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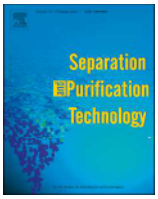

Novel ethyl lactate based ATPS for the purification of rutin and quercetin

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ABSTRACT

The application of new aqueous two-phase systems (ATPS) composed by a hydrophilic green solvent, ethyl lactate, and two low environmental impact salts: sodium and potassium citrate have been studied for the recovery of two flavonoids from aqueous solutions. First, to understand the phase formation process of the ATPS, binodal curves and tie-lines and their respective slopes and tie-line lengths were determined at T = 298.15 K and atmospheric pressure. The degree of consistency of the tie-lines was evaluated using Othmer-Tobias and Bancroft equations. Moreover, the influence of the change of the salt cation on the phase behaviour has been assessed. Furthermore, the UNIversal QUAsiChemical (UNIQUAC) thermodynamic model was applied to describe the phase equilibria presented in this work. Furthermore, the partitioning of two flavonoids, rutin and quercetin, in these ATPS {ethyl lactate (1) + citrate salts (2) + water (3)} was measured at T = 298.15 K and atmospheric pressure. Finally, the obtained results were compared with those found in literature.

1 Introduction

Since Albertsson in 1986 proposed for the first time the use of aqueous two-phase systems (ATPS) as a suitable alternative to conventional liquid–liquid extraction techniques to recover and purify several biomolecules such as proteins and nucleic acids [1], this separation technique has gained a great interest in the field of the separation and purification of a broad variety of biomolecules like: proteins, enzymes, drugs, and other added-value compounds [2–7].

ATPS are composed by two liquid phases that are immiscible from a critical composition. Some of the compounds used on the ATPS formation are polymers (polyethylene glycol, dextran…), salts (phosphates, carbonates…), carbohydrates (glucose, lactose…) or ionic liquids [4]. This separation technique presents many advantages over other conventional extraction processes like: simplicity, biocompatibility, low cost or being an environmentally friendly separation technique due to its high water content. However, their industrial implementation is still scarce due to the lack of knowledge of the mechanisms that rule the partition of biomolecules in this kind of systems [4,7–9].

In the last years, a large amount of researches has focused on the application of ionic liquid-based ATPS in the extraction of biomolecules such as amino acids, proteins, enzymes, among others [2,4,10]. Nevertheless, the most used ionic liquids are toxic and poorly biodegradable and biocompatible [11]. Moreover, inorganic salts (phosphate, carbonate or sulphate salts) in the ATPS formation are widely employed as salting out agents [2,8,9] and the use of these kind of salts at large scale may cause environmental distress. For these reasons, the search of solvents and salting out agents to ATPS formation with better performance regarding environmental and toxicity issues is required.

In view of this, ethyl lactate (EL), a monobasic ester that can be produced from bio-renewable sources as corn fermentation [12,13], has garnered interest as an alternative green solvent in the last years, due to its favourable properties such a low toxicity, high biodegradability, low eco-toxicity, non-corrosiveness or harmless to ozone shield [14,15]. Moreover, several studies reported in literature have presented good results as extraction agent for different biomolecules as caffeine, flavonoids, phenolic compounds or hydrocarbons [16–21].

Regarding the selection of the salting-out agent, recent studies point out the use of organic salts like citrates or tartrates as sustainable alternatives to inorganic salts [22–24]. In this work, two citrate salts, sodium and potassium citrate, were selected, according to their biodegradable and nontoxic nature, as well as, being used as additive for food (E331 sodium citrate and E332 potassium citrate).

Therefore, the extraction of two flavonoids from aqueous solutions using aqueous two-phase systems involving ethyl lactate and the organic salts, sodium and potassium citrate, is presented in this work with

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the aim of getting a more healthful and sustainable separation process.

Flavonoids are a family of antioxidant biomolecules present in colourful fruits and vegetables compounds. This type of phenolic compounds is being increasingly demanded due to its regularly consumption, which has been associated with the reduction in the incidence of different diseases [25,26]. The selected flavonoids, rutin and quercetin, are two of the most representative ones and are widely used in food supplements and cosmetics [27].

For this, the knowledge of the biphasic region of the ATPS, the initial mixture composition and the composition of the individual phases at equilibrium is crucial to any extraction process and to predict the mechanism behind to the partitioning of a solute between the two immiscible aqueous phases. Binodal curves and tie-lines compositions for the ATPS {EL (1) + sodium citrate (2) + water (3)} and {EL (1) + potassium citrate (2) + water (3)} were experimentally determined at $T = 298.15$ K and atmospheric pressure. Merchuk equation [3] was used to fit the binodal curves and the degree of consistency of the obtained tie-lines was tested using the Othmer-Tobias [28] and Brancoft [29] equations. With these systems, it is possible to analyse the influence of the change of the cation of the citrate salt on the phase separation behaviour. Additionally, the experimental data were compared with that found in literature. To our knowledge, only data for the ATPS involving sodium citrate as salting out agent are available to date [20]. Finally, experimental liquid–liquid equilibria data were correlated using the Universal Quasichemical (UNIQUAC) [30] thermodynamic model.

Furthermore, to evaluate the ability of the new ethyl lactate-based ATPS for extraction, partitioning of two flavonoids, rutin and quercetin, was carried out. With these systems, it is possible to analyse the influence of the salt cation on the partitioning and compare the extraction results of the two different flavonoids.

2 Experimental

2.1. Chemicals

(-) Ethyl L-lactate ($C_5H_{10}O_3$) with mass fraction purity higher than 0.98 was purchased from Sigma-Aldrich. Citrate salts: sodium citrate tribasic dihydrate ($Na_3C_6H_5O_7{\cdot}2H_2O$) and potassium citrate tribasic monohydrate ($K_3C_6H_5O_7{\cdot}H_2O$), with mass fraction purity higher than 0.99 were acquired from Sigma-Aldrich. All chemicals were used without any further purification.

Flavonoids: rutin hydrate ($C_{27}H_{30}O_{16}{\cdot}xH_2O$) and quercetin ($C_{15}H_{10}O_7$) were purchased from Sigma-Aldrich with mass fraction purity higher than 0.94. The flavonoids were used without further purification and were stored at $T \leq 281.15$ K in order to prevent their thermal degradation.

2.2. Apparatus

Solutions used in the experimental procedure were prepared by weighing with double distilled water. Their concentrations were obtained gravimetrically on a Mettler AX-205 Delta Range balance with an uncertainty in the measurement of $\pm 3{\cdot}10^{-4}$ g. The physical properties, density (ρ) and refractive index (n_D), of the binary and ternary mixtures were measured using an Anton Paar DSA-5000 M digital vibrating tube densimeter with an uncertainty in the measurement of $\pm 3{\cdot}10^{-5}$ $g{\cdot}cm^{-3}$ and an automatic refractometer Abbemat-HP Dr. Kernchen with an uncertainty in the measurement of $\pm 4{\cdot}10^{-5}$, respectively. The equilibrium temperature was maintained at $T = 298.15$ K using a PoliScience thermostatic bath with a digital temperature controller ASL model F200 with an uncertainty in the measurement of ± 0.01 K. The pH measurements of the phase equilibria were determined using a pHmeter Crison Basic 20 with an uncertainty in the measurement of pH and temperature of ± 0.01 and ± 0.1 K, respectively. Moreover, the absorbance measurements involved in the partitioning studies were performed using an UV–Vis spectrophotometer Thermo Scientific Varioskan flash with an uncertainty of measurement of $\pm 10^{-4}$.

2.3. Phase diagrams determination: binodal curves and tie-lines

2.3.1. Binodal curves

Aqueous solutions of salt (sodium or potassium citrate) of known composition (30.52 wt , weight percentage, for sodium citrate and 26.98 wt , weight percentage, for potassium citrate) were prepared by weight. These concentrations were chosen to obtain the maximum solubility of salt in water, which ensures that the mixture is homogeneous. Then, they were placed into glass tubes and titrated with ethyl lactate (EL) until the phase transition (slight turbidity) in the mixtures was observed. The composition of the mixtures was determined by weighting. Afterwards, water was added dropwise to the cloudy mixtures to get clear homogeneous mixtures again, and this procedure was repeated until enough data was obtained to cover the binodal curve. The method follow for the determination of the compositions of the binodal curves has been described in detail in a previous work [31].

In order to ensure that the whole range of compositions was covered, a known amount of ethyl lactate was titrated with the corresponding salt solution. The measured experimental data were put together to build the complete solubility curve. The equation given by Merchuk *et al.* [3] was used to fit the binodal curves:

$$[EL] \quad A\cdot\exp[B\cdot[salt]^{0\ 5} - C\cdot[salt]^{3}] \tag{1}$$

where [EL] and [salt] are the ethyl lactate and salt compositions in weight percentage, respectively, and A, B and C are the temperature-dependent adjustable parameters obtained by nonlinear least squares regression.

2.3.2. Tie-lines

For the experimental determination of tie-lines compositions, ternary mixtures with known compositions, within the immiscible region, were gravimetrically prepared in glass cells and sealed with silicon covers to avoid losses by evaporation or moisture absorption. Then, they were placed into a thermostatic bath at $T = 298.15$ K and were vigorously stirred for 6 h to allow an intimate contact between the two phases. Thereafter, they were left to settle overnight to guarantee the achievement of the equilibrium. After the phase separation, samples of both phases were taken with syringes and their compositions were determined through the measurement of both density and refractive index at $T = 298.15$ K and atmospheric pressure.

To estimate the tie-lines compositions, a method based on a previous determination of the isolines of the physical properties of density and refractive index was carried out. For this purpose, different binary and ternary mixtures with known composition were prepared within the miscible region of the corresponding phase diagram. The compositions of these samples were calculated paying close attention to have enough points well distributed covering all the miscible region of the phase diagram to best characterize the ternary system. Density and refractive index of each miscible sample were measured at $T = 298.15$ K and atmospheric pressure and a polynomial expression for each physical property versus mixtures composition was obtained as follows:

$$Q \quad \sum_{i}^{n}\sum_{j}^{m} {}_{ij}w_i^{m} \tag{2}$$

where: Q is the physical property (ρ or n_D), n is the number of components of the ternary mixtures, m is the polynomial degree, w_i is the mass fraction of component i and A_{ij} are the adjustable parameters.

Therefore, the tie-lines compositions were obtained from the measurement of density and refractive index of the top and bottom phases for each tie-line by crossing the two corresponding polynomial expressions (Eq. (2)).

Two important characteristics of the tie-lines are the tie-line length, TLL, and tie-lines slope, STL, that can be calculated using the following expressions:

$$TLL = \sqrt{\ {}^{top}_{EL} - {}^{bot}_{EL})^2 \qquad {}^{top}_{salt} - {}^{bot}_{salt})^2} \tag{3}$$

$$STL \qquad {}^{top}_{EL} - {}^{bot}_{EL})/\ {}^{top}_{salt} - {}^{bot}_{salt}) \tag{4}$$

where the subscripts EL and salt denote ethyl lactate and salt compositions in mass fraction and the superscripts top and bot refer to the top and bottom phases, respectively.

Moreover, the degree of consistency of the obtained tie-lines was tested using the equations proposed by Othmer-Tobias (Eq. (5)) and Brancroft (Eq. (6)) which are the most widely used in this kind of systems [28,29]:

$$\log\left[\frac{-\quad {}^{top}_{EL}}{{}^{top}_{EL}}\right] = K \qquad n\cdot\log\left[\frac{-\quad {}^{bot}_{salt}}{{}^{bot}_{salt}}\right] \tag{5}$$

$$\log\left[\frac{-\quad {}^{bot}_{water}}{{}^{bot}_{salt}}\right] = K_2 \qquad r\cdot\log\left[\frac{-\quad {}^{top}_{water}}{{}^{top}_{EL}}\right] \tag{6}$$

where ${}^{top}_{w\ ter}$ and ${}^{bot}_{w\ ter}$ are the mass fraction of water in the top and bottom phases at equilibrium, respectively and where ${}^{top}_{L}$ and ${}^{bot}_{s\ lt}$ stand for the compositions, in mass fraction, of EL in the top phase and salt in the bottom phase, respectively.

pH of the two equilibrium phases (top and bottom phases) were measured to check for possible changes of this property, which can lead to different structures of the flavonoids.

2.4. Partitioning of flavonoids

In order to study the influence of the tie-line length (i.e., the concentration of ethyl lactate and salt on the feed) of the ATPS, three tie lines of the ATPS studied in this work were chosen to carry out the partition studies of the rutin and quercetin. These were the tie-line which has the lowest content of ethyl lactate (TL1), one with an average content of ethyl lactate (TL3) and the tie-line with the highest content of ethyl lactate (TL6).

Aqueous solutions of flavonoids of concentration 0.48 g/l for rutin and 0.09 g/l for quercetin were prepared. Three ternary mixtures with the same composition of the tie-line determination were selected, and 1 ml of stock solution of flavonoid was added to each ternary mixture. All mixtures were prepared by weight with an uncertainty of ± 3·10 − 4 g. Each mixture was shaken vigorously for 6 h and to ensure complete separation, the samples were kept in a water bath at T = 298.15 K. After a careful separation of both phases, samples of each phase were withdrawn and the flavonoid quantification was carried out using a UV-spectroscopic at a wavelength of 368 nm for rutin and 324 nm for quercetin. Calibration curves for rutin and quercetin previously established were used, the range of the calibration was: from 0 to 0.0001185 g/mL for the rutin and to 0.0000117 g/mL for the quercetin.

To check for possible interferences of both ethyl lactate and organic salts, blank samples (with the same composition of tie-line but without flavonoid) were prepared and their absorbance measured in the same wavelength. No interference was found neither for ethyl lactate or for organic salts in the two wavelengths used.

The partition coefficients of rutin and quercetin, ${}_{flav}$, are defined as the ratio of the concentration of the each flavonoid in the top phase (ethyl lactate-rich phase) and bottom phase (organic salt-rich phase) according to Eq. (7):

$$K_{flav} \qquad \frac{{}^{top}_{flav}}{{}^{bottom}_{flav}} \tag{7}$$

where ${}^{top}_{flav}$ and ${}^{bottom}_{flav}$ are the concentration (g/l) of each flavonoid in the top and in the bottom phases, respectively.

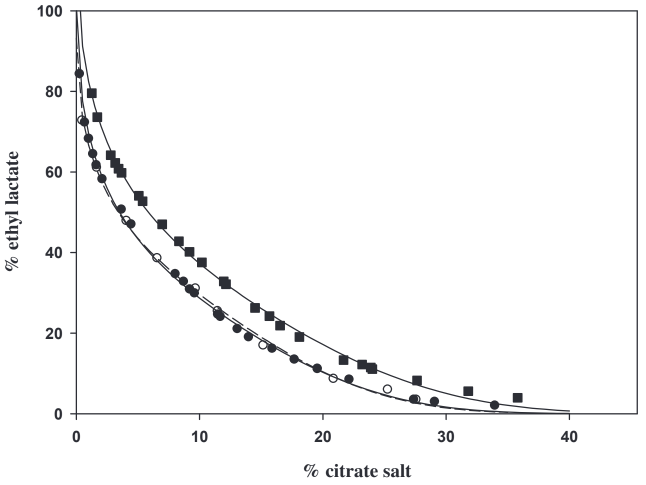

Fig 1 Binodal curves of the ternary systems {ethyl lactate (1) + citrate salts (2) + water (3)} determined at T = 298.15 K and p = 0.1 MPa. Symbols represent the experimental data and the line corresponds to the fit by Eq. (1). (●) sodium citrate (this work); (○) sodium citrate [reference [20]] and (■) potassium citrate (this work).

The percentage extraction, E_{flav} , of flavonoid is defined as the percentage ratio between the amount (grams) of flavonoid in the top phase, g^{top}_{flav} and in the total mixture, g^{total}_{flav}

$$E_{flav} \qquad 100\cdot\frac{g^{top}_{flav}}{g^{total}_{flav}} \tag{8}$$

3 Results and discussion

3.1. Phase diagrams: Binodal curves and tie-lines

Binodal curves for the aqueous two-phase systems containing ethyl lactate and the organic salts, sodium citrate and potassium citrate, have been experimentally determined at *T* = 298.15 K and atmospheric pressure. Experimental phase diagrams are displayed in weight percentage in Fig. 1 and presented in Table 1. It should be noted that the

Table 1
Binodal curve data (weight percentage, w_i) for the ternary systems {EL (1) + salts (2) + water (3)} determined experimentally at *T* = 298.15 K and *p* = 0.1 MPa.[a]

w_1	w_2	w_1	w_2	w_1	w_2
{EL (1) + sodium citrate (2) + water (3)}					
1.9	34.0	20.9	13.1	50.6	3.7
2.9	29.1	24.0	11.7	58.1	2.1
3.4	27.4	24.5	11.5	61.6	1.7
8.4	22.2	29.8	9.6	64.4	1.4
11.0	19.6	30.8	9.2	68.2	1.0
13.4	17.7	32.7	8.7	72.2	0.7
16.1	15.9	34.6	8.1	84.2	0.3
18.9	14.0	46.9	4.5		
{EL (1) + potassium citrate (2) + water (3)}					
4.0	35.8	24.2	15.7	54.1	5.1
5.6	31.8	26.3	14.5	59.8	3.7
8.3	27.6	32.1	12.1	60.8	3.4
11.1	24.0	32.8	12.0	62.2	3.2
11.5	23.9	37.6	10.2	64.2	2.8
12.2	23.2	40.2	9.2	73.6	1.7
13.3	21.7	42.8	8.3	79.6	1.3
19.0	18.1	47.0	7.0		
21.9	16.5	52.7	5.4		

[a] Standard uncertainties, *u*, are: *u*(*T*) = 0.2 K, *u*(*p*) = 10 kPa, *u*(*w*) = 10^{-1}.

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

System	A	B	C	σ[a]
{EL (1) + sodium citrate (2) + water (3)}	101.115	−0.374	$7.64{\cdot}10^{-5}$	0.971
{EL (1) + potassium citrate (2) + water (3)}	116.409	−0.346	$4.61{\cdot}10^{-5}$	0.956

[a] Standard deviation; $\sigma \quad \left\{\sum_{i}^{n_{d\ t}} \quad z - z_{c\ l}))^2/n_{d\ t}\right\}^{1/2}$ (z and z_{cal} are the values of the experimental and calculated property and n_{dat} is the number of experimental data points)

calculations of salt compositions were carried out taking into account the additional water included in their corresponding commercial form and hence added to water composition of each phase diagram.

The adjustable parameters calculated using the Merchuk equation (Eq. (1)) together with the standard deviations are summarized in Table 2. From Fig. 1, it can be visually checked the good fit of Merchuk equation to experimental data for both ternary systems studied in this work. Additionally, the reported data for the ternary system {ethyl lactate (1) + sodium citrate (2) + water (3)} by Kamalanathan *et al.* [20] are also plotted in Fig. 1. As it can be observed, the experimental data for the ATPS involving sodium citrate presented in this work are in good agreement with the values reported in the literature.

Moreover, it is possible to analyse the influence of the salt cation on the binodal curve with experimental data drawn in Fig. 1. The system containing sodium citrate exhibits larger biphasic region than the ATPS involving potassium citrate. Hence, the salting-out ability of the studied citrate salts follows the order: $Na^+ > K^+$ which is in accordance with the well-known Hofmeister series [32] and the results reported by other authors in ionic liquid-based ATPS [2,10,33].

As it has been commented in the previous section, the tie-lines compositions were calculated using a method based on the determination of the physical properties of mixtures within the miscible region of the corresponding phase diagram. These experimental data were fitted to a polynomial expression for each physical property versus composition using Eq. (2) and the obtained adjustable are shown in Table 3.

Feed and tie-lines compositions are depicted in Fig. 2 and shown in Table 4 together with the calculated slopes, lengths of the tie-lines and values of pH for top and bottom phases.

As it can be inferred from Table 4 and Fig. 2, a higher content of ethyl lactate in the initial composition of the tie-line gives rise to higher ethyl lactate and salt concentration in the top and bottom phases, respectively. Therefore, the top phase is the ethyl lactate-rich phase and the bottom one is the salt-rich phase for both studied ATPS. Tie-line lengths (TLL) and tie-line slopes (STL) were calculated from tie-lines compositions. Higher TLL values mean higher degree of separation, that is to say, larger differences between the compositions of top and bottom phases. The highest values of TLL were obtained for the ternary system involving potassium citrate. Regarding the slope of the tie-lines, the slightly difference between the STL values show that the tie-lines are practically parallel and the steeper slopes were obtained for the system {ethyl lactate (1) + sodium citrate (2) + water (3)}.

The consistency of the tie-lines was satisfactorily ascertained using the Othmer-Tobias (Eq. (5)) and Bancroft (Eq. (6)) equations [28,29] and the obtained adjustable parameters and the correlation coefficients are presented in Table 5. The values of pH for the top and bottom phases are very similar for both studied systems. Therefore, the structures of rutin and quercetin remain the same in both liquid phases and the partition coefficients measured correspond to the values for quercetin and rutin.

Finally, a comparison of the calculated tie-line data for the system {ethyl lactate (1) + sodium citrate (2) + water (3)} with values reported in literature [20] was carried out. As it can be observed in Fig. 3 a good agreement is observed between our data and those reported by Kamalanathan *et al.* [20].

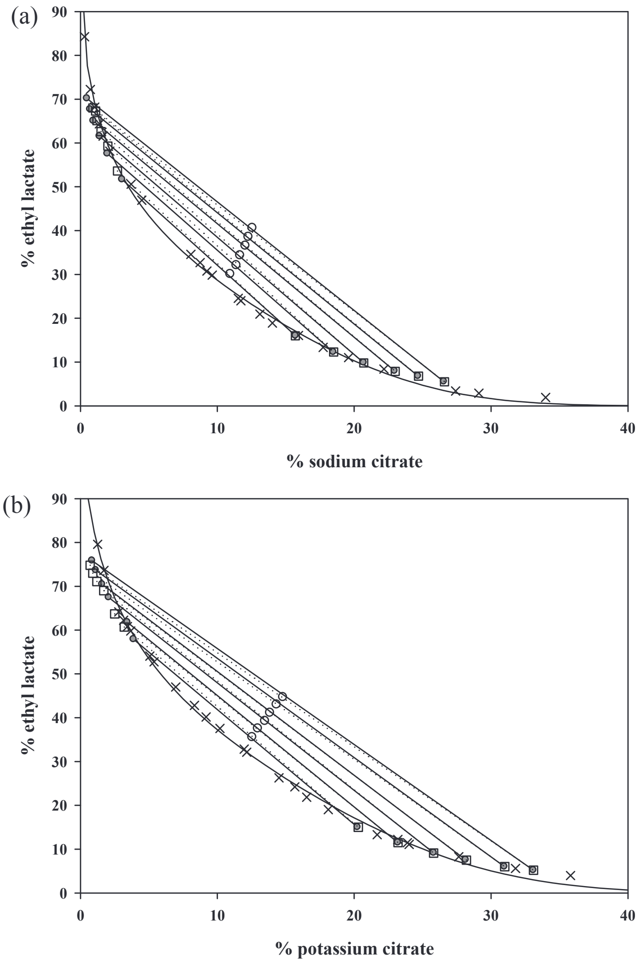

Fig 2 Binodal curves and corresponding tie-lines for the ternary systems containing ethyl lactate, salt and water at T = 298.15 K and p = 0.1 MPa. (○) feed compositions, (X) binodal curve, (–) fit of the binodal data, (●, grey) experimental tie-line compositions and (☐) calculated tie-lines compositions using the UNIQUAC model. (a) {EL (1) + sodium citrate (2) + water (3)}; (b) {EL (1) + potassium citrate (2) + water (3)}.)

3.2. Thermodynamic modelling

There are not studies about the thermodynamic modelling of ATPS with ethyl lactate, although correlation and prediction of experimental data are fundamental to apply the APTS process in the industry because it is impossible to cover the phase behaviours of all conditions through experiments.

For this reason, in this work UNIQUAC [30] thermodynamic model was adopted to describe the liquid–liquid equilibrium phase diagrams. This model was selected since it keeps the simplicity of the Wilsońs equation [34] and allows to extrapolate for multicomponent mixtures of immiscible liquids. Even though this model was not originally developed for systems containing electrolytes, it has provided very adequate correlating ability for aqueous two-phase systems involving ionic liquids or deep eutectic solvents with organic or inorganic salts [35–41] and was also widely used in modelling LLE of polymer-salt systems. The required van der Waals parameters, r_i (volume) and q_i, (surface area) for ethyl lactate and water were taken from literature [42] and for organic salts were calculated in this work. Their values are: $r_{\text{ethyl lactate}} = 4.4555$, $r_{\text{water}} = 0.9200$, $r_{\text{Nacitrate}} = 6.0872$, $r_{\text{Kcitrate}} = 6.8702$

Table 3
Polynomial expressions for the density and refractive index as function of composition for the studied ternary systems.

{EL (1) + sodium citrate (2) + water (3)}
$\rho = -\,0\;0185\cdot\;{}_1\quad 1\;0792\cdot\;{}^2_1 - 0\;0190\cdot\;{}^3_1\quad 0\;2607\cdot\;{}_2\quad 1\;6766\cdot\;{}^2_2\quad 0\;2607\cdot\;{}^3_2\quad 0\;0607\cdot\;{}_3\quad 0\;9817\cdot\;{}^2_3 - 0\;0455\cdot\;{}^3_3$
$n\;=-\,0\;0061\cdot w_1\quad 1\;4665\cdot w_1^2 - 0\;0413\cdot w_1^3\quad 0\;0252\cdot w_2\quad 1\;5414\cdot w_2^2 - 0\;0036\cdot w_2^3\quad 0\;1495\cdot w_3\quad 1\;2431\cdot w_3^2 - 0\;0609\cdot w_3^3$
{EL (1) + potassium citrate (2) + water (3)}
$\rho = -\,0\;0892\cdot\;{}_1\quad 1\;0905\cdot\;{}^2_1\quad 0\;0436\cdot\;{}^3_1\quad 0\;3334\cdot\;{}_2\quad 1\;6399\cdot\;{}^2_2 - 0\;3009\cdot\;{}^3_2 - 0\;0073\cdot\;{}_3\quad 1\;0151\cdot\;{}^2_3 - 0\;0120\cdot\;{}^3_3$
$n\;=-\,0\;0476\cdot w_1\quad 1\;4474\cdot w_1^2\quad 0\;0167\cdot w_1^3\quad 0\;0760\cdot w_2\quad 1\;4907\cdot w_2^2 - 0\;1628\cdot w_2^3\quad 0\;0009\cdot w_3\quad 1\;3268\cdot w_3^2\quad 0\;0041\cdot w_3^3$

and $q_{\text{ethyl lactate}} = 3.9280$, $q_{\text{water}} = 1.3997$, $q_{\text{Nacitrate}} = 5.7890$ and $q_{\text{kcitrate}} = 6.5780$. The six binary adjustable parameters of the UNIQUAC model, Δu_{ij}, together with the values of the root-mean-square deviation of the composition, σx, are shown in Table 6. The root-mean-square deviation, σx, were ascertained as follows:

$$\sigma x = \sqrt{100\left(\frac{\sum^{m}\sum_{j}^{n-1}\; x_j^{\mathrm{I,exp}} - x_j^{\mathrm{I,cal}})^2 \qquad x_j^{\mathrm{II,exp}} - x_j^{\mathrm{II,cal}})^2}{2mn}\right)} \tag{9}$$

where m is the number of the tie-lines and n is the number of components in the mixture. The superscripts I and II refer to the top and bottom phase, respectively, x_i^{xp} and $x_i^{c\;l}$ are the experimental and calculated composition in mole fraction of component i in both phases, respectively. Taking into account the values of the deviations obtained, it can be inferred that the UNIQUAC model describe accurately the experimental data. The high quality of the correlation can also be visually confirmed in Fig. 2, where the experimental and calculated tie-lines using the UNIQUAC model are displayed.

3.3. Partitioning of rutin and quercetin

In order to explore the applicability of the ATPS proposed in the biotechnology industry, the partitioning of two flavonoids: rutin and quercetin in the {ethyl lactate (1) + sodium citrate or potassium citrate (2) + water (3)} ATPS at $T = 298.15$ K and atmospheric pressure was carried out.

The results of the values of the partition coefficients, K_{flav}, and percentage extraction, E_{flav} , together the TLL values are shown in Table 7. Table 7 presents the distribution coefficients for two flavonoids in the two ATPS studied, which are higher than one.

The obtained values reveal that the results of the separation of the rutin and quercetin depend on the composition of ethyl lactate and organic salts present in the ATPS. In our essay, the two flavonoids studied showed higher affinity for the ethyl lactate-rich phase. The flavonoids preferentially migrate to the EL-rich phase, showing that the ethyl lactate-based ATPS are new possibilities for the extraction of rutin and quercetin.

Table 5
Parameters obtained using Othmer-Tobias equation (Eq. (5)) and Brancroft equation (Eq. (6)) with correlation coefficients.

ATPS	Othmer-Tobias			Bancroft		
	n	K_1	R^2	r	K_2	R^2
{EL (1) + sodium citrate + (2) + water (3)}	1.186	−0.895	0.999	0.720	0.681	0.999
{EL (1) + potassium citrate + (2) + water (3)}	1.272	−0.889	0.994	0.688	0.629	0.994

Since the value of K strongly depends on the ATPS composition, the representation of the logarithm of the partition coefficient against tie-line length must be linear. Logarithm of the partition coefficient versus TLL of the two flavonoids in the two systems studied is shown in Fig. 4. As can be seen, there is a linear relationship between the logarithm of the partition coefficient and the value of the TLL, which is according to the following equation:

$$\mathrm{Ln}\qquad {}_{\mathrm{flav}}\qquad \alpha\cdot\mathrm{TLL} \tag{10}$$

where α is a constant value which depends on the ATPS composition. The values of the α together with the squared correlation coefficient, R^2, of the two flavonoids on the two studied ATPS were the following: for quercetin on {ethyl lactate + sodium citrate + water}, α = 0.417 and $R^2 = 0.9667$; for rutin on {ethyl lactate + sodium citrate + water}, α = 0.589 and $R^2 = 0.9928$; for quercetin on {ethyl lactate + potassium citrate + water}, α = 0. 372 and $R^2 = 0.9803$ and for rutin on {ethyl lactate + potassium citrate + water}, α = 0.480

Table 4
Experimental phase equilibria compositions (in weight percentage), together with their corresponding tie-line length (TLL), tie-line slope (STL) and pH values for the systems {EL(1) + citrate salts (2) + water (3)} at $T = 298.15$ K and $p = 0.1$ MPa.[a]

N° TL	feed		top phase			bottom phase			TLL	STL
	$[\mathrm{EL}]_{\mathrm{feed}}$	$[\mathrm{salt}]_{\mathrm{feed}}$	$[\mathrm{EL}]_{\mathrm{top}}$	$[\mathrm{salt}]_{\mathrm{top}}$	pH	$[\mathrm{EL}]_{\mathrm{bot}}$	$[\mathrm{salt}]_{\mathrm{bot}}$	pH		
{EL (1) + sodium citrate (2) + water (3)}										
TL1	30.0	11.0	51.7	3.0	7.00	16.0	15.7	6.98	37.85	−2.81
TL2	32.0	11.4	57.5	2.0	6.98	12.3	18.5	6.96	48.18	−2.74
TL3	34.3	11.7	61.5	1.4	6.98	9.8	20.7	6.97	55.17	−2.68
TL4	36.5	12.1	65.0	1.0	7.00	7.9	23.0	7.00	61.17	−2.59
TL5	38.5	12.3	67.7	0.7	6.98	6.8	24.7	6.97	65.44	−2.54
TL6	40.6	12.6	70.1	0.5	6.98	5.5	26.6	7.00	69.68	−2.48
{EL (1) + potassium citrate (2) + water (3)}										
TL1	35.5	12.6	57.9	3.9	7.21	15.0	20.3	7.39	45.91	−2.62
TL2	37.5	13.0	61.7	3.4	7.22	11.5	23.2	7.41	54.02	−2.54
TL3	39.2	13.5	67.4	2.1	7.19	9.1	25.8	7.37	62.96	−2.46
TL4	41.1	13.9	70.4	1.6	7.23	7.5	28.2	7.41	68.28	−2.37
TL5	43.0	14.3	73.6	1.1	7.12	6.0	31.0	7.39	73.92	−2.27
TL6	44.6	14.8	75.8	0.9	7.22	5.2	33.1	7.43	77.66	−2.19

[a] Standard uncertainties, u, are: $u(T) = 0.2$ K, $u(p) = 10$ kPa, $u(w, \text{weight percentage}) = 10^{-1}$, $u(\mathrm{pH}) = 10^{-2}$.

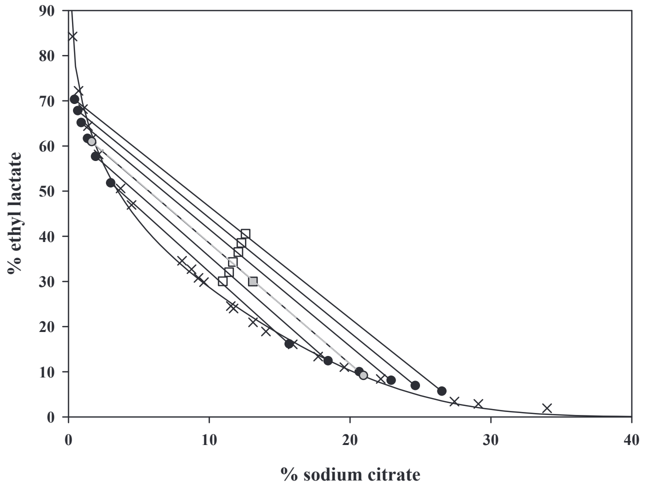

Fig 3 Comparison of tie-line compositions obtained in this work (black) and tie-line compositions reported by Kamalanathan et al. [20] (grey) for the ternary system {ethyl lactate (1) + sodium citrate (2) + water (3)}. Symbols: (□) feed compositions, (X) binodal curve, (●) tie-line data and (–) fit of the binodal data by eq.1 at T = 298.15 K and p = 0.1 MPa.

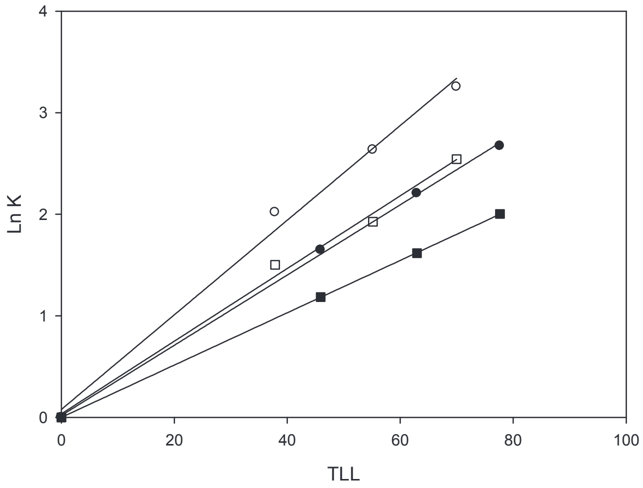

Fig 4 Logarithm of the partition coefficients of the flavonoids versus tie-line length for: (○) quercetin on {EL (1) + sodium citrate (2) + water (3)}, (□) quercetin on {EL (1) + potassium citrate (2) + water (3)}, (●) rutin on {EL (1) + sodium citrate (2) + water (3)}, (■) rutin on {EL (1) + potassium citrate (2) + water (3)} and (—) fits from equation (10) at T = 298.15 K and at p = 101.3 kPa.

Table 6
UNIQUAC parameters, Δu_{ij}, and deviation, σx, obtained from LLE data correlation.

i-j	Δu_{ij}/(kJ·mol^{-1})	Δu_{ji}/(kJ·mol^{-1})	σx
{EL (1) + sodium citrate (2) + water (3)}			
1–2	3127.0	10,000	0.40
1–3	7883.2	8650.0	
2–3	820.00	16,500	
{EL (1) + potasium citrate (2) + water (3)}			
1–2	2889.4	17,580	0.48
1–3	7860.9	717.71	
2–3	352.00	18,925	

and R^2 = 0.9927.

Furthermore, the extraction potential of the two proposed ATPS was evaluated thought the percentage extraction of the rutin and quercetin. In Fig. 5, the values of the percentage extraction as function of TLL for the two flavonoids obtained on the two ATPS are shown.

With the systems chosen, the study of the influence of salt cation on APTS in the separation of flavonoids can be carried out. As can be seen from Fig. 4, Fig. 5 and Table 7, higher K_{flav} and E_{flav} values were achieved for the two flavonoids studied in the ATPS containing sodium citrate as salt, in addition, higher partition coefficient and percentage extraction values were obtained for quercetin than for the routine in both ATPS.

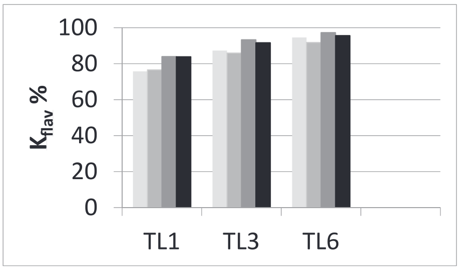

Fig 5 Extraction percentage, K_{flav} of the flavonoids for the three tie-lines. (▩) quercetin on {EL (1) + sodium citrate (2) + water (3)}, (■) quercetin on {EL (1) + potassium citrate (2) + water (3)}, (□) rutin on {EL (1) + sodium citrate (2) + water (3)}, (▩) rutin on {EL (1) + potassium citrate (2) + water (3)}.

Table 7
Partition coefficients (K) and percentage of extraction (E) of flavonoids (rutin and quercetin) in ATPS containing ethyl lactate and sodium citrate or potassium citrate at T = 298.15 K and p = 0.1 MPa.[a]

N° TL	$[EL]_{feed}$	$[salt]_{feed}$	TLL	$^{top}_{flav}$ (g/mL)	$^{bottom}_{flav}$ (g/mL)	K_{flav}	E_{flav}
Quercetin in ATPS: ethyl lactate + sodium citrate + water							
TL1	10.96	30.04	37.85	1.98E−05	2.62E−06	7.53	83.97
TL3	11.68	34.31	55.17	1.71E−05	1.23E−06	13.93	93.29
TL6	12.58	40.55	69.98	1.58E−05	6.10E−07	25.91	97.26
Rutin in ATPS: ethyl lactate + sodium citrate + water							
TL1	10.96	30.04	37.85	9.38E−05	2.09E−05	4.49	75.72
TL3	11.68	34.31	55.17	8.89E−05	1.29E−05	6.87	87.28
TL6	12.58	40.55	69.98	8.36E−05	6.57E−06	12.72	94.56
Quercetin in ATPS: ethyl lactate + potassium citrate + water							
TL1	12.56	35.53	45.91	1.66E−05	3.20E−06	5.19	83.88
TL3	13.50	39.20	62.96	1.58E−05	1.74E−06	9.07	91.65
TL6	14.81	44.64	77.66	1.48E−05	1.02E−06	14.47	95.63
Rutin in ATPS: ethyl lactate + potassium citrate + water							
TL1	12.56	35.53	45.91	8.14E−05	2.49E−05	3.27	76.60
TL3	13.50	39.20	62.96	8.11E−05	1.61E−05	5.04	85.89
TL6	14.81	44.64	77.66	8.00E−05	1.08E−05	7.41	91.80

[a] Standard uncertainties, u, are: $u(T)$ = 0.2 K, $u(p)$ = 10 kPa.

4 Conclusions

In this work, aqueous two-phase systems (ATPS) with ethyl lactate and two organic citrate salts were studied. In order to understand the phase formation process of the ATPS, binodal curves, tie-lines and their respective tie-line lengths and slope tie-line were determined at T = 298.15 K and atmospheric pressure. Besides, Othmer-Tobias and Bancroft equations were using to fit the tie-line compositions. The extraction potential of these new ethyl lactate-based ATPS in the separation of two flavonoids: rutin and quercetin was evaluated through the determination of the partition coefficients and percentage extraction at T = 298.15 K and atmospheric pressure. Furthermore, the UNIQUAC model was used to correlate the liquid–liquid equilibria experimental data and the results from the modelling are in good agreement with the experimental data.

The ability of phase splitting of ATPS follows the order: sodium citrate > potassium citrate, the ATPS with ethyl lactate and sodium citrate showed to have higher biphasic region that system with potassium citrate.

The results obtained for the partitioning of the rutin and quercetin showed that an increase in the value of the tie-line length leads to an increase of the K and percentage extraction values. Besides, the highest distribution coefficients were obtained for the quercetin on the two ATPS studies and on the ATPS involving the organic salt sodium citrate.

The applicability of the ethyl lactate-organic citrate salts ATPS for the separation of rutin and quercetin may be a green approach for the extraction of the biotechnological industry.

CRediT authorship contribution statement

Pedro Velho: Writing - review & editing. **Patricia F Requejo:** Writing - review & editing, Visualization. **Elena Gómez:** Writing - review & editing, Conceptualization, Supervision, Validation. **Eugénia A Macedo:** Conceptualization, Supervision.

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.

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