister's historical papers, Curr. Opin. Colloid Interface Sci. 9 (2004) 19–37.
[31] M. Ide, Y. Maeda, H. Kitano, Effect of hydrophobicity of amino acids on the structure of water, J. Phys. Chem. B 101 (1997) 7022–7026.
[32] S.P.M. Ventura, C.M.S.S. Neves, M.G. Freire, I.M. Marrucho, J. Oliveira, J.A.P. Coutinho, Evaluation of anion in uence on the formation and extraction capacity of ionic-liquid-based aqueous biphasic systems, J. Phys. Chem. B 113 (2009) 9304–9310.