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Extraction and Recovery of Rutin from Acerola Waste using Alcohol-Salt-Based Aqueous Two-Phase Systems

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Extraction of rutin from acerola waste was investigated using alcohol-salt-based aqueous two-phase systems (ATPS). Initially, the partitioning was studied using model systems with pure and commercial rutin. The impact of the ATPS constituents and composition, initial amount of rutin, temperature and addition of electrolytes was evaluated. Rutin can be recovered either in the alcohol-or-salt-rich phase depending on the salt used. To validate the optimization process, rutin extraction from acerola waste was carried out further. The results obtained with the real samples are in close agreement with the model systems and validate the optimization tests and support their applicability in bioresource-related processes.

Keywords acerola waste; aqueous two-phase system; extraction; rutin

INTRODUCTION

Acerola, also known as West Indian cherry or Barbados cherry, is a native plant from Central America. It is also present in South America, mainly in Brazil, due to its versatile adaptation to soil and climate (1, 2). The wide chemical composition of acerola includes a large amount of volatile compounds, such as substances responsible for the aroma, and non-volatile compounds such as vitamin C, anthocyanins, carotenoids, and flavonoids including rutin (3).

The use of acerola as a dietary supplement by humans has positive health effects, namely protective effects against cancer, arteriosclerosis, neurodegenerative diseases, and aging (4–6). Furthermore, according to Zibadi and co-workers (7), the daily consumption of bioflavonoids, phenolic acids, and anthocyanin through fruits and vegetables decreases the risk of degenerative and chronic diseases. For this reason, the consumption of acerola as an *in natura* fruit or in juices and jams is highly recommended for human health maintenance (8).

Nowadays, as a consequence of the industrialization of some fruit-related products, agro-industrial wastes are generated in high quantities resulting in the accumulation of residues with an inherent environmental impact. Besides the fruit pulp itself, peels and seeds do not receive adequate attention and they are the main residues resulting from fruit processing approaches (9). Nevertheless, some works have already demonstrated that some added-value components, such as antioxidant compounds, are present in higher amounts in the residues of certain fruits when compared with the pulp (10,11). According to Freitas and co-workers (6), the Brazilian industry uses 34.4 thousand tons of acerola (7.16% of the total fruits) and produces 18.0 thousand tons of juice and pulp (52.3%) and 16.4 thousand tons of waste (47.7%). In this context, there is large interest in finding sustainable processes to reuse and take the maximum value of those wastes.

Among the antioxidant compounds present in acerola, rutin (3',4',5,7-tetrahydroxyflavone-3-β-D-rutinoside or quercetin-3-rutinosid) is a non-toxic bioflavonoid comprised of the flavonol quercetin and the disaccharide rutinose (12,13). The chemical structure of rutin is depicted in Fig. 1.

Traditionally, rutin is extracted from biomass or complex matrices making use of ethanol (14). More modern techniques, such as supercritical fluid extraction (15), microwave-assisted extraction (16), pressurized liquid extraction (17), solid phase micro-extraction (18), and ultrasound-assisted extraction (19, 20) have also been proposed. However, these alternatives usually require more drastic conditions, namely high temperatures and pressures, and they also depend on more sophisticated equipment turning the current extraction in an expensive and complex process.

In order to eliminate some of the disadvantages mentioned before, aqueous two-phase systems (ATPS) can be regarded as alternative liquid-liquid extraction techniques. Due to their

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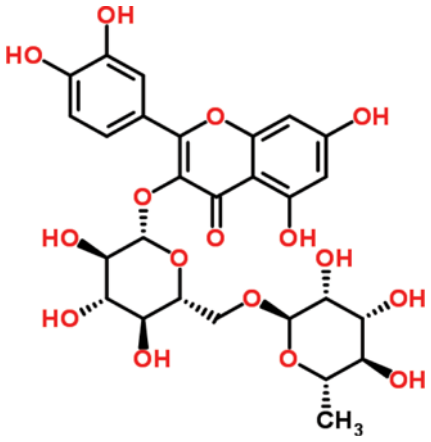

FIG. 1. Molecular structure of rutin.

high content in water, they have been labelled as biocompatible systems. Indeed, they have been successfully applied in the extraction of several biomolecules such as enzymes (21, 22), alkaloids (23), antibiotics (24), dyes (25), lithospermic acid B (26), and aroma compounds (27). This large spectrum of applications is justified by the ease of scaling-up the process, and high extraction efficiencies and high yields are usually attained (28).

ATPS are formed in aqueous media by the dissolution of two incompatible polymers (29) or a polymer and a salt (30). In both examples, there is the formation of two macroscopic liquid phases with water as the major constituent (31). Despite these conventional ATPS, more recent works have shown the possibility of forming liquid-liquid systems by the combination of an organic solvent and a salt (32) or by the addition of an inorganic salt to an aqueous ionic liquid solution (33, 34). Particularly, the use of polymer-polymer and polymer-salt systems has some disadvantages, such as the high cost of the polymer, phases of high viscosity, a slow phase separation, and a challenging recyclability of the phase forming components (35). On the other hand, ATPS formed by alcohols and salts are of low cost, allow the easy recovery of the alcohol by evaporation or distillation approaches, are of low viscosity, and usually lead to high extraction efficiencies and purification levels in a single-step procedure (36).

In this work, the use of ATPS for the extraction and recovery of rutin from acerola wastes was evaluated. Different ATPS composed of alcohol (methanol, ethanol, 1-propanol and 2-propanol) + potassium phosphate salts (K_3PO_4, K_2HPO_4 and potassium phosphate buffer composed of K_2HPO_4/KH_2PO_4), and whose phase diagrams were recently published by our group (32), were used. As a first and preliminary methodology, the partitioning behavior of commercial and high purity rutin was investigated, and the several constituents of each ATPS, the composition of the biphasic mixture, the concentration of rutin, the temperature (278.15 to 308.15 K) and the addition of electrolytes (NaCl) were investigated and optimized. After the optimization step with model systems, the optimized conditions were further employed in the extraction of rutin from acerola waste.

MATERIAL AND METHODS

Materials

Methanol, ethanol, 1-propanol, 2-propanol, dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), and potassium phosphate (K_3PO_4) were purchased at Vetec (Rio de Janeiro, Brazil). All alcohols have purities higher than 99 wt%. The phosphate salts present a purity level higher than 98 wt%. Rutin ($\geq$ 97 wt% pure) was acquired at Acros Organics (New Jersey, USA). Ultrapure and double distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus, was used in all experiments. Acerola at a mature stage was purchased in a regular supermarket in Aracaju, Brazil. The fruit was squeezed using a domestic depulper and the waste generated was kept at 253.15 K until use.

Partitioning Studies of Rutin in Model ATPS

The studied model ATPS are composed of different alcohols (methanol, ethanol, 1-propanol and 2-propanol) and several potassium phosphate salts (K_3PO_4, K_2HPO_4, and the buffer K_2HPO_4/KH_2PO_4). The phosphate buffer is constituted of the mixture of the two salts K_2HPO_4 and KH_2PO_4 in the proper proportions (pH = 7.0; Henderson-Hasselbalch equation equivalents = 1.087).

The biphasic systems were prepared in graduated centrifuge tubes (15 mL) by weighing the appropriate amounts of alcohol (40–60 wt%) and potassium phosphate salts (10–20 wt%) taking into account the phase diagrams previously reported (32) To these systems, aqueous solutions containing rutin at 25, 50, 70, 100, and 200 mg/L were added. The total weight of each ATPS was 14.0 g. After, the mixtures were stirred for 2 min and then centrifuged at 3000 rpm for 10 min., the graduated tubes were then placed at the respective temperature (from 278.15 to 308.15 K), for at least 12 hours and within $\pm$ 1.0 K, using a MARCONI MA-127 thermostatic bath. It should be remarked that the vials were kept closed during this period to avoid alcohol vaporisation. The two phases were then cautiously separated and collected for the determination of their volume and weight. Finally, rutin was quantified in both the top and bottom phases. Details on the quantification of rutin are described below. The quantification of rutin was performed in three assays and the average partition coefficients, average extraction efficiencies, and respective standard deviations were calculated. It should be noted that for all studied ATPS, the top phase is the alcohol-rich phase while the bottom phase corresponds to the salt-rich phase.

The partition coefficient of rutin (K_{rut}) is defined as the ratio between the concentration of rutin in the top phase (C_T) to that in the bottom phase (C_B) according to:

$$K_{rut} = \frac{C_T}{C_B} \quad (1)$$

In order to evaluate the rutin percentage extraction efficiencies (EE_{rut}%) and the volume ratio (R_v) in each ATPS, the following equations were used:

$$R_v = \frac{V_T}{V_B} \tag{2}$$

$$EE_{rut}\% = 100 \times \left(\frac{K_{rut} \times R_v}{1 + K_{rut} \times R_v} \right) \tag{3}$$

where V is the phase volume and T and B correspond to the top and bottom phases, respectively.

Thermodynamic Functions

The thermodynamic functions associated with the migration phenomenon of rutin, namely the Gibbs free energy (ΔG_m°), the enthalpy (ΔH_m°) and the entropy of transfer (ΔS_m°), were determined according to Eqs. (4) and (5):

$$\ln(K_{rut}) = -\frac{\Delta H_{\mathrm{m}}^0}{R} \times \frac{1}{T} + \frac{\Delta S_{\mathrm{m}}^0}{R} \tag{4}$$

$$\Delta G_{\mathrm{m}}^0 = \Delta H_{\mathrm{m}}^0 - T\Delta S_{\mathrm{m}}^0 \tag{5}$$

where T is the temperature, K_{rut} is the partition coefficient of rutin, and R is the universal gas constant ($8.314\,\mathrm{J.mol^{-1} \cdot K^{-1}}$).

Extraction and Partitioning of Rutin from Acerola Waste

Acerola waste samples ($\approx$ 10g of peels and seeds), after a depulping process, were dispersed in 25 mL of an aqueous solution of 1-propanol at 46 or 60 wt%. The vials were kept sealed and under constant agitation at 298.15 K, at 200 rpm and for 24 h, using a Marconi MA-095 shaker. After 24 h, the samples were filtrated through 0.42 μm microporous membranes. The inorganic salts (K_2HPO_4/KH_2PO_4 or K_3PO_4) and water were then added to prepare the respective ATPS in the required concentrations up to a total weight of 14 g. The composition of each component in a given ATPS and the optimized conditions determined with the model systems were used in this step. In particular, the systems composed of 1-propanol (50 wt%) + K_2HPO_4/KH_2PO_4 (15 wt%) + H_2O (35 wt%) and 1-propanol (40 wt%) + K_2HPO_4/KH_2PO_4 (20 wt%) + H_2O (40 wt%) were employed for maximizing the concentration of rutin in the top phase and 1-propanol (50 wt%) + K_3PO_4 (15 wt%) + H_2O (35 wt%) was used for maximizing the partition of rutin into the bottom phase. The mixtures were then stirred for 5 min and finally centrifuged at 3,000 rpm for 10 min. These systems were further placed at 298.15 K for 12 h to reach the equilibrium. Both phases were carefully separated and weighed; the volume of each phase was registered, and the rutin was quantified in the top phase by high-performance liquid chromatography (HPLC) analysis. The quantification of rutin was only determined in the alcohol-rich phase due to the high salt content in the bottom phase. Thus, the rutin concentration in the bottom phase was determined by the mass balance of the rutin concentration on the initial alcoholic extract and its concentration at the top phase.

Rutin Quantification

The concentration of rutin in both phases of the model systems was determined by UV-Vis spectroscopy, using a Varian Cary-50 spectrophotometer UV-visible Bio apparatus, at 350 nm and using a calibration curve that had been previously established. The mass balance of rutin was always confirmed and was within ± 5.5%. Interferences of both the inorganic salt and the alcohol in the analytical method were taken into account and found to be insignificant at the dilutions performed. The quantification of rutin was carried out in three assays and the average partition coefficients, average extraction efficiencies, and respective standard deviations were calculated.

The concentration of rutin extracted from acerola waste was determined by HPLC analysis according to a method initially described by Fang and co-workers (37), with slight modifications. Chromatographic separations were performed on a Discovery® HS C18 (Supelco, USA) column (26.0 cm × 4.6 mm, 5 μm). The HPLC equipment consists of a Varian Prostar (Australia) LC Detector series pumping system with a UV detector set at 360 nm and Galaxie chromatography data system software. Two solvents with a constant flow rate of 1.0 mL/min were used: solvent A, which is composed of 20% acetonitrile and 5% methanol in water (pH 3.0), and solvent B, which is constituted of 55% acetonitrile and 15% methanol in water (pH 3.0). All of the solvents are of HPLC grade. The gradient elution program was as follows for solvent B: 2% from 0–15 min, 2–28% from 15– 28 min, 28–36% from 28–40 min, 36% from 40–44 min, 36-80% from 44–45 min, and 80% from 45–52 min. At least three quantifications were performed for each system and the respective average values and standard deviations were calculated.

RESULTS AND DISCUSSION

In this work, the use of alcohol-salt-based ATPS was investigated as an alternative platform for the extraction and recovery of rutin from acerola waste. As a first approach, the extraction of rutin was studied and optimized using model systems with commercial and high purity rutin. Different parameters, namely the type of alcohol and phosphate salt, the composition of the system, the rutin concentration, the temperature of extraction, and the addition of NaCl as an additional electrolyte, were evaluated regarding the partition coefficients and extraction efficiencies obtained. As a second and validation step, the optimized ATPS were further used for the partitioning of rutin extracted from acerola waste.

For all systems studied, the R_v was higher than 4.1 (data not shown), that is, the volumes of the bottom phase were larger than 2.7 g and top phase were smaller than 11.25 g. A vanillin concentration used (25 to 200 mg/L to model system and 0.54 g/g to real system) does not permit the saturation of any phase, due to vanillin solubility in water

(0.070 M, i.e. 10.65 g/L) and alcohols (between 1.82 M-1-propanol and 4.16 M-methanol, that is, 276.88 g/L–632.86 g/L) (http://www.scm.com/Doc/Doc2013/GUI/GUI_tutorial/page168.html).

Partitioning Studies of Rutin in Model ATPS

The influence of the alcohol and salt, as well as the initial concentration of rutin added to each ATPS, was investigated by means of the partition coefficients and extraction efficiencies obtained. The concentration of rutin used varied between 25 and 200 mg/L. The results obtained at 298.15 K are depicted in Fig. 2.

With the exception of the systems composed of K_3PO_4, rutin preferentially migrates for the alcohol-rich phase ($K_{rut} > 1$). For the ATPS constituted of KH_2PO_4/K_2HPO_4 and K_2HPO_4, the partition coefficients of rutin range between 1.1 and 22.6. On the other hand, for the systems composed of K_3PO_4 the partition coefficients are within 0.03 and 0.07. Thus, the different potassium phosphate salts lead to very different behaviors in the preferential partitioning of rutin. The reduction of the partition coefficient of rutin is inversely proportional to the Hofmeister series (38) which describes the salting-in and salting-out behavior of molecules in aqueous media. The strongest salting-out salt studied is K_3PO_4, whereas KH_2PO_4 tends to fit into the salting-in regime. Therefore, if the salting-out of salts is a dominant phenomenon to force the migration of rutin for the alcohol-rich phase, the enhanced partition coefficients should be obtained with the systems composed of K_3PO_4. Indeed, the opposite trend is observed. Taking into account the pKa of rutin (7.73), it can be deduced that their charged or non-charged forms have strong contributions to the preferential migration observed in the diverse ATPS (39).

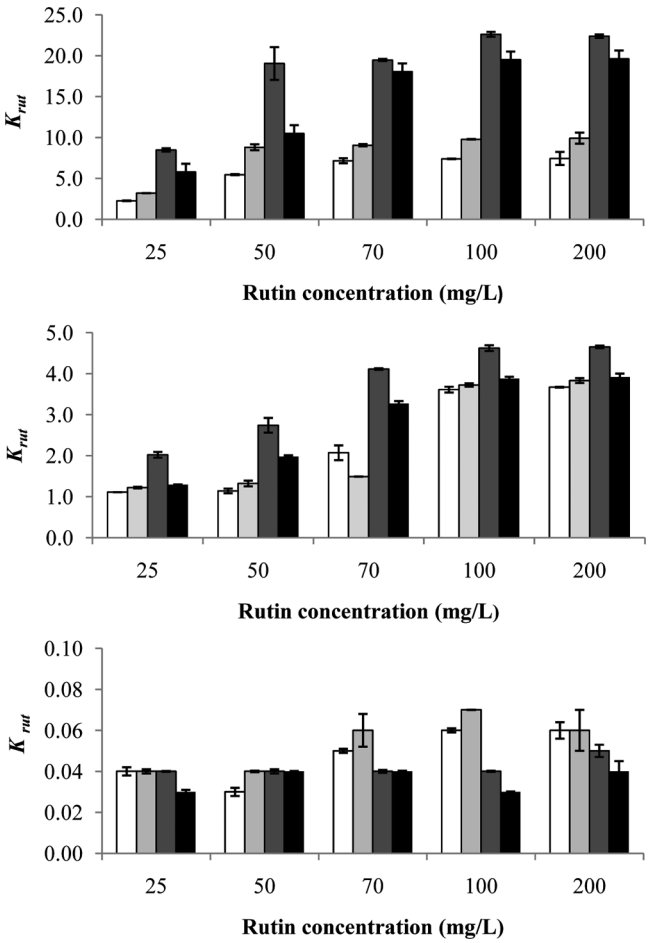

FIG. 2. Partition coefficients of rutin, K_{rut}, in the several alcohol-salt ATPS at 298.15 K as a function of initial rutin concentration. Alcohol: □ - methanol, ■ - ethanol, ■ - 1-propanol, ■ - 2-propanol; potassium phosphate salts: (i) KH_2PO_4/K_2HPO_4, (ii) K_2HPO_4, and (iii) K_3PO_4. All ATPS are composed of 50 wt% of alcohol and 15 wt% of salt.

Previously, we have published (32) the pH values of the coexisting phases of the systems studied here. For the systems composed of K_3PO_4, the media is highly alkaline, with pH values ranging between 12.38 and 13.22, while the pH values for the remaining systems are lower and closer to the pKa of rutin. Thus, at alkaline medium, almost all rutin is negatively charged and preferentially migrates for the most hydrophilic and ionic phase (salt-rich phase). For the remaining systems, the amount of charged rutin is lower and the majority of neutral rutin partitions in the hydrophobic alcohol-rich phase. Indeed, among the ATPS based on KH_2PO_4/K_2HPO_4 and K_2HPO_4, the partition coefficients of rutin are higher in the systems composed of the phosphate buffer – those with the lower pH values and close to 7. The choice of the salts, and subsequently the pH values that they induce in aqueous media, is a dominant parameter in the extraction of biomolecules that suffer speciation as the pH of the solution changes. In the systems considered here, there are two aqueous phases of different natures: a predominant hydrophobic phase composed mainly of alcohol and a more hydrophilic and ionic phase constituted majorly by the inorganic salt. These differences in the phases' polarities, coupled to the charged or non-charged nature of rutin, control the preferential migration for a given phase. Indeed, this trend was already observed in the partition coefficients of gallic acid using ATPS formed by ionic liquids and different inorganic salts (40). In this work (40), it was observed that the neutral and less hydrophilic form of gallic acid present in the acidic media, is more easily extracted into the most hydrophobic ionic-liquid-rich phase. On the other hand, gallate, the charged conjugate base of gallic acid present in neutral or alkaline pH solutions, preferentially migrated into the charged salt-rich phase (40).

In most cases, the ATPS composed of the same salt and different alcohols, the partition coefficients of rutin decrease in the order: 1-propanol > 2-propanol > ethanol < methanol. Rutin is poorly soluble in water and highly soluble in alcohols, thus supporting the preferential migration of rutin for the alcohol-rich phase in most cases. In general, the solubility of rutin increases with the alkyl chain length of the alcohol, for example, with the increase on the alcohol hydrophobicity (41).

This increased solubility in alcohols with longer aliphatic chains supports the higher partition coefficients observed in systems formed by propanol towards the lower partition coefficients observed in the systems constituted by methanol. Regarding the results obtained with ATPS constituted by the two isomers of propanol, 1-propanol and 2-propanol, the partition coefficients of rutin are higher in the 1-propanol-based systems because of its higher hydrophobicity. The higher hydrophobicity of the 1-propanol isomer is sustained by its higher octanol-water partition coefficient ($K_{ow} = 1.78$) compared to that of 2-propanol ($K_{ow} = 1.12$) (42). This straight pattern is less visible in the systems composed of K_3PO_4. Nevertheless, it should be remarked that more complex phenomena take place in these ATPS due to the charged character of rutin at alkaline medium.

The influence of the initial concentration of rutin, ranging from 25 to 200 mg/L, was also evaluated. In general, and as seen in Fig. 2, the partition coefficients of rutin increase with the concentration of the solute. This trend is independent of the alcohol or salt employed. Nonetheless, this pattern is less pronounced in the systems with K_3PO_4. In the ATPS formed by K_2HPO_4 and K_2HPO_4/KH_2PO_4, the improved migration for the alcohol-rich phase with the increase on the initial rutin content can be a main result of solute-solute interactions such as $\pi \cdots \pi$ interactions (non-covalent interactions between the aromatic rings) and improved hydrogen-bonding ability between the hydroxyl groups. This pattern was already observed by Cláudio and co-workers (40) with the partitioning of vanillin in ATPS composed of ionic liquids and salts. Furthermore, Tavagnacco and co-workers (43) have already demonstrated the presence of $\pi \cdots \pi$ interactions in aromatic solutes when dissolved in aqueous media using molecular dynamics simulations.

In summary, it was shown that the preferential migration of rutin for a given phase is versatile and mainly depends on the pH of the aqueous media. For instance, the biomolecule can be recovered in the top phase (with extraction efficiencies ranging between 91.62% and 98.23%) or in the bottom phase (with extraction efficiencies within 8.16% and 23.04%) (data not shown).

In order to infer the effect of the composition of the ATPS on the partitioning of rutin, several experiments were carried out with varying concentrations of 1-propanol in the total mixture, from 40 to 60 wt%, while maintaining the concentration of the KH_2PO_4/K_2HPO_4 mixture of salts at 20 wt%. It should be noted that, according to the phase diagrams published before (32), the lower limit of 1-propanol capable of ensuring the formation of two liquid phases is 40 wt%. The partition coefficient and extraction efficiency results are shown in Fig. 3.

Increasing the amount of 1-propanol leads to a slight decrease in the partition coefficients of rutin, albeit no significant changes were observed in the extraction efficiencies. However, in all cases, rutin was almost completely extracted to the top phase with extraction efficiencies in the order of 98%.

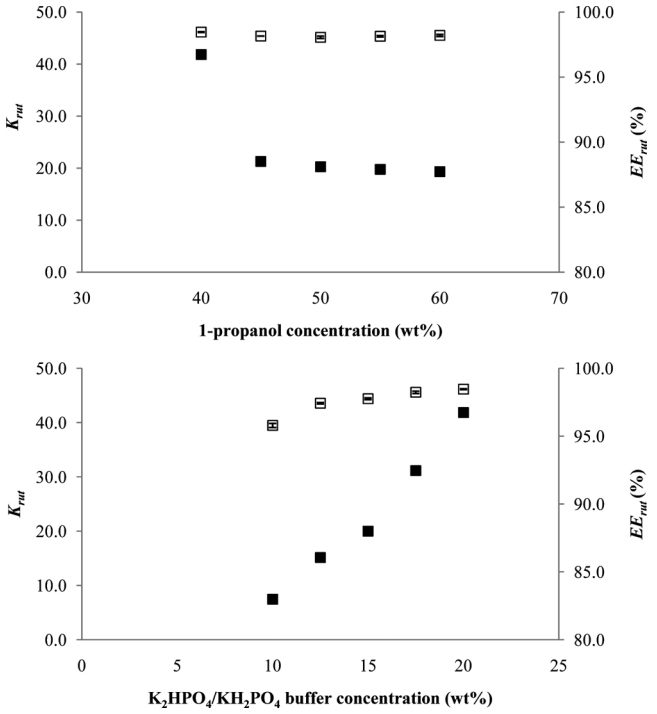

FIG. 3. Influence of the 1-propanol concentration in the ATPS containing K_2HPO_4/KH_2PO_4 at 20 wt% (i) and K_2HPO_4/KH_2PO_4 buffer concentration in the ATPS containing 1-propanol at 40 wt% (ii) at 298.15 K in the partition coefficient (■) and extraction efficiency (□) of rutin.

After the evaluation of the 1-propanol concentration, we further analyzed the effect of the concentration of the mixture of the salts K_2HPO_4/KH_2PO_4 from 10 to 20 wt%. In these studies, the concentration of 1-propanol was maintained at 40 wt%. It should be remarked that the maximum concentration of salt than can be used is 20 wt% since higher values lead to the precipitation of the salt and to fall into the solid-liquid region. The partition coefficients and extraction efficiencies of rutin are depicted in Fig. 3.

Contrarily to what was observed with the alcohol concentration effect, the increase of the salt concentration leads to higher partition coefficients of rutin, that is, to a higher ability of rutin to migrate for the alcohol-rich phase. In accordance, the extraction efficiencies of rutin increased from 95.8 to 98.5%. Rutin was almost completely extracted in the alcohol-rich phase with the higher amount of salt. This phenomenon is a main result of the salting-out effect of the salt over rutin which forces the biomolecule migration for the other phase. Wu and co-workers (36) studied different solvents and reported extraction efficiencies of 13.0% (water), 80% (ionic liquid- $[C_8mim]Cl$) and circa 90.0% (methanol). All of these values are lower than those found in this work with the enhanced ATPS.

The influence of temperature on the rutin extraction was also studied using ATPS composed of 1-propanol at 40 wt% and K_2HPO_4/KH_2PO_4 at 20 wt%. The temperature of equilibrium

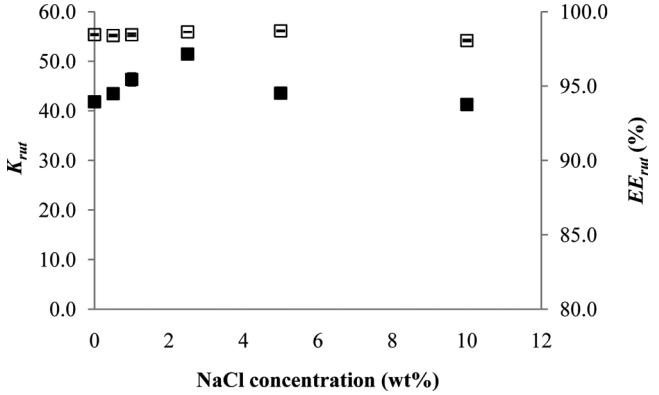

FIG. 4. Effect of the concentration of NaCl in the partition coefficient and the extraction efficiency of rutin in the system composed of 1-propanol (40 wt%) and K_2HPO_4/KH_2PO_4 (20 wt%) at 303.15 K.

was changed from 278.15 to 308.15 K. In general, an increase in temperature slightly favors the extraction of rutin for the alcohol-rich phase. The partition coefficient reaches the average value of 40.15 ± 2.80. On the other hand, the extraction efficiencies were almost constant (98.36 ± 0.16) at all.

In order to calculate the thermodynamic functions of transfer of rutin, namely the molar Gibbs energy (ΔG_m^o), the molar enthalpy (ΔH_m^o) and the molar entropy of transfer (ΔS_m^o), Eqs. (4) and (5) were used. The calculated value for ΔG_m^o (-13.20 KJ/mol) is negative, reflecting therefore the spontaneous and preferential partitioning of rutin for the alcohol rich-phase ($K_{rut} > 1$). The migration process of rutin from the salt-rich phase to the alcohol-rich phase is endothermic (ΔH_m^o = 3.97 KJ/mol) and mainly governed by entropic forces ((ΔS_m^o = 44.30 J/mol.K), since $T \times \Delta S_m^o > \Delta H_m^o$.

ATPS formed by 1-propanol (40 wt%) and K_2HPO_4/KH_2PO_4 (20 wt%) were chosen to study the effect of the addition of further electrolytes. The effect of the addition of NaCl (from 0.5 a 10.0 wt%) in the partition coefficient of rutin was investigated; the results are shown in Fig. 4.

A close examination of the results indicates a slight increase of K_{rut} from 42.76 (without NaCl addition) to 51.47 when 2.5 wt% of NaCl was added. Hence, the addition of an additional electrolyte forces the migration of rutin into the alcohol-rich phase. The addition of the electrolyte is mainly expected to be retained in the salt-rich phase turning this phase into a more hydrophilic and charged one. On the other hand, for concentrations higher than 2.5 wt% of NaCl, the opposite trend is observed. The addition of large amounts of NaCl decreases the partition coefficients of rutin. This result can be a direct consequence of the dissolution of NaCl that tends to partition for the alcohol-rich phase at larger concentrations and blocks the partitioning of the biomolecule for the most hydrophobic phase. In fact, the partitioning of biomolecules depends on the hydrophobicity/hydrophilicity balance of the coexisting phases as well as on the charge of the compounds (44).

Extraction and Partitioning of Rutin from Acerola Waste

To validate the optimized and model extractions using the commercial and high purity rutin, the extraction of rutin from acerola waste (peels and seeds) was further conducted. Besides the optimal conditions gathered for the extraction of rutin to the top phase with the ATPS composed of 40 or 50 wt% of 1-propanol + 20 wt% of K_2HPO_4/KH_2PO_4, the system formed by 50 wt% of 1-propanol + 15 wt% of K_3PO_4 + 35 wt% of water was also studied. The three systems allow us to infer the extraction of rutin for the two different phases. While in the first two systems, rutin preferentially partitions into the alcohol-rich phase, in the third system with K_3PO_4, the opposite trend was observed. These three ATPS ensure that the system conditions can be manipulated and that the biomolecule can be recovered in a preferential phase. The extractions carried out with acerola waste were conducted at 298.15 K.

First, the extraction of rutin from acerola waste was performed with aqueous solutions of 1-propanol at 46 wt% and 60 wt%. Then, the respective ATPS were formed by the addition of the appropriate amounts of water and salts, and the partitioning of rutin obtained from a real sample was analyzed. The results obtained from acerola waste are depicted in Fig. 5.

The extraction yields of rutin from the acerola peels and seeds with the 46 and 60 wt% aqueous solutions of 1-propanol was 0.54 ± 0.01 mg/g and 0.527 ± 0.003 mg/g, respectively. Hence, there is an almost insignificant effect of the alcohol concentration from 40 to 50 wt%. The low influence of the alcohol concentration towards the extraction yields of rutin was already verified by Peng and co-workers (45). For comparison purposes, Nunes and co-workers (46) obtained extraction yields of rutin from acerola (*Malpighia glabra* L.) of the order of 1.5 mg/g, demonstrating that 33.3 wt% of rutin is present in acerola waste.

Comparing the results of K_{rut} obtained for the systems composed of 1-propanol + K_2HPO_4/KH_2PO_4, it is observed that reducing the concentration of 1-propanol and increasing the amount of the salt there is an increase in the partition coefficient of rutin, as observed before with the model systems. For instance, in the system with the optimized

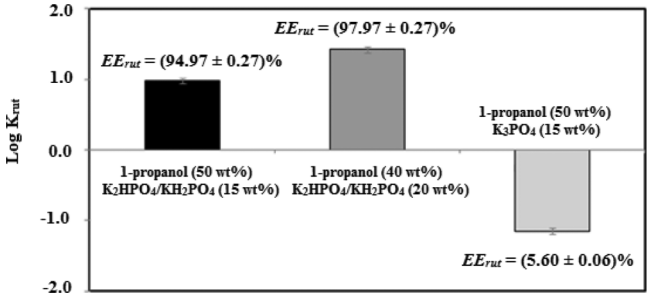

FIG. 5. Partition coefficients and extraction efficiencies of rutin extracted from the peels and seeds of acerola at 298.15 K.

partition coefficients (40 wt% of 1-propanol + 20 wt% of K_2HPO_4/KH_2PO_4), the K_{rut} in the model systems and in those with rutin from the biomass extractions is 26.31 and 45.58, respectively. In the same line, the extraction efficiencies are 97.67% and 98.41%, respectively. Hence, it seems that rutin from acerola waste is more efficiently extracted for the alcohol-rich phase than the commercial rutin in the K_2HPO_4/KH_2PO_4-based ATPS. The extraction efficiencies obtained with the acerola waste are also slightly superior, although the results are very close and in the same order of magnitude. In this context, the optimization tests carried out with the model systems apply to the rutin extracted from the acerola samples.

In order to tailor the extraction of rutin for the salt-rich phase, the system constituted by 1-propanol (50 wt%) + K_3PO_4 (15 wt%) + water (35 wt%) was also investigated. K_{rut} and EE_{rut}% are 0.072 and 5.60% with the model systems, whereas with rutin extracted from acerola waste these parameters are 0.068 and 12.49%, respectively. These values are in close agreement and, in general, the optimization investigations and optimal conditions gathered with the model systems can be applied to the extraction of rutin from bioresource samples.

CONCLUSIONS

In this work, the ability of ATPS composed of alcohols and potassium phosphate salts to extract and recover rutin in one of the coexisting phases was evaluated. It was observed that the studied ATPS are versatile, since the biomolecule can be recovered either in the alcohol- or in the salt-rich phase. This trend is mainly dependent on the salt employed, which leads to different pH values in solution. The differences in the phases' polarities coupled to the charged or non-charged nature of rutin control the preferential migration for a given phase. Indeed, it was observed that the neutral and less hydrophilic form of rutin, present in the lower pH value media, is more easily extracted into the most hydrophobic alcohol-rich phase whereas the charged conjugate base of rutin, present in highly alkaline pH solutions, preferentially migrates to the salt-rich phase. Regarding the effect of the alcohol employed, and in general, an increase in the alkyl chain length of the alcohol or an increase in its hydrophobicity conducts to higher extraction efficiencies of rutin into the top phase. The highest partition coefficient (51.47) and extraction efficiency (98.64%) were obtained in ATPS consisting of 1-propanol (40 wt%) + K_2HPO_4/KH_2PO_4 (20 wt%) + water (40 wt%) and 2.5 (wt%) NaCl at 298.15 K. To validate the optimization tests conducted with the model systems using commercial rutin, the extraction of rutin from acerola wastes (peels and seeds) was initially carried out with alcohol aqueous solutions, which were further applied in the composition of a given ATPS. The results obtained with rutin extracted from the real samples are in close agreement with the model systems and validate all of the optimization investigations and support their further applicability.

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