

Study of Liquid–Liquid Equilibrium of Aqueous Two-Phase Systems Based on Ethyl Lactate and Partitioning of Rutin and Quercetin

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Cite This: *Ind. Eng. Chem. Res.* 2020, 59, 21196–21204

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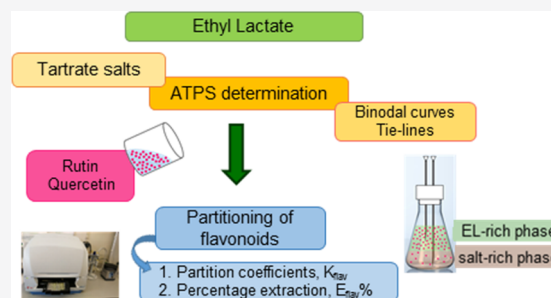
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ABSTRACT: Two ethyl lactate (EL)-based aqueous two-phase systems (ATPSs) were studied at $T = 298.15$ K and atmospheric pressure. Sodium tartrate and potassium sodium tartrate organic salts have been used to form biphasic systems. To assess the phase splitting, binodal curves and tie-lines were obtained experimentally by the cloud point method and a technique, which consist of the density and refractive index, respectively. Binodal curves were fitted with the Merchuk equation obtaining a good fit. Moreover, modeling with the universal quasichemical thermodynamic equation was carried out to accurately represent the two-biphasic systems. Furthermore, experimental binodal curves obtained in this work and literature results for the same systems and for systems with EL and other salts were analyzed regarding their similarity. Finally, the application of the adopted ATPS in the partitioning of two flavonoids, rutin and quercetin, was studied in terms of the partition coefficients and the percentage of extraction. Rutin and quercetin showed better affinity to the EL-rich phase, evidencing that the use of the proposed ATPS may be a successful technique in the biotechnological industry for the separation of each flavonoid.



1. INTRODUCTION

In this work, new aqueous two-phase systems (ATPSs) composed of a novel biosolvent, ethyl lactate (EL) with two biodegradable salts, sodium and potassium sodium tartrate, were evaluated regarding the recovery of flavonoids.

Flavonoids, which are antioxidants and exist in food and several biological products, have gained increasing interest mainly because of their potential nutritional and medical value.¹ These substances have very good properties, such as anti-inflammatory, antiosteoporotic, antiviral, and antioxidant properties.^{2,3} Therefore, flavonoids can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxidative species, which affect many biological functions.¹

Synthetic antioxidants are commonly used in the food industry because of their effectiveness and low price. Nevertheless, their toxicity and the increasing search for safe nutritional additives by consumers have increased the need to identify natural and safe sources. Thus, the recovery of flavonoids from natural sources is a hot topic of improving health through food, mainly fresh fruits and vegetables and also with natural supplements.⁴

The main process to obtain antioxidants for biotechnological applications is solvent extraction and more modern techniques, such as extraction with supercritical fluids. However, these extraction methods are laborious, require operation conditions with high temperatures and pressures, are time consuming, and require abundant volatile and hazardous organic solvents, such as ethanol, methanol, and others;⁵ therefore, in this context, it

is necessary to search new solvents and new techniques that are more environmentally friendly. The application of ATPSs is a separation technique with very low environmental impact and considered accurate and very adequate for the extraction of biomolecules because both liquid phases are mostly composed of water.⁶ ATPSs have been applied to the separation of proteins, enzymes, amino acids, and antibiotics because of their convenient advantages: a better effectiveness and performance of the extraction process can be achieved, as well as greater purity of the selected product and therefore a lower cost of the process.^{7–10} Taking all referred before, the application of ATPSs can be regarded as an alternative liquid–liquid extraction technique that is more environmentally friendly.

ATPSs are formed in aqueous media by the dissolution of two polymers¹¹ or a polymer and a salt.¹² The use of conventional ATPSs (formed by polymer–polymer and polymer–salt systems) has some weak points, as the polymer is expensive and presents high viscosity of phases.¹³

In this work, ATPSs containing EL and tartrate salts were chosen, because EL can be produced by fermentation,¹⁴ and has attracted interest as a novel green solvent in the last few

Received: May 28, 2020

Revised: November 5, 2020

Accepted: November 5, 2020

Published: November 17, 2020



years¹⁰ because of its positive characteristics: biodegradability and low toxicity and ecotoxicity; in addition, it is noncorrosive and does not damage the ozone shield,^{14,15} and regarding the chosen salts, tartrate salts are biodegradable and nontoxic compared to the other inorganic salts.

The use of EL in ATPSs has only been reported in two publications^{16,17} with three inorganic and three organic salts with good results about separation of phases, but only one paper¹⁶ used these ATPSs for the recovery of biomolecules. Therefore, it is interesting and vital to study more ATPSs based on EL to enlarge and evaluate the possibility of using these systems for the partitioning of biomolecules.

From the abovementioned, in this work, the separation of two flavonoids, rutin and quercetin, using a green and mild environment of a new EL-based ATPS containing tartrate salts has been studied. Within the wide variety of flavonoids, in this work, rutin and quercetin were selected among flavonoids because they are flavonoids existing in fresh fruits and vegetables.

Binodal curves and tie-lines have been experimentally determined for EL-based ATPSs with two organic salts: disodium tartrate and potassium sodium tartrate at $T = 298.15$ K and atmospheric pressure. The binodal data have been fitted to the Merchuk equation, and the results are in accordance with the experimental data measured in this work. The binodal curves were compared with reported data for the same systems presented and with systems with EL and other organic salts.

The composition of both equilibrium phases has been calculated using a technique consisting of measurement of the density and refractive index of the homogeneous mixture with phase diagrams. Bancroft and Othmer–Tobias equations have been adopted to evaluate the degree of consistency of the tie-line compositions.

In addition, modeling of the liquid–liquid equilibrium (LLE) data using the universal quasichemical thermodynamic (UNIQUAC) equation has been carried out with successful results.

The partitioning of rutin and of quercetin was carried out in the abovementioned ATPS, with the objective of exploring the applicability of the proposed ATPS. The partition coefficients and extraction percentages obtained for the two flavonoids in the EL-based ATPS show that these systems are suitable for use in the biotechnology industry.

2. EXPERIMENTAL PROCEDURE

2.1. Chemicals and Aqueous Solutions. (–) Ethyl L-lactate (CAS 687-47-8) with purity higher than 98% was supplied by Sigma-Aldrich. Disodium L (+) tartrate dihydrate (CAS 6106-24-7) and potassium-sodium tartrate tetrahydrate (CAS 6381-59-5) with purity higher than 99% were acquired

from VRW-Chemical and Merck, respectively. Rutin hydrate (CAS 207671-50-9) and quercetin (CAS 117-39-5) are from Sigma-Aldrich and present a purity >94%. Table 1 shows the chemicals used together with their purities and suppliers. No purification was performed in the chemicals.

All aqueous solutions required for the experimental assays were gravimetrically prepared using a Mettler AX-205 Delta Range balance with an uncertainty in the measurement of $\pm 3 \cdot 10^{-4}$ g.

2.2. Binodal Curves. The application of the presented ATPSs as an alternative liquid–liquid extraction technique for the extraction of flavonoids from aqueous solutions was compared to the conventional liquid–liquid extraction using EL. A previous study of the miscibility of mixtures of EL and water was carried out at $T = 298.15$ K and atmospheric pressure. The results showed that water and EL are completely miscible in the whole range of compositions. Hence, a conventional extraction is not possible to perform given that a partial or total immiscibility between the two liquid components is an essential requirement to carry it out. Therefore, the addition of a third component, tartrate salts, to obtain the phase splitting is needed. This fact highlights the importance of the application of ATPSs in the separation of the studied flavonoids from aqueous solutions.

The cloud point method was used for the experimental determination of the binodal curves of the ternary systems composed of EL, one of the two organic salts disodium tartrate ($\text{Na}_2\text{tartrate}$) or potassium-sodium tartrate (KNatartrate) and water at $T = 298.15$ K and atmospheric pressure. Drops of pure EL were added dropwise to a known amount of aqueous salt solution until a slight turbidity appears in the mixture. The composition of the aqueous salt solutions employed was 26.5 and 29.7 in weight percentage for $\text{Na}_2\text{tartrate}$ and KNatartrate , respectively. The cloudy mixture composition was determined by weighing. Then, drops of water were added until one homogenous mixture was achieved again. This procedure was repeated to collect enough data to complete the phase diagram. To ensure the correct measurement of the binodal curve, an inverse procedure was performed, which means aqueous solution of the corresponding salt with known composition (previously mentioned) was added dropwise to a known amount of pure EL.

Experimental data of the binodal curves were fitted using the following equation, widely employed for this kind of system, proposed by Merchuk et al.¹⁸

$$[\text{EL}] = A^{\text{B} \cdot [\text{salt}]^{0.5} - \text{C} \cdot [\text{salt}]^3} \quad (1)$$

where $[\text{EL}]$ and $[\text{salt}]$ mean the concentration of EL and the corresponding salt in weight percentage, and A , B , and C are parameters fitted by nonlinear least squares regression. It should be mentioned that the water included in the commercial form of the tartrate salts used was taken into account for the calculations of all compositions.

2.3. Tie-Line Determination. Prior to the experimental tie-line determination, some binary and ternary mixtures with concentration within the homogenous region of each diagram were prepared by mass. Then, the physical properties of these systems—density (ρ) and refractive index (n_D)—were determined at $T = 298.15$ K and atmospheric pressure using an Anton Paar DSA-5000 M digital vibrating tube densimeter with an uncertainty in the measurement of $\pm 3 \cdot 10^{-5}$ g·cm⁻³ and an automatic refractometer Abbemat-HP Dr. Kernchen with an uncertainty in the measurement of $\pm 4 \cdot 10^{-5}$. Experimental

Table 1. Purity and Suppliers of Pure Compounds Used in This Work

chemicals	supplier	purity (% wt) ^{a,b}
(–) ethyl- (L) lactate	Sigma-Aldrich	≥98.0%
di-sodium L (+) tartrate dihydrate	VWR-Chemicals	≥99.0%
potassium-sodium tartrate tetrahydrate	Merck	≥99.0%
rutin hydrate	Sigma-Aldrich	≥94.0%
quercetin	Sigma-Aldrich	≥95.0%

^a% wt: weight percent. ^bGiven by the suppliers.

data of density and refractive data obtained for these mixtures were fitted to a polynomial equation versus the composition of the mixtures using the following expression:

$$Q = \sum_{i=1}^n \sum_{j=1}^m A_{ij} w_i^m \quad (2)$$

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

Equation 2 was used separately for the refractive index and for density; therefore, two polynomials (one for each property) were obtained.

For the tie-line determination, different immiscible ternary mixtures with known compositions were prepared by weighing in glass equilibrium cells. These mixtures were introduced into a thermostatic bath, PoliScience thermostatic bath with a digital temperature controller ASL model F200 with an uncertainty in the measurement of ± 0.01 K, to maintain the temperature at $T = 298.15$ K. Then, these ternary mixtures were stirred for 6 h to achieve contact among all components and settled more than 12 h to ensure an adequate phase splitting. Afterward, samples for top and bottom phases were collected with syringes, and the density and refractive index were measured for each phase. Finally, tie-line compositions were calculated combining the polynomial equations (eq 2) of density and refractive index versus composition of each system.

Once the tie-line compositions were determined, the tie-line length, TLL, was calculated as follows:

$$\text{TLL} = \sqrt{(w_{\text{EL}}^{\text{top}} - w_{\text{EL}}^{\text{bot}})^2 + (w_{\text{salt}}^{\text{top}} - w_{\text{salt}}^{\text{bot}})^2} \quad (3)$$

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

The Othmer–Tobias (eq 4) and Bancroft (eq 5) equations were adopted to fit tie-line data because they are the most widely used for this kind of system.^{19,20}

$$\log \left[\frac{1 - w_{\text{EL}}^{\text{top}}}{w_{\text{EL}}^{\text{top}}} \right] = K_1 + n \cdot \log \left[\frac{1 - w_{\text{salt}}^{\text{bot}}}{w_{\text{salt}}^{\text{bot}}} \right] \quad (4)$$

$$\log \left[\frac{1 - w_{\text{water}}^{\text{bot}}}{w_{\text{salt}}^{\text{bot}}} \right] = K_2 + r \cdot \log \left[\frac{1 - w_{\text{water}}^{\text{top}}}{w_{\text{EL}}^{\text{top}}} \right] \quad (5)$$

where w_{water} , w_{EL} , and w_{salt} are the mass fraction of water, EL, or salt, respectively, and the superscripts top and bot denote top and bottom phases, respectively.

2.4. Partitioning of Flavonoids. The partition values of rutin and quercetin were experimentally obtained, for each one, in the previously established tie-lines of the ternary systems {EL (1) + Na₂tartrate (2) + water (3)} and {EL (1) + KNatartrate (2) + water (3)} at $T = 298.15$ K and atmospheric pressure.

Aqueous solutions of each flavonoid, with compositions of 5×10^{-4} and 1×10^{-4} g/mL for rutin and for quercetin, respectively, were prepared by weighing. These compositions correspond to the maximum solubility of rutin and of quercetin in water at room temperature and atmospheric pressure.

Feed compositions of the tie-lines of each ternary system were gravimetrically prepared in glass cells. Then, 1 mL of the flavonoid stock solution was added to each tie-line. To

maintain the same composition of the tie-lines, water from stock solutions of flavonoids (1 mL) was taken into account in the feed composition calculations. Following, all mixtures were stirred for at least 6 h and settled down during 12 h in a temperature-controlled bath at $T = 298.15$ K and atmospheric pressure. After reaching equilibrium, samples from the two phases were withdrawn with syringes and weighed.

Partition coefficients (K) and percentage extraction (%E) were calculated using the following equations:

$$K = \frac{[\text{flavonoid}]_{\text{top}}}{[\text{flavonoid}]_{\text{bot}}} \quad (6)$$

$$\%E = \frac{g_{\text{flavonoid}}^{\text{top}}}{g_{\text{flavonoid}}^{\text{bot}}} \quad (7)$$

where [flavonoid] is the concentration of flavonoids in g/mL, $g_{\text{flavonoid}}$ refers to grams of flavonoids in the indicated phase, and the subscripts top and bot indicate top and bottom phases, respectively, on both equations.

In eq 6, the concentration of flavonoids is given in g/mL; therefore to calculate the grams of flavonoids in each phase (eq 7), it is necessary to know the volume of each phase. Because we weigh both phases, we know the total mass of each phase and to convert to volume, it is necessary to divide the mass by density.

The concentrations of the flavonoid in each phase were calculated from measurements of the absorbances of each phase with a spectrophotometer model Thermo Scientific Varioskan Flash UV–vis. The used wavelengths were 368 nm for rutin and 324 nm for quercetin and using the calibration curves previously established. The uncertainty of the measurement of absorbance is $\pm 10^{-4}$. To reduce possible interferences from salt or EL on the absorbance values of mixtures, all samples were analyzed against blanks, that is to say, the same phase of each tie-line but without the presence of the corresponding flavonoid. Interferences of the other components were found to be insignificant.

To determine the extraction percentage, density of each sample was measured at $T = 298.15$ K and atmospheric pressure to enable the calculation of the volume of each phase (eq 7). In addition, with the aim to ensure the reliability of the results, mass balance calculations were carried out.

Finally, pH values of samples of all phases were measured at $T = 298.15$ K and atmospheric pressure using a pH meter Crison Basic 20, with an uncertainty in the measurement of pH and temperature of ± 0.01 and ± 0.1 K, respectively.

3. RESULTS AND DISCUSSION

3.1. Binodal Curves. Experimental binodal curve data for the ternary systems {EL (1) + salts (2) + water (3)} measured at $T = 298.15$ K and atmospheric pressure are shown in Figure 1 and Table S1 (available in the Supporting Information). The fitted parameters in eq 1 together with the respective standard deviation (σ) obtained for the two systems are shown in Table 2. As it can be inferred from this table, the Merchuk equation adequately represents the experimental values of the binodal curves presented in this work. The fitting capacity can be observed from Figure 1, where experimental and calculated data are shown.

In addition, analyzing Figure 1, it is possible to conclude that the phase diagram involving Na₂tartrate presents a larger immiscible region than that obtained with KNatartrate. This behavior may be due to a higher kosmotropic effect driven by

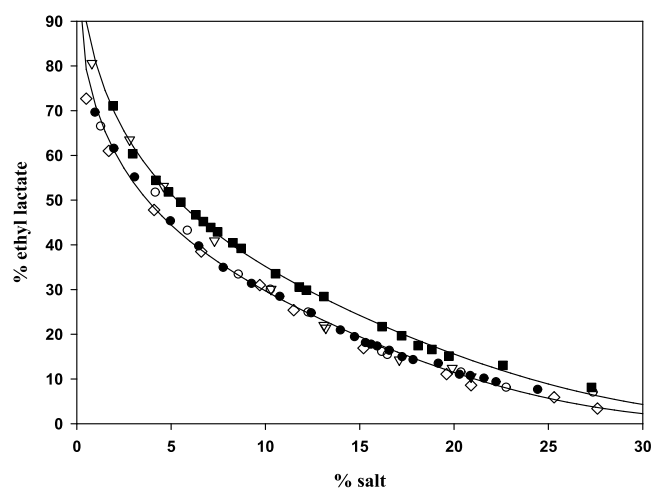


Figure 1. Phase diagrams for the EL-based ternary systems: {EL (1) + Na₂tartrate (2) + water (3)} (this work) (solid box), {EL (1) + KNatartrate (2) + water (3)} (this work) (solid circle), {EL (1) + Na₂tartrate (2) + water (3)} (empty diamond),¹⁶ {EL (1) + Na₃citrate (2) + water (3)} (empty inverted triangle),¹⁶ and {EL (1) + Na₂succinate (2) + water (3)} (empty inverted triangle)¹⁶ determined at $T = 298.15$ K and atmospheric pressure. Lines correspond to the fit of the binodal curve using eq 1 for the corresponding ternary diagram.

Table 2. Adjustable Parameters (A–C) Obtained with the Merchuk Equation (eq 1), Standard Deviation, and R^2 for the Experimental Binodal Curve Data

system	A	B	C	σ^a	R^2
{EL (1) + Na ₂ tartrate (2) + water (3)}	103.14	-0.373	$6.61 \cdot 10^{-5}$	0.754	0.998
{EL (1) + KNatartrate (2) + water (3)}	116.18	-0.362	$4.86 \cdot 10^{-5}$	0.790	0.998

^aStandard deviation; $\sigma = \left\{ \sum_i^{n_{\text{dat}}} ((z - z_{\text{cal}}))^2 / n_{\text{dat}} \right\}^{1/2}$ (z and z_{cal} are the values of the experimental and calculated properties, and n_{dat} is the number of experimental data points).

sodium cations over potassium cations, which is related to the establishment of water–ion complexes.^{21,22} Therefore,

Na₂tartrate acts as a salting-out agent because of its preferential hydration over EL.

Furthermore, literature data¹⁶ for the phase diagram for {EL (1) + Na₂tartrate (2) + water (3)} are also shown in Figure 1. The data measured in this work agree well with the reported data from the data reported by Kamalanathan.¹⁶

A comparison of the experimental data of the binodal curves determined in this work and the published data for binodal curves of ternary systems involving ethyl-lactate and other organic salts¹⁶ is also displayed in Figure 1, where both data are drawn. The ability of phase separation of the organic salts in EL-based systems follows the order: Na₃citrate \geq Na₂tartrate \geq Na₂succinate $>$ KNatartrate. Comparing the phase diagrams of the systems containing organic salts with the same cation (Na⁺), it can be observed that citrate salt exhibits stronger salting-out ability because Na₃citrate (trivalent) presents a higher ion charge versus tartrate and succinate salts (divalent). This trend is in agreement with the Hofmeister series²³ and other published results for ATPS with this kind of salt^{16,21,24–26} in which the dominant role of the ion charge in the phase splitting is evidenced. Regarding the behavior observed for tartrate (Na₂C₄H₄O₆) and succinate (Na₂C₄H₄O₄) salts, though both salts are divalent, succinate anions present a more polar character because of two hydroxyl (–OH) groups in their structure. On the other hand, the presence of potassium cations leads to a decrease in the ability of phase separation. This fact could be explained by the weaker salting-out ability of potassium cations, in comparison with sodium cations as it is established in the well-known Hofmeister series.^{21,23}

3.2. Tie-Lines. The behavior of the biphasic region, the initial concentration, and the composition of the individual phases at equilibria are vital to any separation process and for knowing how a solute is partitioned among the two immiscible aqueous phases. Different tie-lines were experimentally determined for the EL-based ATPS studied in this work.

Experimental feed and tie-line compositions together with the tie-line length calculated from these compositions are summarized in Table 3 and are depicted in Figure 2. These tie-line compositions were calculated using the previously determined polynomial equations that relate the composition of mixture components to the physical properties of the density and refractive index as mentioned before in the

Table 3. Experimental Phase Equilibrium Compositions (in Weight Percentage), Together with Their Corresponding TLLs for the Systems {EL (1) + Tartrate Salts (2) + Water (3)} as well as pH Values of each Phase at $T = 298.15$ K and $p = 0.1$ MPa^a

tie-line (TL)	feed		top phase			bottom phase			TLL
	[EL] _{feed}	[salt] _{feed}	[EL] _{top}	[salt] _{top}	pH _{top}	[EL] _{bot}	[salt] _{bot}	pH _{bot}	
{EL (1) + Na ₂ tartrate (2) + water (3)}									
TL1	28.0	12.5	51.4	3.7	6.18	15.5	17.0	6.10	38.33
TL2	30.0	13.0	57.5	2.7	6.13	11.6	19.8	6.17	49.02
TL3	32.5	13.3	63.1	2.0	6.13	9.0	22.3	6.18	57.82
TL4	35.5	13.8	68.9	1.5	6.15	7.0	24.8	6.18	66.14
TL5	38.0	14.0	72.4	1.3	6.11	6.0	26.2	6.17	70.90
{EL (1) + KNatartrate (2) + water (3)}									
TL1	32.0	13.0	53.8	4.2	6.62	13.7	20.0	6.61	43.10
TL2	34.3	13.3	59.0	3.1	6.71	10.8	22.9	6.56	52.13
TL3	36.5	13.7	63.7	2.3	6.64	8.2	25.5	6.56	60.15
TL4	38.5	13.9	66.2	2.0	6.65	6.9	27.7	6.56	64.59
TL5	41.4	14.2	70.0	1.6	6.61	5.4	30.5	6.56	70.73

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

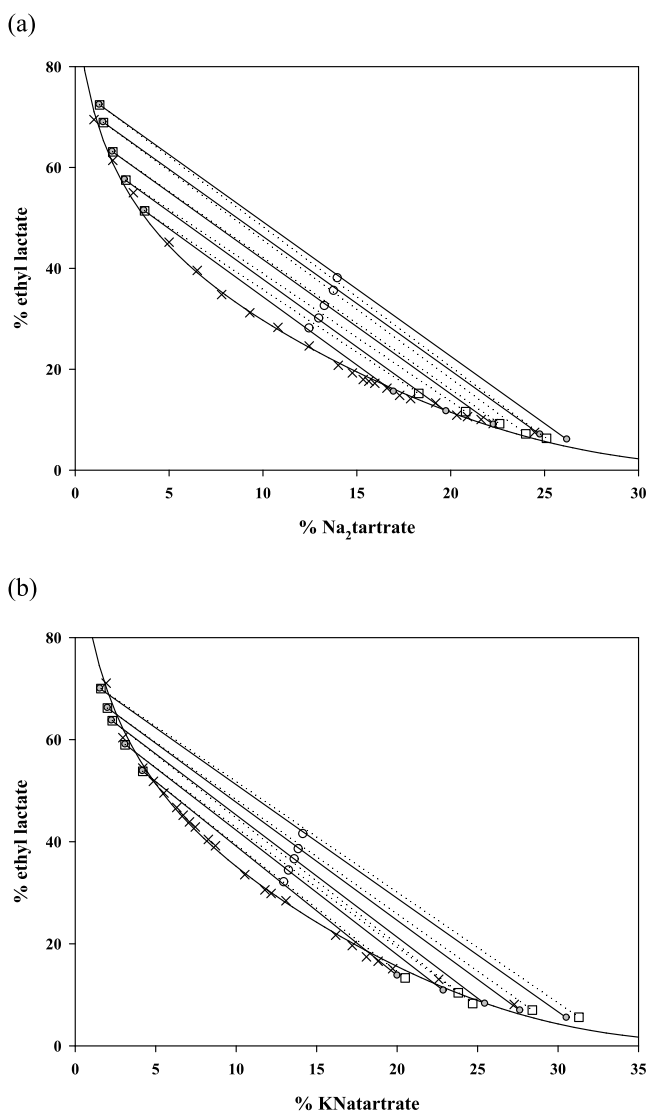


Figure 2. Binodal curves and corresponding tie-lines for the ternary systems containing EL, salt, and water at $T = 298.15$ K and $p = 0.1$ MPa. (a) {EL (1) + $\text{Na}_2\text{tartrate}$ (2) + water (3)}; (b) {EL (1) + KNatartrate (2) + water (3)}. (empty circle) feed compositions, (X) binodal curve, (line) fit of the binodal data, (solid circle) experimental tie-line compositions, and (empty box) calculated tie-line compositions using the UNIQUAC model. Solid lines and full gray points indicate experimental tie-lines, and dotted lines and empty squares indicate calculated using the UNIQUAC model.

Table 4. UNIQUAC Parameters, Δu_{ij} , and Deviation, σx , Obtained from LLE Modeling

$i-j$	$\Delta u_{ij}/(\text{kJ}\cdot\text{mol}^{-1})$	$\Delta u_{ji}/(\text{kJ}\cdot\text{mol}^{-1})$	σx
{EL (1) + $\text{Na}_2\text{tartrate}$ (2) + water (3)}			
1-2	2235.5	13,200	0.07
1-3	2586.9	1235.2	
2-3	180.00	18,020	
{EL (1) + KNatartrate (2) + water (3)}			
1-2	2289.0	15,120	0.05
1-3	6167.4	1033.1	
2-3	159.21	16,520	

Experimental procedure section. The polynomial equations obtained from experimental data are shown in Table S2.

The TLL is commonly used to evaluate the composition difference between the two phases and to study the solute partitioning among the coexisting phases.²⁷ An increase of TLL values means an increase of differences between the bottom and top phase compositions, hence, a greater degree of separation. As it can be observed from Figure 2 and Table 3, a higher EL composition in the feed leads to a higher EL and salt composition in the top and bottom phases, respectively. Therefore, these translate into higher TLL values, and the top phase can be named the EL-rich phase and the bottom phase as the tartrate salt-rich phase. The TLL values obtained were similar for both ternary systems presented in this work.

Furthermore, Othmer–Tobias (eq 4) and Bancroft (eq 5) equations^{19,20} were used to confirm the consistency of the tie-lines. The adjustable parameters and their corresponding R^2 values are summarized in Table S3 given as the Supporting Information.

Regarding the pH values of the EL-rich phases and tartrate salt-rich phases listed in Table 3, slightly higher pH values were obtained for the ternary system {EL (1) + KNatartrate (2) + water (3)}. All pH values are around pH = 6, so, flavonoids will be in their acid form in these tie-lines, which is the pH of these flavonoids in the natural sources, and accordingly, there is no need to modify the pH for the partitioning studies.

3.3. UNIQUAC Thermodynamic Modeling. Thermodynamic modeling is important to describe the phase behavior under different conditions of temperature and pressures. There are many studies on ATPSs with polymer/polymer, polymer/salt, polymer/ionic liquid, and ionic liquid/salt but not all of these studies present modeling work.

In this essay, the UNIQUAC²⁸ model was used to represent the tie-line compositions. This model was selected because although it was not developed for systems containing electrolytes, it has been demonstrated to be very suitable for ATPSs containing ionic liquids with organic or inorganic salts^{7,29–33} and LLE of polymer–salt systems. The UNIQUAC model requires the volume, r_i , and surface area q_i , parameters for each pure component i . In this study, for EL and water, the values were taken from the literature,³⁴ and for organic salts, the values were calculated, and they are presented in Table S4. According to the UNIQUAC model, the six parameters obtained from the experimental LLE data, Δu_{ij} , and the corresponding root-mean-square deviation of the composition, σx , are given in Table 4. The root-mean-square deviations in compositions, σx , were ascertained as follows:

$$\sigma x = \sqrt{100 \left(\frac{\sum_i^m \sum_j^{n-1} (x_{ij}^{I,\text{exp}} - x_{ij}^{I,\text{cal}})^2 + (x_{ij}^{II,\text{exp}} - x_{ij}^{II,\text{cal}})^2}{2mn} \right)} \quad (8)$$

where m represents the number of tie-lines and n means the number of components in the mixture. The superscripts I and II are the top and bottom phase, respectively, and x_i^{exp} and x_i^{cal} are the experimental and calculated composition in the molar fraction of component i in both phases, respectively. The tie-line compositions predicted from the UNIQUAC model for the two ATPSs studied in this work are shown in Figure 2. The obtained deviations of compositions, σx , given in Table 4 and the results presented in Figure 2 show that the UNIQUAC model adequately describes the ATPS formed by EL and $\text{Na}_2\text{tartrate}$ or KNatartrate salts.

Table 5. Partition Coefficients (K), Percentage of Extraction (% E) and Concentrations on Top ($[\text{flavonoid}]_{\text{top}}$), and Bottom ($[\text{flavonoid}]_{\text{bot}}$) Phases of Flavonoids (Rutin and Quercetin) in ATPSs Containing Ethyl Lactate and Sodium or Potassium Sodium Tartrate at $T = 298.15$ K and $p = 0.1$ MPa^a

N° TL	$[\text{EL}]_{\text{feed}}$	$[\text{salt}]_{\text{feed}}$	TLL	$[\text{flavonoid}]_{\text{top}}$ (g/mL)	$[\text{flavonoid}]_{\text{bot}}$ (g/mL)	K	% E
quercetin in ATPS: EL + Na ₂ tartrate + water							
TL1	28.0	12.5	38.33	2.08E-05	4.25E-06	4.89	72.90
TL2	30.0	13.0	49.02	2.05E-05	3.20E-06	6.42	81.60
TL3	32.5	13.3	57.82	1.95E-05	2.41E-06	8.08	87.07
TL4	35.5	13.8	66.14	1.90E-05	1.89E-06	10.05	91.25
rutin in ATPS: EL + Na ₂ tartrate + water							
TL1	28.0	12.5	38.33	8.96E-05	3.20E-05	2.80	60.60
TL2	30.0	13.0	49.02	9.06E-05	2.71E-05	3.35	69.82
TL3	32.5	13.3	57.82	9.03E-05	2.14E-05	4.22	77.85
TL4	35.5	13.8	66.14	9.00E-05	1.65E-05	5.46	85.00
quercetin in ATPS: EL + KNatartrate + water							
TL1	32.0	13.0	43.10	1.63E-05	4.51E-06	3.62	76.58
TL2	34.3	13.3	52.13	1.63E-05	3.78E-06	4.32	80.51
TL3	36.5	13.7	60.15	1.62E-05	3.00E-06	5.45	85.91
TL4	38.5	13.9	64.59	1.58E-05	2.46E-06	6.42	88.93
TL5	41.4	14.2	70.73	1.53E-05	1.94E-06	7.88	91.80
rutin in ATPS: EL + KNatartrate + water							
TL1	32.0	13.0	43.10	7.39E-05	3.41E-05	2.17	66.19
TL2	34.3	13.3	52.13	7.58E-05	3.27E-05	2.32	68.89
TL3	36.5	13.7	60.15	7.74E-05	2.92E-05	2.65	74.80
TL4	38.5	13.9	64.59	7.82E-05	2.28E-05	3.43	81.10
TL5	41.4	14.2	70.73	7.93E-05	1.85E-05	4.28	85.86

^aStandard uncertainties, u , are: $u(T) = 0.2$ K, $u(p) = 10$ kPa.

3.4. Partitioning of Rutin and Quercetin. The application of the proposed ATPSs was evaluated as an alternative liquid–liquid extraction technique for the determination of the partition coefficients and extraction percentages of rutin and of quercetin in {EL (1) + Na₂tartrate or KNatartrate (2) + water (3)} at $T = 298.15$ K and atmospheric pressure.

Table 5 lists the values of the partition coefficients, K , and the extraction percentages, % E , together with the TLL. The values of K calculated with eq 6 were obtained from measurements of absorbance by calibration curves previously established.

The distribution coefficients for rutin and for quercetin in the two ATPSs studied are higher than one, K ranges from 2 to 9, and the extraction percentages are among 60 and 90%.

Table 4 shows that the obtained values for K and for % E of two flavonoids are a function of the concentration of the other components existing in the ATPS phases. Each of the two flavonoids studied is found mainly in the EL-rich phase; when the concentration of EL increases, the amount of flavonoids in this phase also increases. This behavior can be observed from Figure 3.

The representation of $\ln K$ versus TLL is shown in Figure 4. A linear relationship between $\ln K$ and TLL is observed in Figure 4, in agreement with the equation:

$$\ln K = \alpha \text{ TLL} \quad (9)$$

where α is a constant which depends on the concentration of the ATPS phases. The values of α and R^2 for each flavonoid on the two studied ATPSs are presented in Table S5 (available in the Supporting Information).

According to the results, a better separation is achieved for the quercetin extraction, when compared with the rutin extraction. This behavior is the same for both ATPSs under

study. A higher K value is obtained for ATPSs containing Na₂tartrate salt than for potassium sodium salt.

Furthermore, the percentage extraction of rutin and of quercetin on the EL-based ATPS has been determined. The percentage extraction values versus the tie-line length, for each flavonoid on the two ATPSs, are shown in Figure 5, and the conclusions are similar to those mentioned for K values. An increase in the TLL means an increase in extraction percentage, and the extraction of quercetin is more effective than the extraction of rutin on the ATPS studied.

The EL-based ATPS with organic tartrate salts may be a green possibility for the purification of rutin and of quercetin to replace conventional liquid–liquid extraction.

4. CONCLUSIONS

Binodal curves and tie-lines were experimentally determined for two EL-tartrate salt ATPSs at $T = 298.15$ K and atmospheric pressure. The Merchuk equation was applied for the binodal curve fitting, and presented a very agreement with the experimental data. The influence of the cation of tartrate salts on the phase formation was analyzed and compared with published data for systems with EL and other organic salts. The ion charge of the salt anion and the presence of potassium in the salt play an important role in phase splitting. The ability of phase separation follows the order: Na₃citrate \geq Na₂tartrate \geq Na₂succinate $>$ KNatartrate.

Compositions of the equilibrium phases were obtained by a technique based on the measurement of the density and refractive index of mixtures within the homogeneous region of the phase diagrams. A higher concentration of EL in the feed means a higher concentration of EL and salt in the top and bottom phases, respectively, hence, a higher phase separation. Moreover, the studied ternary systems {EL (1) + tartrate salts

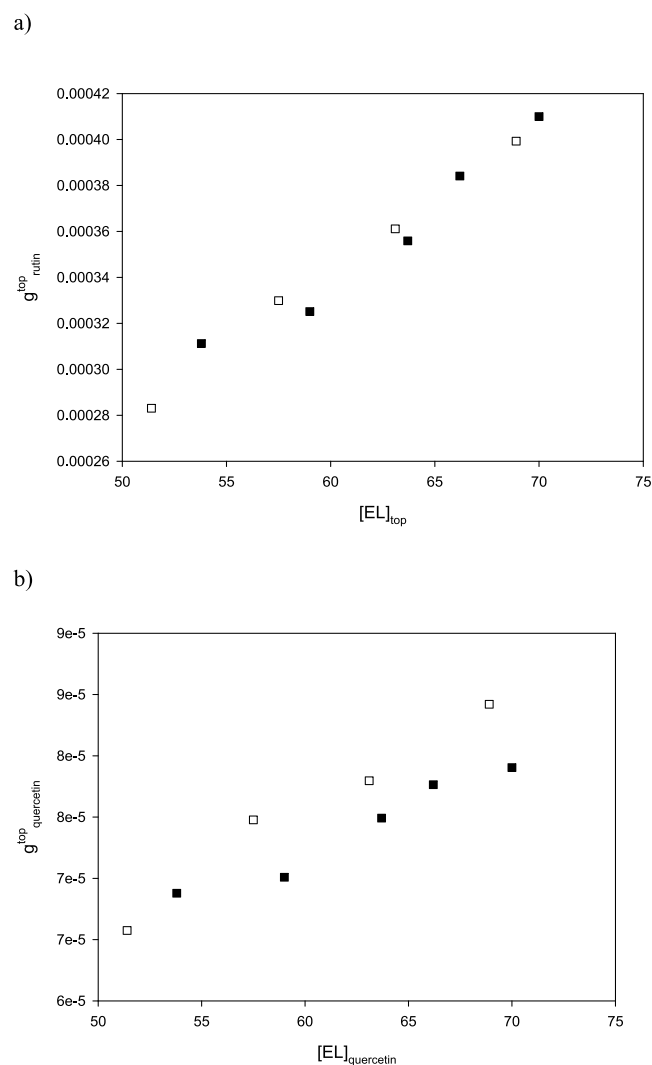


Figure 3. Concentration of EL on the top phase, $[EL]_{top}$, in weight percentage vs grams of flavonoids on the top phase, $g_{flavonoid}^{top}$. Symbols: empty box {EL + Na₂tartrate + water}; solid box {EL + KNatartrate + water}. (a) Rutin and (b) quercetin.

(2) + water (3)} were satisfactorily described using the UNIQUAC thermodynamic model.

The applicability of the two studied ATPSs for the extraction of rutin and of quercetin was evaluated through the determination of partition coefficients and extraction percentage at $T = 298.15$ K and atmospheric pressure.

The highest distribution coefficients were obtained for quercetin on the two ATPSs studied and on the ATPS involving the organic disodium tartrate salt.

The results from this study indicate a novel possibility for the application of EL-based ATPSs with organic tartrate salts for the extraction of rutin and of quercetin in the biotechnological industry.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.iecr.0c02664>.

Experimental binodal curve data (weight percentage, w_i) for the ternary systems {EL (1) + salt (2) + water (3)} determined experimentally at $T = 298.15$ K and $p = 0.1$

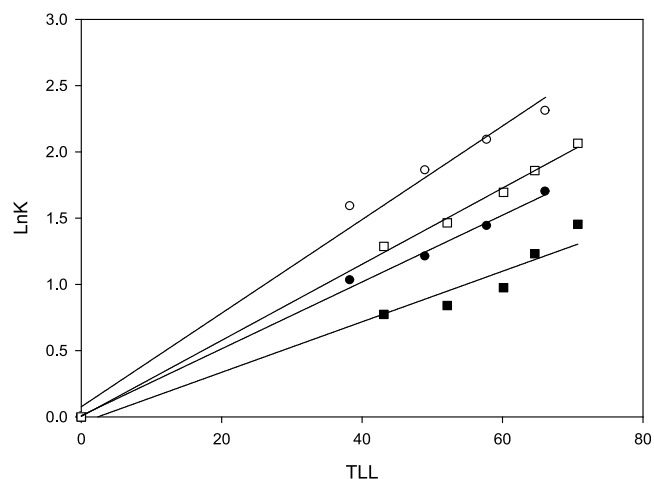


Figure 4. Logarithm of the partition coefficients of the flavonoids vs the tie-line length for (empty circle) quercetin on {EL (1) + Na₂tartrate (2) + water (3)}, (empty box) quercetin on {EL (1) + KNatartrate (2) + water (3)}, (solid circle) rutin on {EL (1) + Na₂tartrate (2) + water (3)}, (solid box) rutin on {EL (1) + KNatartrate (2) + water (3)}, and (line) fits from eq 10 at $T = 298.15$ K and at $p = 101.3$ kPa.

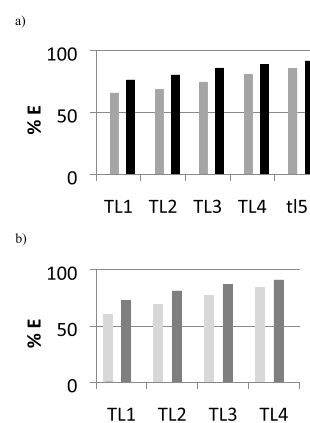


Figure 5. Extraction percentage, % E, of the flavonoids for the tie-lines. (a) (solid black bar) rutin on {EL (1) + KNatartrate (2) + water (3)}, (solid gray bar) quercetin on {EL (1) + KNatartrate (2) + water (3)}; (b) (solid dark gray bar) rutin on {EL (1) + Na₂tartrate (2) + water (3)} and (solid light gray bar) quercetin on {EL (1) + Na₂tartrate (2) + water (3)}.

MPa, polynomial expressions for the density and refractive index as a function of the composition for the studied ternary systems, parameters obtained using the Othmer–Tobias equation (eq 5) and Bancroft equation (eq 6) with the correlation coefficients, UNIQUAC volume, r_i , and surface area, q_i , structural parameters, and values of the α parameter together with R^2 of eq 9 (Table S1–S5) (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was financially supported by Base Funding - UIDB/50020/2020 of the Associate Laboratory LSRE-LCM - funded by national funds through FCT/MCTES (PIDDAC). E.G. acknowledges funding support from Stimulus of Scientific Employment, Individual Support 2017, CEECIND/02646/2017, FCT. P.F.R. is grateful to the Xunta de Galicia (Spain) for her scholarship (ED481B-2017/019).

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