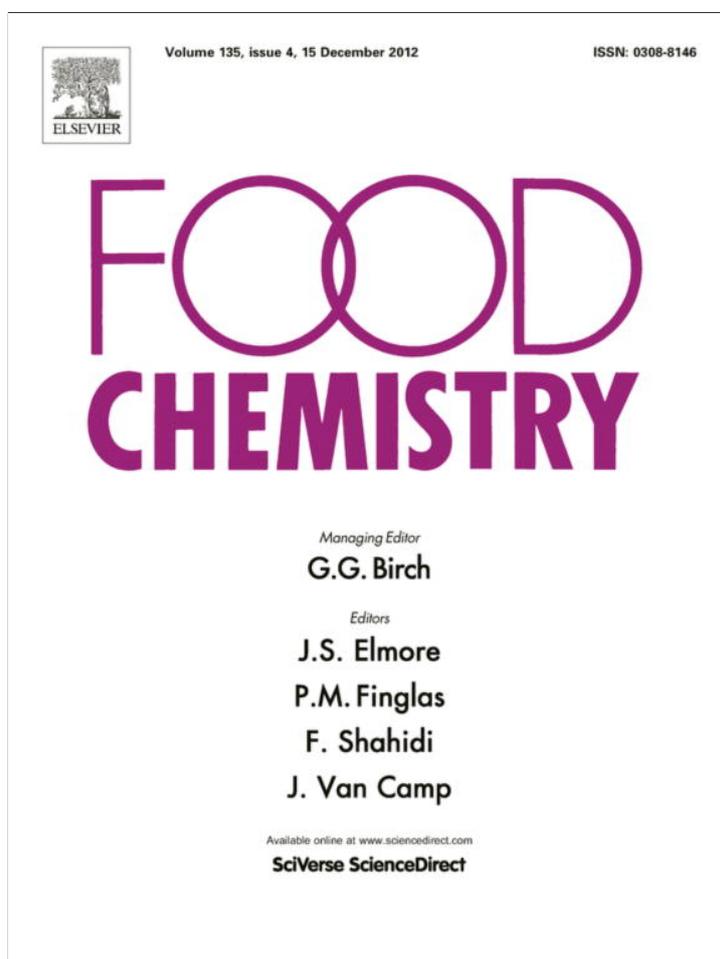


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Increased significance of food wastes: Selective recovery of added-value compounds

Igor A.O. Reis^a, Samuel B. Santos^a, Ludmila A. Santos^a, Naiana Oliveira^a, Mara G. Freire^b, Jorge F.B. Pereira^b, Sónia P.M. Ventura^b, João A.P. Coutinho^b, Cleide M.F. Soares^{a,c}, Álvaro S. Lima^{a,c,*}

^a Programa de Pós-Graduação em Engenharia de Processos, Universidade Tiradentes, Av. Murilo Dantas 300, Farolândia. CEP: 49032-490, Aracaju-SE, Brazil

^b Departamento de Química, CICECO, Universidade de Aveiro, 3810-193 Aveiro, Portugal

^c Instituto de Tecnologia e Pesquisa, Av. Murilo Dantas, 300, CEP: 49032-490, Aracaju-SE, Brazil

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ABSTRACT

A single-step selective separation of two food additives was investigated using alcohol-salt aqueous two-phase systems (ATPS). The selective partitioning of two of the most used additives from a processed food waste material, vanillin and L-ascorbic acid, was successfully accomplished. The results obtained prove that alcohol-salt ATPS can be easily applied as cheaper processes for the selective recovery of valuable chemical products from food wastes and other sources.

As a first approach, the phase diagrams of ATPS composed of different alcohol + inorganic salt + water were determined at 298 (±1) K and atmospheric pressure. The influence of methanol, ethanol, 1-propanol, and 2-propanol and K₃PO₄, K₂HPO₄ or KH₂PO₄/K₂HPO₄ in the design of the phase diagrams was addressed. After the evaluation of the phase diagrams behaviour, the influence of the phase forming constituents was assessed towards the partition coefficients and recovery percentages of vanillin and L-ascorbic acid among the coexisting phases. Both model systems and real processed food waste materials were employed. Using these ATPS as partitioning systems it is possible to recover and separate vanillin, which migrates for the alcohol-rich phase, from L-ascorbic acid, which preferentially partitions for the salt-rich phase.

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1. Introduction

“Roughly one-third of the edible parts of food produced for human consumption gets lost or wasted globally, which is about 1.3 billion ton per year” is one of the main highlights described in the report “Global Food Losses and Food Waste – Extent, Causes and Prevention” presented recently by the Food and Agriculture Organisation of the United Nations (FAO) (Gustavsson, Cederberg, Sonesson, van Otterdijk, & Meybeck, 2011). The management of these food wastes is becoming extremely difficult due to legislative restrictions on landfill. These are however an incredible source of raw materials or added-value compounds and there is, therefore, the need to develop new recovery and reuse technologies, along with the development of sustainable ideas, technologies and processes to avoid those disposals or, at least, to restrain the loss of added-value compounds attached to these wastes.

Processed food that has passed its validity time is an immense source of priceless and valuable chemical compounds, including different sugars, fats, flavours, and antioxidants. Taking this into account, this work aims at the development of a sustainable and

economical process for the recovery of valuable products from food wastes, namely flavours and antioxidants.

An antioxidant compound can be defined as a substance that, when present in low concentrations compared to that of the oxidizable substrate, significantly delay or inhibit the oxidation of that substrate (Atoui, Mansouri, Boskou, & Kefalas, 2005; Moreira & Mancini Filho, 2003). The 3-methoxy-4-hydroxybenzaldehyde, commonly known as vanillin, is one of the most valuable flavour and antioxidant products obtained from waste sources (Kaygorodov, Chelbina, Tarabanko, & Tarabanko, 2010). Indeed, vanillin as a natural flavour, occupies a prominent market place and is commonly used in the preparation of ice creams, chocolates, cakes, soft drinks, pharmaceuticals, and liquors, in the perfumery industry, and in nutraceuticals (Noubigh, Mgaidi, & Abderrabba, 2010; Rana-dive, 1994; Tarabanko, Chelbina, Sokolenko, & Tarabanko, 2007). Since this product has a large range of applications, the development of new techniques for its separation and purification, while keeping its functional characteristics unchanged, is still ongoing. Some publications have demonstrated different approaches to perform the separation of vanillin from different matrices (Converti, Aliakbarian, Domínguez, Bustos-Vázquez, & Perego, 2010; Hocking, 1997; Tarabanko et al., 2007). L-Ascorbic acid is the main biologically active form of Vitamin C. This chemical compound is mostly present in plant cells, where it plays a crucial role in their growth and metabolism. As an effective antioxidant, L-ascorbic acid has the

* Corresponding author at: Programa de Pós-Graduação em Engenharia de Processos, Universidade Tiradentes, Av. Murilo Dantas 300, Farolândia. CEP: 49032-490, Aracaju-SE, Brazil. Tel.: +55 7932182115; fax: +55 7932182190.

E-mail address: alvaro_lima@unit.br (Á.S. Lima).

capacity to eliminate several reactive oxygen species, acts as a cofactor maintaining the activity of a number of enzymes, appears to be the substrate for oxalate and tartrate biosynthesis, and contributes for the stress resistance (Arrigoni & De Tullio, 2002; Davey et al., 2000; Klein & Kurilich, 2000; Lee et al., 2004). Also, given the essential role played in the human diet, L-ascorbic acid (E300) and its salt derivatives (E301–303) are commonly used as food additives due to their antioxidant and flavour enhancing properties. Due to the high economical value attributed to vanillin and L-ascorbic acid, the development of a simple, cheap and compatible recovery process from food wastes is thus of utmost importance.

Aqueous two-phase systems (ATPS) are vital techniques used for the extraction, or even purification, of several compounds and biomolecules, due to their versatility, high effectiveness, high yield, selectivity, low cost and technological simplicity, as well as improved degree of purification. Moreover, ATPS usually allow the combination of the recovery and purification steps (Cláudio, Freire, Freire, Silvestre, & Coutinho, 2010; Malpiedi, Romanini, Picó, & Nerli, 2009). ATPS are generally described as two aqueous liquid phases that are immiscible at critical concentrations of the phase forming components. In the past decades, these systems have been widely applied in the separation/purification of proteins, enzymes, antibiotics, among other biomolecules of interest (Albertsson, 1986; Lima, Alegre, & Meirelles, 2002; Wang, Han, Xu, Hu, & Yan, 2010). To promote the formation of ATPS, several compounds can be used, such as different polymers (Azevedo et al., 2009; Silva & Meirelles, 2000), inorganic salts (Lima et al., 2002; Silva, Coimbra, Rojas, & Teixeira, 2009; Souza et al., 2010), sugars (Chen et al., 2010; Wu, Zhang, & Wang, 2008; Wu, Zhang, Wang, & Yang, 2008), and more recently, ionic liquids (Freire et al., 2012; Gutowski et al., 2003; Neves, Ventura, Freire, Marrucho, & Coutinho, 2009; Ventura et al., 2009). However, several of these ATPS forming components present some crucial disadvantages, when the objective is to apply them as separation systems for products that can easily suffer irreversible chemical alterations, and thus lose their main characteristics (for example, their antioxidant capacity). The high viscosity of polymer-based systems, the low window of potential ATPS based in sugars, and the high cost of ionic liquids, are some of the additional disadvantages that should be taken into account for a number of ATPS (Ooi et al., 2009). Thus, in this work, the use of polar hydroalcoholic ATPS was considered (Broinizi et al., 2007; Roesler et al., 2007; Wang, Han, Xu, Hu, and Yan, 2010). These systems have already shown to be successful in the separation of enzymes, antibiotics, and nucleic acids (Broinizi et al., 2007; Louwrier, 1999).

In this work, four alcohols (methanol, ethanol, 1-propanol and 2-propanol) and three salting-out ionic species (K_3PO_4 , K_2HPO_4 and KH_2PO_4/K_2HPO_4) were studied through the formation of ATPS. Their phase diagrams, tie-lines and tie-line lengths were determined at 298 K and atmospheric pressure. Subsequently, these systems were evaluated toward their application as liquid–liquid separation processes for two antioxidants: vanillin and L-ascorbic acid. Both model systems and food waste materials were employed. The results gathered highlight a selective partitioning of both antioxidants, while maintaining their main chemical characteristics as unchanged. Thus, it is possible to realise that the application of alcohol–salt-based ATPS can be a promising technique for the extraction of valuable compounds from simple or even more complex sources, such as food wastes.

2. Materials and methods

2.1. 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). The alcohols have purities higher than 99 wt.%. The phosphate salts present had purity levels higher than 98 wt.%. The L-ascorbic acid (>98 wt.%) was acquired at Labsynth (São Paulo, Brazil) and vanillin (>99 wt.%) was purchased at Sigma–Aldrich. Ultrapure water, double distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus, was used.

The vanilla diet pudding Dr. Oetker was purchased at a regular supermarket in Aracaju, Brazil (<http://www.oetker.com.br/?actA=2111&produtoID=138>).

2.2. Phase diagrams and tie-lines

The ATPS were formed using aqueous solutions of alcohols (methanol, ethanol, 1-propanol and 2-propanol) at 80 wt.% and distinct aqueous solutions of inorganic potassium phosphate salts (K_3PO_4 , K_2HPO_4 and the phosphate buffer solution KH_2PO_4/K_2HPO_4 - Henderson–Hasselbalch equation equivalents = 1.087) at ca. 40 wt.%. The phase diagrams were determined through two different experimental methodologies well described in literature, the cloud point titration method (Merchuck, Andrews, & Asenjo, 1998; Neves et al., 2009; Ventura et al., 2009; Ventura et al., 2011; Ventura et al., in press) and the turbidometric titration method (“Aqueous Two-Phase Systems: Methods and Protocols (Methods in Biotechnology) (Ed.: R. Hatti-Kaul), Humana Press, Totowa, 2000.”; Freire et al., 2012) at (298 ± 1) K and atmospheric pressure.

The tie-lines (TLs) were obtained using a gravimetric method originally applied by Merchuck and co-workers (Merchuck et al., 1998) and already validated in previous studies (Neves et al., 2009; Ventura et al., 2009). Each tie-line (TL) was determined by the application of the lever-arm rule. For that purpose, the experimental solubility curves were correlated using the following Eq. (1),

$$Y = A \exp[(B X^{0.5}) - (C X^3)] \quad (1)$$

where Y and X , are the alcohol and salt mass fraction percentages, respectively, and A , B and C are the regression constants.

2.3. Partitioning of antioxidants in model systems

The partitioning systems for L-ascorbic acid were prepared using graduated centrifuge tubes by weighing the appropriate amounts of alcohol (at ca. 50 wt.%), inorganic salt (at ca. 15 wt.%) and L-ascorbic acid (2.8 mg). To prepare the vanillin partitioning systems, vials with the same weight fractions of alcohol and inorganic salt were prepared. An aqueous solution of vanillin (concentration of ca. $1.0 \text{ g} \cdot \text{dm}^{-3}$) was used as the aqueous phase. Afterwards, the mixtures were gently stirred and centrifuged at $3,000 \times g$ for 10 min. The extraction systems were placed at (298 ± 1) K, for at least 18 h, to reach the equilibrium and the consequent and complete partitioning of the antioxidants. The vials were closed during this period to avoid the alcohol vaporisation. Finally, both phases were carefully separated and weighed, the volume of each phase was measured, and the biomolecules were quantified in each one of the phases. Their quantification was performed in triplicate and the final concentrations reported are averages of three assays (accompanied by the respective standard deviations).

The quantification of L-ascorbic acid was performed using the volumetric Tillman's method (Lutz, 2008), which is based on the oxidation of 2,6-dichlorophenolindophenol-sodic (DCPIP). The vanillin was quantified through UV-spectroscopy, using a SHIMADZU UV-1700, Pharma-Spec Spectrometer, at two different wavelength values, namely 280 and 347 nm. These two wavelengths

were considered, since the significant variation in the pH of the phases is responsible for alterations on the surface charge of vanillin, and consequently, these differences promote the variation of the maximum peaks of absorption. Thus, to guarantee that the totality of vanillin is quantified, its absorbance was measured in the two maximum peaks of absorption for these pH conditions. Calibration curves were properly established for each wavelength considered, and the mass balance of vanillin was calculated and confirmed in each experiment.

The partition coefficients of each antioxidant were estimated according to Eq. (2)

$$K = \frac{C_T}{C_B} \quad (2)$$

where K is the partition coefficient, C represents the concentration of vanillin or L-ascorbic acid, and the subscripts T and B denote the top and bottom phases, respectively. It should be remarked that in all the extraction systems, the top phase corresponds to the alcohol-rich phase, while the bottom phase is the inorganic-salt-rich phase.

The recovery percentages of each biomolecule for the top (R_{i-T}) and bottom (R_{i-B}) phases were determined accordingly to the following equations,

$$R_{i-T} = \frac{100}{1 + \left(\frac{1}{R_V \times K}\right)} \quad (3)$$

$$R_{i-B} = \frac{100}{1 + R_V \times K} \quad (4)$$

where R_V represents the volume ratio between the volume of the top phase (V_T) and the volume of the bottom phase (V_B), K represents the partition coefficients of each biomolecule, and the subscripts T and B denote the top and bottom phases, respectively. The subscript i describes each biomolecule, being substituted by *van* for vanillin and *AA* for L-ascorbic acid.

2.4. pH determination

The pH (± 0.02) of the top and bottom phases was measured at 298 (± 1) K using an HI 9321 Microprocessor pH meter (HANNA instruments). The compositions adopted at the biphasic region mixture correspond to 50 wt.% of alcohol and 15 wt.% of inorganic salt, and these compositions are similar to those used in the partitioning experiments. All mixtures were gravimetrically prepared within $\pm 10^{-4}$ g. After the separation of the equilibrated phases the pH was measured.

2.5. Selective recovery of vanillin and L-ascorbic acid from a food sample

The selective recovery of both biomolecules (vanillin and L-ascorbic acid) was carried out in a real food waste sample, namely in the vanilla diet pudding Dr. Oetker (<http://www.oetker.com.br/?actA=2111&produtoID=138>). According to the product specifications both vanillin and L-ascorbic acid are mentioned as constituents.

The composition of the preferred ATPS to selectively extract the two antioxidants was chosen by taking into account the partition coefficients and recovery efficiencies obtained with the model systems. Thus, the system composed of ethanol (50 wt.%) + K_2HPO_4 (15 wt.%) + H_2O (35 wt.%) was used with the intent of maximising the concentration of vanillin in the top phase, while the system composed of 2-propanol (50 wt.%) + K_2HPO_4 (15 wt.%) + H_2O (35 wt.%) was employed based on enhanced partition coefficients obtained for L-ascorbic acid at the bottom phase.

The pudding powder samples (5 g of total mass) were dissolved in 23.3 ml of aqueous solution of alcohol (ethanol or 2-propanol at 50 wt.%) and at (298 \pm 1) K. The inorganic salts (K_2HPO_4 or K_3PO_4 at 15 wt.%) and water were then added to prepare the respective ATPS in the required concentrations up to a total volume of 14 ml. Next, the mixtures were gently stirred during 5 min and finally centrifuged at 2,000 rpm for 5 min. The extraction systems were placed at (298 \pm 1) K for 18 h to reach the equilibrium. The vials were closed during this period to avoid the alcohol vaporisation. Finally both phases were carefully separated and weighed, the volume of each phase was measured, and the biomolecules were quantified in each phase by the standard methods described before. The pH of both phases was also measured according to the experimental methodology described above. The biomolecules quantification was performed in triplicate, and the average of the three assays and respective standard deviations are reported.

3. Results and discussion

3.1. Phase diagram and tie-lines

The ATPS formation capacity of four alcohols, using three different potassium inorganic salts (K_3PO_4 , K_2HPO_4 , and K_2HPO_4/KH_2PO_4) was assessed in the present study. All phase diagrams were determined at 298 (± 1) K and at atmospheric pressure. The mass fraction solubility data for all systems are presented in Supporting Information (Tables S1 to S5). The set of solubility curves obtained is depicted in Fig. 1 and Figure S1 (see Supporting Information), according to two different criteria, namely, (a) the effect of alcohols while maintaining the inorganic salt, and (b) the influence of the inorganic salts against one alcohol. All the phase diagrams are presented in molality units to avoid discrepancies in the phase diagrams behaviour which could be a direct result of the differences between the alcohol and salt molecular weights.

According to Fig. 1, it is possible to conclude that alcohols with longer alkyl chains have, in general, a higher ability for ATPS formation, as described by the trend: 1-propanol (370 K) > 2-propanol (356 K) > ethanol (351 K) \geq methanol (337 K). It should be stressed that the boiling temperatures of each alcohol are presented in parenthesis. It is well-known that the solubility of an aliphatic alcohol in water depends on its chain length, and decreases while increasing the number of carbon atoms. Therefore, alcohols with a lower affinity for water are easily separated from aqueous media by the addition of salting-out inorganic salts (Ventura et al., in press). Particularly, for the systems composed of K_2HPO_4 and K_3PO_4 , the alcohol with a branched-alkyl chain, 2-propanol, is less effective for undergoing liquid–liquid demixing, when compared with its isomer, 1-propanol. These results are in good agreement with the literature (Greve & Kula, 1991; Ooi et al., 2009; Shekaari, Sadeghi, & Jafari, 2010; Wang, Wang, Han, Hu, & Yan, 2010; Wang et al., 2010; Zafarani-Moattar, Banisaeid, & Beirami, 2005), where ternary systems based in the same alcohols and organic citrate salts (sodium- and potassium-based) were used. This trend can be explained by the higher hydrophobicity of 1-propanol. Generally, the solvent with the higher hydrophobicity has a lower capacity for dissolving in water, and thus, it is easily excluded from the salt-rich media for an alcohol-rich phase. The higher hydrophobicity of the 1-propanol isomer is also confirmed by its higher octanol–water partition coefficient ($K_{ow} = 1.78$) (Oliferenko et al., 2004) when compared with 2-propanol ($K_{ow} = 1.12$) (Oliferenko et al., 2004). Wang and co-authors (Wang et al., 2010) also pointed out that, despite the idea that the phase separation is driven by the competition of alcohol–water and salt–water interactions, those were still not sufficient to explain the phase formation behaviour. The authors justified the capacity of these four alcohols in promot-

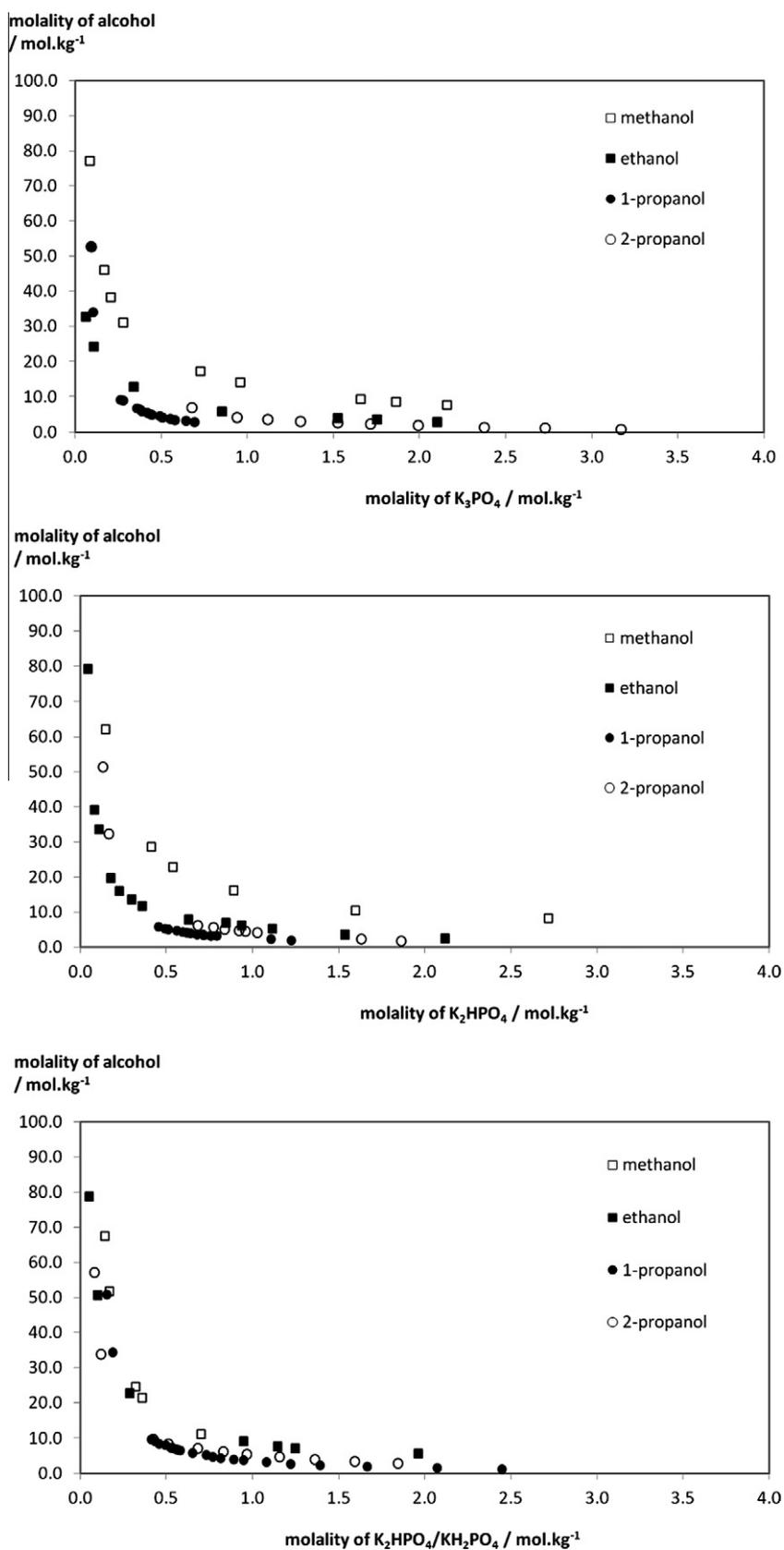


Fig. 1. Phase diagrams of all the ternary systems studied composed of four alcohols: (□) methanol, (■) ethanol, (●) 1-propanol, and (○) 2-propanol; and three inorganic salts: (i) K₃PO₄, (ii) K₂HPO₄, and (iii) KH₂PO₄/K₂HPO₄.

ing the phase formation by showing clear correlation of the acting forces of the alcohol molecules with themselves, and that this con-

dition is well described by their “boiling points” (Lide, 2008) (shown above). The same correlation is obtained here, meaning

that the forces established between the alcohol molecules are also crucial interactions, which rule the phase behaviour. It is also mentioned that the difference of 15 K in the “boiling points” of the isomers reflects the enhanced capacity of 1-propanol to establish van der Waals forces, and which further facilitates the exclusion of this alcohol from the salt- to the alcohol-rich phase (Wang et al., 2010). The same argument is given to explain the small difference on ATPS formation by ethanol and 2-propanol. In fact, these two systems have similar alcohol-alcohol forces described by their close “boiling points”.

For a better understanding of the phenomenon included in the formation of alcohol-salt ATPS, the same binodal curves were also considered aiming to focus the influence of the three inorganic salts on the ATPS formation (Figure S1). The decrease in the capacity of the inorganic salts to promote ATPS formation is as follows:

methanol: $K_2HPO_4/KH_2PO_4 > K_3PO_4 \geq K_2HPO_4$
 ethanol: $K_3PO_4 \approx K_2HPO_4/KH_2PO_4 > K_2HPO_4$
 1-propanol: $K_3PO_4 > K_2HPO_4 > K_2HPO_4/KH_2PO_4$
 2-propanol: $K_3PO_4 \approx K_2HPO_4 \approx K_2HPO_4/KH_2PO_4$

The capacity of these specific inorganic salts to promote the phase separation was already investigated as part of different ternary systems (Ventura et al., 2011; Ventura et al., in press), and, in general, the effect of these inorganic salts follows the Hofmeister series: $K_3PO_4 > K_2HPO_4 > K_2HPO_4/KH_2PO_4$ (Ventura et al., 2011). However, this trend was only verified for systems composed of 1-propanol. The remaining systems do not follow the accepted trend in what concerns the position of K_2HPO_4/KH_2PO_4 . In fact, it is possible to confirm that between K_2HPO_4 and K_3PO_4 , the latter inorganic salt has the highest capacity to induce the phase separation, although in some cases, only a small difference is observed. This behaviour can be easily supported by literature data and it is related to the idea that the strong salting-out inducing anions, PO_4^{3-} and HPO_4^{2-} , exhibit a stronger capability for creating ion-hydration complexes by excluding water from the alcohol-rich phase, and thus favouring the formation of ATPS (He, Li, Liu, Li, & Liu, 2005). Also, according to literature, the K_2HPO_4/KH_2PO_4 salts have a lower ability for the ATPS formation, due to the presence of KH_2PO_4 , which tends towards the salting-in regime. Indeed, it was already described that KH_2PO_4 is not capable by itself to promote the formation of alcohol-based ATPS. Here the “usual” behaviour of K_2HPO_4/KH_2PO_4 was only detected for the 1-propanol system. Searching for an explanation for this behaviour, the pH of both phases of each system were measured (Table 1). According to Table 1, it is observed that the pH is salt-dependent and alcohol-independent. The addition of some of these alcohols is responsible for the destruction of the buffer condition, which is demonstrated by significant differences in the expected pH values of the phases. The buffer condition was lost in most of the systems, with the exception of the 1-propanol. Thus, for the ternary systems with K_2HPO_4/KH_2PO_4 and methanol, ethanol and 2-propanol, the effect is not driven by the phosphate buffer ionic strength and respective interactions, but it is induced by the presence of two different inorganic salts, K_2HPO_4 and KH_2PO_4 , as individual ionic species, and which partition in different directions of the system. Since ATPS making use of K_2HPO_4/KH_2PO_4 were not found in literature, a comparison between our results and those in the literature was not possible. Evidently, the use of these ternary systems for extraction purposes should be cautiously carried out since the pH value is not neutral for systems composed of methanol, ethanol or 2-propanol.

The solubility curves described before, were correlated using the mathematical approach originally described in literature (Merchuck et al., 1998), by the application of Eq. (1). The regression parameters *A*, *B* and *C*, the respective standard deviations (std), and the correlation coefficients (R^2), are reported in Table S6 in

Table 1

pH values of the top (*T*) and bottom (*B*) phases for the extraction systems composed of alcohol + inorganic salt + water, at 298 K and atmospheric pressure.

Ternary System		pH (<i>T</i>) ± 0.02	pH (<i>B</i>) ± 0.02
K_3PO_4	Methanol	12.79	13.20
	Ethanol	12.80	13.22
	1-Propanol	12.38	12.69
K_2HPO_4	2-Propanol	12.61	12.83
	Methanol	10.34	9.76
	Ethanol	9.78	9.52
	1-Propanol	9.69	9.01
K_2HPO_4/KH_2PO_4	2-Propanol	9.85	9.15
	Methanol	8.68	8.99
	Ethanol	7.70	8.01
	1-Propanol	7.88	7.64
	2-Propanol	8.08	7.31

Supporting Information. To complete the phase diagrams, the tie-lines (TLs), and respective tie-line lengths (TLLs), were determined. Their values are reported in Table S7 in Supporting Information, along with the compositions of inorganic salt and alcohol at the top (*T*) and bottom (*B*) phases. The graphical representation of the phase diagrams of all the systems studied is depicted in Supporting Information (Figures S2 to S12). The TLs information is crucial for the extraction process of any compound when using ATPS. Usually, as the concentrations of alcohol and salt used to form the biphasic system increases, the TLL becomes longer, and the top and bottom phases become increasingly different in composition (Guo et al., 2002; Neves et al., 2009; Pereira, Lima, Freire, & Coutinho, 2010; Salabat & Hashemi, 2006; Ventura et al., 2011; Ventura et al., in press; Willauer, Huddleston, & Rogers, 2002). Thus, the partitioning of common molecules in ATPS depends on the TLL considered, which reflects the hydrophilicity/hydrophobicity of the phases (Willauer et al., 2002).

3.2. Partitioning of antioxidants in model systems

In this part of the work we focused on the possibility of using alcohol-salt ATPS to promote the selective partition of two compounds, namely vanillin and L-ascorbic acid, found in some food matrices. Several mixture compositions using alcohol-salt ATPS were prepared according to the following weight percentages: 50 wt.% of alcohol + 15 wt.% of salt + 35 wt.% of biomolecule aqueous solution (L-ascorbic acid or vanillin). The exact mass fraction composition percentages used in the preparation of the mixture points and the respective partition coefficients and corresponding standard deviations are reported in Tables S8 and S9 in the Supporting Information.

The L-ascorbic acid was quantified by the Tillman's method, and the influence of the alcohols and inorganic salts in the antioxidant quantification was assessed before the partition assays. Thus, several saline (40, 20, 10, 5 and 1 wt.%) and alcoholic aqueous solutions (80, 60, 40, 20 and 10 wt.%) were prepared, in combination with three concentrations of L-ascorbic acid (5, 50 and 200 mg L⁻¹). The results suggest that the alcohols' effect in the antioxidant quantification using the Tillman's method is insignificant (results provided in Supporting Information – Figure S13). On the other hand, higher deviations are observed between the real and the quantified concentration of L-ascorbic acid at the salt-rich phase. Thus, the acid concentration was only measured at the alcohol-rich phase (top phase), with its concentration in the other phase estimated by the difference between the initial concentration used to prepare the partition systems, and its concentration in the top phase.

To appreciate the influence of the phase forming components of the ATPS on the vanillin quantification, its UV-Vis spectra were evaluated under different compositions of these alcohols and

inorganic salts. It is well known that vanillin changes its surface charge and chemical structure at different pH values because of the deprotonation of its hydroxyl group (Li, Jiang, Mao, & Shen, 1998) (Figure S14 in Supporting Information). Vanillin has a pK_a of 7.4, indicating that for pH values above 7.4, this biomolecule is preferentially negatively charged. The difference in its structural conformation at different pH values and UV-Vis spectra was already verified by Li and co-workers (Li et al., 1998). Thereby, two different calibration curves were prepared considering the maximum absorbance peaks observed for the biomolecule at different pH values (280 and 347 nm). To achieve the mass balance, the total amount of vanillin was determined using one or both the calibration curves depending on the vanillin structures present in the media.

After the validation of the methods used for the quantification of the biomolecules, their partition coefficients were addressed. The partition analysis of these ATPS was assessed making use of the logarithmic function of the partition coefficient ($\log K$). According to Fig. 2, it is observed that vanillin and L-ascorbic acid preferentially migrate to opposite phases, the top and bottom phases, respectively. While vanillin preferentially migrates to the alcohol-rich phase ($\log K > 0$), L-ascorbic acid has a higher affinity for the salt-rich phase ($\log K < 0$). Aiming at explaining the preference of the acid for the salt-rich phase, some assumptions can be taken into account. The first is related to the L-ascorbic acid chemical structure (depicted in Fig. 2). This biomolecule is highly polar and has the capacity to establish a vast number of hydrogen bonds with water, having more affinity to the more hydrophilic (salt-rich) phase. In an opposite way, vanillin is less polar since it presents a lower number of hydrogen-bond acceptors, and has a consequently higher aptitude for the hydrophobic (alcohol-rich) phase. This trend is also in close agreement with the 1-octanol–water partition coefficients reported in literature for each biomolecule. Reported experimental values of this parameter, $\log K_{ow} = 1.19$ (Noubigh et al., 2010) for vanillin and $\log K_{ow} = -1.85$ (Takács-Novak & Avdeef, 1996) for L-ascorbic acid, show that these molecules have a different hydrophilic/lipophilic aptitude. L-ascorbic acid is more hydrophilic ($\log K_{ow} < 0$), while vanillin is more hydrophobic ($\log K_{ow} > 0$). The partition results obtained here are indeed in good agreement with the $\log K_{ow}$ values (Noubigh et al., 2010; Takács-Novak & Avdeef, 1996), suggesting that the molecules' hydropho-

bicity control the partition nature of these ATPS. Moreover, in order to evaluate the alcohol and salt influence in the partitioning of both biomolecules, the recovery percentages of vanillin in the top phase (R_{van-T}) and L-ascorbic acid in the bottom phase (R_{AA-B}), were also evaluated and are presented in Fig. 3. For all the aqueous systems studied, the recovery of vanillin for the alcohol-rich phase is between $(98.37 \pm 0.08)\%$ and $(99.94 \pm 0.01)\%$, while the recovery of L-ascorbic acid for the salt-rich phase is between $(85.15 \pm 1.27)\%$ and $(95.50 \pm 0.19)\%$. Finally, the recovery results obtained also show that the effect of the alcohol molecular structure on the extraction of both antioxidants is marginal; yet, stronger salting-out inducing inorganic salts, namely K_3PO_4 and K_2HPO_4 , largely enhance the recovery achieved at each phase.

Summing up, the selective partitioning of the two biomolecules is envisaged taking into account the results obtained with the model systems. Therefore, the next stage of this work was to employ the enhanced systems for the selective partitioning of vanillin and ascorbic acid in real food samples.

3.3. Selective recovery of vanillin and L-ascorbic acid from a food sample

The success of a new methodology or process is only proven when the final goal behind the optimisation studies is accomplished. In this context, the capacity of these new alcohol-salt ATPS to simultaneously separate vanillin and L-ascorbic acid from a food waste source was evaluated in this work as a real separation. Thus, the vanilla diet pudding Dr. Oetker was used here as the food waste source of vanillin and L-ascorbic acid. The choice of this food matrix was based on the fact that both biomolecules are present in significant (non-residual) quantities, providing the necessary conditions for their accurate quantification. Since our goal is to demonstrate the separation capacity of the ATPS investigated here for real systems, this part of the investigation was carried out using the best two partition systems identified above, described by the two ATPS with higher partition coefficients and recoveries of both biomolecules into opposite phases. The two systems selected were: ethanol (50 wt.%) + K_2HPO_4 (15 wt.%) + H_2O (35 wt.%) and 2-propanol (50 wt.%) + K_2HPO_4 (15 wt.%) + H_2O (35 wt.%). The ATPS systems were prepared using an alcohol solution of the pudding samples (Table S10).

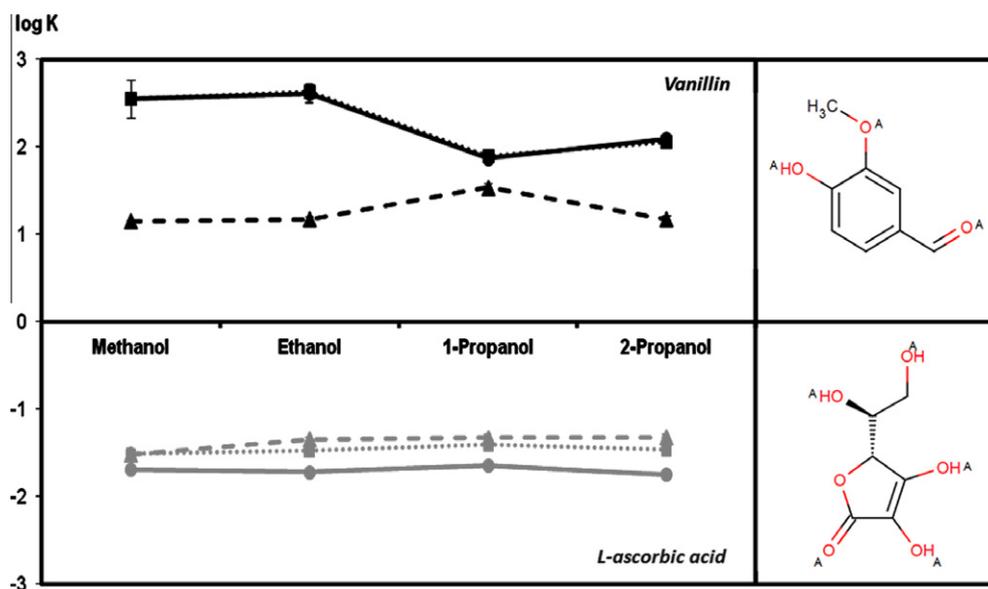


Fig. 2. Logarithm function of the partition coefficients ($\log K$) of the two compounds, vanillin (black lines) and L-ascorbic acid (gray lines), in the ATPS composed of methanol, ethanol, 1-propanol, and 2-propanol, and the inorganic salts K_3PO_4 (circles - solid line), K_2HPO_4 (squares - pointed line) and K_2HPO_4/KH_2PO_4 (triangles - dashed line). The chemical structures of each one of the biomolecules are also shown.

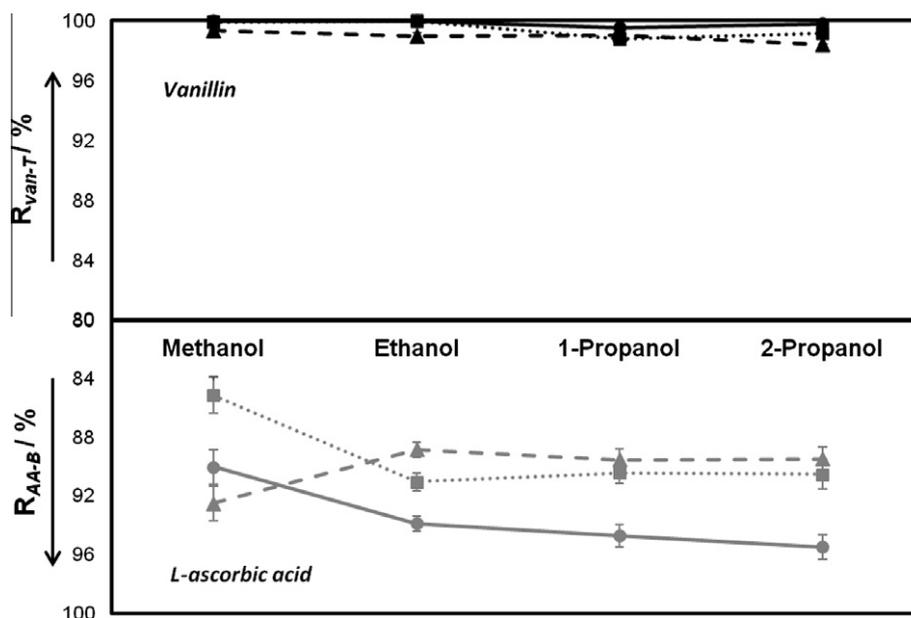


Fig. 3. Recovery percentages of vanillin (dark lines) and *L*-ascorbic acid (gray lines) obtained in each one of the extraction systems studied. The ternary systems are composed of different alcohols, namely methanol, ethanol, 1-propanol, and 2-propanol, with the inorganic salts K_3PO_4 (circles - solid line), K_2HPO_4 (squares - pointed line) and K_2HPO_4/KH_2PO_4 (triangles - dashed line).

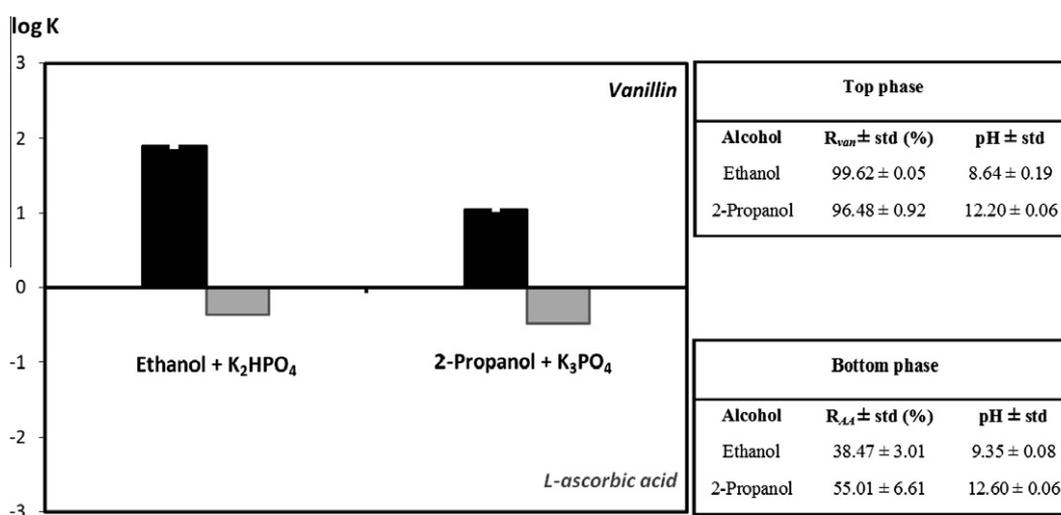


Fig. 4. Extraction parameters (recovery percentages and pH values for the top and bottom phases) and partition coefficients of vanillin (dark bars) and *L*-ascorbic acid (gray bars) from the vanilla diet pudding Dr. Oetker, obtained using the optimised ATPS: 2-propanol (50 wt.%) + K_2HPO_4 (15 wt.%) + H_2O (35 wt.%) and ethanol (50 wt.%) + K_2HPO_4 (15 wt.%) + H_2O (35 wt.%). The pH values together with the respective standard deviations are also reported.

To study the capacity of the selected ATPS in the separation of vanillin and *L*-ascorbic acid from the vanilla diet pudding, the following parameters were evaluated: the partition coefficient logarithmic function, the recovery percentage in the top (vanillin) and bottom (*L*-ascorbic acid) phases, and the pH of each phase. The results are shown in Fig. 4.

Despite the smaller values obtained for K of vanillin and *L*-ascorbic acid, Fig. 4 shows that both systems are capable of promoting the separation of the biomolecules. In this context, it is observed that in the real separation, as in the optimisation study described above, vanillin is migrating almost completely for the top phase ($\log K > 0$ with recovery > 95%) while *L*-ascorbic acid is concentrated in the bottom phase. The smaller values of K_{AA-B} , obtained in the real extraction from the pudding powder, can be explained by the complexity of the pudding sample. Nevertheless the high recovery values obtained for vanillin, and good recoveries (above

50%) for the *L*-ascorbic acid in 2-propanol, prove the success of this selective separation process.

To the best of our knowledge, this is the first time that a selective separation is optimised and successfully applied to simultaneously extract two distinct biomolecules from a food waste raw material into different phases. In this context, alcohol-salt-based ATPS can be envisaged as novel and alternative extractive procedures for the recovery of added-value compounds from several raw materials.

4. Conclusions

Alcohol-salt aqueous two-phase systems (ATPS) were successfully applied to the selective partitioning of two biomolecules, namely vanillin and *L*-ascorbic acid, from a real food waste matrix.

In this context, novel phase diagrams to perform the partitions were determined at 298 (± 1) K and at atmospheric pressure. The main results showed that alcohols with longer aliphatic chains (higher hydrophobicity) enhance the phase separation.

The capacity of these ATPS to be used in the separation of two biomolecules studied was proven, with vanillin being preferentially concentrated in the alcohol-rich phase, whereas L-ascorbic acid migrates for the salt-rich phase. This behaviour is in close agreement with the hydrophilicity/lipophilicity balance of each biomolecule. The optimised systems in what concerns the selective partitioning of vanillin and L-ascorbic acid are: 50 wt.% ethanol + 15 wt.% K_2HPO_4 + 35 wt.% H_2O ($K_{van} = 430 \pm 46$ and $R_{van-T} = (99.93 \pm 0.01\%)$) and 2-propanol (50 wt.%) + K_2HPO_4 (15 wt.%) + H_2O (35 wt.%) ($K_{AA} = 0.018 \pm 0.001$ and $R_{AA-B} = (95.50 \pm 0.19\%)$).

From the application of the optimised ATPS to real food samples, it was concluded that it is possible to design cheaper and simple separation processes capable of promoting the simultaneously separation of two different biomolecules. Thus, this work shows for the first time the successful use of alcohol-salt ATPS in the selective recovery of valuable products from food waste sources, with their application being envisaged in other raw material sources.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2012.07.010>.

References

- Albertsson, P. A. (1986). *Aqueous polymer-phase systems*. New York: Wiley.
- Aqueous two-phase systems: Methods and protocols (Methods in Biotechnology) (Ed.: R. Hatti-Kaul), Humana Press, Totowa, 2000.
- Arrigoni, O., & De Tullio, M. C. (2002). Ascorbic acid: much more than just an antioxidant. *Biochimica et Biophysica Acta*, 1569, 1–9.
- Atoui, A. K., Mansouri, A., Boskou, G., & Kefalas, P. (2005). Tea and herbal infusions: Their antioxidant activity and phenolic profile. *Food Chemistry*, 89, 27–36.
- Azevedo, A. M., Rosa, P. A. J., Ferreira, I. F., Pisco, A. M. M. O., Vries, J., Korporaal, R., et al. (2009). Affinity-enhanced purification of human antibodies by aqueous two-phase extraction. *Separation and Purification Technology*, 65, 31–39.
- Broinizi, P. R. B., Andrade-Wartha, E. R. S., Silva, A. M. O., Novoa, A. J. V., Torres, R. P., Azeredo, H. M. C., et al. (2007). Avaliação da atividade antioxidante dos compostos fenólicos naturalmente presentes em subprodutos do pseudofruto de caju (*Anacardium occidentale* L.). *Food Science and Technology*, 27, 902–908.
- Chen, Y., Meng, Y., Zhang, S., Zhang, Y., Liu, X., & Yang, J. (2010). Liquid–liquid equilibria of aqueous biphasic systems composed of 1-butyl-3-methylimidazolium tetrafluoroborate + sucrose/maltose + water. *Journal of Chemical and Engineering Data*, 55, 3612–3616.
- Cláudio, A. F. M., Freire, M. G., Freire, C. S. R., Silvestre, A. J. D., & Coutinho, J. A. P. (2010). Extraction of vanillin using ionic-liquid-based aqueous two-phase systems. *Separation and Purification Technology*, 75, 39–47.
- Converti, A., Aliakbarian, B., Domínguez, J. M., Bustos-Vázquez, G., & Perego, P. (2010). Microbial production of biovanillin. *Braz. J Microbiol*, 41, 519–530.
- Davey, M. W., Montagu, M. V., Inzé, D., Sanmartin, M., Kanellis, A., Smirnov, N., et al. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of Science Food and Agriculture*, 80, 825–860.
- Freire, M. G., Pereira, J. F. B., Francisco, M., Rodríguez, H., Rebelo, L. P. N., Rogers, R. D., et al. (2012). Insight into the interactions that control the phase behaviour of new aqueous biphasic systems composed of polyethylene glycol polymers and ionic liquids. *Chemistry—A European Journal*, 18, 1831–1839.
- Greve, A., & Kula, M. R. (1991). Phase diagrams of new aqueous phase systems composed of aliphatic alcohols, salts and water. *Fluid Phase Equilibria*, 62, 53–63.
- Guo, Z., Li, M., Willauer, H. D., Huddleston, J. G., April, G. C., & Rogers, R. D. (2002). Evaluation of polymer-based aqueous biphasic systems as improvement for the hardwood alkaline pulping process. *Industrial & Engineering Chemistry Research*, 41, 2535–2542.
- Gustavsson, J., Cederberg, C., Sonesson, U., van Otterdijk, R., & Meybeck, A. (2011). Global Food Losses and Food Waste - Extent, Causes and Prevention, International Congress Save Food!, http://www.fao.org/fileadmin/user_upload/ags/publications/GFL_web.pdf.
- Gutowski, K. E., Broker, G. A., Willauer, H. D., Huddleston, J. G., Swatloski, R. P., Holbrey, J. D., et al. (2003). Controlling the aqueous miscibility of ionic liquids: aqueous biphasic systems of water-miscible ionic liquids and water-structuring salts for recycle, metathesis, and separations. *Journal of American Chemical Society*, 125, 6632–6633.
- He, C., Li, S., Liu, H., Li, K., & Liu, F. (2005). Extraction of testosterone and epitestosterone in human urine using aqueous two-phase systems of ionic liquid and salt. *J Chromatography A*, 1082, 143–149.
- Hocking, M. B. (1997). Vanillin: Synthetic Flavouring for Spent Sulfite Liquor. *J. Chem. Educ.*, 74, 1055–1059. <http://www.oetker.com.br/?actA=2111&produtID=138.1>.
- Kaygorodov, K. L., Chelbina, Y. V., Tarabanko, V. E., & Tarabanko, N. V. (2010). Extraction of vanillin by aliphatic alcohols. *Journal of Siberian Federal University*, 3, 228–233.
- Klein, B. P., & Kurilich, A. C. (2000). Processing effects on dietary antioxidants from plant foods. *HortScience*, 35, 580–584.
- Lee, J. S., Kim, J. W., Han, S. H., Chang, I. S., Kang, H. H., Lee, S., et al. (2004). The stabilization of L-ascorbic acid in aqueous solution and water-in-oil-in-water double emulsion by controlling pH and electrolyte concentration. *Journal of Cosmetic Science*, 55, 1–12.
- Li, R., Jiang, Z., Mao, L., & Shen, H. (1998). Adsorbed resin phase spectrophotometric determination of vanillin or/and its derivatives. *Talanta*, 47, 1121–1127.
- Lide, D. R. (2008). CRC Handbook of Chemistry and Physics, 87th ed., internet version; 2007. <http://www.hbcpnetbase.com>.
- Lima, A. S., Alegre, R. M., & Meirelles, A. J. A. (2002). Partitioning of pectinolytic enzymes in polyethylene glycol/potassium phosphate aqueous two-phase systems. *Carbohydrate Polymers*, 50, 63–68.
- Louwrier, A. (1999). Model isolations of nucleic acids from prokaryotic and eukaryotic sources using an organic/ aqueous biphasic system. *Biotechnology Techniques*, 13, 329–330.
- Lutz, A. (2008). *Normas analíticas do Instituto Adolfo Lutz: Métodos físico-químicos para análise de alimentos* (4ª Ed.). São Paulo: Instituto Adolfo Lutz.
- Malpiedi, L. P., Romanini, D., Picó, G. A., & Nerli, B. B. (2009). Purification of trypsinogen from bovine pancreas by combining aqueous two-phase partitioning and precipitation with charged flexible chain polymers. *Separation and Purification Technology*, 65, 40–45.
- Merchuck, J. C., Andrews, B. A., & Asenjo, J. A. (1998). Aqueous two-phase systems for protein separation: Studies on phase inversion. *Journal of Chromatography B: Biomedical Sciences and Applications*, 711, 285–293.
- Moreira, A. V. B., & Mancini Filho, J. (2003). Atividade antioxidante das especiarias mostarda, canela e erva-doce em sistemas aquosos e lipídicos. *Journal of the Brazilian Society of Food and Nutrition*, 25, 31–46.
- Neves, C. M. S. S., Ventura, S. P. M., Freire, M. G., Marrucho, I. M., & Coutinho, J. A. P. (2009). Evaluation of cation influence on the formation and extraction capability of ionic-liquid-based aqueous biphasic systems. *Journal of Physical Chemistry B*, 113, 5194–5199.
- Noubigh, A., Mgaïdi, A., & Abderrabba, M. (2010). Temperature effect on the distribution of some phenolic compounds: an experimental measurement of 1-octanol/water partition coefficients. *Journal of Chemical and Engineering Data*, 55, 488–491.
- Oliferenko, A. A., Oliferenko, P. V., Huddleston, J. G., Rogers, R. D., Palyulin, V. A., Zefirov, N. S., et al. (2004). Theoretical scales of hydrogen bond acidity and basicity for application in QSAR/QSPR studies and drug design. partitioning of aliphatic compounds. *Journal of Chemical Information and Computer Sciences*, 44, 1042–1055.
- Ooi, C. W., Tey, B. T., Hii, S. L., Kamal, S. M. M., Lan, J. C. W., Ariff, A., et al. (2009). Purification of lipase derived from *Burkholderia pseudomallei* with alcohol/salt-based aqueous two-phase systems. *Proceedings of the Biochemical Society*, 44, 1083–1087.
- Pereira, J. F. B., Lima, A. S., Freire, M. G., & Coutinho, J. A. P. (2010). Ionic liquids as adjuvants for the tailored extraction of biomolecules in aqueous biphasic systems. *Green Chemistry*, 12, 1661–1669.
- Ranadive, A. S. (1994). Vanilla - cultivation, curing, chemistry, technology and commercial products. In G. Charalambous (Ed.), *Spices, herbs and edible fungi* (pp. 517–577). Amsterdam: Elsevier.
- Roesler, R., Malta, L. G., Carrasco, L. C., Holanda, R. B., Sousa, C. A. S., & Pastore, G. M. (2007). Atividade antioxidante de frutos do cerrado. *Food Science and Technology*, 27, 53–60.
- Salabat, A., & Hashemi, M. (2006). Temperature effect on the liquid–liquid equilibria for some aliphatic alcohols + water + K_2CO_3 systems. *Journal of Chemical and Engineering Data*, 51, 1194–1197.
- Shekari, H., Sadeghi, R., & Jafari, S. A. (2010). Liquid–liquid equilibria for aliphatic alcohols + dipotassium oxalate + water. *Journal of Chemical and Engineering Data*, 55, 4586–4591.
- Silva, C. A. S., Coimbra, J. S. R., Rojas, E. E. G., & Teixeira, J. A. C. (2009). Partitioning of glycomacropeptide in aqueous two-phase systems. *Process Biochemistry*, 44, 1213–1216.

- Silva, L. H. M., & Meirelles, A. J. A. (2000). Bovine serum albumin, alfa-lactoalbumin and beta-lactoglobulin partitioning in polyethylene glycol/maltodextrin aqueous two-phase systems. *Carbohydrate Polymers*, *42*, 279–282.
- Souza, R. L., Barbosa, J. M. P., Zanin, G. M., Lobão, M. W. N., Soares, C. M. F., & Lima, A. S. (2010). Partitioning of Porcine Pancreatic Lipase in a Two-Phase Systems of Polyethylene Glycol/Potassium Phosphate Aqueous. *Applied Biochemistry and Biotechnology*, *16*, 288–300.
- Takács-Novak, K., & Avdeef, A. (1996). Interlaboratory study of logP determination by shake-flask and potentiometric methods. *Journal of Pharmaceutical and Biomedical Analysis*, *14*, 1405–1413.
- Tarabanko, V. E., Chelbina, Y. V., Sokolenko, V. A., & Tarabanko, N. V. (2007). A study of vanillin extraction by octylamine. *Solvent Extraction and Ion Exchange*, *25*, 99–107.
- Ventura, S. P. M., Neves, C. M. S. S., Freire, M. G., Marrucho, I. M., Oliveira, J., & Coutinho, J. A. P. (2009). Evaluation of anion influence on the formation and extraction capacity of ionic-liquid-based aqueous biphasic systems. *Journal of Physical Chemistry B*, *113*, 9304–9310.
- Ventura, S. P. M., Sousa, S. G., Freire, M. G., Serafim, L. S., Lima, Á. S., & Coutinho, J. A. P. (2011). Design of ionic liquids for lipase purification. *Journal of Chromatography B*, *879*, 2679–2687.
- Ventura, S. P. M., Sousa, S. G., Serafim, L. S., Lima, Á. S., Freire, M. G., & Coutinho, J. A. P. (2011). Ionic liquids based aqueous two-phase systems with ph controlled by phosphate buffer: The cation effect. *Journal of Chemical and Engineering Data*, *56*, 4253–4260.
- Ventura, S. P. M., Sousa, S. G., Serafim, L. S., Lima, Á. S., Freire, M. G., & Coutinho, J. A. P. (in press). Ionic Liquids Based Aqueous Two-Phase Systems with pH Controlled by Phosphate Buffer: The Anion Effect. *Journal of Chemical and Engineering Data*, *57*, 507–512.
- Wang, Y., Han, J., Xu, X., Hu, S., & Yan, Y. (2010). Partition behaviour and partition mechanism of antibiotics in ethanol/2-propanol-ammonium sulfate aqueous two-phase systems. *Separation and Purification Technology*, *75*, 352–357.
- Wang, Y., Wang, J., Han, J., Hu, S., & Yan, Y. (2010). Liquid-liquid equilibrium of novel aqueous two-phase systems and evaluation of salting-out abilities of salts. *Central European Journal of Chemistry*, *8*, 886–891.
- Willauer, H. D., Huddleston, J. G., & Rogers, R. D. (2002). Solute partitioning in aqueous biphasic systems composed of polyethylene glycol and salt: the partitioning of small neutral organic species. *Industrial & Engineering Chemistry Research*, *41*, 1892–1904.
- Wu, B., Zhang, Y., Wang, H., & Yang, L. (2008). Temperature dependence of phase behaviour for ternary systems composed of ionic liquid + sucrose + water. *Journal of Physical Chemistry B*, *112*, 13163–13165.
- Wu, B., Zhang, Y. M., & Wang, H. P. (2008). Aqueous biphasic systems of hydrophilic ionic liquids + sucrose for separation. *Journal of Chemical and Engineering Data*, *53*, 983–985.
- Zafarani-Moattar, M. T., Banisaeid, S., & Beirami, M. A. S. (2005). Phase diagrams of some aliphatic alcohols + potassium or sodium citrate + water at 25°C. *Journal of Chemical and Engineering Data*, *50*, 1409–1413.