Valorization of expired energy drinks by designed and integrated ionic-liquid-based aqueous biphasic systems

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ABSTRACT

Expired energy drinks are rich in bioactive added-value compounds that can be recovered and reused in order to valorize food waste within a circular economy perspective. However, to accomplish such requirements it is necessary to develop sustainable extraction and recovery processes, which must comprise a decrease in the number of steps required or by developing integrated strategies. In this work, novel aqueous biphasic systems (ABS) composed of ionic liquids (ILs) and a biocompatible polymer polypropylene glycol (400 g.mol^{-1}, PPG 400) were studied for the simultaneous extraction and recovery of three added-value compounds, namely caffeine, taurine and niacin, from expired energy drinks. ILs were designed and synthetized in order to have similar anions to the target compounds, thus allowing enhanced selectivity and biological activity, while avoiding an extra step of separation of these high-value compounds from the IL-rich phase. To this end, cholinium-based ILs comprising the anions lactate, pyruvate, taurate and nicotinate were synthesized and their cytotoxicity and ecotoxicity credentials evaluated. Overall, taurine and niacin are majorly enriched in the IL-rich phase, while caffeine preferentially migrates in the majority of the cases towards the PPG-rich phase. However, caffeine also partitions to the IL-rich phase in the ABS formed by cholinium pyruvate and cholinium nicotinate. The ABS formed by cholinium nicotinate and PPG 400 is the best system identified, allowing the almost complete recovery (recovery efficiencies >82%) of all target compounds into the IL-rich phase in one-step. Furthermore, cholinium nicotinate exhibits marginal cytotoxic potential and is harmless

from an ecotoxicological point of view. This system is thus a promising platform to simultaneously extract, recover and reuse added-value compounds from expired energy drinks without the need of removing the IL or recovering the target compounds from the IL-rich phase, thus contributing to a sustainable and circular food economy.

INTRODUCTION

In developed countries an increasing amount of food is not consumed and ends up as waste along the food value chain, creating a relevant economic and environmental problem.[1] As disclosed by the Food and Agriculture Organization of the United Nations (FAO), one third of the food produced worldwide for human consumption (1.3 billion tonnes per year) is lost or wasted.[2] In the European Union, for instance, food waste is projected to rise to 126 million tons by 2020.[1] Currently, food waste constitutes a relevant concern, being linked to negative economic, societal and environmental impacts.[3] Accordingly, the valorization of food waste is a priority measure to reduce the carbon footprint of the food production chain[4] and to improve economy as the residues generated may contain high-value compounds.[5] In the framework of circular economy, these evidences reinforce the need on the development of cost-effective and sustainable technologies to recover added-value compounds from food waste.

Among food waste, energy drinks, particularly rich in stimulants and additives, play a significant role. These beverages contain high levels of caffeine (up to 500 mg *per* bottle) and are usually supplemented with taurine, glucuronolactone, and complex B vitamins.[6] Most of these high-value compounds maintain their biological activity, even after the expiration dates of energy drinks. In addition to the environmental concerns generated by this type of drinks, their discharge indirectly has an economic impact by increasing their waste management and production costs. Thus, the recovery and reuse of added-value and bioactive compounds from discharged energy drinks has a relevant environmental

68 and economic influence on this market sector that registered global sales over €38 billion

69 in 2015, being expected to reach €53.4 billion in 2020.[7]

70 Several methods, including liquid-liquid extraction,[8] nanofiltration[9] and solid-phase

71 extraction[10] have been reported for the recovery of target compounds from diet samples

72 and beverages. In addition to the extraction step, separation and purification processes are

73 needed, which may comprise numerous drawbacks, including high energy and chemicals

74 consumption.[11, 12] Furthermore, most of these methods are time consuming, labour

75 intensive and use volatile organic solvents, contributing to a relevant environmental

76 impact. Overall, cost-efficient and sustainable techniques able to provide high recovery

77 yields and purity levels of valuable compounds recovered from food waste, ideally

78 combining or integrating several steps, must be developed to meet the current society and

79 environmental standards.

80 Aqueous biphasic systems (ABS) are downstream processing alternatives acting by

81 liquid-liquid extraction, allowing to recover target products from complex samples.[13]

82 Accordingly, this type of systems can be applied both in the extraction and purification

83 steps of high-value compounds from food waste.[14] Moreover, if properly designed, ABS

84 may be biocompatible and of low cost.[15, 16] Typical ABS consist of two immiscible

85 aqueous-rich phases based on polymer/polymer, polymer/salt or salt/salt combinations.

86 In order to improve their separation performance, ABS formed by ionic liquids (ILs) have

87 been extensively investigated.[15] Besides other relevant properties, their fine-tuning by

88 combining adequate IL cations and anions is one of the most significant.[15, 17, 18]

89 ILs are able to form ABS in presence of salts, amino acids, carbohydrates or polymers,

90 and have been successfully used in the extraction of phenolic acids, alkaloids, amino

91 acids, proteins, among others.[19-21] However, several concerns have been raised with the

92 most studied ILs in this field, mainly imidazolium-based.[22, 23] This trend is however

93   changing, with novel classes of ILs being proposed for the creation of ABS.[19, 21] Among

94   these, cholinium-based ILs have been the main target since the IL cation is an important

95   micronutrient source belonging to the B-complex vitamins, which may thus overcome the

96   main drawback on the use of ILs in the food industry[24]. Cholinium chloride ([Ch]Cl) is

97   currently classified as a safe substance by the U.S. Food and Drug Administration

98   (FDA).[25] Previous works reported the synthesis of cholinium-based ILs alongside with

99   their toxicological and biodegradation potential, showing that when combined with

100   appropriate anions, these ILs exhibit low toxicity and high biodegradability.[26]

101   ABS based on cholinium-based ILs were successfully used in the extraction of

102   flavonoids, polysaccharides, amino acids, proteins and enzymes from aqueous solutions

103   and from complex matrices.[19, 27-29] Some publications can be found in the literature

104   regarding the use of IL-based ABS in the valorization of food waste;[25, 27, 30, 31] yet, the

105   majority of these works focused on ABS formed by imidazolium-based ILs and salts, thus

106   compromising their green credentials, and none envisioned the use of ILs with similar

107   biological features to enhance the biological properties of the recovered compounds,

108   while avoiding the use of an additional step to recover the target compounds from the IL-

109   rich phase. The non-volatile nature of ILs, which is valuable when addressing it from an

110   environmental perspective, is indeed a major drawback when attempting the recovery of

111   target compounds from the IL-rich phase since a simple distillation step cannot be

112   applied. Therefore, and although scarcely considered, the recovery of the target

113   compounds from IL-rich phases has been achieved by the addition of anti-solvents, back-

114   extraction steps with organic solvents or by the use of solid-phase extraction.[32, 33]

115   Aiming at developing a sustainable and cost-effective process for the recovery of added-

116   value compounds (caffeine, taurine and niacin) from expired energy drinks, we here

117   demonstrate the potential of ABS formed by cholinium-based ILs (4 synthesized ILs and

3 commercial ILs for comparison purposes) and polypropylene glycol with a molecular weight of 400 g.mol^{-1} (PPG 400) to directly extract the target compounds from the real samples. By being extracted to the IL-rich phase, and by using ILs with similar biological features, the additional separation step to recover the target compounds from the IL-rich phase can be avoided, and may result in an integrated process. The chemical structures and abbreviations of the investigated ILs are depicted in Figure 1.

The synthesized ILs comprise anions derived from natural sources, such as from plant natural acids (lactate and pyruvate), amino acids (taurate) and vitamins (nicotinate). These ILs were designed taking into account the target compounds to recover from expired energy drinks, which could have their biological properties enhanced if combined with IL anions with similar properties. Bearing in mind the potential reuse of the recovered compounds combined with adequate ILs, the ILs cytotoxicity towards human intestinal cell lines and their ecotoxicity by the microtox assay were evaluated. The recovery performance of the investigated ABS for caffeine (alkaloid), taurine (amino acid) and niacin (vitamin B3) from expired energy drinks was finally addressed. The target biomolecules, whose chemical structures are given in Figure 1, are important in the maintenance of the body homeostasis and are widely used by the food, cosmetic and pharmaceutical industries.[34-39]
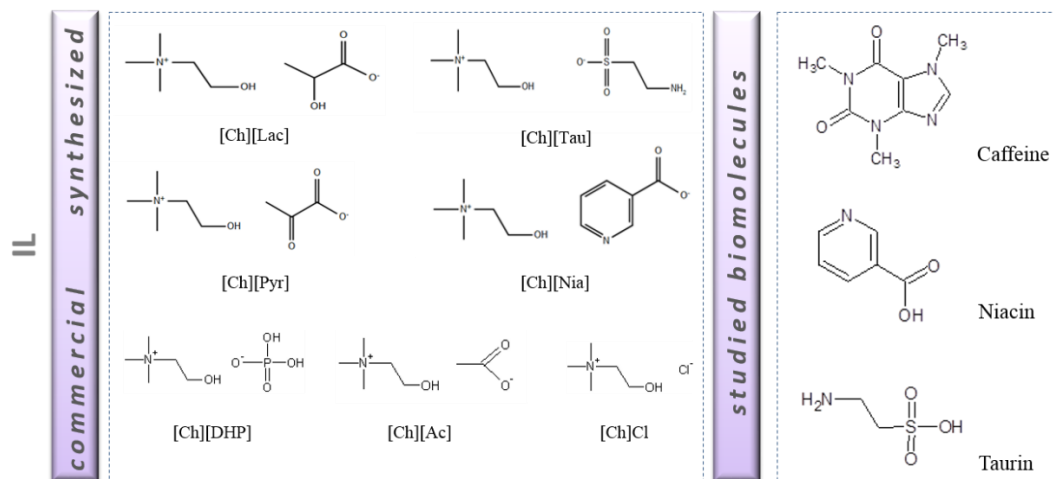
Figure 1. Chemical structures of the investigated ILs and added-value compounds.

EXPERIMENTAL SECTION

Materials. Poly(propylene glycol) with an average molecular weight of 400 g·mol^{-1} (PPG400), cholinium hydroxide ([Ch][OH], 46 wt% in water), taurine (purity ≥99%), lactic acid (purity ≥98%), pyruvic acid (purity ≥98%), nicotinic acid (purity ≥99.5%), 2,4-dinitrofluoro benzene (DNFB purity ≥99%) and dimethyl sulfoxide (DMSO; purity >99.0%) were purchased from Sigma Aldrich (St. Louis, MO, USA). Methanol and acetonitrile (HPLC grade, purity >99.9%) were obtained from Fisher Chemical (Fisher Scientific, USA). Commercial ILs, namely cholinium acetate ([Ch][Ac], >99 wt%) and cholinium dihydrogen phosphate ([Ch][DHP], >98 wt%), were acquired from Iolitec, while cholinium cloride ([Ch]Cl, >98 wt%) was purchased from Acros Organic.

The cell culture medium (Dulbecco's Modified Eagles's Medium – high glucose (HG)) used in the cytotoxicity assays was obtained from Sigma, Fetal Bovine Serum (FBS) from Merck Millipore and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (purity 98%) from ACROS Organics. All chemicals for ecotoxicity assays were purchased from Ambifirst.

The sample of energy drink (sugar free Red Bull®) was acquired from a local market in Aveiro (Portugal); it was used in all experiments with one month after the expiration date. According to the product label it contains caffeine, taurine, B-group vitamins (B3, B5, B6 and B12), aspartame, acesulfame K and water.

ILs Synthesis. Synthesis of cholinium lactate ([Ch][Lac])[40], cholinium nicotinate ([Ch][Nia])[41] and cholinium taurate ([Ch][Tau])[42], were performed according to previously described protocols. Cholinium pyruvate ([Ch][Pyr]) was synthesized in this work for the first time by acid-base titration. The potentiometric acid-base titrations were performed by the slow addition of an aqueous solution of [Ch][OH] (at 1.7524 mol.L^{-1}) to lactic, pyruvic, nicotinic and 2-aminoethanesulfonic acids. [Ch][OH] was added in

small excess and then back titrated by adding the acid solution until the desired inflection point is obtained (pH values for [Ch][Lac] = 9.25, [Ch][Pyr] = 8.20, [Ch][Nia] = 7.95 and [Ch][Tau]= 9.02). Excess water was first removed from the synthesized ILs at 70°C using a rotational evaporator, and then under vacuum until constant weight was achieved. ILs were stored with P_2O_5 under vacuum for the following 72 h. The water content in the ILs was determined by Karl-Fisher titration and found to be $\leq$ 200 ppm in all ILs. The chemical structures and purities of the synthesized ILs, [Ch][Lac] (> 98 wt%), [Ch][Pyr] (>96 wt%), [Ch][Nia] (>99 wt%) and [Ch][Tau] (>99 wt%), were confirmed by ^{1}H and [13]C Nuclear Magnetic Resonance (NMR) and Fourier-transform infrared spectroscopy (FTIR) spectroscopy (Figures S1–S6 in the Supporting Information). NMR spectra were recorded in D_2O at 25°C on a Bruker Advance III 400 MHz spectrometer. Tetramethylsilane was used as internal standard. FTIR spectra were recorded from (4000 to 650) cm^{-1} using a Thermo-Nicolet Nexus 670 spectrometer fitted with a Universal ATR Sampling Accessory. The chemical structures of the investigated ILs are given in Figure 1.

ILs cytotoxicity and ecotoxicity. The cytotoxicity of the synthesized ILs ([Ch][Lac], [Ch][Nia], [Ch][Pyr], [Ch][Tau]) alongside with the commercial ILs [Ch][Ace], [Ch]Cl and [Ch][DHP] was addressed in the human colon epithelial cell line (Caco-2). A stock solution of each IL was prepared in saline aqueous solutions and the test solutions were obtained by successive dilutions of the stock in culture medium, obtaining the final concentrations of 0.1, 1.0, 10, 30, 60, 90 g.L^{-1} of each IL. The epithelial human colon cell line (Caco-2) was grown in high glucose Dulbecco's modified Eagle's medium (DMEM-HG) containing 10% (v/v) fetal bovine serum (FBS), 100 units penicillin, and 50 µg.mL^{-1} streptomycin in a humidified atmosphere of 5% CO_2 at 37°C. Cells were plated on polystyrene cell culture dishes at a density of 1x10^4 cells *per* well in 96 well culture plates.

After 16 h, cells were treated with the different concentrations of the target ILs for 24h. Cytotoxicity was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. This assay is based on the ability of viable cells to convert MTT into a water-insoluble precipitate. Caco-2 cells were incubated with 0.5 mg.dm^{-3} of MTT in medium overnight at 37 °C. The precipitate was then dissolved in 10% (w/v) sodium dodecyl sulfate (SDS), and colorimetrically quantified (at 570 nm) using a microplate spectrophotometer. Each concentration was tested in five replicates of three independent experiments (*n*=3), except for [Ch]Cl for which the values correspond to five replicates of two independent experiments (*n*=2). In all experiments, a negative control corresponding to untreated cells was always included.

The dose response curves and median effective concentration (EC$_{50}$) calculations were performed using the GraphPad PRISM Software (version 8.0.1). The EC$_{50}$ corresponds to the concentration of IL for which 50% of the cells are viable. Cell viability results are expressed as percentage of the control (i.e. unexposed cells).

The ecotoxicity of the synthetized ILs was evaluated using the Standard Microtox liquid-phase assays. This test evaluates the inhibition of the luminescence of the marine bacteria *Vibrio fischeri* and was performed using a range of diluted aqueous solutions (from 0 to 81.9%) of IL, where 100% of the compound corresponds to a known concentration of a stock solution (ca. 10 g.L^{-1}). After 5, 15, and 30 min of exposure to IL, the light output of the luminescent bacteria was determined and compared with the light output of a blank control sample. The toxicity was evaluated on the basis of the percentage decrease in the bacteria luminescence relative to the blank control. The final output of this test is the EC$_{50}$ parameter, which represents the effective concentration of a given IL that produces 50% of inhibition of light emission. Analyses were performed with the MicrotoxOmni^{TM} Software version 4.3.0.1.

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ABS phase diagrams and recovery studies. The phase diagrams of the ABS composed of each IL, PPG 400 and water were determined through the cloud point titration method at (25 ± 1) °C and at atmospheric pressure, as previously described.[43-45] Details regarding phase diagram determination are given in the Supporting Information. Initial tests to address the real samples effect in the binodal curves was carried out, with no significant differences (within the experimental error) obtained between the phase diagrams determined with water or with energy drink samples. This fact is due to the low amount of additives present when compared to the amounts of IL and PPG 400 required to create ABS.

After addressing the ABS phase diagrams and compositions required to create two-phase systems, their recovery capability for the three biomolecules from expired drinks was evaluated at two mixture compositions: (15 wt% IL + 40 wt% PPG400 + 45 wt% expired energy drink) and (30 wt% IL + 30 wt% PPG400 + 40 wt% expired energy drink). The mixture compositions were chosen taking into account two common mixture points in the biphasic region of all systems, while varying the IL and PPG 400 contents to address their effects. Each biphasic system was prepared in 2 mL micro-centrifuge tubes by adding the appropriate amount of PPG 400, IL and energy drink sample to make up a final weight of 1 g. It should be remarked that a liquid energy drink sample is being used directly in the creation of ABS, thus allowing the integration of the extraction and purification steps. Furthermore, by using designed ILs with similar chemical structures to the target compounds, the recovery step can be avoided, allowing to develop and integrated extraction-purification-recovery strategy. All systems were mixed vigorously using a vortex agitator (Reax Top, Heidolph, Germany) and left at 25 °C for 2 h. Each ABS was

240   then centrifuged for 5 min at 2000 rpm to ensure the complete phase separation. The

241   weights of the top and bottom phases were measured.

242   Recovery efficiencies of studied molecules (*RE*%) correspond to the percentage ratio

243   between the amount of each biomolecule in a given phase (IL- or PPG-rich) and that in

244   the total mixture, determined according to Eqs (1) and (2):

245

247    $$RE_{IL}\ (\%) = \frac{[C]_{IL} \cdot w_{IL}}{m_0} \times 100 \qquad\qquad (1)$$

246

248    $$RE_{PPG}\ (\%) = \frac{[C]_{PPG} \cdot w_{PPG}}{m_0} \times 100 \qquad\qquad (2)$$

254

249   where w_{IL}, w_{PPG}, $[C]_{IL}$ and $[C]_{PPG}$ are the weights of the IL- and PPG-rich phases and the

250   concentration of each biomolecule in the IL- and PPG-rich phases, respectively; m_0

251   corresponds to the mass of each biomolecule present in the overall ABS. At the conditions

252   used in this work, the top phase corresponds to the PPG-rich phase while the bottom phase

253   corresponds to the IL-rich phase.

255   According to the product label, the used energy drink contains ca. 320 mg.L^{-1} of caffeine,

256   4000 mg.L^{-1} of taurine and 80 mg.L^{-1} of niacin. The amount of each studied alkaloid

257   (caffeine, niacin and taurine) in Red Bull was confirmed by HPLC using the respective

258   standards and calibration curves. The experimentally determined amounts of these

259   compounds are in accordance with the amounts labelled in the energy drink sample

260   (Figure S9 in the Supporting Information). The stability of caffeine, niacin and taurine in

261   the ABS phases after extraction was also evaluated by HPLC (Figure S10 in the

262   Supporting Information).

263   Caffeine and niacin in each ABS were quantified by HPLC-DAD. The HPLC analysis

264   was performed using an Agilent 1100 liquid chromatograph (USA) with a Zorbax XDB-

265   C18 column (4.6 mm × 250 mm, 3.5 µm particle size). The mobile phase was composed

266   of 18% methanol and 82% water, at a flow rate of 1.5 mL.min^{-1}, with an injection volume

267   of 20 µL and temperature oven at 25°C. Quantification was carried out at 273 nm for

268   caffeine and at 261 nm for niacin. The system was controlled by the Chemstation

269   software. The taurine concentration was determined by HPLC including a pre-column

270   derivatization with DNFB due to low absorption of taurine in the UV-Vis region. The

271   procedure of derivatization is described elsewhere.[46, 47] Briefly, 100 µL of the sample,

272   200 µL of the 0.01 M carbonate buffer (pH 9), 500 µL of DMSO and 10 µL of DNFB

273   were added into an Eppendorf tube, vigorously mixed using a vortex agitator at 2500 rpm

274   for 30 s and placed in a water bath at 40 °C for 15 min. Then, 650 µL of 0.01 M phosphate

275   buffer (pH 6) was added. The mobile phase was a mixture of 0.01 M phosphate buffer pH

276   6 (A) and acetonitrile (B) and the following gradient profile was run: 0–10 min, 90% A

277   and 10% B; 10–15 min, 75% A and 25% B; 15–19 min, 50% A and 50% B; from 19 min,

278   90% A and 10% B at flow rate of 1 mL min^{-1} with a sample injection volume of 20 µL

279   and detection wavelength at 360 nm.

280

281   **RESULTS AND DISCUSSION**

282   In this work, cholinium-based ILs with anions presenting similar characteristics to the

283   ones of the target high-value compounds, to recover from expired energetic drinks, were

284   synthetized and characterized. The ILs were then applied in the creation of ABS to

285   simultaneously extract and recover these compounds directly from the real samples. The

286   ILs selection was based on the premise that the IL-rich phase will have a superior

287   selectivity to the target compounds and that the activity of the recovered compounds could

288   be enhanced, while avoiding a final step to recover these target compounds from the IL-

289   rich phase. The ILs correspond to [Ch][Lac], [Ch][Nia], [Ch][Tau] and [Ch][Pyr]. Three

290   commercial ILs, namely, [Ch][DHP], [Ch][Ac], [Ch]Cl, were also used for comparison

291   purposes. The chemical structures of the investigated ILs and target added-value

292   compounds are depicted in Figure 1.

293

294   **ILs toxicity**

295   Given the potential applications of the recovered ABS phases containing the target

296   bioactive compounds, it is crucial to evaluate the ILs cytotoxicity. For this purpose, the

297   epithelial human colon cell line (Caco-2) was used to study the intestinal cytotoxic effects

298   of the investigated ILs. Figure 2 depicts the cytotoxic profile for the newly synthetized

299   ([Ch][Lac], [Ch][Nia], [Ch][Pyr], [Ch][Tau]) and the commercial ([Ch][Ace], [Ch]Cl and

300   [Ch][DHP]) ILs. The respective dose-response curves and the EC_{50} values are shown in

301   the Supporting Information. Overall, the ILs investigated exhibit EC_{50} values in the $g.L^{-1}$

302   range. [Ch][Lac] has the lowest toxicity (EC_{50}: 96.64 $g.L^{-1}$; 95% Confidence Interval

303   (CI): 74.95-118.3 $g.L^{-1}$), followed by [Ch][Pyr] (EC_{50}: 62.61 $g.L^{-1}$; 95% CI: 54.15-71.08

304    $g.L^{-1}$) and [Ch][Nia] (EC_{50}: 57.59 $g.L^{-1}$; 95% CI: 53.25-62.32 $g.L^{-1}$). Similar results were

305   obtained for the commercial IL [Ch][Ac] with an EC_{50} of 65.11 $g.L^{-1}$ (95% CI: 56.29-

306   73.93 $g.L^{-1}$). [Ch][Tau] exhibits the highest cytotoxicity amongst the synthetized ILs

307   (EC_{50}: 30.05 $g.L^{-1}$; 95% CI: 29.67-30.43 $g.L^{-1}$), being comparable with the ones obtained

308   for the commercial ILs [Ch]Cl (EC_{50}: 30.61 $g.L^{-1}$; 95% CI: 29.85-31.36 $g.L^{-1}$) and

309   [Ch][DHP] (EC_{50}: 28.97 $g.L^{-1}$; 95% CI: 28.50-29.44 $g.L^{-1}$). The toxicity of [Ch][Tau] is

310   approximately twice as high as [Ch][Pyr], [Ch][Nia] or the commercial [Ch][Ac]. A

311   similar profile was obtained for the ecotoxicity tests, with [Ch][Tau] showing the highest

312   toxicity (results and discussion below).
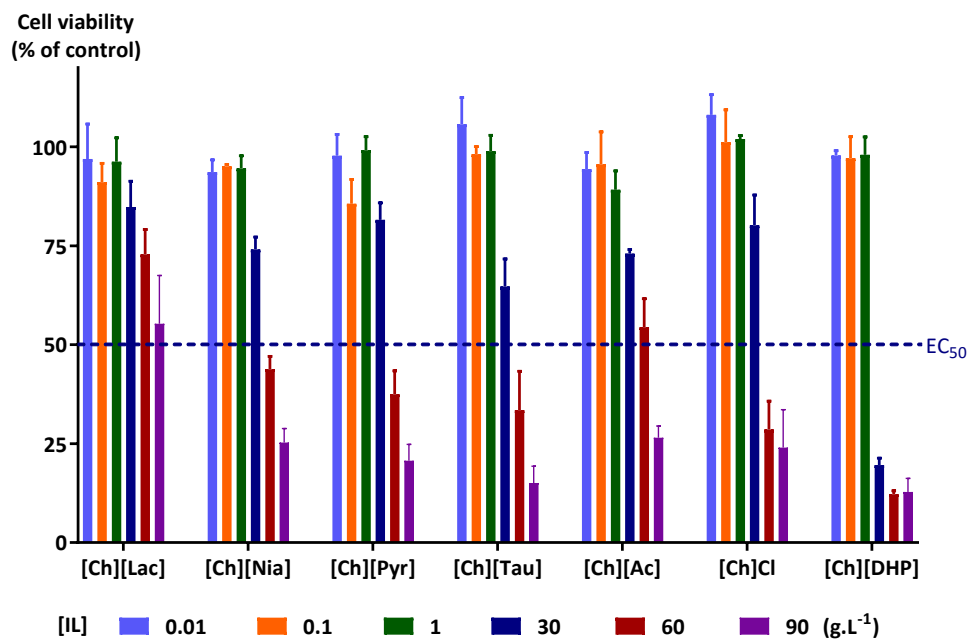
Figure 2. Caco-2 cell viability after 24 h of exposure to the ILs (average values with respective standard error). The dashed line corresponds to the EC_{50}.

In addition to the cytotoxicity, the ecotoxicity of the synthesised ILs was addressed using the bioluminescent bacteria *Vibrio fischeri*. The experimental EC_{50} values were determined by Microtox® bioassays for 5, 15 and 30 min of IL exposure. The detailed results are provided in the Supporting Information. According to the Passino's classification[48], the obtained results reveal that [Ch][Nia] is harmless ($EC_{50} > 1000$ mg.L^{-1}), [Ch][Lac] is practically harmless (100 mg.L$^{-1} < EC_{50} > 1000$ mg.L^{-1}) and [Ch][Tau] is moderately toxic (10 mg.L$^{-1} < EC_{50} > 100$ mg.L^{-1}). [Ch][Pyr] has non-toxic character to the addressed bacteria given that at 60 mg.L^{-1} concentration it was not possible to calculate the EC_{50} value. These results suggest that the investigated anions have a relevant role in defining the cholinium-based ILs toxicity, in agreement with the findings of Ventura et al.[49] showing that [Ch][Ac], [Ch]Cl and [Ch][DHP] are "practically harmless" with EC_{50} values after 30 min of exposure of 673.21 mg.L^{-1} for [Ch][Ac], 469.34 mg.L^{-1} for [Ch]Cl and 572.72 mg.L^{-1} for [Ch][DHP]. [Ch][Tau] is more toxic than these

15

330 commercial ILs. However, [Ch][Lac] is less toxic than [Ch]Cl and has a similar toxicity

331 to [Ch][DHP] and [Ch][Ac]. [Ch][Nia] and [Ch][Pyr] are less toxic than all commercial

332 ILs investigated. Overall, most of the studied ILs have a low environmental impact, where

333 [Ch][Nia] and [Ch][Pyr] stand out as less toxic cholinium-based ILs than those

334 commercially available. This feature is even more relevant if considering the largely

335 investigated imidazolium-based ILs in ABS for separation purposes.[15]

336

ABS phase diagrams and extraction and recovery of added-value compounds from

expired energy drinks

339 By using expired energy drinks, which are liquid samples, to directly create ABS, it is

340 possible to integrate the extraction and recovery steps of the target added-value

341 compounds. With this goal in mind, the ABS phase diagrams were first determined at

342 25°C and atmospheric pressure. The binodal curves experimental data for the ABS

343 composed of each IL, PPG 400 and water are given in the Supporting Information. The

344 respective phase diagrams, given in weight fraction, are shown in Figure 3. Each binodal

345 curve represents the minimum concentration of the system constituents required for the

346 formation of two aqueous phases at 25°C and atmospheric pressure. The binodal curve

347 data were fitted according to the equation proposed by Merchuk et al.,[50] being given in

348 Figure 3. Fitting parameters obtained from the correlation of experimental data and

349 corresponding correlation coefficients are provided in the Supporting Information.

350 Considering that PPG 400 and the cholinium cation are common species to all

351 investigated ABS, the two-phase forming ability of each system is a main result of the IL

352 anion chemical structure. The closer the binodal curve is to the origin of the phase

353 diagram, the lower the concentrations of phase-forming components required for the

354 formation of ABS. The phase-forming ability of the studied ILs in ABS formation, at ca.

25 wt% of PPG 400, follows the order: [Ch][DHP] > [Ch][Ac] > [Ch]Cl > [Ch][Tau] > [Ch][Lac] > [Ch][Pyr] > [Ch][Nia]. [Ch][DHP] shows the highest ability to form ABS, whereas [Ch][Nia] exhibits the lowest.
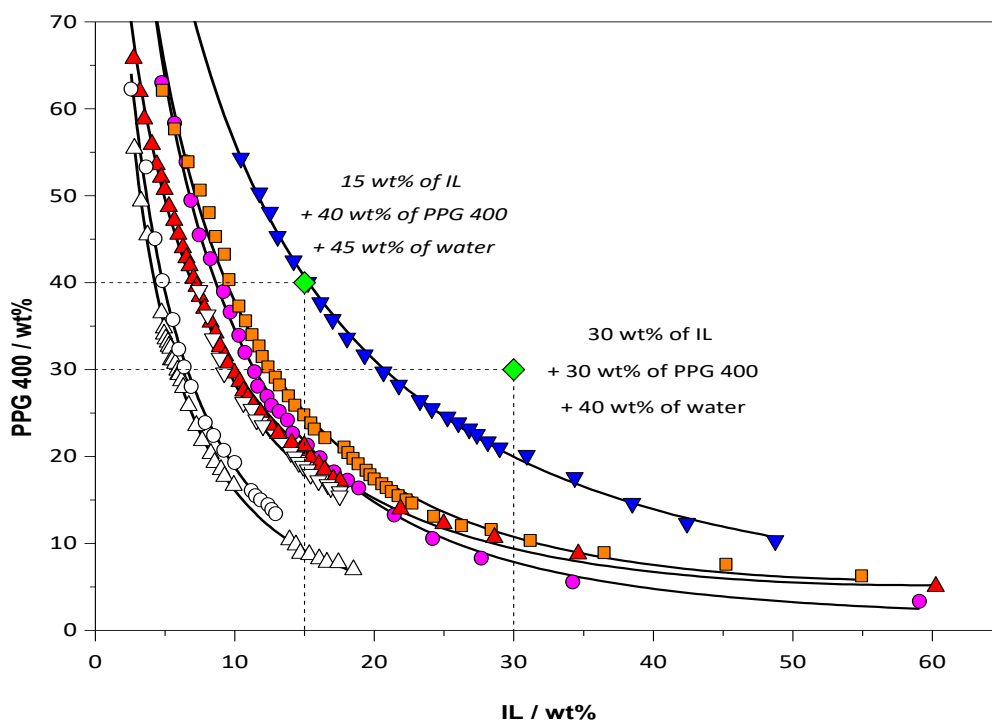
Figure 3. Ternary phase diagrams of the studied ABS (IL + PPG400 + H$_2$O) at 25°C and atmospheric pressure: △, [Ch][DHP]; ○, [Ch][Ac]; ▽, [Ch]Cl; ▲, [Ch][Tau]; ●, [Ch][Lac]; ■, [Ch][Pyr] and ▼, [Ch][Nia]. ◆, ABS compositions used in the extraction studies; continuous line, fitting of the experimental binodal data.

According to the given trend, it seems that the cholinium-based ILs act as the salting-out species over the moderately hydrophobic PPG 400. The salting-out aptitude of ILs is directly related to the hydration capacity of their ions.[32, 51] Since all ILs share a common cation, cholinium, more hydrophilic anions with a higher charge density, such as [DHP]$^-$, have a higher hydration ability and display stronger salting-out effects. However, it should be taken into account that the mechanisms behind the formation of ABS containing

ILs and polymers are far more complex than those observed in ABS formed by salts and ILs.[45] It was previously reported that the ABS formation in systems comprising higher melting temperature cholinium-based ILs, e.g. [Ch][DHP], [Ch][Ac] and [Ch]Cl, is mainly governed by their affinity for water.[52] A higher affinity for water implies a higher ability to promote phase separation when dealing with systems involving polymers, where the ILs acts as the salting-out species. In the case of ABS based on ILs with lower melting temperatures (which will be the case of [Ch][Tau], [Ch][Lac], [Ch][Pyr] and [Ch][Nia]) and polymers, the two-phase formation ability is not a main result of the IL ions to create hydration complexes, but yet the IL-polymer interactions play a significant role.[53]

The anions hydrophobicity may be appraised by the logarithmic values of their octanol-water partition coefficients ($\log K_{ow}$); for conjugated acids of the studied anions they are in the range from –2.77 to 0.22 (detailed values given in the Supporting Information). The most hydrophilic anion is [Tau]$^-$, justifying its higher affinity for water and salting-out ability. On the other hand, the most hydrophobic anion is [Nia]$^-$, which shows the lowest ability to form ABS. However, the obtained trend of ABS formation does not straight follow the $\log K_{ow}$ values; according to this rank, [Ch][Pyr] ($\log K_{ow}$= - 1.24) should be a stronger salting-out agent than [Ch][Lac] ($\log K_{ow}$= - 0.70). On the other hand, the trend of low melting cholinium-based ILs to induce ABS follows the decrease in the anion polar surface, which is the surface sum over all polar atoms (80.39, 60.36, 57.20 and 50.19 Å^2 for [Ch][Tau], [Ch][Lac], [Ch][Pyr] and [Ch][Nia], respectively).[54] These results suggest that the ABS formation of these low melting ILs with PPG is governed by their ability to be solvated by water to act as salting-out species and by specific interactions with the polymer, being in agreement with the literature.[52] For instance, [Ch][Nia] contains π electrons able to establish strong hydrogen bond interactions with

the ether oxygen atoms of PPG, and this could be the major factor for the lower ability of [Ch][Nia] to promote the creation of ABS with PPG.

Tie-line (TL) compositions, tie-lines length (TLL) and slope were additionally determined for ABS formed with the synthesized ILs, being given in the Supporting Information. TL data are particularly relevant to have information on the compositions of the phases at which the extractions are carried out.

After addressing the phase-forming components compositions required to create ABS, the potential of these new ABS formed by cholinium-based ILs and PPG 400 to recover added-value compounds from expired energy drinks, namely caffeine, taurine and niacin (Vitamin B$_3$), was investigated. The synthesized ILs were designed to have similar anions to the target products, which could be beneficial to improve selectivity and biological activity while ideally avoiding the need of the products recovery from the ABS phases. The commercial energy drink contains aspartame, acesulfame K (110 g.L^{-1}), caffeine (320 mg.L^{-1}), taurine (4 g.L^{-1}), vitamin B$_3$ - niacin (80 mg.L^{-1}) and other B complex vitamins (Figure S9 in the Supporting Information). The properties of the targeted compounds which can influence the partitioning in the proposed ABS and their stability in the ABS phases are given in the Supporting Information (Table S6 and Figure S10, respectively). All studied bioactive compounds are stable in the ABS' phases and ILs/PPG 400 aqueous solutions. According to the chromatograms given in Figure S10 in the Supporting Information, it is shown that the retention times of taurine, niacin and caffeine do not change when compared to the standards and that no new peaks appear in addition to those already existing in the energy drink sample.

The extraction of the target compounds from the energy drink was performed at two ternary system compositions (identified in Figure 3): (A) 15 wt% of IL, 40 wt% of PPG 400 and 45 wt% of expired energy drink, and (B) 30 wt% of IL, 30 wt% of PPG 400 and

40 wt% of expired energy drink. The composition of the ternary mixtures was selected based on the phase diagrams previously determined, and close to the binodal curve of the ABS with lowest ability to undergo liquid-liquid demising aiming at improving their sustainability, i.e. requiring lower amounts of the phase-forming components (IL and PPG 400). The obtained recovery efficiencies (*RE*%) in the IL-rich and PPG-rich phases for niacin, caffeine and taurine are depicted in Figure 4 (detailed results are provided in the Supporting Information).

At the conditions investigated, taurine and niacin from the expired drink preferentially migrate to the IL-rich phase in all ABS, whereas caffeine shows an opposite pattern in most of the systems investigated (preferential migration towards the PPG-rich phase, with the exception of [Ch][Pyr]-, [Ch][Nia]- and [Ch]Cl-based ABS) – *cf.* Figure 4. Recovery efficiencies varying from 47.89 to 92.55% for niacin and from 71.68 to 99.87% for taurine to the IL-rich phase, and varying between 5.12 to 83.81% for caffeine to the PPG-rich phase, were obtained. These values support the ILs designer aptitude since the almost complete extraction of the three target compounds is achieved in one-step into the IL-rich phase with the ABS formed by [Ch][Nia], whereas the ABS formed by [Ch][DHP] allows to separate niacin and taurine from caffeine (by their partitioning to opposite phases).
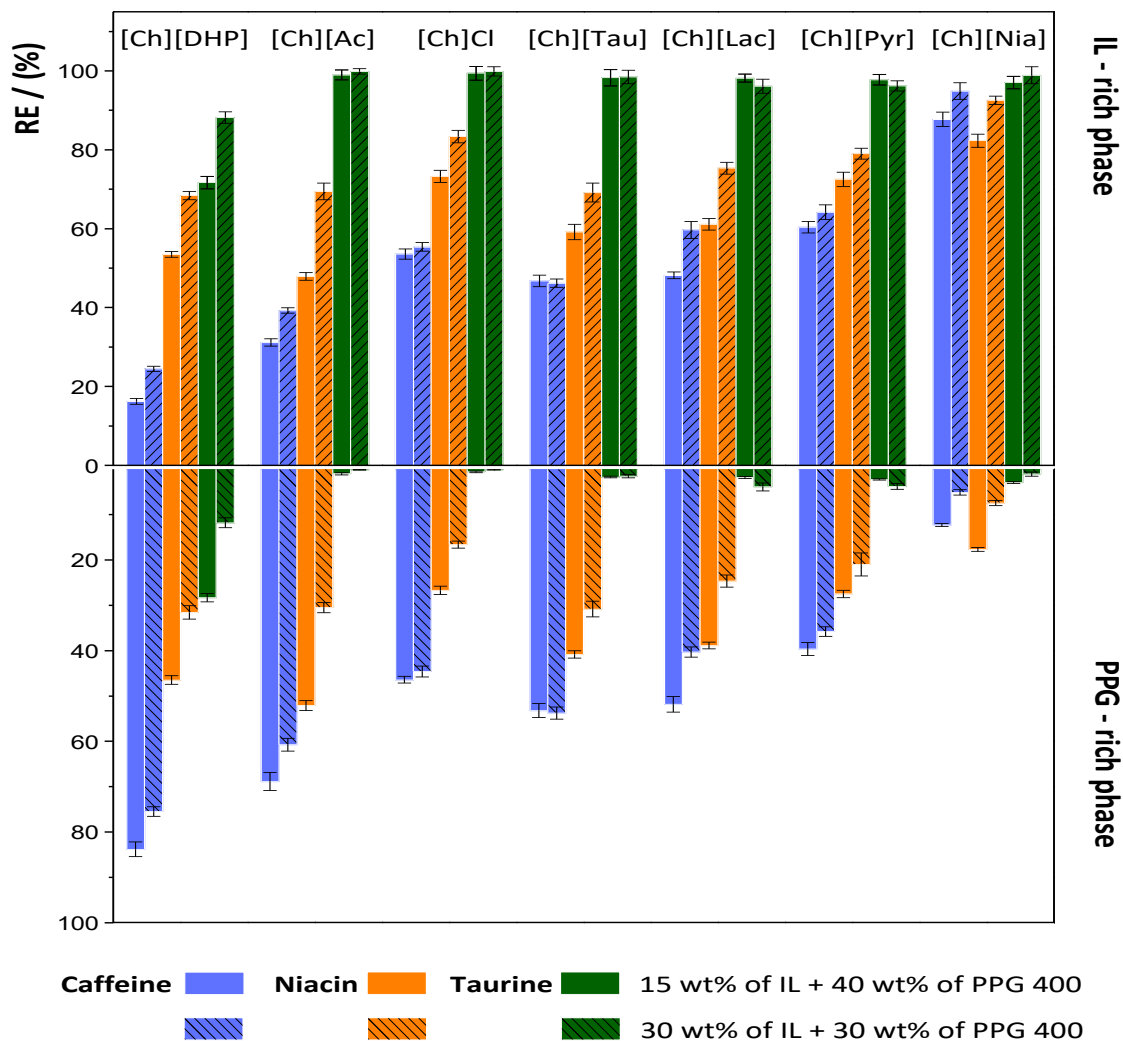
Figure 4. Recovery efficiencies of the caffeine, niacin and taurine in the studied ABS at two mixture compositions at 25°C.

Caffeine can be used as an effective and valuable probe to characterize the relative hydrophobicity of a series of ABS, particularly when hydrophobic type interactions are predominant, since this alkaloid does not suffer speciation in a large range of pH values (pK_{a1}/pK_{a2} = 0.12 / 10.5) while being moderately hydrophilic ($\log K_{ow}$ = –0.63).[55] According to the *RE%* values, caffeine preferentially partitions to the PPG-rich phase at both compositions, except in the case of the ABS composed of [Ch][Nia] and [Ch][Pyr]

446 where caffeine preferentially migrates to the IL-rich phase (Figure 4). Recovery

447 efficiencies of caffeine in the range from 16.19 to 55.38% toward the IL-rich phase and

448 from 5.12 to 83.81% to the PPG-rich phase were obtained, meaning that the enrichment

449 of caffeine in a given phase can be tailored by changing the IL chemical structure. In ABS

450 composed of [Ch][DHP], [Ch]Cl and [Ch][Ac], caffeine is majorly enriched in the PPG-

451 rich phase. On the other hand, when more hydrophobic ILs are applied, such as [Ch][Pyr]

452 and [Ch][Nia], caffeine preferentially partitions to the IL-rich phase. It should be noted

453 that the water content in the PPG400-rich phase in the ABS based on [Ch][Pyr] and

454 [Ch][Nia] is significantly low (3.13-20.52 wt% and 1.85-15.85 wt%, respectively)

455 compared to ABS based on [Ch][Tau] (20.04-33.79%) and [Ch][DHP][56] (17.16-35.87%)

456 (cf. the Supporting Information, tie-line data), thus justifying the inversion on the caffeine

457 partitioning among the coexisting phases, and in agreement with the use of caffeine as a

458 valuable probe to characterize the relative hydrophobicity of ABS phases.[49]

459 Niacin (pKa=4.80)[57], unlike caffeine, is negatively charged in the pH range of the studied

460 ABS (5.8-10.1). Moreover, niacin is the most hydrophilic compound ($\log K_{ow}$ values

461 ranging from –2.03 to –2.93 in the pH range 5.8-10.1) and contains an acidic (–COOH)

462 and a basic group (N from the pyridine core. In the same line, taurine (pKa=-1.49 /9.06)[57]

463 has a high hydrophilic nature ($\log K_{ow}$ values ranging from –5.27 to –6.23 in the pH range

464 5.8-10.1), and exists as a zwitterion or as negatively charged species at the working pH.

465 The results disclosed in Figure 4 show that niacin and taurine preferentially migrate to

466 the IL-rich phase, which is the most hydrophilic phase in the ABS phases, mainly due to

467 their hydrophilic character.

468 In summary, the ABS evaluated are able to remarkably recover added-value compounds

469 directly from expired energy drinks. Furthermore, caffeine, taurine and niacin can be

470 recovered in one phase or caffeine can be separated from the remaining target compounds

only by changing the IL chemical structure. For the first strategy, the best ABS is formed by [Ch][Nia] that allows to recover all compounds in one step with *RE*% higher than 82.32%, whereas the best ABS able to separate caffeine from taurine and niacin is composed of the commercial IL [Ch][DHP]. [Ch][Nia] exibits low citotoxicity and low ecotoxicity, while PPG 400 is approved as a food additive by FDA.[58] Given the enviosioned target applications and assuming that no separation of caffeine from taurine and niacin is aimed, the results obtained reveal that the ABS formed by [Ch][Nia] and PPG 400 is the most efficient system to recover added-value compounds from expired energy drinks, which could be then reused in diverse industrial applications without the need of removing the IL or recovering the target compounds from the IL-rich phase. For instance, these compounds can be used together in food supplements and in cosmetic or pharmaceutical formulations. Despite our promising results, it should be stressed that a thorough risk assessment is required in order to demonstrate the safety use of the IL-rich phase directly by these industries. This assessment includes further *in vitro* and *in vivo* tests in order to evaluate the acute and chronic toxicity towards mammals and towards other test organisms from different trophic levels to guarantee that there are no hazards related with these products.

A schematic overview of the developed integrated platform, and within a circular economy perspective, to extract and recover added-value compounds from expired energy drinks without the need of recovering them from the IL-rich phase is shown in Figure 5. This strategy envisions the direct use of the IL-rich fraction with enhanced biological activity by the food, cosmetics or pharmaceutical industries, although additional tests are required as highlighted above. The reuse of the PPG-rich phase is also envisioned, particularly in the creation of a new ABS with similar samples, at least up to saturation or losses of separation performance.

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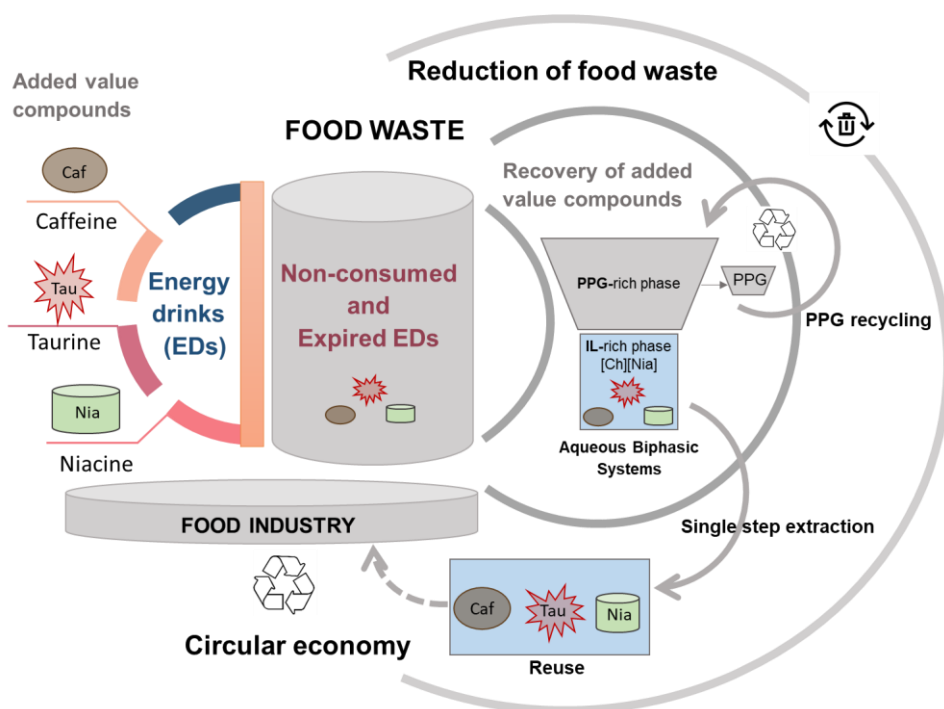
Figure 5. Schematic representation of the proposed integrated platform to extract and recover added-value compounds from expired energy drinks using ABS composed of cholinium-based ILs and PPG 400.

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CONCLUSIONS

In this work, we proposed an integrated approach to extract and recover high-value and bioactive compounds from expired energy drinks using aqueous biphasic systems composed of cholinium-based ILs and PPG 400. A set of cholinium-based ILs was synthesized, characterized and used. [Ch][Lac] exhibits the lowest toxicity towards the human epithelial colon cell line (Caco-2), followed by [Ch][Pyr] and [Ch][Nia]. [Ch][Tau] exhibits the highest cytotoxic character, being comparable to the commercial ILs [Ch]Cl and [Ch][DHP]. A similar profile with [Ch][Tau] exhibiting the highest

toxicity was obtained for the ecotoxicity tests, with this IL being considered moderately toxic. [Ch][Nia] is harmless to the marine bacteria *V. fischeri*, whereas [Ch][Lac] and [Ch][Pyr] were considered as practically harmless.

The ABS phase diagrams were determined at 25°C and atmospheric pressure, in which the ILs in ABS formation followed the order: [Ch][DHP] > [Ch][Ac] > [Ch]Cl > [Ch][Tau] > [Ch][Lac] > [Ch][Pyr] > [Ch][Nia]. In the studied ABS, taurine and niacin preferentially migrate to the IL-rich phase, while caffeine migrates in the majority of the cases towards the PPG-rich phase. However, an opposite behaviour on the partition of caffeine was achieved with ABS formed by [Ch][Pyr] or [Ch][Nia], in which caffeine also partitions to the IL-rich phase. The system composed of [Ch][Nia] and PPG 400 allows the almost complete recovery (*RE*% >82.32%) of all the target compounds into the IL-rich phase in one-step. Given that [Ch][Nia] exhibits lower cytotoxic potential and is considered harmless from an ecotoxicological point of view, this system can be considered a promising platform to simultaneously extract, recover and reuse added-value compounds from expired energy drinks and thus contribute to a more sustainable and circular food economy.

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssusche-meng.xxxxxxx.

NMR and FTIR spectra; information on the determination of the ABS phase diagrams; Caco-2 cytotoxicity dose response curves; Microtox toxicity results; Ternary phase diagrams and binodal weight fraction data of ABS, TLs and TLLs; HPLC chromatograms; Recovery efficiencies.

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Notes

There are no conflicts to declare.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given

approval to the final version of the manuscript.

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559 **REFERENCES**

560 1.　N. Mirabella, V. Castellani and S. Sala, J. Clean. Prod., 2014, 65, 28-41.

561 　　https://doi.org/10.1016/j.jclepro.2013.10.051.

562 2.　J. Gustavsson, C. Cederberg and U. Sonesson, FAO, Global food losses and food

563 　　waste – Extent, causes and prevention, Rome, Italy, 2011.

564 3.　Å. Stenmarck, C. Jensen, T. Quested, G. M., Estimates of European food waste

565 　　levels, Sweden, 2016, ISBN: 978-91-88319-01-2.

566 4.　Y. Virtanen, S. Kurppa, M. Saarinen, J.-M. Katajajuuri, K. Usva, I. Mäenpää, J.

567 　　Mäkelä, J. Grönroos, A. Nissinen, J. Clean. Prod., 2011, 19, 1849-1856.

568 　　https://doi.org/10.1016/j.jclepro.2011.07.001.

569 5.　M. Arshadi, T. M. Attard, R. M. Lukasik, M. Brncic, A. M. da Costa Lopes, M.

570 　　Finell, P. Geladi, L. N. Gerschenson, F. Gogus, M. Herrero, A. J. Hunt, E. Ibáñez,

571 　　B. Kamm, I. Mateos-Aparicio, A. Matias, N. E. Mavroudis, E. Montoneri, A. R.

572 　　C. Morais, C. Nilsson, E. H. Papaioannou, A. Richel, P. Rupérez, B. Škrbić, M.

573 　　Bodroža Solarov, J. Švarc-Gajić, K. W. Waldron and F. J. Yuste-Córdoba, Green

574 　　Chem., 2016, 18, 6160-6204. https://doi.org/10.1039/C6GC01389A.

575 6.　C. J. Reissig, E. C. Strain and R. R. Griffiths, Drug Alcohol. Depen., 2009, 99, 1-

576 　　10. https://doi.org/10.1016/j.drugalcdep.2008.08.001

577 7.　S. Starling, The world's unquenchable thirst for energy drinks,

578 　　https://www.beveragedaily.com/Article/2016/06/14/The-world-s-unquenchable-

579 　　thirst-for-energy-drinks, (accessed 09, 2019).

580 8.   R. Sharif, S. W. Ahmad, H. Anjum, N. Ramzan and S. R. Malik, J. Food Process.

581   Eng., 2014, 37, 46-52. https://doi.org/10.1111/jfpe.12058.

582 9.   Y. K. Ong, H. T. Ng and T.-S. Chung, Ind. Eng. Chem. Res., 2015, 54, 7737-

583   7742. https://doi.org/10.1021/acs.iecr.5b01737.

584 10. M. E. Salinas-Vargas and M. P. Cañizares-Macías, Food Chem., 2014, 147, 182-

585   188. https://doi.org/10.1016/j.foodchem.2013.09.134.

586 11. H. Passos, M. G. Freire and J. A. P. Coutinho, Green Chem., 2014, 16, 4786-4815.

587   https://doi.org/10.1039/C4GC00236A.

588 12. M. Martínez-Aragón, S. Burghoff, E. L. V. Goetheer and A. B. de Haan, Sep.

589   Purif. Technol., 2009, 65, 65-72. https://doi.org/10.1016/j.seppur.2008.01.028.

590 13. B. Y. Zaslavsky, Anal. Chem., 1992, 64. https://doi.org/10.1021/ac00039a709.

591 14. T. Mourão, L. C. Tomé, C. Florindo, L. P. N. Rebelo and I. M. Marrucho, ACS

592   Sustain. Chem. Eng., 2014, 2, 2426-2434. https://doi.org/10.1021/sc500444w.

593 15. M. G. Freire, A. F. M. Claudio, J. M. M. Araujo, J. A. P. Coutinho, I. M.

594   Marrucho, J. N. C. Lopes and L. P. N. Rebelo, Chem. Soc. Rev., 2012, 41, 4966-

595   4995. https://doi.org/10.1039/C2CS35151J.

596 16. D. Mondal, M. Sharma, M. V. Quental, A. P. M. Tavares, K. Prasad and M. G.

597   Freire,   Green   Chem.,   2016,   18,   6071-6081.

598   https://doi.org/10.1039/C6GC01482H.

599 17. A. M. Ferreira, P. D. O. Esteves, I. Boal-Palheiros, A. B. Pereiro, L. P. N. Rebelo

600   and   M.   G.   Freire,   Green   Chem.,   2016,   18,   1070-1079.

601   https://doi.org/10.1039/C5GC01610J.

602 18. Y. Xie, H. Xing, Q. Yang, Z. Bao, B. Su and Q. Ren, ACS Sustain. Chem. Eng.,

603   2015, 3, 3365-3372. https://doi.org/10.1021/acssuschemeng.5b01068.

604   19.   S. P. M. Ventura, F. A. e Silva, M. V. Quental, D. Mondal, M. G. Freire and J. A.

605   P.   Coutinho,   Chem.   Rev.,   2017,   117,   6984-7052.

606   https://doi.org/10.1021/acs.chemrev.6b00550.

607   20.   E. L. P. de Faria, A. M. Ferreira, A. F. M. Cláudio, J. A. P. Coutinho, A. J. D.

608   Silvestre and M. G. Freire, ACS Sustain. Chem. Eng., 2019, 7, 14143-14152.

609   https://doi.org/10.1021/acssuschemeng.9b02808.

610   21.   M. B. Vraneš, J. J. Panić, A. S. Tot, S. M. Ostojić, D. D. Četojević-Simin, N.

611   Janković and S. B. Gadžurić, ACS Sustain. Chem. Eng., 2019, 7, 10773-10783.

612   https://doi.org/10.1021/acssuschemeng.9b01515.

613   22.   G. Chatel, E. Naffrechoux and M. Draye, J. Hazard. Mater., 2017, 324, 773-780.

614   https://doi.org/10.1016/j.jhazmat.2016.11.060.

615   23.   T. Wang, Q. Wang, P. Li and H. Yang, Green Chem., 2019, 21, 4133-4142.

616   https://doi.org/10.1039/C9GC00995G.

617   24.   A. A. C. T. Hijo, G. J. Maximo, M. C. Costa, E. A. C. Batista, and A. J. A.

618   Meirelles,   ACS   Sustain.   Chem.   Eng.,   2016,   4,   5347-5369.

619   https://doi.org/10.1021/acssuschemeng.6b00560

620   25.   A. A. C. Toledo Hijo, G. J. Maximo, M. C. Costa, E. A. C. Batista and A. J. A.

621   Meirelles,   ACS   Sustain.   Chem.   Eng.,   2016,   4,   5347-5369.

622   https://doi.org/10.1021/acssuschemeng.6b00560.

623   26.   M. Petkovic, J. L. Ferguson, H. Q. N. Gunaratne, R. Ferreira, M. C. Leitão, K. R.

624   Seddon, L. P. N. Rebelo and C. S. Pereira, Green Chem., 2010, 12, 643-649.

625   https://doi.org/10.1039/B922247B.

626   27.   R. Wang, Y. Chang, Z. Tan and F. Li, Separ. Sci. Technol., 2016, 51, 1093-1102.

627   https://doi.org/10.1080/01496395

628 28. Z. Li, X. Liu, Y. Pei, J. Wang and M. He, Green Chem., 2012, 14, 2941-2950.

629   https://doi.org/10.1039/C2GC35890E

630 29. D. J. S. Patinha, L. C. Tomé, C. Florindo, H. R. Soares, A. S. Coroadinha and I.

631   M. Marrucho, ACS Sustain. Chem. Eng., 2016, 4, 2670-2679.

632   https://doi.org/10.1021/acssuschemeng.6b00171.

633 30. L. Ran, C. Yang, M. Xu, Z. Yi, D. Ren and L. Yi, Sep. Purif. Technol., 2019, 226,

634   154-161. https://doi.org/10.1016/j.seppur.2019.05.089

635 31. C. M. S. S. Neves, M. Figueiredo, P. M. Reis, A. C. A. Sousa, A. C. Cristóvão,

636   M. B. Fiadeiro, L. P. N. Rebelo, J. A. P. Coutinho, J. M. S. S. Esperança and M.

637   G. Freire, Front. Chem., 2019, 1-12. https://doi.org/10.3389/fchem.2019.00459

638 32. S. Shahriari, C. M. S. S. Neves, M. G. Freire and J. A. P. Coutinho, J. Phys. Chem.

639   B, 2012, 116, 7252-7258. https://doi.org/10.1021/jp300874u.

640 33. M. V. Quental, M. M. Pereira, A. M. Ferreira, S. N. Pedro, S. Shahriari, A.

641   Mohamadou, J. A. P. Coutinho and M. G. Freire, Green Chem., 2018, 20, 2978-

642   2983. https://doi.org/10.1039/C8GC00622A

643 34. A. Herman and A. P. Herman, Skin Pharmacol. Phys., 2013, 26, 8-14.

644   https://doi.org/10.1159/000343174.

645 35. J. L. Temple, C. Bernard, S. E. Lipshultz, J. D. Czachor, J. A. Westphal and M.

646   A.   Mestre,   Front.   Psychiatry,   2017,   8,   80.

647   https://doi.org/10.3389/fpsyt.2017.00080

648 36. R. Lourenco and M. E. Camilo, Nutr. Hosp., 2002, 17, 262-270. ISSN 0212-1611.

649 37. Y. Yamori, T. Taguchi, A. Hamada, K. Kunimasa, H. Mori and M. Mori, J.

650   Biomed. Sci., 2010, 17, S6-S6. https://doi.org/10.1186/1423-0127-17-S1-S6.

651 38. A. Garg, A. Sharma, P. Krishnamoorthy, J. Garg, D. Virmani, T. Sharma, G.

652 Stefanini, J. B. Kostis, D. Mukherjee and E. Sikorskaya, Am. J. Med. Genet.,

653 2017, 130, 173-187. https://doi.org/10.1016/j.amjmed.2016.07.038.

654 39. J. Mielgo-Ayuso, R. Aparicio-Ugarriza, J. Olza, J. Aranceta-Bartrina, A. Gil, R.

655 M. Ortega, L. Serra-Majem, G. Varela-Moreiras and M. Gonzalez-Gross,

656 Nutrients, 2018, 10, 846. https://doi.org/10.3390/nu10070846

657 40. M. Vraneš, J. Panić, A. Tot, M. Popsavin, A. Jocić and S. Gadžurić, J. Chem.

658 Thermodyn., 2019, 131, 80-87. https://doi.org/10.1016/j.jct.2018.10.026.

659 41. M. Vraneš, A. Tot, S. Papović, D. Četojević-Simin, S. Markov, A. Velićanski, M.

660 Popsavin and S. Gadžurić, J. Mol. Liq., 2017, 247, 411-424.

661 https://doi.org/10.1016/j.molliq.2017.10.015.

662 42. M. Vraneš, A. Tot, J. Panić, S. Papović, S. Gadžurić and D. Četojević-Simin, J.

663 Serb. Chem. Soc., 2019. https://doi.org/10.2298/JSC190413047V.

664 43. S. P. M. Ventura, C. M. S. S. Neves, M. G. Freire, I. M. Marrucho, J. Oliveira and

665 J. A. P. Coutinho, J. Phys. Chem. B, 2009, 113, 9304-9310.

666 https://doi.org/10.1021/jp900293v.

667 44. C. L. S. Louros, A. F. M. Claudio, C. M. S. S. Neves, M. G. Freire, I. M. Marrucho,

668 J. Pauly and J. A. P. Coutinho, Int. J. Mol. Sci., 2010, 11, 1777-1791.

669 https://doi.org/10.3390/ijms11041777.

670 45. C. M. S. S. Neves, S. Shahriari, J. Lemus, J. F. B. Pereira, M. G. Freire and J. A.

671 P. Coutinho, Phys. Chem. Chem. Phys., 2016, 18, 20571-20582.

672 https://doi.org/10.1039/C6CP04023C.

673 46. D. L. Orth, J. Chem. Educ., 2001, 78, 791. https://doi.org/10.1021/ed078p791.

674 47. Y. Sawabe, T. Tagami and K. Yamasaki, J. Health Sci., 2008, 54, 661-664.

675 https://doi.org/10.1248/jhs.54.661

676 48. D. R. M. Passino and S. B. Smith, Environ. Toxicol. Chem., 1987, 6, 901-907.

677   https://doi.org/10.1002/etc.5620061111.

678 49. S. P. M. Ventura, F. A. e Silva, A. M. M. Gonçalves, J. L. Pereira, F. Gonçalves

679   and J. A. P. Coutinho, Ecotox. Environ. Safe., 2014, 102, 48-54.

680   https://doi.org/10.1016/j.ecoenv.2014.07.003.

681 50. J. C. Merchuk, B. A. Andrews and J. A. Asenjo, J. Chromatogr. B Biomed. Sci.

682   Appl., 1998, 711, 285-293. https://doi.org/10.1016/s0378-4347(97)00594-x.

683 51. X. Liu, Z. Li, Y. Pei, H. Wang and J. Wang, J. Chem. Thermodyn., 2013, 60, 1-8.

684   https://doi.org/10.1016/j.jct.2013.01.002.

685 52. J. F. B. Pereira, K. A. Kurnia, O. A. Cojocaru, G. Gurau, L. P. N. Rebelo, R. D.

686   Rogers, M. G. Freire and J. A. P. Coutinho, Phys. Chem. Chem. Phys., 2014, 16,

687   5723-5731. https://doi.org/10.1039/C3CP54907K

688 53. M. G. Freire, J. F. B. Pereira, M. Francisco, H. Rodriguez, L. P. N. Rebelo, R. D.

689   Rogers and J. A. P. Coutinho, Chem-Eur J., 2012, 18, 1831-1839.

690   https://doi.org/10.1002/chem.201101780.

691 54. Chemicalize was used for predicting ILs properties. October 2019.

692   https://chemicalize.com/ developed by ChemAxon (http://www.chemaxon.com).

693 55. J. F. B. Pereira, A. Magri, M. V. Quental, M. Gonzalez-Miquel, M. G. Freire and

694   J. A. P. Coutinho, ACS Sustain. Chem. Eng., 2016, 4, 1512-1520.

695   https://doi.org/10.1021/acssuschemeng.5b01466.

696 56. M. V. Quental, M. Caban, M. M. Pereira, P. Stepnowski, J. A. P. Coutinho and

697   M. G. Freire, Biotechnol. J., 2015, 10, 1457-1466.

698   https://doi.org/10.1002/biot.201500003.

699 57. ACD/Labs PhysChem Suite v12, Advanced Chemistry Development, Inc.,

700   Toronto, On, Canada, www.acdlabs.com, 2019.

701   58.   Food and drug administration, Current good manufacturing practice for finished

702   pharmaceuticals Part 173, Chapter I, 2017, 211.

703 **SYNOPSIS**
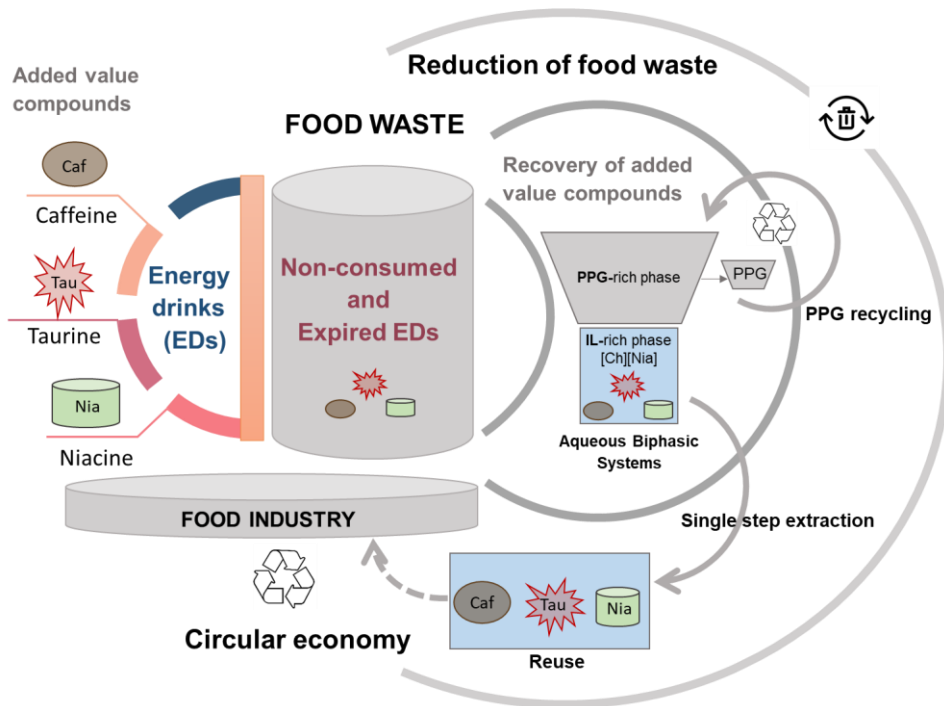
704

705 Ionic-liquid-based aqueous biphasic systems are effective platforms to simultaneously
706 extract, recover and reuse added-value compounds from expired energy drinks,
707 contributing to a sustainable and circular food economy.