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Review

Aqueous Two-Phase Systems Based on Ionic Liquids and Deep Eutectic Solvents as a Tool for the Recovery of Non-Protein Bioactive Compounds—A Review

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Abstract: Aqueous two-phase systems (ATPS) based on ionic liquids (IL) and deep eutectic solvents (DES) are ecofriendly choices and can be used to selectively separate compounds of interest, such as bioactive compounds. Bioactive compounds are nutrients and nonnutrients of animal, plant, and microbial origin that benefit the human body in addition to their classic nutritional properties. They can also be used for technical purposes in food and as active components in the chemical and pharmaceutical industries. Because they are usually present in complex matrices and low concentrations, it is necessary to separate them in order to increase their availability and stability, and ATPS is a highlighted technique for this purpose. This review demonstrates the application of ATPS based on IL and DES as a tool for recovering nonprotein bioactive compounds, considering critical factors, results and the most recent advances in this field. In addition, the review emphasizes the perspectives for expanding the use of nonconventional ATPS in purification systems, which consider the use of molecular modelling to predict experimental conditions, the investigation of diverse compounds in phase-forming systems, the establishment of optimal operational parameters, and the verification of bioactivities after the purification process.

Keywords: bioseparation; green processes; phenolic compounds; alkaloids; natural pigments

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1. Introduction

The global market for bioactive ingredients was estimated to be US $27 billion in 2021 and is estimated to reach approximately US $52 billion in 2027, demonstrating the high demand for bioactive products and compounds, whether of animal, plant, or microbial origin, which are intended for application in the food, beverage, dietary supplement, cosmetic, pharmaceutical, and animal feed sectors [1].

The greater demand for bioactive ingredients is related to the population's greater awareness about health, the search for more adequate diets [2] and, consequently, the use of components with potential health-improving effects, because indicators for diseases, such as obesity, diabetes, stress, sleep disorders, and high blood pressure demonstrate that a large part of the population are affected by such disorders [3,4].

Among the ingredients used in the search for better physiological conditions are bioactive compounds, which are defined as nutrients and nonnutrients that can produce physiological effects beyond their classic nutritional properties [5]. These include proteins, peptides, minerals, carbohydrates, polyphenols, carotenoids, anthocyanins, vitamins, omega-3 fatty acids, organic acids, phytosterols, and others [6–8]. Bioactive compounds can modulate metabolic processes in the body that promote health [9], acting as antioxidants, antimicrobials, and anti-inflammatory and antihypertensive agents. In addition, they have an immune-modulating activity and can reduce damage from cell oxidation and from cardiovascular complications [8].

Bioactive compounds are increasingly being incorporated into food and beverage formulations, increasing their nutritional, sensorial, and technofunctional properties [10,11], in addition to their use in the chemical and pharmaceutical areas, where active ingredients have already been patented by several companies [12]. Bioactive compounds are found in complex matrices with a diversity of compounds that may interfere with their applicability; thus, extraction and purification techniques must be applied to separate the target component from the mixture [2] and to increase the availability and stability of bioactive compounds [13], generating new ingredients or products [14]. As they occur naturally in small amounts in their source matrices [15], isolation procedures and particularly, concentration techniques, also need to be applied.

Among the available separation techniques, aqueous two-phase systems (ATPS) can be used as a tool for the selective separation of target bioactive compounds. The formation of ATPS occurs when two water-soluble compounds are mixed above their critical concentrations, resulting in two immiscible phases. Each phase of the system becomes enriched with one of the compounds giving rise to two aqueous phases of different chemical and physical natures, which propitiates the migration of target compounds into one of the phases [16].

ATPS has the advantage of a high extraction and purification capacity, higher selectivity, and a biocompatible environment [17]. Furthermore, the application of ATPS in bioseparation presents several advantages compared to conventional separation methods, including scale-up potential, simple operation, fast separation, low energy consumption and low cost [18].

Standard ATPS based on polymers and salts are commonly applied in the purification of protein-based molecules [19]. However, there are infinite other possibilities for their use in the separation of nonprotein bioactive compounds, including mainly phenolic compounds [20], alkaloids [21], natural pigments [22] and other components of interest. Options include the use of alternative solvents such as ionic liquids (IL) and deep eutectic solvents (DES), which are a new class of green solvents capable of replacing traditional solvents, thus reducing or eliminating the use or generation of hazardous substances, with a consequential reduction in the environmental impact [23–25]. Advances in this field, including the molecular modelling of IL and DES, could facilitate the expansion and development of purification systems through model prediction, especially when it comes to new and poorly studied alternative solvents, thus also contributing to reducing the time and money involved [26].

In this sense, this review aims to demonstrate the application of ATPS based on IL and DES as a tool for the recovery of nonprotein bioactive compounds, considering important factors for the application, the main results and the most recent advances in this field. To the best of our knowledge, this is the first time that a literature review has explored the application of nonconventional ATPS to a wide range of compounds of nonprotein origin.

2. Bioactive Compounds

Bioactive compounds are nutrients and nonnutrients that exhibit variations in their chemical structures and thus a variety of bioactive properties [5]. They can be obtained from different sources in nature, including animal [27], plant [28], and microbial sources [8,29].

Bioactive compounds from plants present polyphenols, tannins, flavonoids, vitamins, minerals, fatty acids, dietary fibers and other important compounds of different chemical classes, such as carboxylic acids, alcohols, terpenoids, aldehydes, ketones, esters, and pigments [30]. Bioactive compounds from animal sources include bioactive peptides, haem iron, cholesterol, hormones such as melatonin and cortisol, polyunsaturated fatty acids such as omega-3, and collagen [31]. In the case of the dairy industry, the main bioactive compounds are principally extracted from whey and colostrum, highlighting the peptides α-casein, lactorphin, and lactoferricin [32,33].

The demand for bioactive compounds for industries and the consuming public has increased in recent times, due to the technological effects on food and the physiological

effects they produce on the human body, in addition to their classic nutritional properties. Often, the bioactive compound itself is not capable of promoting bioactivity, but produces or induces metabolite effects, or effects on the host's gut microbiome, after consumption [34].

Bioactive compounds are constantly associated with several beneficial properties for the human body, including antihyperglycaemic potential [35], antioxidant, antimicrobial, and angiotensin-converting enzyme inhibitory activity [36], immune-modulating activity, the ability to protect the immune system, and reducing of damage from cell oxidation and from cardiovascular complications [8].

In addition, in technological terms, several bioactive compounds can be used in food products, for example, as a coloring agent, in preparing flours, to increase shelf-life, and as natural antioxidants [13]. Additionally, bioactive compounds improve the nutritional and physicochemical properties, such as texture, and increase the volume of bakery products, e.g., by influencing the viscosity of solutions [15], among other associated technological characteristics.

Among the bioactive compounds, there are three major groups, with varying bioactivity and applications, that will be discussed in this review in relation to purification through ATPS based on IL and DES: (1) phenolic compounds, (2) alkaloids, and (3) natural pigments.

(1) Phenolic compounds. Phenolic compounds comprise a vast and important group of bioactive compounds. These molecules originated as a plant defence mechanism and are considered secondary metabolites [37–39]. Phenolic compounds are generally produced when the plant is subjected to stress conditions, including pathogen infection, excessive light, low temperatures, nutrient deficiency, and others [40]. In plants, phenolic compounds play a role in accelerating the process of pollination and in coloring for camouflage and defence against herbivores, as well as offering activity against bacteria and fungi [37–39]. Within the group of phenolic compounds, we will mainly highlight the simple and complex flavonoids, phenolic acids, and colored anthocyanins, for example [38].

(2) Alkaloids. Alkaloids are also secondary metabolites of plants, housed in different parts of the plant and with differing amounts involving critical biological functions for different species, including the destructive activity of some insect species, storage reservoirs of nitrogen, growth regulators, and substitutes for minerals in plants, for example [41,42]. They are included within the group of nitrogen-containing compounds, which may consist of one or more nitrogen atoms (within the heterocyclic ring) [42], responsible for the alkalinity of these compounds [43], responsible for alkalinity of these compounds [41]. In their pure form, alkaloids are colorless and odorless crystalline solids but can also be yellowish and impart a bitter taste [41]. The group of alkaloids includes molecules such as caffeine, nicotine [44], and capsaicin [45], among others.

(3) Natural pigments. Natural pigments are alternatives to synthetic dyes and offer various promising applications due to their better biodegradability, greater compatibility with the environment and lower adverse health effects [46]. They are applied to compensate for the loss of natural color present in foods, which occurs during industrial processing and storage, to give identity to certain products, to standardize or intensify the color, and to give coloration to original colorless products, among other properties [47,48]. Among the group of natural pigments with potential for application, carotenoids (orange, yellow, and red), chlorophylls (green) and phycobiliproteins (phycoerythrin, red; phycocyanin and allophycocyanin, blue) stand out, having several bioactive properties with a positive impact on products and on human health, in addition to their colorific potential [49].

3. Methods for Obtaining Bioactive Compounds and Bioseparation

In general, bioactive compounds can be obtained as follows: (1) by direct recovery from different biomass in which they are naturally present (e.g., leaves, flowers, fruits, seeds, husks, oilseeds, cereals, mushrooms, and residues from processing plant and animal tissues); (2) by the cultivation of microorganisms (e.g., bacteria, filamentous fungi, yeasts, and microalgae); and (3) through chemical synthesis.

In the first case, the recovery strategy adopted will depend on the intrinsic characteristics of the matrix and the target compound. However, common preparation steps include selection, drying, size reduction, and degreasing. Examples include the collection and selection of leaves [50], drying of seeds to standard humidity [51,52], milling and sieving of plant material [53] and, in some cases, the extraction of fat from oleaginous materials [54]. Afterward, the powder material can be incorporated directly into ATPS, which will act as the target compound's extraction and recovery process [51,54]. In other cases, the matrix is subjected to a solid-liquid extraction step, and then the extract is applied to different ATPS for further purification [52,55–58].

Several metabolites of industrial interest (e.g., proteins, enzymes, carbohydrates, lipids, pigments, phenolic compounds, and chemicals, among others) [59–63] can be produced by the most diverse microorganisms, including wild strains [64], genetically modified strains [65], or microbial consortium [63]. The recovery of target biocompounds will depend on their intrinsic characteristics, the place where they are produced (inside the cell or in the extracellular medium), the type of cultivation (solid or submerged state), and the culture medium (synthetic or containing industrial byproducts). Intracellular biocompounds require a cell disruption step to release to the liquid phase [60,61,65]. An extraction step follows solid-state cultivations to obtain the liquid extract containing the biocompounds [66]. Submerged cultures can rely on a subsequent clarification step, such as centrifugation and filtration, to obtain cell-free extracts with a reduced level of culture medium components, especially in processes where industrial byproducts are used as the source of nutrients [63,64,67]. After all these steps, the extracts can be directly applied to ATPS for selective extraction or partial purification of the compound of interest [61,65].

Unlike bioactive compounds produced by chemical synthesis, extracts from biomass or cultivation processes are complex, containing other compounds and possible contaminants. For example, microbial cultivations can result in highly diluted extracts containing particulate material and undesirable compounds beyond the bioactive compounds, especially those that use medium-containing industrial byproducts. In this sense, purification steps are applied to obtain purer fractions of the bioactive compound. The choice of purification techniques must consider the ability to maintain the bioactivity of the target compounds, in addition to operational aspects (e.g., process parameters, yield, and scale-up) and the degree of purity required. In addition, the bioactivities of the crude extract and purified target compounds should be evaluated when possible. Some techniques used for purification of the most varied bioactive compounds include precipitation by using salt and organic solvents, membrane separation, and chromatographic approaches [68,69]. In this review, we will focus on extracting bioactive compounds from ATPS based on IL and DES as a tool for recovery, due to its operational advantages, as shown below.

4. Aqueous Two-Phase Systems (ATPS)

ATPS are liquid–liquid extraction processes that have an interesting approach for the extraction of biomolecules requiring a high-water content in the coexisting phases. The advantages of this system are related to the high extraction and purification capacity, higher selectivity, and biocompatible environment [17].

In 1896, Beijerinck observed the formation of two liquid phases after mixing aqueous solutions of gelatine and agar. In order to verify a possible generality of the phase separation phenomenon in systems containing macromolecules, in 1947, Dobry and Boyer–Kawenoki, studied the miscibility of water-soluble polymer pairs in organic solvents and the occurrence of phase separation [70]. However, it was only with the work of Albertsson [71] that the great potential of this system for separating macromolecules, such as proteins, became evident to the scientific community.

ATPS for application in bioseparation present several advantages compared to conventional separation methods. These include simple operation, fast separation, high selectivity, low energy consumption, and low cost [72]. The extremely low interfacial tension of this system (between 0.0001 and 0.1 dina/cm) creates a high interfacial contact area of the dis-

persed phases, increasing mass transfer efficiency [73–75]. These systems have considerable potential for application with excellent technical and economic advantages in downstream processes. In addition, they exhibit several advantages, such as simplicity, rapid separation, efficiency, economy, flexibility, and biocompatibility [75]. The variety of chemical species forming ATPS allows combinations to give rise to systems with diverse physicochemical characteristics, enabling the separation of different analytes [76].

The formation of ATPS occurs when two water-soluble compounds are mixed above their critical concentrations, resulting in two immiscible phases (top phase and bottom phase). Each phase of the system becomes enriched with one of the compounds giving rise to two aqueous phases of different chemical and physical natures, which propitiates the migration of target compounds into one of the phases [16].

The formation of the two phases can be explained as a function of the enthalpy of hydration and the system's entropy. Although both components have an affinity for water, their hydration enthalpies are different from each other, making it possible for two different thermodynamic scenarios to exist. If the system's energy is greater than the difference between the enthalpy of hydration and the entropy of the system components, they can coexist in a single phase. However, if the system's energy is lower, the separation of the constituents becomes favorable, and the two phases are formed in equilibrium [77]. Thus, the separation of phases in ATPS results from each component's ability to form hydration complexes with the water of the system. This process is influenced by numerous variables, such as the concentration, type and molar mass of the polymer and salt, the pH and the temperature of the system [78,79]. The interactions between the system-forming components command its formation and will be responsible for all the physicochemical properties of the phases at equilibrium [72].

The composition of the system can be represented in a triangular (Figure 1A) or orthogonal (Figure 1B) phase diagram. The orthogonal representation, where the water concentration is omitted (the origin of the orthogonal axis represents pure water), is the most widely used in the literature because it allows the use of any concentration unit, whereas the concentration units are restricted to mass and/or mole fraction in the triangular representation. In both cases, it is possible to identify the solubility curve, also often referred to as the binodal curve or equilibrium curve, separating the monophasic region from the biphasic area. Knowledge of the two-phase area, initial mixing, and composition of the individual phases are crucial for any extraction process design and operation, as well as for understanding the mechanisms that govern the partitioning of a solute between two phases. Phase diagram data are also necessary to develop models that can predict partitioning between phases [80]. For example, in the biphasic region, an initial mixture of composition E results in two coexisting phases with the composition represented by two endpoints, D and F, on the binodal curve.

Binodal curves can be constructed by determining the concentration of the components by different methods. The use of high-performance liquid chromatography is the most accurate method for determining the composition of the phases that form the system [81]. However, the method most widely used, due to its simplicity and speed is based on the titration process between the system's components. This method is commonly called the cloud-point method. The procedure is performed from the visual observation of the appearance or disappearance of turbidity, which occurs during the titration with another compound, generating experimental points. In general, when the system becomes turbid, there is a change from one to two phases; when changing from two phases to a single phase, the turbidity disappears. System compositions are determined by mass (g) quantification [72]. To correlate the experimental points, different models have been reported in the literature. Tubio, et al. [82] associated the experimental points using an empirical sigmoidal equation (Equation (1)); however, the most proposed model for data

correlation is that of Merchuk, et al. [83], which proposed a mathematical model with three adjustable parameters (Equation (2)). We have

$$[Y] = y_0 + \frac{a}{1 + e^{-\frac{([X]-x_0)}{b}}} \quad (1)$$

$$Y = A\, exp\left[\left(BX^{0.5}\right) - \left(CX^3\right)\right], \quad (2)$$

where Y and X are the percentages in mass fraction of component 1 and component 2, respectively. The fitting parameters a, b, and x_0, and A, B and C are obtained by regressing the data.

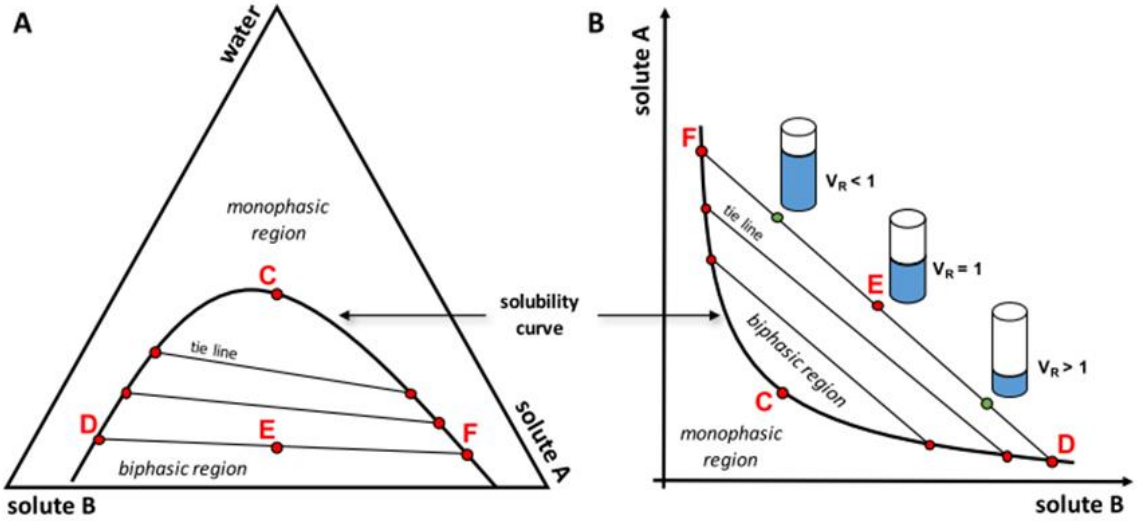


Figure 1. Ternary phase diagram for a hypothetical system composed of solute A + solute B + water. (**A**) triangular phase diagram; (**B**) orthogonal phase diagram; (**C**) binodal curve; (**D**) Composition of solute B-rich phase; (**E**) Composition of initial mixture; (**F**) Composition of solute A-rich phase.

Point E refers to the mixing of the components, and points D and F are the compositions in the top and bottom phase, respectively. The connection between these three points forming a well-defined line is called a tie line (TL). Different mixing points along the same tie line always include two phases with the same compositions but different mass-to-volume ratios. The TL are commonly determined by the method of Merchuk, Andrews, and Asenjo [83]. The masses of the mixture components are gravimetrically determined and thoroughly mixed. After equilibrium is reached, the top and bottom phases are separated, and their masses are determined. Each individual TL is determined by applying the lever rule to the relationship between the mass composition of the top phase and the overall system. Once the TL have been determined, Equation (3) through (6) can be solved [83]. We have

$$Y_T = (Y_M \,/\, \alpha) - ((1-\alpha) \,/\, \alpha)\, Y_F \quad (3)$$

$$X_T = (X_M \,/\, \alpha) - ((1-\alpha) \,/\, \alpha)\, X_F \quad (4)$$

$$Y_T = f(X_T) \quad (5)$$

$$Y_F = f(X_F), \quad (6)$$

where $f(X)$ is the function representing the binodal, the subscript M, T, and F denote the mixture, top phase, and bottom phase, respectively. The value of α is the ratio of the top mass to the total mass of the mixture.

In Figure 1B, the difference between the compositions of the two coexisting phases is numerically described by the tie line length. Higher values of TL length reflect more significant differences between the composition of the lower and upper phases and are often

used to correlate the partitioning of solutes between the two phases. Gutowski, et al. [84] showed that the more hydrophobically rich phase becomes increasingly hydrophobic as the divergence between the two phases increases, i.e., with an increase in the tie line length. This thermodynamic parameter is generally used as the determining variable in partitioning processes. The length of the tie line can be calculated by applying Equation (7) [85],

$$TLL = \sqrt{(\Delta Y)^2 + (\Delta X)^2}, \quad (7)$$

where ΔY and ΔX are the concentration differences of component 1 and 2 between the phases, respectively.

Point C on the solubility curve is known as the critical point and represents the mixture at which the composition of the coexisting phases has become equal, and the two-phase system ceases to exist ($TLL = 0$). The determination of the critical point for ternary systems is estimated by applying Equation (8) [72],

$$Y = f + gX, \quad (8)$$

where Y and X are the compositions of component 1 and 2, respectively. f and g are fitting parameters.

The phase-forming constituents are traditionally formed by aqueous solutions of two polymers (e.g., dextran, maltodextrin, polyethylene glycol-PEG) [86,87] or polymer + salt (e.g., PEG and phosphate-, sulphate- or citrate-based salts) [88–90]. Despite these systems having been extensively applied to partition different biomolecules, they present high viscosity, which hinders mass transfer and a limited polarity range between the two phases [17,91].

To improve the characteristics of the systems and increase the extraction efficiency of several biomolecules, other less traditional phase-forming constituents, such as organic solvents [92–94], IL [84,95], and DES [96,97] have been reported in the literature.

4.1. ATPS Phase-Forming Components

4.1.1. Ionic Liquid (IL)-Based ATPS

In 2003, Gutowski, Broker, Willauer, Huddleston, Swatloski, Holbrey, and Rogers [84] were the first to show that a mixture of an aqueous solution of 1-butyl-3-methylimidazolium chloride ([C4mim]Cl) and K_3PO_4 could form ATPS with an IL-rich (top) phase and a salt-rich (bottom) phase. This system has additional advantages over polymer-based ATPS, such as low viscosity, fast phase separation, high-extraction efficiencies, and high selectivity, contributing to more cost-effective processes if the proper IL are chosen. IL are ionic compounds belonging to the group of molten salts and are composed of organic cations and inorganic or organic anions, which melt at temperatures below 100 °C [98]. The low melting temperatures of IL are typically associated with the lack of an ordered crystalline structure resulting from weak intermolecular interactions derived from the large size ions and their charge distribution [99]. The ionic nature of IL is responsible for some unique properties: negligible vapor pressure under atmospheric conditions, low flammability, high thermal and chemical stability, enhanced selectivity, high ionic conductivity, and a strong solvation capacity for organic and inorganic compounds [100].

IL can be classified into two main categories: aprotic ionic liquids (AIL) and protic ionic liquids (PIL), which are based on proton donation [101]. AIL forms hydrogen bonds and are IL, consisting mainly of organic cations based on imidazolium, pyridinium, ammonium, and phosphonium. They have high synthesis cost, which hinders their industrial application [102]. PIL are readily synthesized by using an equimolar ratio of a Brønsted acid and a Brønsted base, via proton transfer, and have a highly mobile proton. These reagents (acid and base) are low in cost, and the synthesis is straightforward; thus, PIL are more economical than their aprotic analogues. In addition, AIL can usually be recovered and reused by distillation [103,104]. Some examples of cations and anions can be seen in Figure 2. The term "design solvents" has been used to describe one of the essential advan-

tages of IL: the fact that their physicochemical properties can be tailored by manipulating the chemical structure of their ions and it is therefore able to synthesize an IL for a specific application [105]. Therefore, IL cover the entire range of hydrophilicity and hydrophobicity by tunability. Their adaptability is extendable to IL-based ATPS, supporting the great interest in this type of system in recent years.

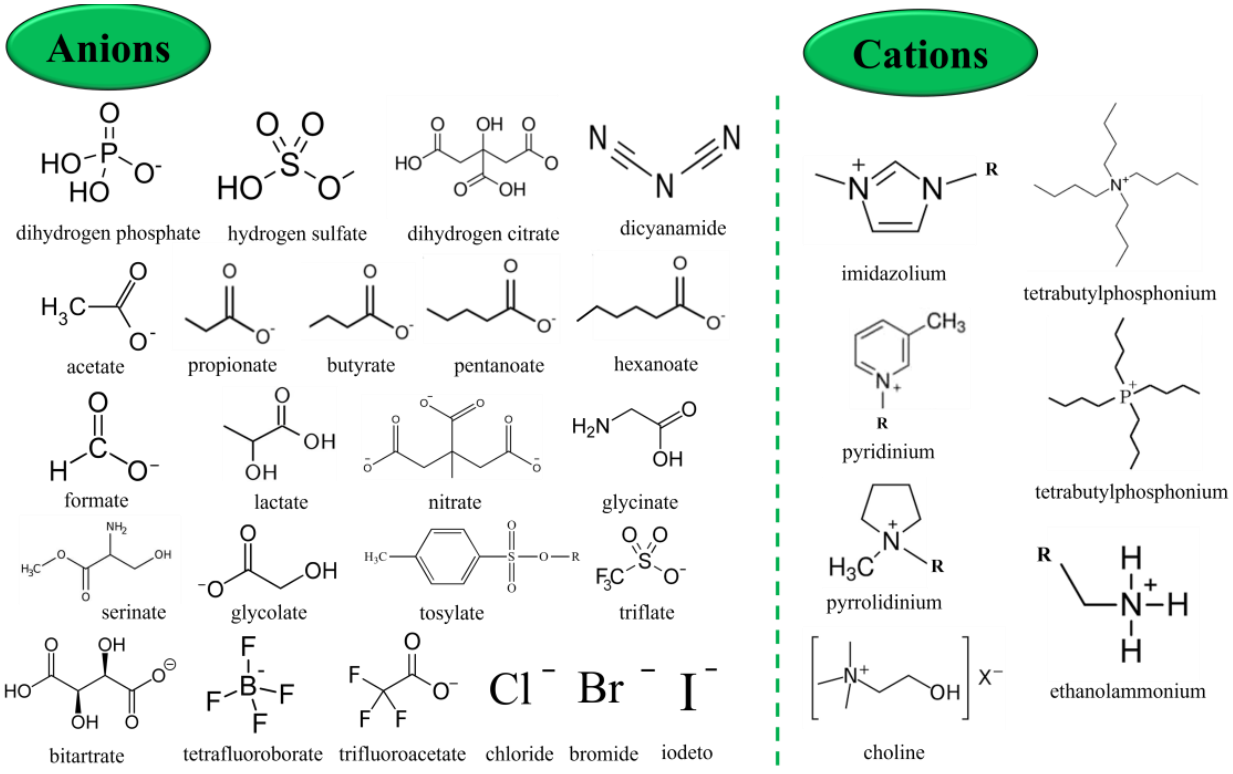


Figure 2. Structures of anions and cations that can be combined to form IL.

IL-based ATPS can be formed when salts, amino acids, carbohydrates, or polymers, are added to an aqueous IL solution [84,106–108]. The ability of these compounds to induce ATPS formation depends on their nature. To find these trends, some authors have studied the abilities of different IL to induce phase separation by evaluating their cationic and anionic nature, as well as the length of the alkyl chain.

In general, the formation mechanism in IL-based ATPS (except for systems composed of polymers and IL) is dominated by a salting-out effect on the IL. In these ATPS, the solvent is excluded from an IL-rich phase due to the preferential hydration of the second phase-forming component, which acts as a salting-out species. Thus, the greater the ability to form hydration complexes, the greater the ability to induce phase separation [17,84,106,107].

The effect of the chemical structure of the IL shows a behavior in opposition to that of the salting-out inducing species, i.e., the lower its hydrogen bonding capacity with water, the higher its ability to induce the formation of the system [109]. The influence of the cationic alkyl chain length was extensively studied by evaluating the trends observed in the literature, such as the increased hydrophobicity of the IL (decreased water solubility with increasing alkyl chain length) and the ability of IL with chains longer than hexyl to self-aggregate in aqueous solution. As the alkyl chain length of the cation increases, the ability of the IL to induce ATPS formation also increases. However, when this self-aggregation occurs (chains longer than C_6), a change is this trend observed, and the ability to form ATPS is reduced [17,110,111]. Regarding the effect of anions, several works [109,112,113] have suggested that the anionic ability to form ATPS correlates inversely with its hydrogen bond acceptor nature, i.e., the greater its ability to interact with water by hydrogen bonds, the lower its ability to separate phases in aqueous media.

ATPS formed by a polymer and an IL seem to result from more complex mechanisms. Specific interactions are established not only with water but also between these constituents, influencing the salting-in and salting-out effects. Freire, Pereira, Francisco, Rodríguez, Rebelo, Rogers, and Coutinho [108] and Pereira, et al. [114] reported that the ability to form systems increases as the immiscibility between IL and polymer increases. The miscibility between the polymer-IL can be adjusted to influence ATPS formation by changing the nature of the phase-forming components. It has been shown that the effect of the anion is directly related to its ability to be solvated by water. If IL are composed of anions with high hydrogen bond basicity, adding water to the polymer-IL blends will destroy the hydrogen bonds between these constituents and, consequently, ATPS formation. However, the influence of the cation is more complex, and the specific interactions (electrostatic and/or hydrophobic) that can affect the solubility of the compounds also need to be taken into account [108,114].

Several works have been published reporting the application of IL-based ATPS in the extraction of various compounds, identifying alkaloids (caffeine, theobromine, and theophylline) [101], drugs (amoxicillin, ampicillin, diclofenac, naproxen, ketoprofen, ibuprofen, and hormones) [115–118], proteins (albumin, trypsin, cytochrome, and bovine serum albumin) [119,120], enzymes [88,121], metals (Cr (IV), Cr (III) and Cd2+) [122,123], and phenolic compounds [124,125].

One of the advantages of ATPS is their ability to integrate processes. These systems have been applied to improve partitioning, either by the difference in hydrophobicity or by the polarity of the compounds. In addition to the application of IL as the main constituents of ATPS, the use of small amounts in polymer-based ATPS as adjuvants (5 wt% of the total mass of the system) appears to be an alternative system due to the adaptability of IL to cover a more comprehensive hydrophilic–hydrophobic range. Moreover, these systems overcome the high concentrations of IL in the ATPS, making this technique less expensive and more sustainable.

Some studies have reported that IL can be used as an adjuvant. Pereira, et al. [126] were the first to propose using IL as an adjuvant in a conventional ATPS composed of PEG and salt to separate L-tryptophan. The results indicated that the use of IL as an adjuvant showed modifications in the polarities of both phases, leading to better phase separations. Regarding amino acid partitioning, the adjuvant was found to increase typical solute–solvent interactions (van der Waals forces, electrostatic interactions, hydrogen bonds, and π-π-type interactions) and L-tryptophan migrated preferentially to the PEG-rich phase. These results proved that IL as adjuvants could modify the characteristics of the polymer phase representing an alternative to the limitations of PEG/salt-based ATPS. Souza, et al. [127] studied IL as an adjuvant in an ATPS composed of polymer + salt to increase the biphasic region, and Yang, et al. [128] evaluated the use of IL as adjuvants in polymer + salt ATPS to modify the system's polarity. Almeida, et al. [129] proposed a system of PEG 200, 300, 400, and 600, sodium sulphate, water, and IL-based adjuvants (imidazolium, piperidinium, and pyrrolidinium at concentrations of 5 or 10%) in the extraction of gallic, vanillic, and syringic acids. The authors reported that all investigated antioxidants preferred the PEG-rich phase and that the adjuvants played a role in adjusting the polarity of the preferred phase.

More recently, an alternative that has been explored is the use of mixtures of IL, which can further increase the range of properties of these compounds and allow properties to be fine tuned. This alternative allows improved flexibility in using these solvents without needing to change their chemical structure, thus avoiding the need to synthesize new compounds [130–132].

4.1.2. Deep Eutetic Solvent (DES)-Based ATPS

DES are an emerging class of solvents introduced by Abbott, et al. [133] and are formed from an association of two compounds with a high capacity to form hydrogen bonds (hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD)). The mixture of

these compounds gives rise to a decrease in the entropy associated with phase transitions and, consequently, to a significant reduction in the melting temperature of the mixture compared to the melting temperatures of the two separate constituents [134].

DES are simple and fast to prepare (simple mixture of the starting compounds at a given temperature) [135]. Moreover, the starting compounds of a DES are usually of low cost, biocompatible, biodegradable, and, in many cases, derived from renewable resources, allowing development applications with a low cost and reduced environmental impact [136,137]. The combination of different HBDs and HBAs capable of originating eutectic mixtures are numerous. DES have different chemical properties to conventional IL, but have similar physical properties, namely their potential as designer solvents, and are even more flexible. Because no reaction occurs between the HBD:HBA, it is possible to obtain DES in a wide range of relative molar compositions, typically by using ratios of 1:2, 1:1, and 2:1 [137].

Choi and coauthors introduced natural deep eutectic solvents (NADES), where only natural, nontoxic compounds are used (such as organic acids, amino acids, or sugars) [138]. The scientific community has widely accepted NADES because they are the ideal green solvent, with 100% atomic efficiency, prepared from renewable, nontoxic, biocompatible, biodegradable, and inexpensive compounds. Because they are present in cells, NADES are related to health applications, such as in the pharmaceutical, cosmetic, and food industries. Some studies have aimed to extract phenolic compounds from coproducts [139–141], there are various studies on the use of NADES/DES to extract phenolic compounds from coffee seeds [142], rosemary [143], grapefruit peel [144], and mulberry leaves [145].

Zeng, et al. [146] were the first authors to propose the application of DES in ATPS. In this work, the applicability of DES is due to their similarity with IL, which can be advantageous due to the relatively simple process of obtaining these solvents. Thus, these solvents were described as being able to form ATPS when combined with the inorganic salt K_2HPO_4. The authors believe that the phase formation of DES-salt systems is directly linked to the affinity of DES with water, i.e., the lower this affinity, the lower the amount of salt required to obtain the ATPS. Thus, the authors suggest that, there is a competition in these system between DES and salt for the water of the system, where the salt has a high affinity for the water of the system resulting in a phase rich in DES and another phase rich in salt and water.

Pang, Sha, Chao, Chen, Han, Zhu, Li, and Zhang [96] evaluated the use of PEG polymer in the synthesis of DES to be applied in ATPS. DES composed of PEG 2000 and choline chloride showed the ability to form phases with different sodium salts. The DES-Na_2CO_3 system was chosen for the separation processes. The authors related that the evaluated systems did not result in any type of degeneration of the biomolecules target due to the characteristics of the chosen DES, i.e., acting as a biodegradable solvent.

The literature presented here mainly shows the potential application of DES-based ATPS in the extraction of biomolecules. However, in the cited works, no reference was made regarding the stability of DES applied in ATPS. The high-water content in these systems should be considered, as it can weaken or even break the hydrogen bonds forming the DES [138]. In this context, Passos, Tavares, Ferreira, Freire, and Coutinho [97] reported the stability of DES (choline chloride + organic acids) when applied to ATPS composed of PPG 400. The authors used the nuclear magnetic resonance (^{1}H NMR) technique to evaluate the distribution of DES-forming compounds among the phases. It was found that the initial stoichiometry between the HBA and HBD molecules of the DES did not hold in the phases of the systems. Thus, the hydrogen donor molecules (organic acids) were preferentially partitioned to the PPG-rich phase, whereas the hydrogen receptor was partitioned preferentially to the other phase. In these systems, the phase separation was governed by the salting-out effect of the quaternary ammonium salt on the polymer. The authors further reported that DES-based ATPS are, in reality, quaternary systems [97].

Coutinho and collaborators (2018) clarified that the definition of DES could not be generalized because these are not new compounds or pseudopure compounds, but rather

that these should be considered mixtures, avoiding any mistaken purposes. These authors also reinforce that the characteristics conferred to DES (green solvents, economical, and easy to obtain) depend on the compounds used to get them [147].

4.1.3. Organic Solvent-Based ATPS

Organic solvents have great potential in the isolation of target compounds, presenting favorable solubility for the extraction of the matrix sample due to their various properties, such as temperature range, vapor pressure, density, viscosity, surface tension, and chemical and thermal stability. To classify a solvent, the solute–solvent interactions, polarity, and solvent effect are confirmed through spectroscopic, kinetic, or equilibrium measurements [148]. The critical criteria to consider when choosing a solvent as an extraction agent are the solubility of the target compound to be extracted, the affinity toward the solute, and the ease of phase separation [149].

Aqueous solutions of organic solvents and inorganic salts are immiscible at specific concentrations and will form two liquid phases. They are lower in cost than traditional systems formed by polymer–polymer and polymer–salt, are simple to operate, easy to remove from the extracts obtained, easy to scale up, and have low viscosity, rapid phase separation, low toxicity, little interference with analytes, low interfacial tension, and high yield [150].

Salts are used in two-phase aqueous systems due to their salting-out effect, because the addition of electrolytes causes a decrease in the aqueous solubility of organic solvents, such as ethanol, propanol, acetonitrile, tetrahydrofuran, and acetone, among others. Thus, the difference in the acting forces between the ion–water and alcohol–water pairs leads to alcohol exclusion or salt crystallization [19].

Organic solvents have been used as phase-forming components of ATPS, for example, acetone + inorganic salt [151], acetone + potassium salts [152], acetone + PIL [153], acetonitrile + carbohydrates [154], acetonitrile + polyols [155], acetonitrile + polyvinylpyrrolidone (PVP) [124], acetonitrile + IL [101,156], acetonitrile + maltodextrin [157], tetrahydrofuran + salt [93], and tetrahydrofuran + carbohydrates [92,95]. Alcohols have also been reported in the literature as a constituent of ATPS, such as in the study by Ooi, et al. [158] who evaluated the ability of ethanol to induce phase formation with ammonium sulphate, sodium phosphate, and sodium citrate. Reis, et al. [159] reported systems formed by ethanol with potassium salts, and Lo, et al. [160] studied the formation of ATPS composed of ethanol + citrate salts. Farias, et al. [161] evaluated the formation of systems based on ethanol + phosphate salts, carbonate, and citrate + IL (adjuvant), and Chong and Brooks [162] reported the effect of ethanol and propanol as phase-forming components in alcohol–carbohydrate systems.

Several works in the literature have applied organic solvent-based ATPS in the partitioning/extraction/purification of various target biomolecules. Ran, et al. [163] reported the formation of ATPS composed of ethanol/$(NH_4)_2SO_4$ by using IL $[C_6mim]BF_4$ as an adjuvant, evaluating the recovery of proanthocyanins which are antioxidants present in seeds by varying the concentration of the phase-forming components. The extraction efficiency and partition coefficient were observed with increasing organic solvent and salt. Both salting-out effects were the major driving force for the enrichment of solutes in the ethanol-rich phase (top phase), because increasing the salt concentration favored the recovery of proanthocyanins in the organic solvent. Nainegali, et al. [164] studied the ATPS formed by 1-propanol/$(NH_4)_2SO_4$ in the partitioning of bioactive compounds (anthocyanins, garcinol, isogarcinol, and hydroxy citric acid) from *Garcinia indica* peels. The authors identified that hydrophobic biomolecules (garcinol and isogarcinol) were partitioned to the alcohol-rich phase. In contrast, hydrophilic biomolecules (anthocyanins and hydroxy citric acid) migrated preferentially to the salt-rich phase. This behavior is due to the hydrophobic interactions and hydrogen bonds between the biomolecules and the salting-out effect of the system.

Regarding the effect of temperature on alcohol-based ATPS, Han, et al. [165] studied systems formed by 1-propanol, 2-propanol, and ethanol + sodium hydroxide and water at different temperatures (283.15 K, 298.15 K, and 303.15 K). The authors observed that the increase in temperature led to the expansion of the two-phase region and the ability to form phases following the order 1-propanol > 2-propanol > ethanol. In this study, the authors also compared the formation aspects of alcohol-NaOH and alcohol–salt systems, where they reported that phase separation may be related to the ionic charge of NaOH ($\pm$1) being lower than the ATPS-forming inorganic salts, which have the charge $\pm$2 and $\pm$3. Some authors, such as Hu, et al. [166] and Wang, et al. [167], observed no significant difference in the effect of temperature on the two-phase region of alcohol–salt-based ATPSs. However, for the 1-propanol/2-propanol/2-butanol + diammonium hydrogen citrate system [168] and the 1-propanol/2-propanol/2-methyl-2-propanol/2-butanol + dipotassium oxalate system [169] the effect of temperature on the binodal curve showed that the phase separation ability of these ATPS increased when the temperature was increased. Similarly, Robles, et al. [170] studied the effect of temperature on the system formed by water + ethyl acetate + ethanol at four different temperatures (293.15 K, 298.15 K, 303.15 K, and 308.15 K) and found that there was an increase in the immiscibility region at the lowest evaluated temperatures of 293.15 K and 298.15 K.

The effect of pH has also been described in the literature as a variable that affects the liquid surface charge of the medium. It can be adjusted by aiming at the partitioning of the target compound [115]. Nainegali, et al. [171] studied ATPS formed by alcohols and ammonium sulphate and observed that pH 6 favored the system's selectivity, where hydrophobic biomolecules were partitioned to the top phase and hydrophilic biomolecules migrated to the bottom phase.

A system derived from ethanol-based ATPS was reported by Buarque, Soares, de Souza, Pereira, and Lima [94] and Buarque, et al. [172]. The authors reported developing a new type of system, the ethanolic two-phase system (ETPS), based on the same principles as traditional ATPS, where water is replaced by ethanol. Thus, ETPS was reported to overcome the main limitation of ATPS, the extraction and purification of highly hydrophobic biocompounds (null water-soluble). The systems were formed by polypropylene glycol 2000 (PPG 2000) + mono-, di-, tri-ethylene glycol + ethanol, showing promising results with remarkable extraction values and selectivity of bixin (hydrophobic model biomolecule) and ascorbic acid (hydrophilic model biomolecule). In the other work, the systems were formed by using PPG of different molecular weights (425, 725, 1000, 2000 g mol^{-1}) + ethylene glycol + ethanol, PPG + IL based on imidazolium + ethanol and PPG + ethylene glycol + IL (as adjuvant) + ethanol (quaternary system). These systems were used to partition curcumin and caffeic acid from saffron (*Crocus sativus* L.). In the system composed of PPG 2000 and ethylene glycol, a selectivity was observed in which the biomolecules were partitioned to opposite phases with a maximum extraction efficiency of 90.3% for curcumin and 89.1% for caffeic acid. In systems using IL as a constituent or adjuvant, the interactions between the IL and the biocompounds propitiate the migration of curcumin and caffeic acid to the same phase, interfering with the selectivity of the systems. However, the IL-based ETPS showed a complete partition of curcumin (EE = 100%), and caffeic acid reached an EE value of 98.0% for longer alkyl chain IL ([C_6min]Cl).

5. Computational Modelling Related to ATPS and Applied to Bioactive Compounds

Because the driving forces in the process of separation and purification of biocompounds are multimodal in nature, and knowing that process optimization is usually done empirically, fundamental knowledge at the molecular level has become a challenging task for research groups. Screening efforts can be reduced by applying design of experiment (DoE) techniques. Klamt and Eckert [173] proposed a new prediction method for the thermodynamic equilibria of fluids and liquid mixtures, named the conductor-like screening model for real solvents (COSMO-RS). This method combines the electrostatic advantages and computational efficiency of the quantum continuous solvation model of the chemical

dielectric. COSMO-RS is a method based on unimolecular quantum calculations of the individual species in the system, in which the local deviations of the dielectric behavior and hydrogen bonding are evaluated [174].

In the literature, some studies have reported the application of COSMO-RS as a screening tool for the choice of IL or DES in the process of separation and purification of bioactive compounds. Wojeicchowski, et al. [175] employed COSMO-RS to predict the activity coefficient at infinite dilution and the solubility of phenolic compounds (carnosic acid and carnosol) considering 28 HBA and 49 HBD (1372 possible mixtures). Plácido, Carlos, Galvão, Souza, Soares, Mattedi, Fricks, and Lima [101] reported the use of COSMO-RS in predicting the hydrophobicity, hydrophilicity, and hydrogen bonding data for alkaloids (caffeine, theobromine, and theophylline) and PIL (four cations and three anions) in order to aid in the partition study of these bioactives in PIL- and acetonitrile-based ATPS. Pereira, et al. [176] also reported predictive data by using COSMO-RS for alkaloids (caffeine, theobromine, and theophylline) and IL (choline acetate, [Ch][OAc]) in ATPS formed by PEG + choline-based salts. COSMO-RS has also been used in predicting water activity coefficient values in aqueous IL solutions based on choline [177] and pyridinium, pyrrolidinium, or piperidinium cations (chloride anion in common) [178]. The behavior of IL + water is very important for designing the appropriate phase-forming components in extraction and purification processes.

Other molecular simulation models that have been reported as a viable route to green solvents at the atomic level involve the combination of fixed charge models and preset transferable force fields. This type of model systematically calculates the solvation free energies of a spectrum of solutes with various structural and chemical characteristics in various green solvents. In addition, the ratio of water + ILs or DES and the mass density are also important factors in the application of these solvents in separation and purification processes [26]. Sun, et al. [179] and Sun, et al. [180] performed large-scale molecular simulations of solvation free energies for IL based on a 1-ethyl-3-methylimidazolium cation + dicyanamide, bis(trifluoromethylsulphonyl)imide or trifluoromethylsulphonate [180]; 1-butyl-3-methylimidazolium and 1-hexyl-3-methylimidazolium + bis(trifluoromethylsulphonyl)imide and hexafluorophosphate [179]. The authors mentioned that the solvation thermodynamics were formed by the competition of the electrostatic and dispersive–repulsive contributions to the total solvation free energy. Thus, solvation is actually the average of the behaviors of all solute–solvent pairs in the dataset. The hydration free energy was another calculated parameter, of which it has been stated that the angle = bending potential also introduces nonnegligible errors in some cases. However, the torsional term, which is the most influential part determining the intramolecular conformational preference, agrees quite well with the ab initio calculation.

6. Application of ATPS Based on Ionic Liquids (IL) and Deep Eutectic Solvents (DES) for Bioseparation of Bioactive Compounds

Tables 1–5 present the use of IL, DES, and organic solvents in the construction of systems for separating synthetic or natural compounds from animal, vegetable, and microbial sources, including phenolic compounds, alkaloids, and natural pigments. The ATPS available in the literature prove that it is possible to efficiently, selectively, and sustainably isolate, purify, and concentrate the bioactive compounds in highly complex matrices from the selective partition of the molecules, with high yield [181]. In addition, they demonstrate that it is feasible to reduce the environmental impact caused by the use of volatile solvents in obtaining these compounds [182].

It is important to highlight that studies that contemplate the influence of separation on the maintenance or alteration of bioactivity related to target molecules are not routinely verified, a factor that must be better investigated. Most of the studies were conducted to evaluate the antioxidant activity and pigment recovery [129,183]. However, bioactive compounds can exert multiple biological activities affecting the matrix to which they are added or applied. This potential is almost always ignored, which includes not verifying

the activity or maintaining the properties such as antihyperglycaemic [35], antimicrobial, and angiotensin-converting enzyme inhibitory activity [36], immune-modulating activity, the ability to protect the immune system, reducing the damage from cell oxidation, and reducing cardiovascular complications [8].

Table 1. Nonconventional ATPS in the extraction and recovery of phenolic compounds.

Phenolic Compounds	Raw Material	Phase-Forming	Reference
Geniposide	Gardenia fruit (*Gardenia jasminoides*)	PE62 (ethylene oxide-propylene oxide, 20:80) copolymer + Phosphate (KH_2PO_4, NaH_2PO_4) and sulfate ($(NH_4)_2SO_4$, and $MgSO_4$) salts + ethanol (0–10 wt%)	[184]
Salvianolic acid B	*Salvia miltiorrhiza*	Ethanol or n-propanol + $(NH_4)_2SO_4$, NaCl, and K_2HPO_4/NaH_2PO_4	[185]
Vanillin	Synthetic	IL (alkylmethylimidazolium cations, and Cl, Br, dicyanamide, methylsulfate, methanesulfonate, triflate, acetate anions) + K_3PO_4	[186]
Anthocyanins	Mulberry (*Morus atropurpurea*)	Ethanol + $(NH_4)_2SO_4$	[187]
Vanillin and L-ascorbic acid	synthetic	Methanol, ethanol, 1-propanol and 2-propanol + K_3PO_4, K_2HPO_4 and KH_2PO_4/K_2HPO_4	[159]
Lithospermic acid B	*Salvia miltiorrhiza*	Ethanol + $(NH_4)_2SO_4$	[188]
Gallic acid	Synthetic	IL (alkylmethylimidazolium cations, and Cl, Br, dicyanamide, methylsulfate, ethylsulfate, octylsulfate, triflate anions) + Na_2SO_4, K_3PO_4, and KH_2PO_4/K_2HPO_4	[189]
Vanillin	Synthetic	Acetonitrile + carbohydrates (glucose, maltose, galactose, xylose, arabinose, fructose, sucrose and mannose)	[154]
Vanillin	Synthetic	Acetonitrile + dextran (6000, 40,000 and 100,000 $g{\cdot}mol^{-1}$)	[190]
Vanillin	Synthetic	Acetonitrile + polyols (glycerol, erythritol, xylitol, sorbitol and maltitol)	[155]
Total phenolics, flavonoids and proanthocyanidins	Grape seeds (*Vitis vinifera*, cultivar Riesling)	Acetone + $(NH_4)_3$Citrate	[51]
Rutin	Acerola waste (*Malpighia glabra*)	Methanol, ethanol, 1-propanol and 2-propanol + K_3PO_4, K_2HPO_4 and K_2HPO_4/KH_2PO_4	[191]
Anthocyanins	Grape juice (*Vitis vinifera*)	Ethanol + NaH_2PO_4 or $(NH_4)_2SO_4$	[192]
Gallic, vanillic and syringic acids	Synthetic	IL (1-butyl-3-methylimidazolium cation, and Br, dicyanamide, methylsulfate, ethylsulfate, triflate anions) + Na_2SO_4, Na_2CO_3	[193]
Gallic, vanillic and syringic acids	Synthetic	PEG 200–600 + Na_2SO_4 + IL (1-butyl-3-methylimidazolium, 1-butyl-1-methylpiperidinium, 1-butyl-1-methylpyrrolidinium cations, and acetate, thiocyanate, tosylate, Cl, dicyanamide anions)	[129]
α-Cyclohexylmandelic acid enantiomers	Synthetic	IL (alkylmethylimidazolium cation, and dicyanamide, triflate anions) + $(NH_4)_2SO_4$, Na_2SO_4, K_2HPO_4	[194]
Secoisolariciresinol diglucoside	Flaxseed (*Linum usitatissimum*)	IL (alkylmethylimidazolium cation, and dicyanamide, BF_4 anions) + $(NH_4)_2SO_4$	[54]
Gallic acid	Guava (*Psidium guajava*)	Methanol, ethanol, 1-propanol, and 2-propanol + K_3PO_4, K_2HPO_4 and K_2HPO_4/KH_2PO_4	[195]
Eugenol and propyl gallate	Synthetic	IL (alkylmethylimidazolium, 1-butyl-1-methylpiperidinium, [N_{4444}], 1-butyl-1-methyl-pyrrolidinium cation, and chloride anion) + PEG 1500 or 8000 + K_3Citrate/Citric acid or K_2HPO_4/KH_2PO_4	[196]
Caffeoylquinic acids	*Lonicera japonica* flowers	IL (1-butyl-3-methylimidazolium bromide) + K_3PO_4, K_2HPO_4, KOH, Na_2CO_3,	[197]
Lignans	*Schisandra chinensis*	Ethanol + $(NH_4)_2SO_4$	[198]
Lithospermic acid B	*Salvia miltiorrhiza*	*n*-butyl alcohol + KH_2PO_4	[199]
Total phenolics, and flavonoids	*Aronia melanocarpa* pomace	Ethanol + $(NH_4)_2SO_4$	[200]

Table 1. *Cont.*

Phenolic Compounds	Raw Material	Phase-Forming	Reference
Caffeoylquinic acid, parishin, and forsythoside isomers, phyllirin, salidroside, gastrodin	Synthetic	Ethanol/1-Butanol + $(NH_4)_2SO_4$ and K_2HPO_4	[201]
Hydroxycinnamic acid derivatives	Carrots (*Daucus carota*)	Ethanol, n-Butanol or IL ([C_2mim]Acetate) + $(NH_4)_2SO_4$, KH_2PO_4/K_2KPO_4	[57]
Vanillic acid, vanillin, gallic acid, caffeic acid, syringaldehyde	Synthetic	PEG 8000 + Sodium Polyacrylate 8000 + Electrolyte (sodium dodecylbenzenesulfonate, sodium dodecylsulfate, [$N_{111\ 14}$]Br, [$N_{111\ 16}$]Br, C_{16}pyridinium chloride, C_{16} pyridinium bromide, [C_{12}mim]Cl, [C_{14}mim]Cl)	[58]
Chlorogenic acid	Ramie (*Boehmeria nivea*)	IL (1-butyl-3methylimidazolium cation, and triflate, acetate, propionate, hydrogen sulfate, dihydrogen phosphate anions) + Na_2SO_4, $(NH_4)_2SO_4$	[202]
Anthocyanins	Grape pomace	IL (1-ethyl-3-methylimidazolium Acetate) + K_3PO_4, K_2CO_3	[56]
Total phenolics, and xylooligosaccharides	Wheat chaff (*Triticum* sp.)	Ethanol + $(NH_4)_2SO_4$	[55]
Anthraquinones	*Rheum officinalis*	DES (ChCl, [N_{11110}]Br, [N_{11112}]Br, [$N_{111\ 14}$]Br, as HBA, and hexafluoroisopropanol as HBD) + $(NH_4)_2SO_4$, Na_2SO_4, K_2HPO_4, Na_2HPO_4	[203]
Phloridzin	Crabapple (*Malus micromalus*)	Ethanol + $(NH_4)_2SO_4$	[204]
Chlorogenic acid	Blueberry (*Vaccinium* spp.) leaves	DES (ChCl as HBA, and ethylene glycol, glycerol, 1,3-butanediol, citric acid, oxalic acid, sucrose, glucose, maltose as HBD) + K_2HPO_4	[53]
Gallic acid	Synthetic	DES (ChCl as HBA, and sucrose, glucose, fructose as HBD) + K_2HPO_4	[205]
Flavonoids	*Sophora japonica* flower buds	IL (alkylimidazolium cations, and propionate, lactate anions) + K_3Citrate	[206]
Gallic acid, caffeine, and tryptophan	Synthetic	DES ([N_{4444}]Cl as HBA, and ethanol, n-propanol as HBD) + K_3Citrate/Citric Acid	[207]
Vanillin	Synthetic	Acetonitrile + maltodextrins	[157]
Total phenolics, and lutein	Marigold (*Tagetes erecta*) flowers	Ethanol + $(NH_4)_2SO_4$	[208]
Mandelic acid enantiomers	Synthetic	IL (1-methyl quininium, N,N-dimethyl-L-proline methyl ester, N,N-diethyl-L-proline ethyl ester, N,N,N-trimethyl-L-valinolium cations, and methylsulfate, Br, I anions) + K_3PO_4, K_2HPO_4, K_2CO_3	[209]
Psoralen	Fig (*Ficus carica*) leaves	IL (alkylmethylimidazolium cation, and Br, Cl, nitrate, hydrogensulfate, tetrafluoroborate anions) or Ethanol + Citric acid	[210]
Cyanidin-3-O-glucoside, gallic acid and quercetin.	Synthetic	Acetonitrile + polyvinylpyrrolidone (10,000, 29,000 and 40,000 g·mol^{-1})	[124]
Cyanidin-3-O-glucoside, garcinol, isogarcinol and hydroxycitric acid	Kokum (*Garcinia indica*)	Ethanol, and 1-propanol + $(NH_4)_2SO_4$, $MgSO_4$, Na_2SO_4, $ZnSO_4$, Na_3Citrate, NaH_2PO_4, and K_2HPO_4	[171]
Gallic and protocatechuic acids	Synthetic	PEG (400, 1000, 1500, 4000, 6000, 8000, 10,000 and 20,000 g·mol^{-1}) + K_3PO_4 + acetonitrile	[125]
Proanthocyanidins	Grape seeds	Ethanol + $(NH_4)_2SO_4$, Na_2CO_3, and K_2HPO_4 + IL (alkylmethylimidazolium cation and [TOS], [HSO_4], [CH_3SO_3], [NO_3], [Br], [BF_4] anions)	[163]
Paeonol	*Paeonia suffruticosa* root bark	Ethanol or Ethyl Acetate + NaH_2PO_4, $(NH_4)_2SO_4$, KCl, NaCl, Na_2SO_4, $MgSO_4$	[211]
Rutin and quercetin	Synthetic	IL (cholinium cation, and alaninate, glycinate, serinate anions) + K_2HPO_4, K_3PO_4	[20]

Table 1. *Cont.*

Phenolic Compounds	Raw Material	Phase-Forming	Reference
Lignin alkaline	-	IL (alkylmethylimidazolium cations, and Cl anion) + Na_2CO_3	[212]
Kraft lignin	*Eucalyptus globulus*	PIL (2-Hydroxyethylammonium, bis(2-hydroxyethyl)ammonium, tris(2-hydroxyethyl)ammonium cations, and formate, acetate, propionate, glycolate, lactate anions) + acetone	[153]
Total phenolics	Peppermint leaves (*Mentha* × *piperita*) and lemon balm leaves (*Melissa officinalis*)	DES (ChCl as HBA, and ethylene glycol, glycerol, glucose, citric acid as HBD) + K_2HPO_4	[213]
Lignans	*Schisandra chinensis*	Ethanol + $(NH_4)_2SO_4$	[67]
Cyanidin-3-O-glucoside, garcinol, isogarcinol and hydroxycitric acid	Kokum (*Garcinia indica*) rinds	1-propanol + $(NH_4)_2SO_4$	[164]
Genipin	Genipap (*Genipa americana*)	IL (monoethanolammonium, di-ethanolammonium, triethanol-ammonium cations, and Cl, dihydrogen citrate, dihydrogen phosphate, hydrogen sulfate, nitrate anions) + Acetonitrile	[156]
Total phenolics, flavonoids, and chlorogenic acid	Haskap leaves (*Lonicera caerulea*)	Ethanol, 1-Propanol + $(NH_4)_2SO_4$, NaH_2PO_4, glucose, and maltose	[162]
Vanillin and L-tryptophan	Synthetic	IL (1-decyl-3-methyl imidazolium chloride) + 2-propanol + K_2HPO_4	[214]
Anthocyanins	Grape pomace	IL (bis(2-hydroxyethyl)ammonium hydrogen sulfate) + Acetonitrile	[215]
Genipin	Genipap (*Genipa americana*)	IL (monoethanolammonium, di-ethanolammonium, triethanol-ammonium cations, and Cl, dihydrogen phosphate, hydrogen sulfate, nitrate anions) + PEG 300–750 or PPG 400	[216]
Geniposidic acid and aucubin	*Eucommia ulmoides*	IL (alkylmethylimidazolium cations, and Cl, Br, acetate, nitrate, dicyanamide anions) + K_2CO_3, K_2HPO_4, $(NH_4)_2SO_4$	[217]
Acteoside	*Cistanche tubulosa*	IL (alkylmethylimidazolium cations, and Cl, Br, BF_4, triflate anions) + NaCl, K_2HPO_4, NaH_2PO_4, $(NH_4)_2SO_4$, Na_2SO_4, Na_3Citrate	[218]
Syringic and caffeic acids	Synthetic	IL (1-butyl-3-methylimidazolium triflate) + NaCl, K_3PO_4, K_2HPO_4, NaH_2PO_4, Na_2SO_4, Na_2CO_3	[219]
Vanillic and shikimic acids	Synthetic	DES (ChCl as HBA, and sucrose, glucose, fructose, xylose as HBD) + n-propanol	[220]
Flavonoids	Yam (*Dioscorea alata*) peel.	IL (alkyl-(2-hydroxyethyl)-dimethylammonium cations, 4-sulfonatooxy-2,2,6,6-tetramethyl piperidine-1-yloxyl anion) + K_3PO_4	[221]
Lignin derivatives	Synthetic	PEG 8000 + Sodium Polyacrylate 8000 + Electrolyte (NaCl, Na_2SO_4, IL ([C_2mim cation, and Cl, dicyanamide, methanesulfonate, triflate, tosylate anions)	[222]
Gallic, ferulic, protocatechuic, caffeic and chlorogenic acids	Red apple, yellow pear, purple grape, and native banana	PEG 600 + Na_2CO_3 + PEG bis (methylimidazolium) ditetrachloroferrate	[223]
Syringic acid and eugenol	Synthetic	DES (betaine as HBA, and xylitol, tartaric acid, glycolic acid, urea as HBD) + *t*-butanol	[224]

Although it is essential to understand the mechanism of migration between the phases of the system used, the maintenance of the biological activity of the target molecule is a critical factor for the success of its separation and, mainly, its application in food, chemical, or pharmaceutical products [18].

Table 2. Nonconventional ATPS in the extraction and recovery of alkaloids.

Alkaloids	Raw Material	Phase-Forming	Reference
Codeine and papaverine	Opium (*Pericarpium papaveris*)	IL (1-butyl-3-methylimidazolium chloride) + K_2HPO_4	[225]
Caffeine, β-carotene, and L-tryptophan	Synthetic	IL (triisobutyl(methyl)-phosphonium tosylate, tributyl-(methyl)phosphonium methylsulphate and tetrabutyl-phosphonium bromide) + K_3PO_4	[226]
Puerarin	*Pueraria lobata*	IL (1-carboxymethyl-3-methylimidazolium, 1-hydroxyethyl-3-methylimidazolium and 1-butyl-3-methylimidazolium cations, and Br, Hydroxide, tetrafluoroborate anions) + K_2HPO_4	[227]
Caffeine, nicotine, and xanthine	Synthetic	IL (alkylmethylimidazolium cations, and Cl, acetate, CH_3SO_3, HSO_4, $(CH_3)_2PO_4$ anions) + PEG 2000	[228]
Caffeine, nicotine, theobromine, and theophylline	Synthetic	IL (alkylmethylimidazolium cations, and Cl anion) + K_3Citrate/Citric Acid	[229]
Caffeine and nicotine	Synthetic	IL (alkylimidazolium cation, and Cl, triflate, trifluoroacetate, acetate, methanesulfonate anions) + K_3PO_4	[230]
Matrine and oxymatrine	*Sophora flavescens*	Ethanol + $(NH_4)_2SO_4$ or K_2HPO_4	[231]
Glaucine	*Glaucium flavum*	IL (1-butyl-3-methylimidazolium acesulfamate) + Na_2CO_3, $(NH_4)_2SO_4$, $MgSO_4$, NaH_2PO_4	[232]
Caffeine	Guaraná seeds (*Paullinia cupana*), and coffee beans (*Coffea canephora*, *Coffea arabica*)	Methanol, ethanol, 1-propanol and 2-propanol + K_3PO_4, K_2HPO_4 and K_2HPO_4/KH_2PO_4	[233]
Capsaicin	*Capsicum frutescens* var. *malagueta*	Acetonitrile + IL (cholinium cation, and Cl, bitartrate, dihydrogen citrate anions)	[234]
Monoester- and diester-diterpenoid aconitines	*Aconitum carmichaeli*	IL (alkylmethylimidazolium cation, and Br, tetrafluoroborate anions) + K_2HPO_4	[235]
Caffeine	Synthetic	IL ($[C_4mim]BF_4$) + Na_2SO_4, $NaNO_3$	[236]
Caffeine, theobromine, and theophylline	Synthetic	PEG 600 + IL (cholinium cation, and Cl, acetate, bicarbonate, dihydrogencitrate, dihydrogenphosphate anions)	[176]
Capsaicin	*Capsicum chinense* var. cumari-do-Pará.	Ethanol + NaH_2PO_4, $Na_2S_2O_3$, Na_2SO_4, and Na_2CO_3	[237]
Capsaicin	capsicum oleoresin	Ethanol + Na_2CO_3, $(NH_4)_2SO_4$, NaH_2PO_4, Na_3Citrate	[45]
Caffeine, nicotine, gallic acid, vanillic acid, amino acids	Synthetic	Choline chloride/Glucose + PPG 400	[238]
Caffeine, codeine, and vanillin	Synthetic	Ethanol, 1-propanol, and 2-propanol + carbohydrates (xylose, L-arabinose, glucose, galactose, fructose, sucrose, maltitol, xylitol, and maltose)	[239]
Matrine, sophocarpine and oxymatrine	*Sophora tonkinensis*	Ethanol + Na_2HPO_4, NaH_2PO_4, and Na_3PO_4	[240]
Sinomenine	*Sinomenium acutum*	Ethanol + $(NH_4)_2SO_4$ + ionic liquids ($[C_4mim]Br$, $[C_6mim]Br$, $[C_8mim]Br$, $[C_4mim]FeCl_3Br$, $[C_2OHmim]FeCl_4$, $[C_6mim]$ $FeCl_3Br$ and $[C_8mim]$ $FeCl_3Br$)	[241]
Caffeine, theobromine, and theophylline	Synthetic	PIL (2-hydroxyethylammonium, bis(2-hydroxyethyl) ammonium, *N*-methyl-2-hydroxyethyl-ammonium cations, and acetate, propionate, butyrate, pentanoate anions) + Acetonitrile	[101]
Caffeine, nicotine, gallic acid, vanillic acid, amino acids, β-carotene	Synthetic	Choline chloride + alcohols (ethanol, n-propanol, ethylene glycol, 1,2-propanediol) + K_2HPO_4	[242]

Table 2. *Cont.*

Alkaloids	Raw Material	Phase-Forming	Reference
Caffeine, nicotine, gallic acid, vanillic acid, amino acids, eugenol	Synthetic	PEG 400 + IL (1-butyl-3-methylimidazolium, 1-butyl-1-methylpyrrolidinium, 1-butyl-3-methylpiperidinium, tetrabutylammonium, tetrabutylphosphonium cations, and chloride anion) + K_3Citrate/Citric Acid	[243]
Caffeine and theobromine	Synthetic	IL (N-methyl-N-alkyl-N,N-di(hydroxyethyl)ammonium cations, and Br anion) + K_2CO_3, K_3Citrate, and K_3PO_4	[244]
Berberine HCl	Rhizoma coptidis (*Coptis chinensis*, *C. deltoidea*, or *C. teeta*)	IL (alkyl-(2-hydroxyethyl)-dimethylammonium cations, 4-sulfonatooxy-2,2,6,6-tetramethyl piperidine-1-yloxyl anion) + K_3PO_4, K_2CO_3, Na_3Citrate, K_3Citrate, K_2HPO_4	[245]
Caffeine and nicotine	Synthetic	Ethanol + K_2CO_3, K_3Citrate, K_2HPO_4, NaH_2PO_4, and K_3PO_4	[246]
Caffeine, nicotine, gallic acid, vanillic acid, amino acids, eugenol	Synthetic	PEG 400 + IL (1-butyl-3-methylimidazolium, 1-butyl-1-methylpyrrolidinium, 1-butyl-3-methylpiperidinium, tetrabutylammonium, tetrabutylphosphonium, cholinium cations, and chloride anion) + $(NH_4)_2SO_4$	[247]
Codeine and caffeine	Synthetic	IL (1-butyl-3-methylimidazolium tetrafluoroborate) + sugars (xylose, arabinose, fructose, glucose, maltose, sucrose)	[21]
Caffeine	Synthetic	Tetrabutylphosphonium bromide + sorbitol	[248]
Caffeine, nicotine, amino acids (L-tryptophan, L-phenylalanine and L-tyrosine) and phenolic compounds (gallic acid and vanillic acid)	Synthetic	Ethanol + $[N_{111(2}OH)]Cl$ + NaH_2PO_4, K_2HPO_4, K_3PO_4, K_2CO_3, and K_3Citrate	[161]
Caffeine, nicotine, theobromine, and theophylline	Synthetic	Pluronic PE6200 + IL(cholinium cation, and Cl, acetate, propionate butanoate, lactate, bitaratrate, dihydrogenphosphate, dihydrogencitrate, bicarbonate anions)	[44]
Caffeine, nicotine, amino acids (L-tryptophan, L-phenylalanine and L-tyrosine) and phenolic compounds (gallic acid and vanillic acid)	Synthetic	Zwitterions (4-(triethylammonio)-butane-1-sulfonate, 4-(1-methylimidazolium-3-yl)butane-1-sulfonate, 4-(1-vinylimidazolium-3-yl)butane-1-sulfonate, 4-(1-methylpyrrolidinium-1-yl)butane-1-sulfonate, 4-(1-ethyl-piperidinium-1-yl))butane-1-sulfonate) + K_2CO_3	[249]
Caffeine	Synthetic	IL (tetrabutylammonium cátion, and amino acids anions) + K_3PO_4, K_2HPO_4 and K_2CO_3	[250]
Caffeine	Synthetic	Choline chloride/saccharose + polyethylene glycol dimethyl ether 250	[251]

The diversity of ATPS in the literature demonstrates its versatility, robustness, ease of operation, and reproducibility [18]. Furthermore, the use of APTS for the extraction, purification, and concentration of bioactive compounds is advantageous in not needing to use severe operating conditions (high temperatures and pressures or the need for sophisticated equipment), which contribute to the cost and complexity of the process [182] and even lead to the deactivation of the target molecules, with loss of their bioactivities. Thus, such characteristics make the technique necessary to separate and concentrate bioactive compounds [8,18].

Table 3. Non-conventional ATPS in the extraction and recovery of natural pigments.

Pigments	Raw Material	Phase-Forming	Reference
Crocins	*Crocus sativus* stigmas	IL (alkylmethylimidazolium cation, acetate and BF_4) or Ethanol + NaH_2PO_4, K_2HPO_4/KH_2PO_4	[252]
Chlorophyll	silkworm excrement	Ethanol + NaOH	[253]
Curcuminoids	*Curcuma longa* powder	IL (1,3-Diethylimidazole, 1,3-dioctylimidazole, 1,3-dimethylimidazole, 1,3-dibutylimidazole cations, and Br, I anions) + K_2HPO_4	[254]
Betanin	*Opuntia ficus-indica*	Tetrahydrofuran + Na_2CO_3 and Na_3Citrate	[255]
C-Phycocyanin	Spirulina (*Arthrospira platensis*)	IL (alkylmethylimidazolium cations, and bromide anion) + K_2HPO_4, K_2HPO_4, K_2CO_3	[183]
Curcumin	synthetic	Tetrabutylphosphonium bromide + sorbitol, fructose	[256]
Astaxanthin	Shrimp waste	IL (tetrabutylammonium,tetrabutylphosphonium, tributyloctylphosphonium cations, and Cl, Br anions) + K_3PO_4	[22]
Curcumin	*Curcuma longa* rhizomes	Pluronic F68/McIlvaine buffer (pH 6.0; K_2HPO_4/Citric Acid) + IL (cholinium cation, and Cl, acetate, propanoate, butanoate, hexanoate anions)	[257]
R-Phycoerythrin	*Porphyra yezoensis*	DES (ChCl as HBA, and glycerol, ethylene glycol, sorbitol, urea, glucose, fructose as HBD) + K_2HPO_4	[258]
C-Phycocyanin	*Arthrospira platensis*	DES (ChCl as HBA, and glycerol, ethylene glycol, urea, glucose, fructose as HBD) + K_2HPO_4	[259]
Betanin	Beetroot (*Beta vulgaris*)	Tributylmethylammonium hexanoate + Trioctylmethylammonium adipate	[260]
Curcumin	synthetic	1-ethylpiperazinium tetrafluoroborate + sodium dodecyl sulfate, and 1-phenylpiperazinium tetrafluoroborate + sodium dodecyl benzene sulfonate	[261]
Crocin	synthetic	DES (ChCl as HBA, and urea, ethylene glycol as HBD) + Acetonitrile	[262]

Table 4. Nonconventional ATPS in the extraction and recovery of other plant, marine, and algae metabolites.

Metabolites	Raw Material	Phase-Forming	Reference
Glycyrrhizin	*Glycyrrhiza uralensis*	2-Propanol or ethanol + $(NH_4)_2SO_4$ or K_2HPO_4	[263]
DNA	Salmon testes	DES ($[N_{4444}]$Br as HBA, and ethylene glycol, propylene glycol, butylene glycol, n-butanol as HBD) + Na_2CO_3, K_2HPO_4, NaH_2PO_4, Na_2SO_4	[264]
Polysaccharides	*Gentiana scabra*	Ethanol + NaH_2PO_4, K_2HPO_4, $(NH_4)_2SO_4$, Na_3Citrate, and K_2CO_3	[50]
Ginsenosides	*Panax ginseng*	IL (n-alkyl-tropinium and n-alkylquinolinium bromide) + Na_3Citrate, K_3Citrate, K_2CO_3, K_3PO_4, K_2HPO_4, and NaH_2PO_4	[52]
Polysaccharides	*Lilium davidii* var. *unicolor*	Ethanol + Na_2CO_3, K_2HPO_4, $(NH_4)_2SO_4$, and NaH_2PO_4	[265]
Ginsenosides	Kang'ai injection (*Astragalus membranaceus*, *Panax ginseng* and kushenin)	DES (ChCl as HBA, and glycerol, ethylene glycol, 1,4-butanediol, glucose as HBD) + K_2HPO_4	[266]
Carbohydrates, Proteins	*Neochloris oleoabundans* and *Tetraselmis suecica.*	IoliLyte 221 PG + K_3Citrate/Citric Acid	[60]
Proteins, Arabinan and glucan	*Isochrysis galbana*	IL (alkylmethylimidazolium cations, and Cl, acetate, triflate, dicyanamide anions) + K_3PO_4, K_2HPO_4	[267]
DNA	Salmon testes	$[N_{4444}]$Br/PPG 400 + IL (betainium cation, and formate, acetate, propionate, butyrate anions) or DES (betaine as HBA, and glucose, xylitol, sucrose, sorbitol as HBD), or salts (Na_2CO_3, K_2HPO_4, NaH_2PO_4, Na_3Citrate)	[268]

Table 4. *Cont.*

Metabolites	Raw Material	Phase-Forming	Reference
Pristimerin	*Mortonia greggii*	IL (1-ethyl-3-methylimidazolium acetate, 1-butyl-3-methylimidazolium tetrafluoroborate), or Ethanol + Sodium Phosphates	[269]
Ursolic acid	*Cynomorium songaricum*	Ethanol + DES (ChCl as HBA, and acetamide, glycerol, urea, $ZnCl_2$, mannitol, organic acids as HBD) + K_3PO_4, $(NH_4)_2SO_4$, Na_2SO_4	[270]
Ursolic acid	*Cynomorium songaricum*	DES (ChCl as HBA, and glycerol, ethylene glycol, urea, glucose as HBD) + K_2HPO_4	[271]
Ginsenosides	*Panax quinquefolius*	Ethanol + Na_2CO_3	[272]
Ginsenoside CK	*Panax notoginseng*	DES (ChCl as HBA, and glycerol, ethylene glycol, urea, glucose as HBD) + K_2HPO_4	[273]
Carbohydrates, proteins, lutein and chlorophyll	*Neochloris oleoabundans*	PEG 8000 + Sodium polyacrylate 8000 + electrolyte (sodium dodecyl sulfate, 1-methyl-3-tetradecylimidazolium chloride, tributyl-1-tetradecylphosphonium chloride, 1-dodecyltrimethyl-ammonium bromide)	[61]
Carbohydrates, proteins, lutein and chlorophyll	*Neochloris oleoabundans*	PPG 400 + IL (cholinium cation, and Cl, dihydrogencitrate, acetate, dihydrogenphosphate, bicarbonate, bitartrate anions)	[274]
Starch, glucose, proteins	*Neochloris oleoabundans*	PEG 400 + IL (choline dihydrogen phosphate), and IoliLyte 221 PG + K_3Citrate/Citric Acid	[275]
α-Tocopherol and β-carotene	*Tetraselmis suecica*	IL (cholinium glycinate, cholinium phenylalaninate, cholinium dipeptide, 1-ethyl-3-methylimidazolium methylsulfate) + K_3PO_4	[276]
DNA	Fish sperm	IL (alkylhydroxyammonium, alkylammonium cations, and bromide anion) + K_2HPO_4/KH_2PO_4	[277]
Lipids, lutein	*Neochloris oleoabundans*	PEG 400 + IL (choline dihydrogen phosphate), and IoliLyte 221 PG + K_3Citrate/Citric Acid	[278]

Table 5. Nonconventional ATPS in the extraction and recovery of microbial bioproducts.

Bioproducts	Raw Material	Phase-Forming	Reference
Nucleic acids	*Escherichia coli* K-12, *Saccharomyces cerevisiae*	Ethanol + KH_2PO_4	[279]
2,3-butanediol	*Klebsiella pneumoniae* CICC	Ethanol + K_2HPO_4	[280]
2,3-butanediol	*Klebsiella pneumoniae* DSM2026	Methanol, ethanol, isopropanol + NaCl, K_2CO_3, K_3PO_4, and $(NH_4)_2SO_4$	[281]
1,3-propanediol	*Klebsiella pneumoniae* CGMCC 2028	Ethanol + Na_2CO_3	[282]
Red colorant	*Penicillium purpurogenum* DPUA 1275	Tetraethylammonium bromide, tetrabutylammonium bromide, and 1-butyl-3-methylimidazolium chloride + citric acid/K_3Citrate	[62]
Succinic acid	*Actinobacillus succinogenes* CGMCC1593	Ethanol, Isopropanol, Acetone + K_2CO_3, K_3PO_4, K2HPO4, NaH_2PO_4, $(NH_4)_2SO_4$	[151]
Succinic acid	Simulated broth	Alcohols (ethanol, 1-propanol, 2-propanol, *t*-butanol) or IL (alkymethylimidazolium cations, and bromide anion) + K_2HPO_4, Na_3Citrate, Na_2SO_4	[283]
RNA	Yeast	DES ($[N_{4444}]Cl$, $[N_{2222}]Cl$, $[N_{4444}]Br$, $[N_{2222}]Br$ as HBA, and PEG 200–4000 as HBD) + Na_2CO_3, K_2HPO_4, NaH_2PO_4, Na_3Citrate, $(NH_4)_2SO_4$, $MgSO_4$, K_3PO_4, K_2CO_3, NaCl, NH_4NO_3	[284]
Rubropunctamine and monascorubramine	*Monascus anka* CICC 5013	1-butyl-3-methylimidazolium chloride + Triton X-100	[285]
sRNA	*Escherichia coli*	IL (cholinium cation, and amino acids anions) + PPG 400	[286]

Table 5. *Cont.*

Bioproducts	Raw Material	Phase-Forming	Reference
Ectoine	*Halomonas salina*	IL ($[C_4mim]BF_4$) + $(NH_4)_2SO_4$, Na_3Citrate	[287]
Acetoin	*Bacillus subtilis* CGMCC 13141	IL (ethanolammonium, isopropanolammonium cations, and acetate, propionate, butyrate, lactate anions) + K_3PO_4	[67]
Violacein	*Yarrowia lipolytica*	Tween 20 + IL (cholinium cation, Cl, acetate, dihydrogenphosphate, dihydrogencitrate, bicarbonate anions)	[65]
Roseoflavin	*Streptomyces davaonensis*	1-ethyl-3-methylimidazolium acetate or Ethanol + NaH_2PO_4/Na_2HPO_4	[59]
ε-Polylysine	*Bacillus licheniformis*	PPG-400 + IL (2-hydroxyethylammonium, cholinium cations, and formate, acetate anions)	[64]
Lactic acid	Microbial consortium	IL (alkylmethylimidazolium cations, and BF_4, triflate anions) + glucose, xylose	[63]

7. Conclusions and Future Perspectives

ATPS-based on IL and DES are ecofriendly choices that can be used as tools for the selective recovery and separation of bioactive compounds. However, due to the complexity of the matrices in which these compounds are found, it is necessary to advance more efficient systems that allow their separation. Investigations of phase-forming, nonconventional ATPS processes should be further explored and optimal operational parameters should be established. Molecular simulation as a prescreening tool is also promising for the expansion of nonconventional ATPS in separating and purifying bioactive compounds. It is also necessary to expand the number and diversity of compounds used in the construction of ATPS, providing greater versatility and wider options for their effective application in the purification of bioactive compounds. The increase, maintenance or loss of the bioactivities of bioactive compounds must be verified after separation by nonconventional ATPS, thus ensuring the viability and functionality of these compounds in their future applications.

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Nomenclature

AIL—Aprotic Ionic Liquid; ATPS—Aqueous Two-phase System; COSMO-RS—Conductor-like Screening Model for Real Solvents; HBA—Hydrogen Bond Acceptor; HBD—Hydrogen Bond Donor; IL—Ionic Liquid; NADES—Natural Deep Eutectic Solvent; PEG—Polyethylene Glycol; PIL—Protic Ionic Liquid; TL—Tie Line.

References

1. MDF. Bioactive Ingredients Market. Available online: https://www.marketdataforecast.com/market-reports/global-bioactive-ingredients-market (accessed on 18 October 2022).
2. Lemes, A.C.; Braga, A.R.C.; Gautério, G.V.; Fernandes, K.F.; Egea, M.B. Application of membrane technology for production of bioactive peptides. In *Bioactive Peptides*; John Oloche Onuh, M., Selvamuthukumaran, P.Y.V., Eds.; CRC Press: Boca Raton, FL, USA, 2021; p. 27.

3. Powell-Wiley, T.M.; Poirier, P.; Burke, L.E.; Després, J.P.; Gordon-Larsen, P.; Lavie, C.J.; Lear, S.A.; Ndumele, C.E.; Neeland, I.J.; Sanders, P.; et al. Obesity and Cardiovascular Disease: A Scientific Statement from the American Heart Association. *Circulation* **2021**, *143*, e984–e1010. [CrossRef] [PubMed]
4. WHO. Noncommunicable Diseases. 2022. Available online: https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases (accessed on 18 October 2022).
5. Cazarin, C.B.B.; Bicas, J.L.; Pastore, G.M.; Marostica Junior, M.R. Chapter 1—Introduction. In *Bioactive Food Components Activity in Mechanistic Approach*; Cazarin, C.B.B., Bicas, J.L., Pastore, G.M., Marostica, M.R., Jr., Eds.; Academic Press: Cambridge, MA, USA, 2022; pp. 1–3.
6. Kamiloglu, S.; Tomas, M.; Ozdal, T.; Yolci-Omeroglu, P.; Capanoglu, E. Chapter 2—Bioactive component analysis. In *Innovative Food Analysis*; Galanakis, C.M., Ed.; Academic Press: Cambridge, MA, USA, 2021; pp. 41–65.
7. Hayes, M.; Flower, D. Bioactive peptides from marine processing byproducts. In *Bioactive Compounds from Marine Foods*; Wiley: Hoboken, NJ, USA, 2013; pp. 57–71.
8. Lemes, A.C.; Egea, M.B.; Oliveira Filho, J.G.d.; Gautério, G.V.; Ribeiro, B.D.; Coelho, M.A.Z. Biological Approaches for Extraction of Bioactive Compounds From Agro-industrial By-products: A Review. *Front. Bioeng. Biotechnol.* **2022**, *9*, 1413. [CrossRef] [PubMed]
9. Galanakis, C.M.; Drago, S.R. Chapter 1—Introduction. In *Nutraceutical and Functional Food Components*, 2nd ed.; Galanakis, C.M., Ed.; Academic Press: Cambridge, MA, USA, 2022; pp. 1–18.
10. Egea, M.B.; Bolanho, B.C.; Lemes, A.C.; Bragatto, M.M.; Silva, M.R.; Carvalho, J.C.M.; Danesi, E.D.G. Low cost cassava, peach palm and soy by-products for the nutritional enrichment of cookies: Physical, chemical and sensorial characteristics. *Int. Food Res. J.* **2018**, *25*, 1204–1212.
11. Guimarães, R.M.; Pimentel, T.C.; de Rezende, T.A.M.; Silva, J.d.S.; Falcão, H.G.; Ida, E.I.; Egea, M.B. Gluten-free bread: Effect of soy and corn co-products on the quality parameters. *Eur. Food Res. Technol.* **2019**, *245*, 1365–1376. [CrossRef]
12. Madureira, J.; Margaça, F.M.A.; Santos-Buelga, C.; Ferreira, I.; Verde, S.C.; Barros, L. Applications of bioactive compounds extracted from olive industry wastes: A review. *Compr. Rev. Food Sci. Food Saf.* **2022**, *21*, 453–476. [CrossRef] [PubMed]
13. Fernandes, I.d.A.A.; Maciel, G.M.; Maroldi, W.V.; Bortolini, D.G.; Pedro, A.C.; Haminiuk, C.W.I. Bioactive compounds, health-promotion properties and technological applications of Jabuticaba: A literature overview. *Meas. Food* **2022**, *8*, 100057. [CrossRef]
14. Lemes, A.C.; Gautério, G.V.; Folador, G.O.; Sora, G.T.S.; Paula, L.C. Reintrodução de resíduos agroindustriais na produção de alimentos. In *Realidades e Perspectivas em Ciência Dos Alimentos*; Nogueira, W.V., Ed.; Pantanal Editora: Nova Xavantina, Brazil, 2020; Volume II, pp. 67–79.
15. Makowski, B.; Rosicka-Kaczmarek, J.; Nebesny, E. Bioactive compounds in cereals. In *Biotechnology of Bioactive Compounds*; John Wiley & Sons: Hoboken, NJ, USA, 2015; pp. 103–122.
16. Dinis, T.B.V.; Passos, H.; Lima, D.L.D.; Esteves, V.I.; Coutinho, J.A.P.; Freire, M.G. One-step extraction and concentration of estrogens for an adequate monitoring of wastewater using ionic-liquid-based aqueous biphasic systems. *Green Chem.* **2015**, *17*, 2570–2579. [CrossRef]
17. Freire, M.G.; Cláudio, A.F.M.; Araújo, J.M.M.; Coutinho, J.A.P.; Marrucho, I.M.; Lopes, J.N.C.; Rebelo, L.P.N. Aqueous biphasic systems: A boost brought about by using ionic liquids. *Chem. Soc. Rev.* **2012**, *41*, 4966–4995. [CrossRef]
18. Lemes, A.C.; Machado, J.R.; Brites, M.L.; Luccio, M.D.; Kalil, S.J. Design Strategies for Integrated β-Galactosidase Purification Processes. *Chem. Eng. Technol.* **2014**, *37*, 1805–1812. [CrossRef]
19. Yau, Y.K.; Ooi, C.W.; Ng, E.-P.; Lan, J.C.-W.; Ling, T.C.; Show, P.L. Current applications of different type of aqueous two-phase systems. *Bioresour. Bioprocess.* **2015**, *2*, 49. [CrossRef]
20. Gómez, E.; Requejo, P.F.; Tojo, E.; Macedo, E.A. Recovery of flavonoids using novel biodegradable choline amino acids ionic liquids based ATPS. *Fluid Phase Equilibria* **2019**, *493*, 1–9. [CrossRef]
21. Jamehbozorg, B.; Sadeghi, R. Extractions of Alkaloids Codeine and Caffeine with [Bmim][BF4]/Carbohydrate Aqueous Biphasic Systems as a Novel Class of Liquid–Liquid Extraction Systems. *J. Chem. Eng. Data* **2019**, *64*, 916–925. [CrossRef]
22. Gao, J.; Fang, C.; Lin, Y.; Nie, F.; Ji, H.; Liu, S. Enhanced extraction of astaxanthin using aqueous biphasic systems composed of ionic liquids and potassiumphosphate. *Food Chem.* **2020**, *309*, 125672. [CrossRef] [PubMed]
23. Afonso, C.A.M.; Crespo, J.G. *Green Separation Processes: Fundamentals and Applications*; Wiley-VCH Verlag: Weinheim, Germany, 2005; p. 363.
24. García-Serna, J.; Pérez-Barrigón, L.; Cocero, M.J. New trends for design towards sustainability in chemical engineering: Green engineering. *Chem. Eng. J.* **2007**, *133*, 7–30. [CrossRef]
25. Tao, J.A.; Kazlauskas, R. *Biocatalysis for Green Chemistry and Chemical Process Development*; John Wiley & Sons: Hoboken, NJ, USA, 2011; p. 496.
26. Sun, Z.; Kayal, A.; Gong, Z.; Zheng, L.; He, Q. Molecular modelling of ionic liquids: Physical properties of species with extremely long aliphatic chains from a near-optimal regime. *J. Mol. Liq.* **2022**, *367*, 120492. [CrossRef]
27. Kulczyński, B.; Sidor, A.; Gramza-Michałowska, A. Characteristics of Selected Antioxidative and Bioactive Compounds in Meat and Animal Origin Products. *Antioxidants* **2019**, *8*, 335. [CrossRef] [PubMed]
28. Ullah, H.; Wilfred, C.D.; Shaharun, M.S. Ionic liquid-based extraction and separation trends of bioactive compounds from plant biomass. *Sep. Sci. Technol.* **2019**, *54*, 559–579. [CrossRef]

29. Lemes, A.C.; Álvares, G.T.; Egea, M.B.; Brandelli, A.; Kalil, S.J. Simultaneous production of proteases and antioxidant compounds from agro-industrial by-products. *Bioresour. Technol.* **2016**, *222*, 210–216. [CrossRef]
30. Gutierrez-Macias, P.; Hernandez de Jesus, M.d.L.; Barragan-Huerta, B.E. The production of biomaterials from agro-industrial waste. *Fresenius Environ. Bull.* **2017**, *26*, 4128–4152.
31. Baiano, A. Recovery of biomolecules from food wastes—A review. *Molecules* **2014**, *19*, 14821–14842. [CrossRef]
32. Vargas, D.; Nörnberg, J.; Ritt, L.; Scheibler, R.; Antunes Rizzo, F.; Milani, M. Potencialidades funcionais e nutracêuticas das proteínas do leite bovino. *Rev. Eletrônica Em Gestão Educ. E Tecnol. Ambient.* **2014**, *18*, 25–35. [CrossRef]
33. Clare, D.A.; Swaisgood, H.E. Bioactive milk peptides: A prospectus. *J. Dairy Sci.* **2000**, *83*, 1187–1195. [CrossRef] [PubMed]
34. Weaver, C.M. Bioactive foods and ingredients for health. *Adv. Nutr.* **2014**, *5*, 306s–311s. [CrossRef] [PubMed]
35. Silva Pinto, M.; Kwon, Y.I.; Apostolidis, E.; Lajolo, F.M.; Genovese, M.I.; Shetty, K. Functionality of bioactive compounds in Brazilian strawberry (Fragaria x ananassa Duch.) cultivars: Evaluation of hyperglycemia and hypertension potential using in vitro models. *J. Agric. Food Chem.* **2008**, *56*, 4386–4392. [CrossRef]
36. Oliveira Filho, J.G.; Rodrigues, J.M.; Valadares, A.C.F.; Almeida, A.B.; Valencia-Mejia, E.; Fernandes, K.F.; Lemes, A.C.; Alves, C.C.F.; Sousa, H.A.d.F.; Silva, E.R.; et al. Bioactive Properties of Protein Hydrolysate of Cottonseed Byproduct: Antioxidant, Antimicrobial, and Angiotensin-Converting Enzyme (ACE) Inhibitory Activities. *Waste Biomass Valorization* **2021**, *12*, 1395–1404. [CrossRef]
37. Abdul, G.M.; Shama, M. Phenolic compounds. In *Bioactive Compounds*; Leila Queiroz, Z., Tatiele Casagrande do, N., Eduardo, J.-L., Eds.; IntechOpen: Rijeka, Croatia, 2021; p. 13.
38. Swanson, B.G. Tanninsand and polyphenols. In *Encyclopedia of Food Sciences and Nutrition*, 2nd ed.; Caballero, B., Ed.; Academic Press: Oxford, UK, 2003; pp. 5729–5733.
39. Lin, D.; Xiao, M.; Zhao, J.; Li, Z.; Xing, B.; Li, X.; Kong, M.; Li, L.; Zhang, Q.; Liu, Y.; et al. An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. *Molecules* **2016**, *21*, 1374. [CrossRef]
40. Lattanzio, V. Phenolic compounds: Introduction. In *Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes*; Ramawat, K.G., Mérillon, J.-M., Eds.; Springer: Berlin/Heidelberg, Germany, 2013; pp. 1543–1580.
41. Joanna, K. Introductory chapter: Alkaloids—Their importance in nature and for human life. In *Alkaloids*; Joanna, K., Ed.; IntechOpen: Rijeka, Croatia, 2019; p. 1.
42. Waller, G.R.; Nowacki, E.K. The role of alkaloids in plants. In *Alkaloid Biology and Metabolism in Plants*; Waller, G.R., Nowacki, E.K., Eds.; Springer US: Boston, MA, USA, 1978; pp. 143–181.
43. Debnath, B.; Singh, W.S.; Das, M.; Goswami, S.; Singh, M.K.; Maiti, D.; Manna, K. Role of plant alkaloids on human health: A review of biological activities. *Mater. Today Chem.* **2018**, *9*, 56–72. [CrossRef]
44. Dimitrijević, A.; Tavares, A.P.M.; Jocić, A.; Marić, S.; Trtić-Petrović, T.; Gadžurić, S.; Freire, M.G. Aqueous biphasic systems comprising copolymers and cholinium-based salts or ionic liquids: Insights on the mechanisms responsible for their creation. *Sep. Purif. Technol.* **2020**, *248*, 117050. [CrossRef]
45. Fan, Y.; Lu, Y.M.; Yu, B.; Tan, C.P.; Cui, B. Extraction and purification of capsaicin from capsicum oleoresin using an aqueous two-phase system combined with chromatography. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2017**, *1063*, 11–17. [CrossRef]
46. Lemes, A.C.; Reis, D.F.; Ores, J.C.; Braga, A.R.C. Microalgas como fontes de pigmentos naturais para aplicação industrial. In *A Biodiversidade Como Fonte de Compostos Bioativos: Moléculas e Aplicações*, 1st ed.; Egea, M.B., Marcionilio, S.M.L.O., Eds.; Editora IF Goiano: Rio Verde, Brasil, 2022; pp. 47–63.
47. Martins, N.; Roriz, C.; Morales, P.; Barros, L.; Ferreira, I. Food colorants: Challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. *Trends Food Sci. Technol.* **2016**, *52*, 1–15. [CrossRef]
48. Aberoumand, A. A Review Article on Edible Pigments Properties and Sources as Natural Biocolorants in Foodstuff and Food Industry. *World J. Dairy Food Sci.* **2011**, *6*, 71–78.
49. Ferreira, V.S.; Sant'Anna, C. Impact of culture conditions on the chlorophyll content of microalgae for biotechnological applications. *World J. Microbiol. Biotechnol.* **2017**, *33*, 20. [CrossRef] [PubMed]
50. Cheng, Z.; Song, H.; Cao, X.; Shen, Q.; Han, D.; Zhong, F.; Hu, H.; Yang, Y. Simultaneous extraction and purification of polysaccharides from *Gentiana scabra* Bunge by microwave-assisted ethanol-salt aqueous two-phase system. *Ind. Crops Prod.* **2017**, *102*, 75–87. [CrossRef]
51. Dang, Y.-Y.; Zhang, H.; Xiu, Z.-L. Microwave-assisted aqueous two-phase extraction of phenolics from grape (*Vitis vinifera*) seed. *J. Chem. Technol. Biotechnol.* **2014**, *89*, 1576–1581. [CrossRef]
52. He, A.; Dong, B.; Feng, X.; Yao, S. Extraction of bioactive ginseng saponins using aqueous two-phase systems of ionic liquids and salts. *Sep. Purif. Technol.* **2018**, *196*, 270–280. [CrossRef]
53. Wang, T.; Xu, W.-J.; Wang, S.-X.; Kou, P.; Wang, P.; Wang, X.-Q.; Fu, Y.-J. Integrated and sustainable separation of chlorogenic acid from blueberry leaves by deep eutectic solvents coupled with aqueous two-phase system. *Food Bioprod. Process.* **2017**, *105*, 205–214. [CrossRef]
54. Tan, Z.J.; Wang, C.Y.; Yang, Z.Z.; Yi, Y.J.; Wang, H.Y.; Zhou, W.L.; Li, F.F. Ionic Liquid-Based Ultrasonic-Assisted Extraction of Secoisolariciresinol Diglucoside from Flaxseed (*Linum usitatissimum* L.) with Further Purification by an Aqueous Two-Phase System. *Molecules* **2015**, *20*, 17929–17943. [CrossRef]
55. Đorđević, T.; Antov, M. Ultrasound assisted extraction in aqueous two-phase system for the integrated extraction and separation of antioxidants from wheat chaff. *Sep. Purif. Technol.* **2017**, *182*, 52–58. [CrossRef]

56. Lima, Á.S.; Soares, C.M.F.; Paltram, R.; Halbwirth, H.; Bica, K. Extraction and consecutive purification of anthocyanins from grape pomace using ionic liquid solutions. *Fluid Phase Equilibria* **2017**, *451*, 68–78. [CrossRef]
57. Sánchez-Rangel, J.C.; Jacobo-Velázquez, D.A.; Cisneros-Zevallos, L.; Benavides, J. Primary recovery of bioactive compounds from stressed carrot tissue using aqueous two-phase systems strategies. *J. Chem. Technol. Biotechnol.* **2016**, *91*, 144–154. [CrossRef]
58. Santos, J.H.P.M.; Martins, M.; Silvestre, A.J.D.; Coutinho, J.A.P.; Ventura, S.P.M. Fractionation of phenolic compounds from lignin depolymerisation using polymeric aqueous biphasic systems with ionic surfactants as electrolytes. *Green Chem.* **2016**, *18*, 5569–5579. [CrossRef]
59. Ibarra-Herrera, C.C.; Meza-Dorantes, A.; Mora-Lugo, R.; González-Valdez, J.; Mack, M.; Mata-Gómez, M.A. Recovery of roseoflavin from a recombinant *Streptomyces davaonensis* strain by using biphasic aqueous systems. *J. Chem. Technol. Biotechnol.* **2021**, *96*, 2529–2536. [CrossRef]
60. Garcia, E.S.; Suarez Ruiz, C.A.; Tilaye, T.; Eppink, M.H.M.; Wijffels, R.H.; van den Berg, C. Fractionation of proteins and carbohydrates from crude microalgae extracts using an ionic liquid based-aqueous two phase system. *Sep. Purif. Technol.* **2018**, *204*, 56–65. [CrossRef]
61. Ruiz, C.A.S.; Martins, M.; Coutinho, J.A.P.; Wijffels, R.H.; Eppink, M.H.M.; Berg, C.v.d.; Ventura, S.P.M. *Neochloris oleoabundans* biorefinery: Integration of cell disruption and purification steps using aqueous biphasic systems-based in surface-active ionic liquids. *Chem. Eng. J.* **2020**, *399*, 125683. [CrossRef]
62. Ventura, S.P.; Santos-Ebinuma, V.C.; Pereira, J.F.; Teixeira, M.F.; Pessoa, A.; Coutinho, J.A. Isolation of natural red colorants from fermented broth using ionic liquid-based aqueous two-phase systems. *J. Ind. Microbiol. Biotechnol.* **2013**, *40*, 507–516. [CrossRef]
63. Zhou, X.; Sun, Y.; Zhan, H.; Liu, H.; Wang, X.; Xu, Y.; Li, Y.; Xiu, Z.; Tong, Y. Ionic liquid-based multi-stage sugaring-out extraction of lactic acid from simulated broth and actual lignocellulosic fermentation broth. *Bioresour. Bioprocess.* **2021**, *8*, 123. [CrossRef]
64. Sequeira, R.A.; Sharma, M.; Pereira, M.M.; Singh, N.; Bhattacharya, S.; Chudasama, N.A.; Prasad, K. One step selective partition of ε-polylysine present in broth cultures in ionic liquid-based aqueous biphasic systems. *Sep. Sci. Technol.* **2021**, *56*, 631–639. [CrossRef]
65. Kholany, M.; Trébulle, P.; Martins, M.; Ventura, S.P.; Nicaud, J.-M.; Coutinho, J.A. Extraction and purification of violacein from *Yarrowia lipolytica* cells using aqueous solutions of surfactants. *J. Chem. Technol. Biotechnol.* **2020**, *95*, 1126–1134. [CrossRef]
66. Silva, O.S.; Gomes, M.H.G.; Oliveira, R.L.; Porto, A.L.F.; Converti, A.; Porto, T.S. Partitioning and extraction protease from Aspergillus tamarii URM4634 using PEG-citrate aqueous two-phase systems. *Biocatal. Agric. Biotechnol.* **2017**, *9*, 168–173. [CrossRef]
67. Li, Y.; Dai, J.-Y.; Xiu, Z.-L. Salting-out extraction of acetoin from fermentation broths using hydroxylammonium ionic liquids as extractants. *Sep. Purif. Technol.* **2020**, *240*, 116584. [CrossRef]
68. Amarante, M.C.A.d.; Braga, A.R.C.; Sala, L.; Moraes, C.C.; Kalil, S.J. Design strategies for C-phycocyanin purification: Process influence on purity grade. *Sep. Purif. Technol.* **2020**, *252*, 117453. [CrossRef]
69. Xiong, Y.W.; Ju, X.Y.; Li, X.W.; Gong, Y.; Xu, M.J.; Zhang, C.M.; Yuan, B.; Lv, Z.P.; Qin, S. Fermentation conditions optimization, purification, and antioxidant activity of exopolysaccharides obtained from the plant growth-promoting endophytic actinobacterium Glutamicibacter halophytocola KLBMP 5180. *Int. J. Biol. Macromol.* **2020**, *153*, 1176–1185. [CrossRef] [PubMed]
70. Zafarani-Moattar, M.T.; Nasiri, S. (Liquid + liquid) and (liquid + solid) equilibrium of aqueous two-phase systems containing poly ethylene glycol di-methyl ether 2000 and di-sodium hydrogen phosphate. *J. Chem. Thermodyn.* **2010**, *42*, 1071–1078. [CrossRef]
71. Albertsson, P.E.R.Å. Partition of Proteins in Liquid Polymer–Polymer Two-Phase Systems. *Nature* **1958**, *182*, 709–711. [CrossRef] [PubMed]
72. Zaslavsky, B.Y. Aqueous Two-Phase Partitioning—Physical Chemistry and Bioanalytical Applications. *Ber. Der Bunsenges. Für Phys. Chem.* **1995**, *99*, 694. [CrossRef]
73. Raja, S.; Murty, R.; Thivaharan, V.; Rajasekar, V.; Ramesh, V. Aqueous Two Phase Systems for the Recovery of Biomolecules –A Review. *Sci. Technol.* **2012**, *1*, 7–16. [CrossRef]
74. Soares, R.R.; Azevedo, A.M.; Van Alstine, J.M.; Aires-Barros, M.R. Partitioning in aqueous two-phase systems: Analysis of strengths, weaknesses, opportunities and threats. *Biotechnol. J.* **2015**, *10*, 1158–1169. [CrossRef]
75. Phong, W.N.; Show, P.L.; Chow, Y.H.; Ling, T.C. Recovery of biotechnological products using aqueous two phase systems. *J. Biosci. Bioeng.* **2018**, *126*, 273–281. [CrossRef]
76. Vieira, A.W.; da Cruz Silva, K.; Mageste, A.B.; Rodrigues, G.D.; de Lemos, L.R. Lycopene partition in new aqueous two-phase systems. *J. Mol. Liq.* **2021**, *339*, 116755. [CrossRef]
77. Benavides, J.; Rito-Palomares, M.; Asenjo, J. Aqueous two-phase systems. In *Comprehensive Biotechnology*, 2nd ed.; Moo-Young, M., Ed.; Elsevier: Amsterdam, The Netherlands, 2011; Volume 2, pp. 697–713.
78. Albertsson, P.-Å.; Cajarville, A.; Brooks, D.E.; Tjerneld, F. Partition of proteins in aqueous polymer two-phase systems and the effect of molecular weight of the polymer. *Biochim. Et Biophys. Acta (BBA)-Gen. Subj.* **1987**, *926*, 87–93. [CrossRef]
79. Rogers, R.D.; Bond, A.H.; Bauer, C.B.; Zhang, J.; Griffin, S.T. Metal ion separations in polyethylene glycol-based aqueous biphasic systems: Correlation of partitioning behavior with available thermodynamic hydration data. *J. Chromatogr. B Biomed. Sci. Appl.* **1996**, *680*, 221–229. [CrossRef] [PubMed]
80. Smith, J.M.; Van Ness, H.C.; Abbott, M.M. *Introdução à Termodinâmica da Engenharia Química*, 7th ed.; LTC: New York, NY, USA, 2007; p. 584.
81. Albertsson, P.A. *Partition of Cell Particles and Macromolecules*, 3rd ed.; John Wiley & Sons: New York, NY, USA, 1986; p. 346.

82. Tubio, G.; Nerli, B.B.; Picó, G.A.; Venâncio, A.; Teixeira, J. Liquid–liquid equilibrium of the Ucon 50-HB5100/sodium citrate aqueous two-phase systems. *Sep. Purif. Technol.* **2009**, *65*, 3–8. [CrossRef]
83. Merchuk, J.C.; Andrews, B.A.; Asenjo, J.A. Aqueous two-phase systems for protein separation: Studies on phase inversion. *J. Chromatogr. B Biomed. Sci. Appl.* **1998**, *711*, 285–293. [CrossRef] [PubMed]
84. Gutowski, K.E.; Broker, G.A.; Willauer, H.D.; Huddleston, J.G.; Swatloski, R.P.; Holbrey, J.D.; Rogers, R.D. Controlling the Aqueous Miscibility of Ionic Liquids: Aqueous Biphasic Systems of Water-Miscible Ionic Liquids and Water-Structuring Salts for Recycle, Metathesis, and Separations. *J. Am. Chem. Soc.* **2003**, *125*, 6632–6633. [CrossRef]
85. Molino, J.V.; Viana Marques Dde, A.; Júnior, A.P.; Mazzola, P.G.; Gatti, M.S. Different types of aqueous two-phase systems for biomolecule and bioparticle extraction and purification. *Biotechnol. Prog.* **2013**, *29*, 1343–1353. [CrossRef]
86. Azevedo, A.M.; Rosa, P.A.J.; Ferreira, I.F.; Pisco, A.M.M.O.; de Vries, J.; Korporaal, R.; Visser, T.J.; Aires-Barros, M.R. Affinity-enhanced purification of human antibodies by aqueous two-phase extraction. *Sep. Purif. Technol.* **2009**, *65*, 31–39. [CrossRef]
87. Rito-Palomares, M. Practical application of aqueous two-phase partition to process development for the recovery of biological products. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2004**, *807*, 3–11. [CrossRef]
88. Souza, R.L.; Barbosa, J.M.; Zanin, G.M.; Lobão, M.W.; Soares, C.M.; Lima, A.S. Partitioning of porcine pancreatic lipase in a two-phase systems of polyethylene glycol/potassium phosphate aqueous. *Appl. Biochem. Biotechnol.* **2010**, *161*, 288–300. [CrossRef]
89. Biazus, J.P.M.; Santana, J.; Souza, R.; Jordão, E.; Tambourgi, E. Continuous extraction of α- and β-amylases from Zea mays malt in a PEG4000/CaCl2 ATPS. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2007**, *858*, 227–233. [CrossRef]
90. Silva, C.A.S.; Coimbra, J.S.R.; Rojas, E.E.G.; Teixeira, J.A.C. Partitioning of glycomacropeptide in aqueous two-phase systems. *Process Biochem.* **2009**, *44*, 1213–1216. [CrossRef]
91. Iqbal, M.; Tao, Y.; Xie, S.; Zhu, Y.; Chen, D.; Wang, X.; Huang, L.; Peng, D.; Sattar, A.; Shabbir, M.A.B.; et al. Aqueous two-phase system (ATPS): An overview and advances in its applications. *Biol. Proc. Online* **2016**, *18*, 18. [CrossRef] [PubMed]
92. Sousa, K.M.; Maciel, G.E.L.O.; Buarque, F.S.; Santos, A.J.; Marques, M.N.; Cavalcanti, E.B.; Soares, C.M.F.; Lima, Á.S. Novel phase diagrams of aqueous two-phase systems based on tetrahydrofuran + carbohydrates + water: Equilibrium data and partitioning experiments. *Fluid Phase Equilibria* **2017**, *433*, 1–9. [CrossRef]
93. Sousa, K.M.; Merlo, L.H.Z.; Marques, M.N.; Cavalcanti, E.B.; Souza, R.L.; Soares, C.M.F.; Lima, A.S. Partitioning of diuron in a novel aqueous two-phase system based on polyols and tetrahydrofuran. *Fluid Phase Equilibria* **2016**, *429*, 325–330. [CrossRef]
94. Buarque, F.S.; Soares, C.M.F.; de Souza, R.L.; Pereira, M.M.; Lima, Á.S. Development of an ethanolic two-phase system (ETPS) based on polypropylene glycol 2000 + ethylene glycol + ethanol for separation of hydrophobic compounds. *Chem. Commun.* **2021**, *57*, 2156–2159. [CrossRef] [PubMed]
95. Buarque, F.S.; Soares, C.M.F.; Marques, M.N.; Miranda, R.d.C.M.; Cavalcanti, E.B.; Souza, R.L.; Lima, Á.S. Simultaneous concentration and chromatographic detection of water pesticides traces using aqueous two-phase system composed of tetrahydrofuran and fructose. *Microchem. J.* **2019**, *147*, 303–310. [CrossRef]
96. Pang, J.; Sha, X.; Chao, Y.; Chen, G.; Han, C.; Zhu, W.; Li, H.; Zhang, Q. Green aqueous biphasic systems containing deep eutectic solvents and sodium salts for the extraction of protein. *RSC Adv.* **2017**, *7*, 49361–49367. [CrossRef]
97. Passos, H.; Tavares, D.J.P.; Ferreira, A.M.; Freire, M.G.; Coutinho, J.A.P. Are Aqueous Biphasic Systems Composed of Deep Eutectic Solvents Ternary or Quaternary Systems? *ACS Sustain. Chem. Eng.* **2016**, *4*, 2881–2886. [CrossRef]
98. Wang, J.; Wang, H.; Zhang, S.; Zhang, H.; Zhao, Y. Conductivities, Volumes, Fluorescence, and Aggregation Behavior of Ionic Liquids [C4mim][BF4] and [Cnmim]Br (n = 4, 6, 8, 10, 12) in Aqueous Solutions. *J. Phys. Chem. B* **2007**, *111*, 6181–6188. [CrossRef]
99. Earle, M.J.; Esperança, J.M.S.S.; Gilea, M.A.; Canongia Lopes, J.N.; Rebelo, L.P.N.; Magee, J.W.; Seddon, K.R.; Widegren, J.A. The distillation and volatility of ionic liquids. *Nature* **2006**, *439*, 831–834. [CrossRef]
100. Rogers, R.D.; Seddon, K.R. Chemistry. Ionic liquids—Solvents of the future? *Science* **2003**, *302*, 792–793. [CrossRef]
101. Plácido, N.S.O.; Carlos, A.L.S.; Galvão, J.U.S.; Souza, R.L.; Soares, C.M.F.; Mattedi, S.; Fricks, A.T.; Lima, Á.S. Protic ionic liquids as a constituent of biphasic systems based on acetonitrile: Phase diagram and alkaloid partitioning. *Sep. Purif. Technol.* **2018**, *200*, 318–326. [CrossRef]
102. Yan, X.-J.; Li, S.-N.; Zhai, Q.-G.; Jiang, Y.-C.; Hu, M.-C. Physicochemical Properties for the Binary Systems of Ionic Liquids [Cnmim]Cl + N,N-Dimethylformamide. *J. Chem. Eng. Data* **2014**, *59*, 1411–1422. [CrossRef]
103. Álvarez, V.H.; Dosil, N.; Gonzalez-Cabaleiro, R.; Mattedi, S.; Martin-Pastor, M.; Iglesias, M.; Navaza, J.M. Brønsted Ionic Liquids for Sustainable Processes: Synthesis and Physical Properties. *J. Chem. Eng. Data* **2010**, *55*, 625–632. [CrossRef]
104. Álvarez, V.H.; Mattedi, S.; Martin-Pastor, M.; Aznar, M.; Iglesias, M. Synthesis and thermophysical properties of two new protic long-chain ionic liquids with the oleate anion. *Fluid Phase Equilibria* **2010**, *299*, 42–50. [CrossRef]
105. Deetlefs, M.; Seddon, K.R. Assessing the greenness of some typical laboratory ionic liquid preparations. *Green Chem.* **2010**, *12*, 17–30. [CrossRef]
106. Domínguez-Pérez, M.; Tomé, L.I.N.; Freire, M.G.; Marrucho, I.M.; Cabeza, O.; Coutinho, J.A.P. Extraction of biomolecules using aqueous biphasic systems formed by ionic liquids and aminoacids. *Sep. Purif. Technol.* **2010**, *72*, 85–91. [CrossRef]
107. Quental, M.V.; Pereira, M.M.; Ferreira, A.M.; Pedro, S.N.; Shahriari, S.; Mohamadou, A.; Coutinho, J.A.P.; Freire, M.G. Enhanced separation performance of aqueous biphasic systems formed by carbohydrates and tetraalkylphosphonium- or tetraalkylammonium-based ionic liquids. *Green Chem.* **2018**, *20*, 2978–2983. [CrossRef]

108. Freire, M.G.; Pereira, J.F.; Francisco, M.; Rodríguez, H.; Rebelo, L.P.; Rogers, R.D.; Coutinho, J.A. Insight into the interactions that control the phase behaviour of new aqueous biphasic systems composed of polyethylene glycol polymers and ionic liquids. *Chemistry* **2012**, *18*, 1831–1839. [CrossRef]
109. Ventura, S.P.M.; Neves, C.M.S.S.; Freire, M.G.; Marrucho, I.M.; Oliveira, J.; Coutinho, J.A.P. Evaluation of Anion Influence on the Formation and Extraction Capacity of Ionic-Liquid-Based Aqueous Biphasic Systems. *J. Phys. Chem. B* **2009**, *113*, 9304–9310. [CrossRef]
110. Pei, Y.; Li, Z.; Liu, L.; Wang, J. Partitioning behavior of amino acids in aqueous two-phase systems formed by imidazolium ionic liquid and dipotassium hydrogen phosphate. *J. Chromatogr. A* **2012**, *1231*, 2–7. [CrossRef]
111. Marchel, M.; João, K.G.; Marrucho, I.M. On the use of ionic liquids as adjuvants in PEG-(NH4)2SO4 aqueous biphasic systems: Phase diagrams behavior and the effect of IL concentration on myoglobin partition. *Sep. Purif. Technol.* **2019**, *210*, 710–718. [CrossRef]
112. Mourão, T.; Cláudio, A.F.M.; Boal-Palheiros, I.; Freire, M.G.; Coutinho, J.A.P. Evaluation of the impact of phosphate salts on the formation of ionic-liquid-based aqueous biphasic systems. *J. Chem. Thermodyn.* **2012**, *54*, 398–405. [CrossRef]
113. Patinha, D.J.S.; Alves, F.; Rebelo, L.P.N.; Marrucho, I.M. Ionic liquids based aqueous biphasic systems: Effect of the alkyl chains in the cation versus in the anion. *J. Chem. Thermodyn.* **2013**, *65*, 106–112. [CrossRef]
114. Pereira, J.; Kurnia, K.; Cojocaru, O.; Gurau, G.; Rebelo, L.P.N.; Rogers, R.; Freire, M. Molecular interactions in aqueous biphasic systems composed of polyethylene glycol and crystalline vs. liquid cholinium-based salts. *Phys. Chem. Chem. Phys.* **2014**, *16*, 5723–5731. [CrossRef] [PubMed]
115. Soto, A.; Arce, A.; Khoshkbarchi, M.K. Partitioning of antibiotics in a two-liquid phase system formed by water and a room temperature ionic liquid. *Sep. Purif. Technol.* **2005**, *44*, 242–246. [CrossRef]
116. Almeida, H.F.D.; Marrucho, I.M.; Freire, M.G. Removal of Non-Steroidal Anti-Inflammatory Drugs from Aqueous Environments with Reusable Ionic-Liquid-based Systems. *ACS Sustain. Chem. Eng.* **2017**, *5*, 2428–2436. [CrossRef]
117. Silva, F.A.; Caban, M.; Kholany, M.; Stepnowski, P.; Coutinho, J.A.P.; Ventura, S.P.M. Recovery of Nonsteroidal Anti-Inflammatory Drugs from Wastes Using Ionic-Liquid-Based Three-Phase Partitioning Systems. *ACS Sustain. Chem. Eng.* **2018**, *6*, 4574–4585. [CrossRef]
118. Buarque, F.S.; Barreto, V.S.; Soares, C.M.F.; Souza, R.L.; Pereira, M.M.; Lima, Á.S. Selective extraction of female hormones using aqueous two-phase system composed of double protic ionic liquid + acetonitrile. *Fluid Phase Equilibria* **2020**, *508*, 112443. [CrossRef]
119. Quental, M.V.; Caban, M.; Pereira, M.M.; Stepnowski, P.; Coutinho, J.A.; Freire, M.G. Enhanced extraction of proteins using cholinium-based ionic liquids as phase-forming components of aqueous biphasic systems. *Biotechnol. J.* **2015**, *10*, 1457–1466. [CrossRef]
120. Pereira, M.M.; Cruz, R.A.P.; Almeida, M.R.; Lima, Á.S.; Coutinho, J.A.P.; Freire, M.G. Single-step purification of ovalbumin from egg white using aqueous biphasic systems. *Process Biochem.* **2016**, *51*, 781–791. [CrossRef]
121. Lee, S.Y.; Khoiroh, I.; Ling, T.C.; Show, P.L. Aqueous Two-Phase Flotation for the Recovery of Biomolecules. *Sep. Purif. Rev.* **2016**, *45*, 81–92. [CrossRef]
122. Akama, Y.; Ito, M.; Tanaka, S. Selective separation of cadmium from cobalt, copper, iron (III) and zinc by water-based two-phase system of tetrabutylammonium bromide. *Talanta* **2000**, *53*, 645–650. [CrossRef] [PubMed]
123. Akama, Y.; Sali, A. Extraction mechanism of Cr(VI) on the aqueous two-phase system of tetrabutylammonium bromide and (NH(4))(2)SO(4) mixture. *Talanta* **2002**, *57*, 681–686. [CrossRef] [PubMed]
124. Oliveira, B.S.; D'Anzicourt, C.M.d.S.; Soares, C.M.F.; Souza, R.L.; Lima, Á.S. Liquid-Liquid extraction of phenolic compounds in systems based on acetonitrile + water + polyvinylpyrrolidone at 298.15 K. *Data Brief* **2018**, *20*, 2045–2053. [CrossRef] [PubMed]
125. Rosário, R.; Souza, R.; Farias, F.; Mafra, M.; Soares, C.; Passos, H.; Coutinho, J.; Lima, Á. Acetonitrile as adjuvant to tune polyethylene glycol + K_3PO_4 aqueous two-phase systems and its effect on phenolic compounds partition. *Sep. Purif. Technol.* **2019**, *223*, 41–48. [CrossRef]
126. Pereira, J.F.B.; Lima, Á.S.; Freire, M.G.; Coutinho, J.A.P. Ionic liquids as adjuvants for the tailored extraction of biomolecules in aqueous biphasic systems. *Green Chem.* **2010**, *12*, 1661–1669. [CrossRef]
127. Souza, R.L.; Campos, V.C.; Ventura, S.P.M.; Soares, C.M.F.; Coutinho, J.A.P.; Lima, Á.S. Effect of ionic liquids as adjuvants on PEG-based ABS formation and the extraction of two probe dyes. *Fluid Phase Equilibria* **2014**, *375*, 30–36. [CrossRef]
128. Yang, H.; Zhang, L.; Chen, L.; Zhou, C.; Yu, X.; Yagoub, A.E.-G.A.; Ma, H. Effect of ionic liquid based imidazolium as an additive on the formation of polymer/salt aqueous biphasic systems. *J. Mol. Liq.* **2018**, *256*, 1–8. [CrossRef]
129. Almeida, M.R.; Passos, H.; Pereira, M.M.; Lima, Á.S.; Coutinho, J.A.P.; Freire, M.G. Ionic liquids as additives to enhance the extraction of antioxidants in aqueous two-phase systems. *Sep. Purif. Technol.* **2014**, *128*, 1–10. [CrossRef]
130. Hallett, J.P.; Welton, T. Room-Temperature Ionic Liquids: Solvents for Synthesis and Catalysis. 2. *Chem. Rev.* **2011**, *111*, 3508–3576. [CrossRef]
131. Oliveira, M.B.; Domínguez-Pérez, M.; Cabeza, O.; Lopes-da-Silva, J.A.; Freire, M.G.; Coutinho, J.A.P. Surface tensions of binary mixtures of ionic liquids with bis(trifluoromethylsulfonyl)imide as the common anion. *J. Chem. Thermodyn.* **2013**, *64*, 22–27. [CrossRef]

132. Buarque, F.S.; Camêlo, L.C.A.; Soares, C.M.F.; Mattedi, S.; Ferreira, A.F.B.; Feitosa, F.X.; Souza, R.L.; de Sant'Ana, H.B.; Lima, Á.S. Binary Mixture of Double Protic Ionic Liquid: Density, Viscosity, Refractive Index, Surface Tension, and Derivative Properties. *J. Chem. Eng. Data* **2021**, *66*, 4309–4325. [CrossRef]
133. Abbott, A.P.; Capper, G.; Davies, D.L.; Rasheed, R.K.; Tambyrajah, V. Novel solvent properties of choline chloride/urea mixtures. *Chem. Commun.* **2003**, *1*, 70–71. [CrossRef] [PubMed]
134. Smith, E.L.; Abbott, A.P.; Ryder, K.S. Deep Eutectic Solvents (DESs) and Their Applications. *Chem. Rev.* **2014**, *114*, 11060–11082. [CrossRef]
135. Francisco, M.; van den Bruinhorst, A.; Kroon, M.C. Low-transition-temperature mixtures (LTTMs): A new generation of designer solvents. *Angew. Chem. (Int. Ed. Engl.)* **2013**, *52*, 3074–3085. [CrossRef]
136. Abbott, A.P.; Boothby, D.; Capper, G.; Davies, D.L.; Rasheed, R.K. Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. *J. Am. Chem. Soc.* **2004**, *126*, 9142–9147. [CrossRef]
137. Francisco, M.; van den Bruinhorst, A.; Kroon, M.C. New natural and renewable low transition temperature mixtures (LTTMs): Screening as solvents for lignocellulosic biomass processing. *Green Chem.* **2012**, *14*, 2153–2157. [CrossRef]
138. Dai, Y.; Witkamp, G.-J.; Verpoorte, R.; Choi, Y.H. Natural Deep Eutectic Solvents as a New Extraction Media for Phenolic Metabolites in *Carthamus tinctorius* L. *Anal. Chem.* **2013**, *85*, 6272–6278. [CrossRef]
139. Choi, Y.H.; Verpoorte, R. Green solvents for the extraction of bioactive compounds from natural products using ionic liquids and deep eutectic solvents. *Curr. Opin. Food Sci.* **2019**, *26*, 87–93. [CrossRef]
140. Cvjetko Bubalo, M.; Vidović, S.; Radojčić Redovniković, I.; Jokić, S. New perspective in extraction of plant biologically active compounds by green solvents. *Food Bioprod. Process.* **2018**, *109*, 52–73. [CrossRef]
141. Vanda, H.; Dai, Y.; Wilson, E.G.; Verpoorte, R.; Choi, Y.H. Green solvents from ionic liquids and deep eutectic solvents to natural deep eutectic solvents. *Comptes. Rendus Chim.* **2018**, *21*, 628–638. [CrossRef]
142. Ahmad, I.; Pertiwi, A.S.; Kembaren, Y.H.; Rahman, A.; Munâim, A. Application of Natural Deep Eutectic Solvent-Based Ultrasonic Assisted Extraction of Total Polyphenolic and Caffeine Content from Coffe Beans (*Coffea Beans* L.) For Instant Food Products. *J. Appl. Pharm. Sci.* **2018**, *8*, 138–143.
143. Barbieri, J.B.; Goltz, C.; Batistão Cavalheiro, F.; Theodoro Toci, A.; Igarashi-Mafra, L.; Mafra, M.R. Deep eutectic solvents applied in the extraction and stabilization of rosemary (*Rosmarinus officinalis* L.) phenolic compounds. *Ind. Crops Prod.* **2020**, *144*, 112049. [CrossRef]
144. El Kantar, S.; Rajha, H.N.; Boussetta, N.; Vorobiev, E.; Maroun, R.G.; Louka, N. Green extraction of polyphenols from grapefruit peels using high voltage electrical discharges, deep eutectic solvents and aqueous glycerol. *Food Chem.* **2019**, *295*, 165–171. [CrossRef] [PubMed]
145. Gao, C.; Cai, C.; Liu, J.; Wang, Y.; Chen, Y.; Wang, L.; Tan, Z. Extraction and preliminary purification of polysaccharides from Camellia oleifera Abel. seed cake using a thermoseparating aqueous two-phase system based on EOPO copolymer and deep eutectic solvents. *Food Chem.* **2020**, *313*, 126164. [CrossRef]
146. Zeng, Q.; Wang, Y.; Huang, Y.; Ding, X.; Chen, J.; Xu, K. Deep eutectic solvents as novel extraction media for protein partitioning. *Analyst* **2014**, *139*, 2565–2573. [CrossRef] [PubMed]
147. Martins, M.A.R.; Pinho, S.P.; Coutinho, J.A.P. Insights into the Nature of Eutectic and Deep Eutectic Mixtures. *J. Solut. Chem.* **2019**, *48*, 962–982. [CrossRef]
148. Poole, C.F. Chapter 2—Solvent selection for liquid-phase extraction. In *Liquid-Phase Extraction*; Poole, C.F., Ed.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 45–89.
149. Grundtvig, I.P.R.; Heintz, S.; Krühne, U.; Gernaey, K.V.; Adlercreutz, P.; Hayler, J.D.; Wells, A.S.; Woodley, J.M. Screening of organic solvents for bioprocesses using aqueous-organic two-phase systems. *Biotechnol. Adv.* **2018**, *36*, 1801–1814. [CrossRef]
150. Grecco, C.; Miranda, L.; Cruz, J.; Queiroz, M.E. Extração líquido-líquido assistida pelo efeito salting out para análise de amostras biológicas. *Sci. Chromatogr.* **2018**, *10*, 99–111. [CrossRef]
151. Gu, B.H.; Zheng, P.; Yan, Q.; Liu, W. Aqueous two-phase system: An alternative process for recovery of succinic acid from fermentation broth. *Sep. Purif. Technol.* **2014**, *138*, 47–54. [CrossRef]
152. Xie, S.; Song, W.; Fu, C.; Yi, C.; Qiu, X. Separation of acetone: From a water miscible system to an efficient aqueous two-phase system. *Sep. Purif. Technol.* **2018**, *192*, 55–61. [CrossRef]
153. Dias, R.M.; Netto, G.C.A.; Petrin, L.C.G.; Pelaquim, F.P.; Sosa, F.H.B.; Costa, M.C.D. Aqueous two-phase system formed by alkanolammonium-based Protic Ionic Liquids and acetone: Experimental data, thermodynamic modeling, and Kraft lignin partition. *Sep. Purif. Technol.* **2020**, *250*, 117207. [CrossRef]
154. Cardoso, G.B.; Mourão, T.; Pereira, F.M.; Freire, M.G.; Fricks, A.T.; Soares, C.M.F.; Lima, Á.S. Aqueous two-phase systems based on acetonitrile and carbohydrates and their application to the extraction of vanillin. *Sep. Purif. Technol.* **2013**, *104*, 106–113. [CrossRef]
155. Cardoso, G.B.; Souza, I.N.; Mourão, T.; Freire, M.G.; Soares, C.M.F.; Lima, Á.S. Novel aqueous two-phase systems composed of acetonitrile and polyols: Phase diagrams and extractive performance. *Sep. Purif. Technol.* **2014**, *124*, 54–60. [CrossRef]
156. Camêlo, L.C.A.; Souza Dias Santos, G.; Lucena de Souza, R.; Faria Soares, C.M.; Brandão Pereira, J.F.; Lima, Á.S. Protic ionic liquids as constituent of aqueous two-phase system based on acetonitrile: Synthesis, phase diagrams and genipin pre-purification. *Fluid Phase Equilibria* **2020**, *507*, 112425. [CrossRef]

157. Souza, I.N.; Soares, C.M.F.; Souza, R.L.; Freire, M.G.; Lima, A.S. Aqueous two-phase systems formed by maltodextrin and acetonitrile: Phase diagrams and partitioning studies. *Fluid Phase Equilibria* **2018**, *476*, 179–185. [CrossRef]
158. Ooi, C.W.; Tey, B.T.; Hii, S.L.; Kamal, S.M.M.; Lan, J.C.W.; Ariff, A.; Ling, T.C. Purification of lipase derived from Burkholderia pseudomallei with alcohol/salt-based aqueous two-phase systems. *Process Biochem.* **2009**, *44*, 1083–1087. [CrossRef]
159. Reis, I.A.O.; Santos, S.B.; Santos, L.A.; Oliveira, N.; Freire, M.G.; Pereira, J.F.B.; Ventura, S.P.M.; Coutinho, J.A.P.; Soares, C.M.F.; Lima, Á.S. Increased significance of food wastes: Selective recovery of added-value compounds. *Food Chem.* **2012**, *135*, 2453–2461. [CrossRef]
160. Lo, S.C.; Ramanan, R.N.; Tey, B.T.; Tan, W.S.; Show, P.L.; Ling, T.C.; Ooi, C.W. Purification of the recombinant enhanced green fluorescent protein from *Escherichia coli* using alcohol+salt aqueous two-phase systems. *Sep. Purif. Technol.* **2018**, *192*, 130–139. [CrossRef]
161. Farias, F.O.; Oliveira, G.; Leal, F.C.; Wojeicchowski, J.P.; Yamamoto, C.I.; Igarashi-Mafra, L.; Mafra, M.R. Cholinium chloride effect on ethanol-based aqueous biphasic systems: Liquid-liquid equilibrium and biomolecules partition behavior. *Fluid Phase Equilibria* **2020**, *505*, 112363. [CrossRef]
162. Chong, K.Y.; Brooks, M.S.-L. Effects of recycling on the aqueous two-phase extraction of bioactives from haskap leaves. *Sep. Purif. Technol.* **2021**, *255*, 117755. [CrossRef]
163. Ran, L.; Yang, C.; Xu, M.; Yi, Z.; Ren, D.; Yi, L. Enhanced aqueous two-phase extraction of proanthocyanidins from grape seeds by using ionic liquids as adjuvants. *Sep. Purif. Technol.* **2019**, *226*, 154–161. [CrossRef]
164. Nainegali, B.S.; Iyyaswami, R.D.; Belur, P. Partitioning of bio-active compounds from rinds of *Garcinia indica* using aqueous two-phase system: Process evaluation and optimization. *Sep. Purif. Technol.* **2020**, *253*, 117520. [CrossRef]
165. Han, J.; Wu, Y.; Xiang, Y.; Wang, Y.; Ma, J.; Hu, Y. Liquid–liquid equilibria of hydrophilic alcohol+sodium hydroxide+water systems: Experimental and correlation. *Thermochim. Acta* **2013**, *566*, 261–267. [CrossRef]
166. Hu, M.; Zhai, Q.; Liu, Z. Phase Diagram of the Cesium Carbonate + Ethanol + Water Ternary System at (0, 20, and 40) °C. *J. Chem. Eng. Data* **2004**, *49*, 717–719. [CrossRef]
167. Wang, Y.; Han, J.; Xu, X.; Hu, S.; Yan, Y. Partition behavior and partition mechanism of antibiotics in ethanol/2-propanol–ammonium sulfate aqueous two-phase systems. *Sep. Purif. Technol.* **2010**, *75*, 352–357. [CrossRef]
168. Nemati-Kande, E.; Shekaari, H.; Jafari, S.A. Liquid–liquid equilibrium of 1-propanol, 2-propanol, 2-methyl-2-propanol or 2-butanol+sodium sulfite+water aqueous two phase systems. *Fluid Phase Equilibria* **2012**, *329*, 42–54. [CrossRef]
169. Shekaari, H.; Sadeghi, R.; Jafari, S.A. Liquid−Liquid Equilibria for Aliphatic Alcohols + Dipotassium Oxalate + Water. *J. Chem. Eng. Data* **2010**, *55*, 4586–4591. [CrossRef]
170. Robles, P.A.; Lourenço, N.I.; Igarashi, E.M.S.; Sousa, M.N.; Arce, P.F. Thermodynamic Behavior of the Phase Equilibrium of Ethyl Acetate + Ethanol + Water Systems at Atmospheric Pressure: Experiment and Modeling. *J. Chem. Eng. Data* **2020**, *65*, 1402–1410. [CrossRef]
171. Nainegali, B.S.; Iyyaswami, R.; Belur, P.D. Simultaneous extraction of four different bioactive compounds from *Garcinia indica* and their enrichment using Aqueous Two-Phase Systems. *Food Bioprod. Process.* **2019**, *114*, 185–195. [CrossRef]
172. Buarque, F.S.; Guimarães, D.E.M.; Soares, C.M.F.; Souza, R.L.; Pereira, M.M.; Lima, Á.S. Ethanolic two-phase system formed by polypropylene glycol, ethylene glycol and/or ionic liquid (phase-forming or adjuvant) as a platform to phase separation and partitioning study. *J. Mol. Liq.* **2021**, *344*, 117702. [CrossRef]
173. Klamt, A.; Eckert, F. COSMO-RS: A novel and efficient method for the a priori prediction of thermophysical data of liquids. *Fluid Phase Equilibria* **2000**, *172*, 43–72. [CrossRef]
174. Klamt, A. Conductor-like Screening Model for Real Solvents: A New Approach to the Quantitative Calculation of Solvation Phenomena. *J. Phys. Chem.* **1995**, *99*, 2224–2235. [CrossRef]
175. Wojeicchowski, J.P.; Ferreira, A.M.; Abranches, D.O.; Mafra, M.R.; Coutinho, J.A.P. Using COSMO-RS in the Design of Deep Eutectic Solvents for the Extraction of Antioxidants from Rosemary. *ACS Sustain. Chem. Eng.* **2020**, *8*, 12132–12141. [CrossRef]
176. Pereira, J.F.B.; Magri, A.; Quental, M.V.; Gonzalez-Miquel, M.; Freire, M.G.; Coutinho, J.A.P. Alkaloids as Alternative Probes To Characterize the Relative Hydrophobicity of Aqueous Biphasic Systems. *ACS Sustain. Chem. Eng.* **2016**, *4*, 1512–1520. [CrossRef]
177. Khan, I.; Taha, M.; Pinho, S.P.; Coutinho, J.A.P. Interactions of pyridinium, pyrrolidinium or piperidinium based ionic liquids with water: Measurements and COSMO-RS modelling. *Fluid Phase Equilibria* **2016**, *414*, 93–100. [CrossRef]
178. Khan, I.; Kurnia, K.A.; Sintra, T.E.; Saraiva, J.A.; Pinho, S.P.; Coutinho, J.A.P. Assessing the activity coefficients of water in cholinium-based ionic liquids: Experimental measurements and COSMO-RS modeling. *Fluid Phase Equilibria* **2014**, *361*, 16–22. [CrossRef]
179. Sun, Z.; Gong, Z.; Zheng, L.; Kalhor, P.; Huai, Z.; Liu, Z. Molecular modelling of ionic liquids: General guidelines on fixed-charge force fields for balanced descriptions. *J. Ion. Liq.* **2022**, *2*, 100043. [CrossRef]
180. Sun, Z.; Wang, M.; He, Q.; Liu, Z. Molecular Modeling of Ionic Liquids: Force-Field Validation and Thermodynamic Perspective from Large-Scale Fast-Growth Solvation Free Energy Calculations. *Adv. Theory Simul.* **2022**, *5*, 2200274. [CrossRef]
181. Chong, K.Y.; Stefanova, R.; Zhang, J.; Brooks, M.S.-L. Aqueous two-phase extraction of bioactive compounds from haskap leaves (*Lonicera caerulea*): Comparison of salt/ethanol and sugar/propanol systems. *Sep. Purif. Technol.* **2020**, *252*, 117399. [CrossRef]
182. Gomes, P.G.C. *Processo Integrativo de Extração e Purificação de Compostos Bioativos Presentes No Alecrim (Rosmarinus Officinalis)*; Universidade Tiradentes—UNIT: Aracajú, Brasil, 2019.

183. Chang, Y.-K.; Show, P.-L.; Lan, J.C.-W.; Tsai, J.-C.; Huang, C.-R. Isolation of C-phycocyanin from Spirulina platensis microalga using Ionic liquid based aqueous two-phase system. *Bioresour. Technol.* **2018**, *270*, 320–327. [CrossRef]
184. Pan, I.H.; Chiu, H.H.; Lu, C.H.; Lee, L.T.; Li, Y.K. Aqueous two-phase extraction as an effective tool for isolation of geniposide from gardenia fruit. *J. Chromatogr. A* **2002**, *977*, 239–246. [CrossRef] [PubMed]
185. Zhi, W.; Deng, Q. Purification of salvianolic acid B from the crude extract of *Salvia miltiorrhiza* with hydrophilic organic/salt-containing aqueous two-phase system by counter-current chromatography. *J. Chromatogr. A* **2006**, *1116*, 149–152. [CrossRef] [PubMed]
186. Cláudio, A.F.M.; Freire, M.G.; Freire, C.S.R.; Silvestre, A.J.D.; Coutinho, J.A.P. Extraction of vanillin using ionic-liquid-based aqueous two-phase systems. *Sep. Purif. Technol.* **2010**, *75*, 39–47. [CrossRef]
187. Wu, X.; Liang, L.; Zou, Y.; Zhao, T.; Zhao, J.; Li, F.; Yang, L. Aqueous two-phase extraction, identification and antioxidant activity of anthocyanins from mulberry (*Morus atropurpurea* Roxb.). *Food Chem.* **2011**, *129*, 443–453. [CrossRef]
188. Guo, Y.X.; Han, J.; Zhang, D.Y.; Wang, L.H.; Zhou, L.L. An ammonium sulfate/ethanol aqueous two-phase system combined with ultrasonication for the separation and purification of lithospermic acid B from *Salvia miltiorrhiza* Bunge. *Ultrason. Sonochem.* **2012**, *19*, 719–724. [CrossRef]
189. Cláudio, A.F.M.; Ferreira, A.M.; Freire, C.S.R.; Silvestre, A.J.D.; Freire, M.G.; Coutinho, J.A.P. Optimization of the gallic acid extraction using ionic-liquid-based aqueous two-phase systems. *Sep. Purif. Technol.* **2012**, *97*, 142–149. [CrossRef]
190. Cardoso, G.d.B.; Souza, I.N.; Pereira, M.M.; Freire, M.G.; Soares, C.M.F.; Lima, Á.S. Aqueous two-phase systems formed by biocompatible and biodegradable polysaccharides and acetonitrile. *Sep. Purif. Technol.* **2014**, *136*, 74–80. [CrossRef]
191. Reis, I.A.O.; Santos, S.B.; Pereira, F.D.S.; Sobral, C.R.S.; Freire, M.G.; Freitas, L.S.; Soares, C.M.F.; Lima, Á.S. Extraction and Recovery of Rutin from Acerola Waste using Alcohol-Salt-Based Aqueous Two-Phase Systems. *Sep. Sci. Technol.* **2014**, *49*, 656–663. [CrossRef]
192. Wu, Y.; Wang, Y.; Zhang, W.; Han, J.; Liu, Y.; Hu, Y.; Ni, L. Extraction and preliminary purification of anthocyanins from grape juice in aqueous two-phase system. *Sep. Purif. Technol.* **2014**, *124*, 170–178. [CrossRef]
193. Cláudio, A.F.M.; Marques, C.F.C.; Boal-Palheiros, I.; Freire, M.G.; Coutinho, J.A.P. Development of back-extraction and recyclability routes for ionic-liquid-based aqueous two-phase systems. *Green Chem.* **2014**, *16*, 259–268. [CrossRef]
194. Chen, L.-L.; Li, F.-F.; Tan, Z.-J. Chiral separation of α-cyclohexylmandelic acid enantiomers using ionic liquid/salt aqueous two-phase system. *Chem. Pap.* **2015**, *69*, 1465–1472. [CrossRef]
195. Reis, I.A.O.; Campos, A.F.; Santos, P.H.S.; Santos, S.B.; Soares, C.M.F.; Lima, Á.S. Potassium Phosphate Salts-Based Aqueous Two-Phase Systems Applied in the Extraction of Gallic Acid from Guava. *Sep. Sci. Technol.* **2015**, *50*, 520–528. [CrossRef]
196. Santos, J.H.; e Silva, F.A.; Ventura, S.P.; Coutinho, J.A.; de Souza, R.L.; Soares, C.M.; Lima, Á.S. Ionic liquid-based aqueous biphasic systems as a versatile tool for the recovery of antioxidant compounds. *Biotechnol. Prog.* **2015**, *31*, 70–77. [CrossRef] [PubMed]
197. Tan, T.; Lai, C.J.; OuYang, H.; He, M.Z.; Feng, Y. Ionic liquid-based ultrasound-assisted extraction and aqueous two-phase system for analysis of caffeoylquinic acids from Flos Lonicerae Japonicae. *J. Pharm. Biomed. Anal.* **2016**, *120*, 134–141. [CrossRef] [PubMed]
198. Cheng, Z.; Cheng, L.; Song, H.; Yu, L.; Zhong, F.; Shen, Q.; Hu, H. Aqueous two-phase system for preliminary purification of lignans from fruits of *Schisandra chinensis* Baill. *Sep. Purif. Technol.* **2016**, *166*, 16–25. [CrossRef]
199. Guo, Y.X.; Shi, C.Z.; Zhang, L.; Lv, L.; Zhang, Y.Y. Extraction and isolation of lithospermic acid B from *Salvia miltiorrhiza* Bunge using aqueous two-phase extraction followed by high-performance liquid chromatography. *J. Sep. Sci.* **2016**, *39*, 3624–3630. [CrossRef]
200. Xu, Y.Y.; Qiu, Y.; Ren, H.; Ju, D.H.; Jia, H.L. Optimization of ultrasound-assisted aqueous two-phase system extraction of polyphenolic compounds from Aronia melanocarpa pomace by response surface methodology. *Prep. Biochem. Biotechnol.* **2017**, *47*, 312–321. [CrossRef]
201. Liu, D.; Hong, Z.; Gao, M.; Wang, Z.; Gu, M.; Zhang, X.; Xiao, H. Polarity, selectivity and performance of hydrophilic organic/salt-containing aqueous two-phase system on counter-current chromatography for polar compounds. *J. Chromatogr. A* **2016**, *1448*, 49–57. [CrossRef]
202. Yang, Z.; Tan, Z.; Li, F.; Li, X. An effective method for the extraction and purification of chlorogenic acid from ramie (*Boehmeria nivea* L.) leaves using acidic ionic liquids. *Ind. Crops Prod.* **2016**, *89*, 78–86. [CrossRef]
203. Deng, W.-W.; Zong, Y.; Xiao, Y.-X. Hexafluoroisopropanol-Based Deep Eutectic Solvent/Salt Aqueous Two-Phase Systems for Extraction of Anthraquinones from Rhei Radix et Rhizoma Samples. *ACS Sustain. Chem. Eng.* **2017**, *5*, 4267–4275. [CrossRef]
204. Zhang, Z.; Liu, F.; He, C.; Yu, Y.; Wang, M. Optimization of Ultrasonic-Assisted Aqueous Two-Phase Extraction of Phloridzin from Malus Micromalus Makino with Ethanol/Ammonia Sulfate System. *J. Food Sci.* **2017**, *82*, 2944–2953. [CrossRef] [PubMed]
205. Farias, F.O.; Sosa, F.H.B.; Igarashi-Mafra, L.; Coutinho, J.A.P.; Mafra, M.R. Study of the pseudo-ternary aqueous two-phase systems of deep eutectic solvent (choline chloride:sugars) + K2HPO4 + water. *Fluid Phase Equilibria* **2017**, *448*, 143–151. [CrossRef]
206. Wang, R.; Chang, Y.; Tan, Z.; Li, F. A novel combined process for extracting, separating and recovering flavonoids from flos sophorae immaturus. *Sep. Purif. Technol.* **2017**, *172*, 422–432. [CrossRef]
207. Farias, F.O.; Passos, H.; Coutinho, J.A.P.; Mafra, M.R. pH Effect on the Formation of Deep-Eutectic-Solvent-Based Aqueous Two-Phase Systems. *Ind. Eng. Chem. Res.* **2018**, *57*, 16917–16924. [CrossRef]

208. Fu, X.-Q.; Ma, N.; Sun, W.-P.; Dang, Y.-Y. Microwave and enzyme co-assisted aqueous two-phase extraction of polyphenol and lutein from marigold (Tagetes erecta L.) flower. *Ind. Crops Prod.* **2018**, *123*, 296–302. [CrossRef]
209. Silva, F.A.; Kholany, M.; Sintra, T.E.; Caban, M.; Stepnowski, P.; Ventura, S.P.M.; Coutinho, J.A.P. Aqueous Biphasic Systems Using Chiral Ionic Liquids for the Enantioseparation of Mandelic Acid Enantiomers. *Solvent Extr. Ion Exch.* **2018**, *36*, 617–631. [CrossRef]
210. Wang, T.; Gu, C.-B.; Wang, S.-X.; Kou, P.; Jiao, J.; Fu, Y.-J. Simultaneous extraction, transformation and purification of psoralen from fig leaves using pH-dependent ionic liquid solvent based aqueous two-phase system. *J. Clean. Prod.* **2018**, *172*, 827–836. [CrossRef]
211. Yi, X.; Mu, X.; Chen, G.; Gou, Y.; Wang, C.; Yuan, X. Water-insoluble cosolvent in aqueous two-phase system improved the extraction of paeonol from cortex moutan. *J. Chem. Technol. Biotechnol.* **2019**, *94*, 2229–2237. [CrossRef]
212. Gogoi, G.; Hazarika, S. Ionic liquid-mediated aqueous two-phase system to enhance the partitioning of lignin. *Can. J. Chem. Eng.* **2019**, *97*, 2527–2535. [CrossRef]
213. Miličević, N.; Panić, M.; Valinger, D.; Cvjetko Bubalo, M.; Benković, M.; Jurina, T.; Gajdoš Kljusurić, J.; Radojčić Redovniković, I.; Jurinjak Tušek, A. Development of continuously operated aqueous two-phase microextraction process using natural deep eutectic solvents. *Sep. Purif. Technol.* **2020**, *244*, 116746. [CrossRef]
214. Arzideh, S.M.; Movagharnejad, K.; Pirdashti, M. Ion-solvent interaction of 1-decyl-3-methylimidazolium chloride and isopropanol in a quaternary aqueous two phase system for the efficient partitioning of vanillin and L-tryptophan. *J. Mol. Liq.* **2021**, *332*, 115860. [CrossRef]
215. Lima, Á.S.; Oliveira, B.S.d.; Shabudin, S.V.; Almeida, M.; Freire, M.G.; Bica, K. Purification of anthocyanins from grape pomace by centrifugal partition chromatography. *J. Mol. Liq.* **2021**, *326*, 115324. [CrossRef]
216. Cristina Alves Camêlo, L.; de Souza Dias Santos, G.; Lucena de Souza, R.; Faria Soares, C.M.; Brandão Pereira, J.F.; Silva Lima, Á. Pre-purification of genipin from genipap using aqueous-two-phase systems composed of protic ionic liquids + polymers + water at 298 K and atmospheric pressure. *Sep. Purif. Technol.* **2021**, *256*, 117843. [CrossRef]
217. Wang, Y.; Wang, S.; Liu, L. Extraction of geniposidic acid and aucubin employing aqueous two-phase systems comprising ionic liquids and salts. *Microchem. J.* **2021**, *169*, 106592. [CrossRef]
218. Xu, H.; Li, X.; Hao, Y.; Zhao, X.; Cheng, Y.; Zhang, J. Highly selective separation of acteoside from Cistanche tubulosa using an ionic liquid based aqueous two–phase system. *J. Mol. Liq.* **2021**, *333*, 115982. [CrossRef]
219. Li, M.; Yu, X.; Zhou, C.; Yagoub, A.E.A.; Sun, Y.; Yang, H.; Chen, L. Development of back-extraction recyclability of IL-ATPS for the efficient recovery of syringic and caffeic acid. *J. Mol. Liq.* **2021**, *328*, 115390. [CrossRef]
220. Liu, F.; Chen, L.; Yin, K.; Fan, T.; Yan, Z. Sugars as hydrogen-bond donors tune the phase behavior in a novel liquid–liquid biphasic system formed by hydrophilic deep eutectic solvents and n-propanol. *Fluid Phase Equilibria* **2022**, *556*, 113393. [CrossRef]
221. Wang, Y.; Wang, F.; Jia, M.; Huang, L.; He, Y.; Dong, S. Rapid Extraction of Yam Peel Total Flavonoids in A Cholinium-Based Magnetic Ionic Liquid Aqueous Biphasic System. *Nat. Environ. Pollut. Technol.* **2022**, *21*, 349–354. [CrossRef]
222. Rocha, I.L.D.; da Costa Lopes, A.M.; Ventura, S.P.M.; Coutinho, J.A.P. Selective Separation of Vanillic Acid from Other Lignin-Derived Monomers Using Centrifugal Partition Chromatography: The Effect of pH. *ACS Sustain. Chem. Eng.* **2022**, *10*, 4913–4921. [CrossRef]
223. Mumcu, T.; Seyhan Bozkurt, S. Simultaneous extraction of five phenolic acids in fruits using ultrasound assisted aqueous two phase system based on polyethylene glycol-ionic liquid-sodium carbonate. *Sep. Sci. Technol.* **2022**, *57*, 433–443. [CrossRef]
224. Yin, K.; Chen, L.; Liu, F.; Fan, T.; Yan, Z. Pseudoternary Systems of Deep Eutectic Solvents + tert-Butanol + Water at T = 288.15 K, 298.15 K, and 308.15 K: Liquid–Liquid Equilibrium and Ability for Syringic Acid/Eugenol Separation. *J. Chem. Eng. Data* **2022**, *67*, 200–211. [CrossRef]
225. Li, S.; He, C.; Liu, H.; Li, K.; Liu, F. Ionic liquid-based aqueous two-phase system, a sample pretreatment procedure prior to high-performance liquid chromatography of opium alkaloids. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2005**, *826*, 58–62. [CrossRef] [PubMed]
226. Louros, C.L.; Cláudio, A.F.; Neves, C.M.; Freire, M.G.; Marrucho, I.M.; Pauly, J.; Coutinho, J.A. Extraction of biomolecules using phosphonium-based ionic liquids + K(3)PO(4) aqueous biphasic systems. *Int. J. Mol. Sci.* **2010**, *11*, 1777–1791. [CrossRef]
227. Fan, J.-P.; Cao, J.; Zhang, X.-H.; Huang, J.-Z.; Kong, T.; Tong, S.; Tian, Z.-Y.; Zhu, J.-H.; Ouyang, X.-K. Extraction of Puerarin using Ionic Liquid Based Aqueous Two-Phase Systems. *Sep. Sci. Technol.* **2012**, *47*, 1740–1747. [CrossRef]
228. Pereira, J.F.B.; Ventura, S.P.M.; e Silva, F.A.; Shahriari, S.; Freire, M.G.; Coutinho, J.A.P. Aqueous biphasic systems composed of ionic liquids and polymers: A platform for the purification of biomolecules. *Sep. Purif. Technol.* **2013**, *113*, 83–89. [CrossRef]
229. Passos, H.; Trindade, M.P.; Vaz, T.S.M.; da Costa, L.P.; Freire, M.G.; Coutinho, J.A.P. The impact of self-aggregation on the extraction of biomolecules in ionic-liquid-based aqueous two-phase systems. *Sep. Purif. Technol.* **2013**, *108*, 174–180. [CrossRef]
230. Abdolrahimi, S.; Nasernejad, B.; Pazuki, G. Prediction of partition coefficients of alkaloids in ionic liquids based aqueous biphasic systems using hybrid group method of data handling (GMDH) neural network. *J. Mol. Liq.* **2014**, *191*, 79–84. [CrossRef]
231. Zhang, W.; Zhu, D.; Fan, H.; Liu, X.; Wan, Q.; Wu, X.; Liu, P.; Tang, J.Z. Simultaneous extraction and purification of alkaloids from *Sophora flavescens* Ait. by microwave-assisted aqueous two-phase extraction with ethanol/ammonia sulfate system. *Sep. Purif. Technol.* **2015**, *141*, 113–123. [CrossRef]
232. Keremedchieva, R.; Svinyarov, I.; Bogdanov, M.G. Ionic Liquid-Based Aqueous Biphasic Systems—A Facile Approach for Ionic Liquid Regeneration from Crude Plant Extracts. *Processes* **2015**, *3*, 769–778. [CrossRef]

233. Santos, S.B.; Reis, I.A.O.; Silva, C.P.C.; Campos, A.F.; Ventura, S.P.M.; Soares, C.M.F.; Lima, Á.S. Selective partition of caffeine from coffee bean and guaraná seed extracts using alcohol–salt aqueous two-phase systems. *Sep. Sci. Technol.* **2016**, *51*, 2008–2019. [CrossRef]
234. Santos, P.L.; Santos, L.N.S.; Ventura, S.P.M.; de Souza, R.L.; Coutinho, J.A.P.; Soares, C.M.F.; Lima, Á.S. Recovery of capsaicin from *Capsicum frutescens* by applying aqueous two-phase systems based on acetonitrile and cholinium-based ionic liquids. *Chem. Eng. Res. Des.* **2016**, *112*, 103–112. [CrossRef]
235. Wang, X.; Li, X.; Li, L.; Li, M.; Wu, Q.; Liu, Y.; Yang, J.; Jin, Y. Green determination of aconitum alkaloids in *Aconitum carmichaeli* (Fuzi) by an ionic liquid aqueous two-phase system and recovery of the ionic liquid coupled with in situ liquid–liquid microextraction. *Anal. Methods* **2016**, *8*, 6566–6572. [CrossRef]
236. Sampaio, D.A.; Sosa, F.H.B.; Martins, A.D.; Igarashi-Mafra, L.; Yamamoto, C.I.; Souza, M.O.; Castilhos, F.; Mafra, M.R. Assessment of Sodium Salt Anions (SO42- and NO3-) Influence on Caffeine Partitioning in Polyethylene Glycol and 1-Butyl-3-Methylimidazolium Tetrafluoroborate Based ATPS. *J. Solut. Chem.* **2016**, *45*, 1857–1878. [CrossRef]
237. Cienfuegos, N.E.C.; Santos, P.L.; García, A.R.; Soares, C.M.F.; Lima, A.S.; Souza, R.L. Integrated process for purification of capsaicin using aqueous two-phase systems based on ethanol. *Food Bioprod. Process.* **2017**, *106*, 1–10. [CrossRef]
238. Farias, F.O.; Passos, H.; Lima, Á.S.; Mafra, M.R.; Coutinho, J.A.P. Is It Possible to Create Ternary-like Aqueous Biphasic Systems with Deep Eutectic Solvents? *ACS Sustain. Chem. Eng.* **2017**, *5*, 9402–9411. [CrossRef]
239. Ebrahimi, N.; Sadeghi, R. Propanol—Sugar aqueous biphasic systems as a suitable platform for biomolecules extraction. *J. Chromatogr. A* **2018**, *1581–1582*, 156–167. [CrossRef]
240. Zhou, S.; Wu, X.; Huang, Y.; Xie, X.; Lin, Y.; Fan, H.; Luo, L.; Zhang, W.; Tang, J.Z. Microwave-assisted aqueous two-phase extraction of alkaloids from Radix Sophorae Tonkinensis with an ethanol/Na2HPO4 system: Process optimization, composition identification and quantification analysis. *Ind. Crops Prod.* **2018**, *122*, 316–328. [CrossRef]
241. Li, F.; Li, Q.; Wu, S.; Sun, D.; Tan, Z. Salting-out extraction of sinomenine from *Sinomenium acutum* by an alcohol/salt aqueous two-phase system using ionic liquids as additives. *J. Chem. Technol. Biotechnol.* **2018**, *93*, 1925–1930. [CrossRef]
242. Farias, F.O.; Passos, H.; Sanglard, M.G.; Igarashi-Mafra, L.; Coutinho, J.A.P.; Mafra, M.R. Designer solvent ability of alcohols in aqueous biphasic systems composed of deep eutectic solvents and potassium phosphate. *Sep. Purif. Technol.* **2018**, *200*, 84–93. [CrossRef]
243. Sousa, R.d.C.S.; Pereira, M.M.; Freire, M.G.; Coutinho, J.A.P. Evaluation of the effect of ionic liquids as adjuvants in polymer-based aqueous biphasic systems using biomolecules as molecular probes. *Sep. Purif. Technol.* **2018**, *196*, 244–253. [CrossRef] [PubMed]
244. Ren, J.; Li, Z.; Liu, J.; Pei, Y.; Wang, H.; Wang, J. Choline derivative ionic liquids-based aqueous two-phase systems: Phase diagrams and partition of purine alkaloids. *J. Chem. Thermodyn.* **2018**, *118*, 51–57. [CrossRef]
245. Nie, L.-r.; Song, H.; Yohannes, A.; Liang, S.; Yao, S. Extraction in cholinium-based magnetic ionic liquid aqueous two-phase system for the determination of berberine hydrochloride in Rhizoma coptidis. *RSC Adv.* **2018**, *8*, 25201–25209. [CrossRef] [PubMed]
246. Toledo, M.O.; Farias, F.O.; Igarashi-Mafra, L.; Mafra, M.R. Salt Effect on Ethanol-Based Aqueous Biphasic Systems Applied to Alkaloids Partition: An Experimental and Theoretical Approach. *J. Chem. Eng. Data* **2019**, *64*, 2018–2026. [CrossRef]
247. Neves, C.; Sousa, R.C.S.; Pereira, M.M.; Freire, M.G.; Coutinho, J.A.P. Understanding the Effect of Ionic Liquids as Adjuvants in the Partition of Biomolecules in Aqueous Two-Phase Systems Formed by Polymers and Weak Salting-Out Agents. *Biochem. Eng. J.* **2019**, *141*, 239–246. [CrossRef]
248. Yazdabadi, A.; Shahriari, S.; Salehifar, M. Extraction of caffeine using aqueous two-phase systems containing ionic liquid and sorbitol. *Fluid Phase Equilibria* **2019**, *502*, 112287. [CrossRef]
249. Basaiahgari, A.; Passos, H.; Coutinho, J.A.P.; Gardas, R.L. The influence of zwitterions on the partition of biomolecules in aqueous biphasic systems. *Sep. Purif. Technol.* **2020**, *253*, 117537. [CrossRef]
250. Basaiahgari, A.; Priyanka, V.P.; Ijardar, S.P.; Gardas, R.L. Aqueous biphasic systems of amino acid-based ionic liquids: Evaluation of phase behavior and extraction capability for caffeine. *Fluid Phase Equilibria* **2020**, *506*, 112373. [CrossRef]
251. Jafari, P.; Jouyban, A. Partitioning behavior of caffeine, lamotrigine, clonazepam and oxcarbazepine in a biodegradable aqueous two-phase system comprising of polyethylene glycol dimethyl ether 250 and choline chloride/saccharose deep eutectic solvent. *J. Mol. Liq.* **2021**, *323*, 115055. [CrossRef]
252. Montalvo-Hernández, B.; Rito-Palomares, M.; Benavides, J. Recovery of crocins from saffron stigmas (*Crocus sativus*) in aqueous two-phase systems. *J. Chromatogr. A* **2012**, *1236*, 7–15. [CrossRef]
253. Han, J.; Wang, Y.; Ma, J.; Wu, Y.; Hu, Y.; Ni, L.; Li, Y. Simultaneous aqueous two-phase extraction and saponification reaction of chlorophyll from silkworm excrement. *Sep. Purif. Technol.* **2013**, *115*, 51–56. [CrossRef]
254. Shu, Y.; Gao, M.; Wang, X.; Song, R.; Lu, J.; Chen, X. Separation of curcuminoids using ionic liquid based aqueous two-phase system coupled with in situ dispersive liquid–liquid microextraction. *Talanta* **2016**, *149*, 6–12. [CrossRef] [PubMed]
255. Santos, R.P.; Souza, L.M.; Balieiro, A.L.; Soares, C.M.F.; Lima, Á.S.; Souza, R.L. Integrated process of extraction and purification of betanin from *Opuntia ficus-indica* using aqueous two-phase systems based on THF and sodium salts. *Sep. Sci. Technol.* **2018**, *53*, 734–744. [CrossRef]
256. Ghasemzadeh, B.; Shahriari, S.; Pazuki, G. Efficient separation of curcumin using tetra butyl phosphonium bromide / carbohydrates (sorbitol, fructose) aqueous two-phase system. *Fluid Phase Equilibria* **2019**, *498*, 51–58. [CrossRef]

257. Kurnik, I.S.; Noronha, M.A.; Câmara, M.C.C.; Mazzola, P.G.; Vicente, A.A.; Pereira, J.F.B.; Lopes, A.M. Separation and purification of curcumin using novel aqueous two-phase micellar systems composed of amphiphilic copolymer and cholinium ionic liquids. *Sep. Purif. Technol.* **2020**, *250*, 117262. [CrossRef]
258. Xu, Y.; Wang, Q.; Hou, Y. Efficient Purification of R-phycoerythrin from Marine Algae (*Porphyra yezoensis*) Based on a Deep Eutectic Solvents Aqueous Two-Phase System. *Mar. Drugs* **2020**, *18*, 618. [CrossRef]
259. Wang, Q.; Wei, N.; Wang, Y.; Hou, Y.; Ren, X.; Wei, Q. Single-step purification of C-phycocyanin from *Arthrospira platensis* using aqueous two-phase system based on natural deep eutectic solvents. *J. Appl. Phycol.* **2020**, *32*, 3873–3883. [CrossRef]
260. Morales-García, P.; Calvillo-Muñoz, E.Y.; Lijanova, I.V.; Likhanova, N.V.; Olivares-Xometl, O.; Arellanes-Lozada, P. Synthesis of Ammonium-Based Ionic Liquids for the Extraction Process of a Natural Pigment (Betanin). *Molecules* **2021**, *26*, 5458. [CrossRef]
261. Lu, X.; Lu, Z.; Zhang, R.; Zhao, L.; Xie, H. Distribution of pigments in the aqueous two-phase system formed with piperazinium-based ionic liquid and anionic surfactant. *J. Mol. Liq.* **2021**, *330*, 115677. [CrossRef]
262. Moradi, M.; Mohammadian, M.; Pazuki, G.; Shahriari, S. Partitioning of Crocin in a Novel Aqueous Two-Phase System Composed of a Deep Eutectic Solvent and Acetonitrile. *J. Chem. Eng. Data* **2022**, *67*, 1205–1213. [CrossRef]
263. Tianwei, T.; Qing, H.; Qiang, L. Purification of glycyrrhizin from *Glycyrrhiza uralensis* Fisch with ethanol/phosphate aqueous two phase system. *Biotechnol. Lett.* **2002**, *24*, 1417–1420. [CrossRef]
264. Li, N.; Wang, Y.; Xu, K.; Wen, Q.; Ding, X.; Zhang, H.; Yang, Q. High-performance of deep eutectic solvent based aqueous bi-phasic systems for the extraction of DNA. *RSC Adv.* **2016**, *6*, 84406–84414. [CrossRef]
265. Zhang, X.; Teng, G.; Zhang, J. Ethanol/salt aqueous two-phase system based ultrasonically assisted extraction of polysaccharides from *Lilium davidiivar. unicolor* Salisb: Physicochemical characterization and antiglycation properties. *J. Mol. Liq.* **2018**, *256*, 497–506. [CrossRef]
266. Li, P.; Zhao, P.; Liu, W.; Jiang, Y.; Wang, W.; Bao, L.; Jin, Y.; Li, X. Determination of common ginsenosides in Kang'ai injection by aqueous two-phase extraction with deep eutectic solvents and HPLC-UV/DAD. *Microchem. J.* **2018**, *137*, 302–308. [CrossRef]
267. Santos, J.H.P.M.; Trigo, J.P.; Maricato, É.; Nunes, C.; Coimbra, M.A.; Ventura, S.P.M. Fractionation of Isochrysis galbana Proteins, Arabinans, and Glucans Using Ionic-Liquid-Based Aqueous Biphasic Systems. *ACS Sustain. Chem. Eng.* **2018**, *6*, 14042–14053. [CrossRef]
268. Xu, P.; Wang, Y.; Chen, J.; Wei, X.; Xu, W.; Ni, R.; Meng, J.; Zhou, Y. A novel aqueous biphasic system formed by deep eutectic solvent and ionic liquid for DNA partitioning. *Talanta* **2018**, *189*, 467–479. [CrossRef]
269. Mejía-Manzano, L.A.; Barba-Dávila, B.A.; Vázquez-Villegas, P.; Serna-Saldívar, S.O.; González-Valdez, J. Improved extraction of the natural anticancerigen pristimerin from Mortonia greggii root bark using green solvents and aqueous two-phase systems. *Sep. Purif. Technol.* **2019**, *211*, 667–672. [CrossRef]
270. Cai, C.; Wu, S.; Wang, C.; Yang, Y.; Sun, D.; Li, F.; Tan, Z. Deep eutectic solvents used as adjuvants for improving the salting-out extraction of ursolic acid from Cynomorium songaricum Rupr. in aqueous two-phase system. *Sep. Purif. Technol.* **2019**, *209*, 112–118. [CrossRef]
271. Zhang, X.; Teng, G.; Zhang, J. Deep eutectic solvents aqueous two-phase system based ultrasonically assisted extraction of ursolic acid (UA) from Cynomorium songaricum Rupr. *Chem. Eng. Commun.* **2019**, *206*, 419–431. [CrossRef]
272. Wei, Y.; Hou, B.; Fang, H.; Sun, X.; Ma, F. Salting-out extraction of ginsenosides from the enzymatic hydrolysates of Panax quinquefolium based on ethanol/sodium carbonate system. *J. Ginseng Res.* **2020**, *44*, 44–49. [CrossRef]
273. Han, X.; Li, W.; Ma, X.; Fan, D. Enzymatic hydrolysis and extraction of ginsenoside recovered from deep eutectic solvent-salt aqueous two-phase system. *J. Biosci. Bioeng.* **2020**, *130*, 390–396. [CrossRef] [PubMed]
274. Ruiz, C.A.S.; Kwaijtaal, J.; Peinado, O.C.; van den Berg, C.; Wijffels, R.H.; Eppink, M.H.M. Multistep Fractionation of Microalgal Biomolecules Using Selective Aqueous Two-Phase Systems. *ACS Sustain. Chem. Eng.* **2020**, *8*, 2441–2452. [CrossRef]
275. Ruiz, C.A.S.; Baca, S.Z.; van den Broek, L.A.M.; van den Berg, C.; Wijffels, R.H.; Eppink, M.H.M. Selective fractionation of free glucose and starch from microalgae using aqueous two-phase systems. *Algal Res.* **2020**, *46*, 101801. [CrossRef]
276. Morandeira, L.; Sanromán, M.Á.; Deive, F.J.; Rodríguez, A. Cholinium dipeptide as the cornerstone to build promising separation processes: A simultaneous recovery strategy for microalgae biorefineries. *Sep. Purif. Technol.* **2020**, *250*, 117288. [CrossRef]
277. Athira, K.K.; Gardas, R.L. Insights into the Partitioning of DNA in Aqueous Biphasic System Containing Ammonium-based Ionic Liquid and Phosphate Buffer. *Fluid Phase Equilibria* **2022**, *558*, 113463. [CrossRef]
278. Ruiz, C.A.S.; Cabau-Peinado, O.; van den Berg, C.; Wijffels, R.H.; Eppink, M.H.M. Efficient Fractionation of Lipids in a Multiproduct Microalgal Biorefinery by Polymers and Ionic Liquid-Based Aqueous Two-Phase Systems. *ACS Sustain. Chem. Eng.* **2022**, *10*, 789–799. [CrossRef]
279. Louwrier, A. Model isolations of nucleic acids from prokaryotic and eukaryotic sources using an organic/aqueous biphasic system. *Biotechnol. Tech.* **1999**, *13*, 329–330. [CrossRef]
280. Jiang, B.; Li, Z.-G.; Dai, J.-Y.; Zhang, D.-J.; Xiu, Z.-L. Aqueous two-phase extraction of 2,3-butanediol from fermentation broths using an ethanol/phosphate system. *Process Biochem.* **2009**, *44*, 112–117. [CrossRef]
281. Li, Z.; Teng, H.; Xiu, Z. Aqueous two-phase extraction of 2,3-butanediol from fermentation broths using an ethanol/ammonium sulfate system. *Process Biochem.* **2010**, *45*, 731–737. [CrossRef]
282. Li, Z.-G.; Sun, Y.-Q.; Zheng, W.-L.; Teng, H.; Xiu, Z.-L. A novel and environment-friendly bioprocess of 1,3-propanediol fermentation integrated with aqueous two-phase extraction by ethanol/sodium carbonate system. *Biochem. Eng. J.* **2013**, *80*, 68–75. [CrossRef]

283. Pratiwi, A.I.; Yokouchi, T.; Matsumoto, M.; Kondo, K. Extraction of succinic acid by aqueous two-phase system using alcohols/salts and ionic liquids/salts. *Sep. Purif. Technol.* **2015**, *155*, 127–132. [CrossRef]
284. Zhang, H.; Wang, Y.; Zhou, Y.; Xu, K.; Li, N.; Wen, Q.; Yang, Q. Aqueous biphasic systems containing PEG-based deep eutectic solvents for high-performance partitioning of RNA. *Talanta* **2017**, *170*, 266–274. [CrossRef] [PubMed]
285. Zhao, L.; Lu, F.; Zhang, X.; Wang, Z. Isolation of ionizable red Monascus pigments after extractive fermentation in nonionic surfactant micelle aqueous solution. *Process Biochem.* **2017**, *61*, 156–162. [CrossRef]
286. Quental, M.V.; Pedro, A.Q.; Pereira, P.; Sharma, M.; Queiroz, J.A.; Coutinho, J.A.P.; Sousa, F.; Freire, M.G. Integrated Extraction-Preservation Strategies for RNA Using Biobased Ionic Liquids. *ACS Sustain. Chem. Eng.* **2019**, *7*, 9439–9448. [CrossRef]
287. Ng, H.S.; Wan, P.K.; Ng, T.-C.; Lan, J.C.-W. Primary purification of intracellular *Halomonas salina* ectoine using ionic liquids-based aqueous biphasic system. *J. Biosci. Bioeng.* **2020**, *130*, 200–204. [CrossRef]

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