Deep Eutectic Solvents for the Extraction of Bioactive Compounds from Natural Sources and Agricultural By-Products

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Featured Application: Application of circular economy and green chemistry principles to bioactive compound extraction.

Abstract: In this work, a review about the applicability of eutectic solvents, mainly deep eutectic solvents (DES) and natural deep eutectic solvents (NADES), for the extraction of bioactive compounds from natural products has been carried out. These alternative solvents have shown not only to have high extraction yields but also to be environmentally friendly, exhibiting very low or almost no toxicity, compared to conventional organic solvents. The last trends and main extraction methods that have been most widely used in studies using these emerging solvents have been reviewed, as well as the varied natural sources in which they have been used, including agro-food by-products. Besides the toxicity, biodegradability of these solvents is reviewed. Likewise, different reported bioactivity tests have been included, in which extracts obtained with these ecological solvents have been tested from antioxidant activity analysis to in vivo studies with rats, through in vitro cytotoxicity tests.

1. Introduction

The valorization of agricultural by-products by extracting their bioactive compounds is a very interesting alternative to their incineration or even composting, as addressed by FAO [1] in their proposed food waste recovery hierarchy. Bioactive compounds have been studied intensively due to their biological properties, capable of providing multiple health benefits. Obtaining these components is not limited to the extraction from by-products of agricultural, food, and fishing industries, such as crustaceans' shells, but also from plants, algae, or microalgae by-products, which can correctly contribute to a circular economy based on zero waste. The agro-food industry generates significant amounts of by-products that are normally discarded and can be a severe environmental problem. However, these by-products are a great source of bioactive compounds, including phenolics, proteins, alkaloids, carotenoids, sugars, or lipids, among others. Many of these bioactive compounds have properties that provide multiple health benefits, such as antihypertensive, anticancer, anti-inflammatory, hypoglycemic, antimicrobial, antiviral, antitumor, antithrombotic, hypocholesterolemic, etc.

For applications, mainly in the food, cosmetic and pharmaceutical industries, these compounds are generally extracted using organic solvents or also known as conventional solvents (methanol, acetone, benzene, chloroform, petroleum ether, and hexane), which many times are rejected for being flammable, explosive, poorly biodegradable and for the toxicity they can produce to the final consumer. For these reasons, the use of Generally Recognized as Safe solvents (GRAS) has been proposed. Only a few conventional solvents

are recognized as GRAS, besides, most of them are polar, not capable of solubilizing apolar molecules, making extractions very limited. In addition, due to the climatic drift observed in recent years, there is a significant concern for the environment. Therefore, there is a desire to reduce energy, water and solvent consumption, and carbon emissions, increasing the demand of ecological extraction processes. Green technologies are being tested, taking into account the need to be effective and efficient, increasing throughput, improving process selectivity, and reducing energy consumption. A solution to this problem is the use of alternative solvents: ionic liquid (ILs) solvents, deep eutectic solvents (DESs), and natural eutectic solvents (NADESs), generally reported as green solvents.

Some authors have discussed the application of these solvents for the extraction of bioactive compounds, but they have been focused on specific applications or compounds. This review attempts to explain the role of DESs and NADESs, to extract bioactive compounds from natural products, which have shown not only to have high yields but also to be eco-friendly and to present low or almost zero toxicity and will provide an updated discussion of the most relevant trends in this area [2].

Eutectic solvents are homogenous liquids whose melting points are lower than the individual melting points of the individual components in the mixture. In fact, the name comes from the Greek words "εύ" (eu = well) and "τήξις" (tēxis = melting).

In this category of liquids, we can find mainly two types: ILs and DESs.

Ionic liquids are molten salts that remain liquid at room temperature. Generally, they are the result of the combination of organic cations with organic or inorganic anions. More than 10^8 possible combinations are estimated [3]. Their main characteristic is having lower melting points than their elements. These substances have physicochemical properties that can be easily modified by the appropriate cation/anion combination, providing ideal properties such as thermal stability, polar variability, high boiling points, good dissolving, and extractability. Besides, ILs have low volatility and flammability, which make them safer than organic solvents. Despite this, ILs have faced difficulties since they have been regulated by the Food and Drug Administration (FDA), the Codex Alimentarius, the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO) or the European Food Safety Authority (EFSA), because of the toxicity observed in some anions and cations managed, low biodegradability, and complex purification [2].

Fortunately, emerging solvents, ILs analogs, called DESs, appeared some years ago. They show the interesting characteristics of ILs but present unique advantages. In this sense, they are not always formed through ionic interactions but also hydrogen bonds and other kinds of interactions. DESs include salts and also carbohydrates, amino acids, polyols, and others. DESs easily integrate, are less toxic, and more environmentally friendly than ILs. They are usually composed of hydrogen bond acceptors (HBA), such as quaternary ammoniums, and hydrogen bond donors (HBD) as, for example, urea, carboxylic acids, or amines. Its main advantage above ILs is that they can be easily eliminated. It is because when forming eutectic solvents, no chemical reaction occurs, so their interactions can be broken under appropriated conditions without the necessity of applying complex procedures. DESs are generally composed of asymmetric components, which reduce energy, characterizing the ionic structure's stability, resulting in a lower melting temperature. DESs are characterized by being more viscous than water and conventional organic solvents [4].

When DESs are formed of natural eutectic compounds from plant metabolites or their derivatives, they are referred to as NADESs. The most common are choline chloride (ChCl), citric acid, malic acid, maleic acid, acetic acid, glucose, fructose, sucrose, trehalose, terpenoids or water, among others. NADESs are readily biodegradable and have less toxicity, if not almost zero. NADESs can act as natural solvents (as it happens in natural matrices) [2]. Some examples of NADESs can be seen in Figure 1.

In a global view, DESs and NADESs are prepared by HBDs and HBAs with a specific molar ratio. Each mixture is placed in a flask at temperatures ranging around 50–85 °C, with continuous stirring until a homogeneous liquid appears. After the formation of the NADES, it generally needs water to reduce the viscosity and help the extraction process, as

water facilitates mass transfer. It needs conditioning such as drying and grinding, or even high voltage electric discharges can be used as a pretreatment. In some articles NADESs are prepared in a different way, dissolving individual components in water, then mixing and removing water or not, depending on the subsequent use.
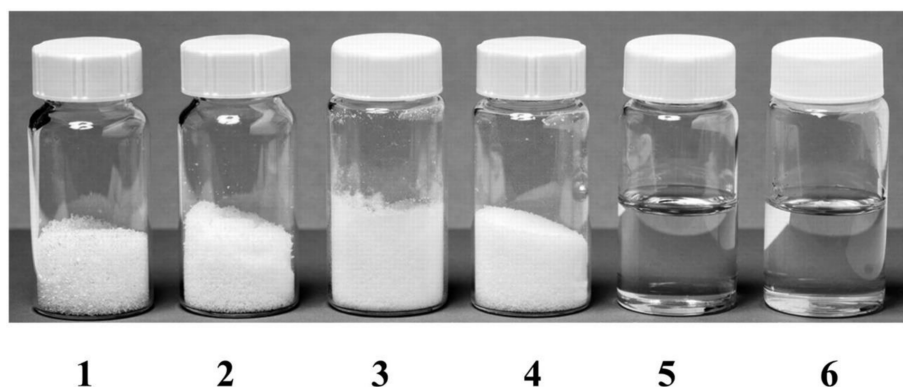

Figure 1. Some examples of natural eutectic solvents (NADESs) and NADESs components. Vial 1, sucrose; vial 2, fructose; vial 3, glucose; vial 4, malic acid; vial 5, sucrose:fructose:glucose (1:1:1, molar ratio); vial 6, sucrose:malic acid (1:1, molar ratio. Reprinted from Choi, Y.H.; van Spronsen, J.; Dai, Y.; Verberne, M.; Hollmann, F.; Arends, I.W.C.E.; Witkamp, G.J.; Verpoorte, R. Are Natural Deep Eutectic Solvents the Missing Link in Understanding Cellular Metabolism and Physiology? Plant Physiol., 2011, 156 (4), 1701–1705 with permission from Oxford University Press [5].

The viscosity of DESs/NADESs also changes significantly depending on their composition and both parameters, nature of components and water can be modified to reach the most adequate extraction conditions. For example, in the study in which the extraction efficiency of phenolic compounds in leaves of *C. cajan* was valuated [6]. Fourteen NADESs were prepared with a molar ratio (1:2) and, for increasing mass transfer, 20% (*v/v*) of water was used in the NADESs solutions. In the experiment, it was found to contain 6978 mg/g stilbenes cajan in stilbene and 4370 mg/g of acid and longistilin C. However, a high excess of water addition can negatively impact the solvent's interactions and the compounds. Changing the water content makes NADESs more polar or less polar if desired. Likewise, the viscosity of these solvents can also change with temperature. When temperature increases, it can cause a decrease in viscosity and better extraction performances. Nevertheless, this increase in temperature must be moderate because it can damage the thermolabile compounds.

2. Toxicity, Bioactivity and Biodegradability

An essential characteristic of DESs, and especially NADEs, is their low toxicity. These solvents can be applied directly in food and pharmaceutical formulations if made with primary metabolites of cells, which have recently been called NADESs [7]. According to Paiva et al. [8], no cytotoxic effect is detected in the components of NADESs.

Proof of their low toxicity is that many of them even lack antimicrobial activity, as demonstrated by the work of Huang et al. [9] in which the toxicity of NADESs was verified against two Gram positive bacteria (*S. aureus* and *L. monocytogenes*) and two Gram negative (*E. coli* and *S. enteritidis*) and no inhibition of microbial growth was detected. However, this is highly dependent on the compounds forming NADESs. In the study by Rodrigues et al. [10], the NADESs used were mono- and diterpenic-based and showed that all the combinations had some toxicity in Caco-2 cells but could also inhibit the proliferation of HT-29 cells. Additionally, all terpenoid-based NADESs were able to inhibit the growth of *S. aureus* and *E. coli*. Likewise, in the study by Zhao et al. [11], there were specific DESs, especially those containing organic acids, which demonstrated an antimicrobial effect against Gram negative and Gram positive bacteria due to their low pH.

However, the application of NADESs in foods must be controlled and deeply studied. Benlebna et al. [12] investigated the toxicity of a NADES composed by betaine:glycerol in rats. The rats were randomized into two groups of six animals each and fed by gavage for 14 days at 9:00 and 17:00 h either with 3 mL of water or 3 mL of NaDES extract (containing 28.01 mg of phenolic compound and 5.89 mg of caffeine dissolved in betaine:glycerol). The selected NADES provided the higher extraction rate. They observed specific adverse effects of oral administration of a high dose of betaine:glycerol. The absorption of the mixture induced mortality in two rats out of six. Additionally, dietary restriction, excessive water consumption, weight loss, adipose tissue loss, hepatomegaly, plasmatic oxidative stress, and increased blood lipid levels were observed. All this occurred because the extract was rich in chlorogenic acids, which have proved beneficial effects. The authors proposed a decomposition into its original constituents in the stomach or while being absorbed, the overall effect results from the individual positive and/or negative effects of each constituent (glycerol, betaine, phenolic compounds). In this case, glycerol is associated with an increase of liver glycogen [13]. Therefore, careful selection of NADES components and their doses is necessary.

One important aspect of DESs to take into account is their own bioactivity. For example, protecting the extracted compounds, thus maintaining the initial biological activity. The study by Na Guo et al. [14] shows how blackberry extracts extracted with ChCl:glucose can behave as a protective agent for anthocyanins. The eutectic solvent behaves as an antioxidant for compounds that are sensitive to the environment (oxygen, light, temperature, and pH). The proposed form of protection is based on the hydrogen bond donor (HBD) interaction and the hydrogen bond acceptor (HBA) that form the NADES which can behave as a substitute in the anion or cation exchange when the compounds are exposed to extreme conditions. As a result of this study, a decrease in unstable substances' degradation rate was observed compared to extracts obtained using conventional solvents.

From an environmental point of view, in general, DESs and NADESs are considered green solvents due to their high biodegradability and lower bacterial toxicity compared to traditional solvents. For example, ChCl, the most used in the research of DESs as an HBA, has very biodegradable characteristics, it is not toxic, and it is low cost since we can find this amino acid salt naturally in the lipid membrane of many cells. The biodegradability of NADESs was studied by Zhao et al. [11]. With this aim, the DESs were added to an aqueous medium containing oxygen. The mixture was inoculated in microorganisms present in the lake water in order to be able to measure the biodegradation value in a set time interval. Based on the closed bottle test, the biodegradability of sodium benzoate (reference substance) was 62.8% on day 14, while that of all DESs tested was >69.3% after 28 days. The order of biodegradability found was DESs based on amines ≈ DEs based on sugars > DESs based on alcohols > DESs based on acids. Therefore, the DESs considered in this study could be considered green or biodegradable solvents.

3. DESs for the Extraction of Bioactive Compounds from Plants, Fruit and Vegetables

Most of studies using DESs as extraction solvents to recover bioactive phytochemicals are mainly based on plants, although other natural sources such as vegetables, fruits and by-products from food and agro-food industry have also been investigated. In this section some recent applications of DESs to extract bioactive compounds from plants, as well as fruit and vegetables will be reviewed. As summarized in Table 1, several combinations of DESs have been tested for their ability to extract bioactive compounds from different plants, covering a broad range of phytochemicals such as flavonoids, phenolic acids, anthocyanins, and alkaloids as they will be seen below. It is important to remark that most of these applications have not been developed as analytical tools prior to analysis. Otherwise, the aim of these works is directed to test the possibilities of DES and NADES as extraction solvents to achieve greenest separations. In this section, the main bioactive compounds extracted using DESs are summarized along with the best extraction conditions for each use.

Table 1. Some examples of recent applications of DESs for the extraction of bioactive compounds from plants, fruits and vegetables.

Sample	Bioactive Compounds	DES (Molar Ratio; Amount; Water Content)	Extraction Procedure	Analytical Technique		Comments	Ref.
Pueraria lobata Kudzu root	Puerarin, daidzein, genistein, vitexin, 4-hydroxyflavone	ChCl: citric acid (−)	UAE	HPLC-UV	-	Evaluation of bioactivity against diarrhea, diabetes and cardiovascular diseases	[15]
Platycladi Cacumen	Flavonoid glycosides (micricitrin and quercitrin) and biflavone aglycones (amentoflavone and hinokiflavone)	ChCl:laevulinic acid (1:2; -; -)	UAE	HPLC-UV	-	Study of therapeutic efficacy: spasmolytic, antiphlogistic, antioxidant, antiallergic and diuretic properties	[16]
Ginkgo biloba leaves	Quercetin, kaempferol, and isorhamnetin	ChCl/levulinic acid (1:2; -; 40% (*w/w*) water)	SLE and UAE	HPLC	-	Study of the inhibition of the onset of dementia, slowing down of cognitive decline, reduction of the incidence of cardiovascular diseases	[17]
Herba Epimedii	Flavonoids: Icarrin, IcarisdII, Epidimcdin A, Epimcdin B, and Epimcdin C	L-proline: ethylene glycol (1:4; -;-)	UAE	HPLC-UV	-	Evaluation of anti-osteoporosis, antioxidant and antitumor activities	[18]
Peumus boldus leaves	Alkaloids and phenolic compounds	ChCl-lactic acid and proline:oxalic acid(−)	UAE	HPLC-PDA-ESI-IT/MS HPLC-QTOF-MS/MS	-	Evaluation of renoprotective activity and anticancer properties	[19]
Sophora japonica L. Flos sophorae—dried flowers of *Sophora japonica* L.	Quercetin, kaempferol and isorhamnetin glucoside	L-proline:glycerol (2:5; -: -)	SLE and UAE	LC-UV UHPLC-QTOF-MS	-	Evaluation of the vasodilation, antitumor and antiplatelet bioactivity	[20]
Lycium barbarum L. fruits	Chlorogenic acid, morine, luteolin, coumaric acid, ferulic acid, hyperoside, rutin, myricetin, quercitrin, apigenin	ChCl:p-toluene sulfonic acid (1:2; -)	SLE and UAE	HPLC-UV	-	Evaluation of antioxidant, anticancer, anti-inflammatory, antimicrobial, antiviral, antitumor, and neuroprotective properties	[21]
Carthamus tinctorius L.	Yellow hydroxysafflor, cartormin, carthamin, tripcoumaroylspermidines	75% (*v/v*) proline:malic acid (-;-; 75% (*w/w*) water); sucrose: ChCl (-;-; 75% (*w/w*) water)	SLE	HPLC, NMR, and MS Analysis	-	Evaluation of antioxidant, antidepressant, antiviral activity	[22]

Table 1. *Cont.*

Sample	Bioactive Compounds	DES (Molar Ratio; Amount; Water Content)	Extraction Procedure	Analytical Technique	Comments	Ref.
Catharanthus roseus	Anthocyanins	Lactic acid:glucose, and 1,2-propanediol:ChCl(−)	UAE and UAEH	HPLC-DAD, UHPLC-TOF-MS	- Evaluation of antioxidant and anti-inflammatory agents, prevention of certain cardiovascular diseases and cancer	[23]
Rosmarinus officinalis L.	Rosmarinic acid, caffeic acid, 7-ethylrosmanol, rutin, naringin, ferulic acid	Glycerol:ChCl (1:2; -; 10% (*w/w*) water); lactic acid:ChCl (1:3; -; 10% (*w/w*) water); 1,2-propanediol:ChCl (1:2; -; 10% (*w/w*) water); oxalic acid:ChCl (1:1; -; 10% (*w/w*) water)	UAE	HPLC-DAD	- Evaluation of antioxidant, anti-inflammatory, anticancer, antiviral properties	[24]
Fructus Mori Fresh mulberry (*Fructus Mori*)	Vitamins, minerals, phenolic acids, flavanols and anthocyanins: cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside	ChCl:citric acid:glucose (1:1:1; -; 10% (*w/w*) water)	HSH-CBE	HPLC-UV	- Evaluation of antioxidants and hypocholesterolemic properties	[14]
Leaves of *Cajanus cajan*	Polar, polar-weak phenolic compounds	ChCl:maltose (1:2; -; 20% (*w/w*) water)	MAE	UHPLC-UV	- Study of therapeutic effects on sickle cell anemia, plasmodiosis, and diabetes	[6]
Sophora japonica flower buds	Routin	ChCl:triethylene glycol (1:4; -; -)	SLE	HPLC-UV	- Extract used to treat hypertensive brain hemorrhage	[11]

DAD: diode array detector; ESI: electrospray ionization; HPLC: high performance liquid chromatography; HSH-CBE: high-speed homogenization and cavitation-burst extraction; IT: ion trap; LC: liquid chromatography; MAE: microwaves assisted extraction; MS: mass spectrometry; MS/MS: tandem mass spectrometry; NMR: nuclear magnetic resonance; PDA: photo diode array; QTOF: quadrupole time-of-flight; SLE: solid–liquid extraction; UAE: ultrasound-assisted extraction; UAEH: ultrasound-assisted extraction with heating; UHPLC: ultra-high performance liquid chromatography.

Among the most studied compounds to be extracted using DESs are flavonoids, they are a family of polyphenols with well-known health promoting properties. Typical organic solvents for flavonoids extraction are methanol, ethanol, acetone, and ethyl acetate, as these compounds are barely soluble in water. In particular, isoflavones are dietary phytoestrogens that have been in the limelight as potential antidiabetic therapeutics. Extraction of isoflavones such as puerarin, daidzein, genistein, vitexin and 4-hydroxyflavone from dried kudzu root was carried out using a NADES comprising ChCl and citric acid (1:2, mol/mol) in an ultrasonic bath at 50 °C for 3 h [14]. Flavonoid glycosides and aglycones were also extracted using different combinations of ChCl-, betaine-, and l-proline-based DESs, showing greater extraction yield with ChCl-laevulinic acid (ChCl/LA) than using conventional solvents. The recovery of extracted flavonoids from DESs was carried out using macroporous resin LX-38 [16]. Similarly, ChCl/LA mixtures containing 40% (*w/w*) water were used to extract Ginkgo flavonoids at a solvent to solid ratio of 10:1 (*v/w*) with stirring at 50 °C. Under this greener extraction approach, higher extraction yields were obtained compared to the traditional hydroalcoholic extraction mixture (70% ethanol in water). This time, the recovery of Ginkgo flavonoids in the DES extraction solution was efficiently achieved using macroporous resin AB-8 (yield: 93.7%) [17]. Other ChCl-based DESs, in combination with various hydrogen-bond donors were investigated systematically to extract rutin from the flower buds of *Sophora japonica*. ChCl/triethylene glycol containing 20% water was the most efficient combination [11]. Alternative tailor-made DES based on L-proline and glycerol (2:5) was more effective than methanol for extraction of quercetin, kaempferol, and isorhamne-tin glycosides from Flos sophorae, whereas L-proline and ethylene glycol mixture (1:4) was suitable for extraction of the main active compounds from *Herba Epimedii*, including flavonoids such as icariin, icarisid II, epimedin A, epimedin B and epimedin C [18]. Despite phenolic compounds, other bioactive phytochemicals such as alkaloids have been obtained from plant sources using DESs. Thus, proline-oxalic acid (1:1) with 20% water was the most promising solvent to attain higher extraction yields of boldine, one of the most representative alkaloids in P. boldus leaves [19].

Regarding extraction techniques used in most of these works, solid–liquid extraction assisted by ultrasonication (UAE) or microwaves (MAE) is the most commonly used extraction procedure. UAE is an energetic extraction procedure that can easily break down the structure of cell walls, releasing intracellular substances containing the bioactive compounds. This method is easy and inexpensive and has many advantages such as improved efficiency, reduced extraction time, low solvent consumption, low process temperature, and a high mechanization level compared to conventional extraction methods [25]. DESs have also been used in MAE for active compounds extraction [6], however, this technique is not satisfactory for the extraction of unstable substances because they may cause local overtemperature and require expensive equipment or high energy demand. Alternative extraction techniques based on integrative high-speed homogenization and cavitation-burst extraction (HSH-CBE) technique have been successfully applied to the pretreatments of mulberry to extract anthocyanins [14]. HSH-based procedures can smash fresh plant materials under continuous strong shear and thrust forces, leading to the release of intracellular constituents. Other sample preparation techniques such as homogenizer (HAE), and high hydrostatic pressure (HHPAE) assisted extractions were also applied to phenolic compounds recovery from olive pomace [26].

The last step in all these works is the analysis. In this sense, DESs are normally separated from the target compounds to avoid chromatographic interferences. After extraction with the convenient DES combination, samples are frequently centrifuged and filtered before analysis and bioactivity testing. However, the recovery of extracted bioactive compounds is challenging. Various methods such as supercritical carbon dioxide, antisolvents, and recrystallisation have been studied for the recovery of extracted compounds [20]. Other works reported the recovery of extracted flavonoids from DESs using ultra-pure water as an efficient antisolvent. After separation, water was evaporated by heating at 50 °C under high vacuum to obtain the DESs for subsequent uses [21].

Sometimes, centrifuged samples are evaporated to remove water and even the NADESs or DESs themselves [14]. However, very often DESs cannot be evaporated due to the high viscosity and extremely low volatility of their components. When necessary, target compounds can be recovered from DESs using macroporous resin column chromatography (e.g., LX-38, AB-8, HPD-450, HP-20) [15,16,22]. Thus, the obtained extracts are passed through a prehydrated column and after adsorption, the loaded column is first washed with sufficient deionized water until all the DESs are washed away, then the phenolic compounds can be eluted with a small volume of the most appropriate organic solvent (e.g., methanol). Alternatively, DES extracts are frequently diluted with deionized water to facilitate chromatographic analysis and subsequent spectroscopic or spectrometric measurement by reducing sample viscosity and ionic strength [27]. In fact, many studies do not report solvent removal, performing the analysis and the bioactive characterization of the extracted compounds together with the DESs. Thus, NADESs containing sugars are reported to showed negligible interference in the Folin–Ciocalteu and DPPH assays [26].

HPLC-based methods are the most widespread used in literature for the analysis of DES extracts from plants and vegetables. In particular, methods based on reverse-phase LC with C_{18} reverse-phase columns are the most reported. Although UV detector is the most popular choice for quantitative analysis, MS detectors based on IT-MS/MS and QTOF (high resolution MS) are powerful alternatives for phytochemical profiling and characterization of bioactive compounds. For instance, Torres et al. evaluated the presence of the main alkaloids and phenolic compounds present in *P. boldus* leaf DES extracts by HPLC-DAD-IT-MS/MS and HPLC-(QTOF)-MS/MS [19]. In another work, Dai et al. used HPLC chromatograms at 520 nm of some of the tested NADESs to obtain the qualitative profiles of anthocyanins of purple and orange petals DES extracts, which were further investigated for major anthocyanis by UPLC-MS [23]. Similarly, LC-UV analysis was used for quantification of extracted flavonoids whereas UHPLC-Q-TOF-MS was performed for qualitative analysis of natural products existing in *Flos sophorae* [20].

However, not only chemical analyses are carried out for extracts obtained using DESs or NADESs, also functional analyses are done to test their bioactivity. Regarding the bioactivity testing reported for DES extracts, the antioxidant activity assays (i.e., TPC, DPPH and FRAP) are the most widespread used to evaluate the phenolic content and antioxidant capacity, mainly due to the extraction of antioxidant polyphenols. Barbieria et al. demonstrated that chloride-based DESs can be used as green solvents in the extraction and stabilization of phenolic compounds from *Rosmarinus officinalis* L. The capacity of DESs to stabilize phenolic compounds can be explained by its intermolecular interactions, mainly due to the hydrogen bonds between the phenolic acids found in rosemary extracts and the solvent. This interaction reduces oxidative degradation by decreasing the movement of solute molecules and reducing contact with oxygen at the DES and air interface [24]. In this line, Na Guo et al. also showed how blackberry extracts with NADESs can behave as a protective agent for anthocyanins extracted from blackberry. As a result of this study, a decrease in unstable substances' degradation rate was observed compared to extracts made with conventional solvents [14]. DES extracts have also been reported to exhibit several therapeutic effects. For instance, Duru et al. evaluated the antidiabetic effect of kudzu (*Pueraria lobata*) extracts obtained with a ChCl and citric acid NADES, with 20% water. These isoflavones-rich extracts, mainly containing puerarin, daidzein and denistein were administered to diabetic Wistar rats treated at 100 or 200 mg/kg for 28 days. There was a general improvement in weight in diabetic rats treated with the extract at a 200 mg/kg dose. The isoflavone-rich extract stimulated the pancreatic β-cell regeneration in a dose-dependent effect and showed no observable toxic impact on either the kidney or the pancreas. Furthermore, extraction with NADESs had the advantage of being able to be administered directly without the need to evaporate the residual solvent [14].

4. Valorization of Agricultural By-Products Using DESs

As it has been indicated in the introduction section, agricultural by-products have surged as an important source of bioactive compounds which bring about the possibility of valorization of these products to be applied in the food industry, for cosmetic, nutraceutical or pharmacological applications [28]. In this context, research has been done in order to develop suitable procedures to effectively extract the desired bioactive compounds from these sources. However, most of these procedures involve conventional extractions in which high quantities of toxic solvents are used. For this reason, important effort is directed towards the design and development of greener methodologies that not only contribute with sustainability from a wide perspective but also that do not compromise the suitability of the obtained products to be subsequently used in the mentioned sectors by the use of toxic materials that can risk the safety of consumers [29,30]. Different action lines based on the principles of green chemistry have been carried out. Among them, the application of DESs as innovative green solvents has sharply increased in recent years as a consequence of the already mentioned advantages of the use of DESs as extraction solvents.

As can be seen in Table 2, in which some examples of recent applications of DESs for the extraction of bioactive compounds from agricultural by-products have been compiled, most applications developed so far are based on NADESs due to the lower or absent toxicity and higher biocompatibility of this group of DESs. The applications have been focused on the extraction of phenolic compounds [30–36], although others such as proteins, polysaccharides, flavonoids, carotenoids, lignans and organic acids have been also investigated [30–33,35,37,38] in vegetable, fruit and plant by-products.

Considering the solid nature of most samples evaluated, solid–liquid extraction (SLE) has been the preferred procedure, although the assistance of ultrasounds (UAE), or microwaves, MAE, were necessary in most cases in order to favor the interaction of analytes–solvent. As an example, Bosiljkov et al. [38] proposed the application of UAE using ChCl-based NADESs for the effective extraction of anthocyanins from wine lees providing a reduction of energy consumption, higher yields, and lower time than conventional nonassisted extraction. In addition, the authors considered the addition of water and the increase of the temperature in order to decrease NADES viscosity and favor the extraction process since the high viscosity difficult cavitation process in UAE resulted in a lower extraction efficiency [33]. In this case the temperature was set at 35 °C to avoid analytes degradation whereas the water content was 50% since it enhances the extraction efficiency, however, higher amount resulted to be unfavorable due to the weakening of the solvent stability. Apart from the advantages of using these solvents instead of conventional toxic ones, NADESs also have shown to provide a positive effect on the application of the assisted procedure in comparison with commonly used solvents. It is the case of the work developed by Kantar et al. [36] in which the extraction of polyphenols from grapefruit peels was investigated using high voltage electrical discharges (HVED) followed by SLE at 50 °C using NADESs and conventional solvents, prior to determination by HPLC-DAD. Results showed that, among the different solvents evaluated (i.e., water, water/ethanol, glycerol, water/glycerol and NADESs based on ChCl, lactic and tartaric acids, glycine, glucose and sodium and ammonium acetates) when glycerol or lactic acid:glucose in a molar ratio (5:1) were applied, the energy applied at the HVED could be reduced up to six times, obtaining the same extraction kinetic and yields, which clearly favor the green character of the methodology.

Table 2. Some examples of recent applications of DESs for the extraction of bioactive compounds from agricultural and food industry by-products.

Sample (Amount)	Bioactive Compound	DES (Molar Ratio; Amount; Water Content)	Extraction Procedure	Analytical Technique	Extraction Efficiency	Comments	Ref.
Aegle marmelos Bael pulp	10 phenolic acids, 6 flavonoids and cinnamic acid	ChCl:oxalic acid (1:1; 25 mL; 25% water (v/v))	UAE	HPLC-DAD	>60 [b]	- Other molar ratios were tested: 1:2 and 2:1. - Comparison with conventional extraction. - DES removal prior to analysis.	[33]
Bamboo shoots tip, basal and sheath bamboo shoots	Proteins	ChCl:levulinic acid (1:6; 40% (w/w) water; -)	SLE	Spectrophotometry	39 [a]	- Higher or similar extraction yields than using conventional solvents. - Sample:solvent ratio (30–70 mg/mL).	[39]
Camellia oleifera Abel. seed cake	Polysaccharides	ChCl:EtGly (1:2; 3 mL; 30% (w/w) water)	UAE	Spectrophotometry	85 [b]	- Re-extraction in EOPO. - Seventeen DESs were investigated. - Evaluation of antioxidant activity.	[37]
Eucalyptus leaves	26 phenolic compounds (tentatively identified)	ChCl:EtGly (1:2; 20 mL; 20% water (v/v))	SLE	UHPLC-(ToF)-MS/MS	-	- SLE, EAE, MAE and UAE with conventional solvents were evaluated. - Other DESs were investigated: ChCl:xylitol (5:1), ChCl:glucose (1:1) and citric acid:glucose (1:1). - Evaluation of TPC and TFC. - Evaluation of antioxidant capacity.	[30]
Grape and olive pomace	2 phenolic acids, 2 phenolic alcohols, vanillin (phenolic aldehyde), 11 flavonoids and pinoresinol	ChCl:citric acid (2:1; 10 mL; 30% water (v/v))	UAE/MAE	HPLC-DAD	-	- Evaluation of biological activity. - Comparison with ethanol extracts.	[34]

Table 2. *Cont.*

Sample (Amount)	Bioactive Compound	DES (Molar Ratio; Amount; Water Content)	Extraction Procedure	Analytical Technique	Extraction Efficiency	Comments	Ref.
Hojiblanca olive leaves Hojiblanca cultivar (by-products)	48 phenolic compounds	ChCl:EtGly (1:2; 1.5 mL; 43% (*w/w*) water)	MAE	HPLC-DAD-(ToF)-MS		- Total phenol index was evaluated. - Other HBDs were evaluated: polyalcohols, three organic acids, one sugar and urea. - Comparison with conventional extraction.	[28]
Manilkara zapota Sapodilla pulp	7 phenolic acids and 4 flavonoids	ChCl based-DESs (1:1; 7 mL; 25% water (*v/v*))	UAE	HPLC-UV	71–86 [b]	- Different HBDs were tested: carboxylic acids, alcohols and urea. - Evaluation of TPC, TFC and TAC. - Evaluation of antioxidant and antimicrobial activities. - Comparison with conventional extraction. - The DES was reused (three times).	[32]
Olive, onion, tomato, and pear food by-products	4 phenolic acids, 2 phenolic alcohols, 6 flavonoids, oleuropein (phenolic secoiridoids) and cinnamic acid	Lactic acid:glucose (5:1; -; 15% water (*v/v*))	UAE	HPLC-DAD	82–110 [b]	- Other DESs composed of citric acid, glucose and fructose were tested. - Sample:solvent ratio: 75 mg/mL was used. - Stability tests were carried out. - Comparison to conventional extraction.	[35]
Punica granatum pomegranate peel	Proteins and other phenolic compounds	ChCl:acetic acid:water (1:1:10; 5 mL; -)	UAE	HPLC-(Q-ToF)-MS	-	- Comparison with PLE. - Evaluation of antioxidant, hypocholesterolemia, and antihypertensive capacities.	[31]

Table 2. *Cont.*

Sample (Amount)	Bioactive Compound	DES (Molar Ratio; Amount; Water Content)	Extraction Procedure	Analytical Technique	Extraction Efficiency	Comments	Ref.
Vitis vinifera Grapefruit peels	Polyphenols	Lactic acid:glucose (5:1; -;-)	HVED, SLE	HPLC-DAD		- Comparison with other solvents. - Liquid:solid ratio: 10. - Other DESs were tested. - Consumption. - Evaluation of Zp polyphenol extraction index and TPC.	[36]
Wine lees	Anthocyanins	ChCl:malic acid (1:1; -; 35.4% (*w/w*) water)	UAE	HPLC-DAD		- Comparison with conventional extraction. - Ratio sample: solvent (33.3 mg/L). - Other HBDs were tested: citric acid, oxalic acid, glucose, fructose, xylose, glycerol.	[38]

[a] yield g of analytes/g of matrix; [b] recovery (%). BTBAB: benzyl tributyl ammonium bromide; BTBAC: benzyl tributyl ammonium chloride; BTEAC: benzyltriethylammonium chloride; BTMAC: benzyl trimethyl ammonium chloride; ChCl: ChCl; COSMO-RS: conductor-like screening model for real solvents; DAD: diode array detector; DES: deep eutectic solvent; EAE: enzyme assisted extraction; EDS: energy-dispersive X-ray spectrometry; EOPO: ethylene oxide-propylene oxide; EtGly: ethylene glycol; FTIR: Fourier transform infrared spectroscopy; GC: gas chromatography; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor; HPLC: high performance liquid chromatography; HVDE: high voltage electrical discharge; ICP: inductively coupled plasma; LLE: liquid–liquid extraction; MAE: microwave assisted extraction; MS: mass spectrometry; MS/MS: tandem mass spectrometry; OES: optical emission spectroscopy; PLE: pressurized liquid extraction; PDA: photodiode array detector; Q: single quadrupole; QqQ: triple quadrupole; SEC: size exclusion chromatography; SEM: scanning electron microscopy; SLE: solid–liquid extraction; TAC: total anthocyanin content; TBAB: tetrabutylammonium bromide; TBAC: tetrabutylammonium chloride; TFC: total flavonoid content; TG: thermogravimetric analysis; ToF: time of flight; TOMAC: trioctylmethylammonium chloride; TPC: total phenolic content; UAE: ultrasound assisted extraction; UA-LLME: ultrasound-assisted-liquid–liquid microextraction; UA-SLE: ultrasound-assisted-solid–liquid extraction; UHPLC: ultra-high performance liquid chromatography; UV: ultraviolet.; VA-DLLME: vortex assisted dispersive liquid–liquid microextraction; XRD: X-ray diffraction.

Regarding the type of DES applied in this area, the use of ChCl-based NADESs has been the most extended combined with different types of HBDs, although combinations of lactic acid and glucose also have demonstrated their suitability for the extraction of phenolic and acidic compounds [35,36]. In those works, in which the nature of the DES was evaluated, results demonstrated the relevance of the type of DES on the extraction performance, as well as the activity of the obtained extracted. An exhaustive study in this sense was developed by Saha et al. [33]. In this case, the authors evaluated the influence of the HBD (i.e., carboxylic acid, polyol and amide) components in ChCl-based NADESs on the UAE of phenolic acids and flavonoids from Sapodilla pulp (natural chewing gum from *Manilkara zapota* tree), as well as on the storage stability, radical-scavenging and antimicrobial properties of the extracts. Results indicated a larger extraction yield for acid HBDs due to the lower pH and higher polarity of the resulting DESs which favored their interaction with phenolic compounds and provided extraction efficiency higher than for conventional acetone/water (5/5, *v*/*v*) solvent. Moreover, it was observed that the higher viscosity of DESs with respect to conventional solvents, which reduced air incorporation, and the H-bonding interaction of polyphenols with DESs, allow further stability of the extracts when acid-based DESs were applied. Antioxidant activity resulted in agreement with the extraction capacity of the evaluated DESs. Therefore, the higher activity was found for oxalic-based NADES that was also the one with the highest extraction efficiency. With respect to antimicrobial activity, the authors found that in this case both the phenolic content and the solvent provided antimicrobial capacity, independently. Besides, acid and urea based NADES extracts showed considerably higher activity and it was fundamentally associated with the own solvents instead the phenolic content of the extract. Similar results were found by Hernández-Corroto et al. [31] for the antioxidant capacity of pomegranate (*Punica granatum*) peel extract obtained using ChCl:acetic acid:water (1:1:10). In this work the authors also highlight the influence of the biological activity provided by the organic acid-based DES on the extract containing the bioactive compounds, which support the application of this sustainable strategy with respect to others also applied such as pressurized liquid extraction (PLE) using conventional solvents. Thus, these results reinforce the suitability of the products to be used in food or pharmaceutical applications without demanding further purification steps.

However, despite the advantages that this aspect could provide in terms of application on the mentioned fields, the innate bioactivity of the own NADESs can be a limitation to evaluate the activity associated with the extracted bioactive compounds. In these cases, the application of chromatographic techniques results essential to evaluate the content of the analytes of interest. Additionally, on some occasions the solvent was removed at the end of the extraction in order to avoid not only their influence on the activity studies, but also due to the incompatibility with commonly used chromatographic and detection systems. With this aim, solid phase extraction clean-up process or thermoseparating aqueous two-phase system using a copolymer have been applied [33,37]. In both cases, bioactive compounds were collected in aqueous or aqueous-organic solvents prior to being analyzed by chromatographic techniques [33] or using activity studies [37].

5. Using DES to Extract Bioactive Compounds from Other Natural Sources

An exhaustive revision of the literature shows that, apart from vegetables, plants, fruits or agricultural by-products, other natural matrices have also served as source of valuable compounds and as occur with previous sources, the use of NADESs as extraction solvents has been also carried out. In this sense, oils obtained from citric, cereals, olives, and palm [40–46] have been the products most widely evaluated using DESs, although flour [47], eggs [48] and milk [49] as well as nonagricultural by-products [10,50] have been also investigated. As can be seen in Table 3, the studies were focused on the search of different and varied types of molecules including phenolic compounds [40–42,46], flavonoids [40,43], terpenoids [10,42,44], proteins [48], lignans [45] or minerals [50].

Table 3. Some examples of recent applications of DESs for the extraction bioactive compounds from other natural sources.

Sample (Amount)	Bioactive Compound	DES (Molar Ratio; Amount; Water Content)	Extraction Procedure	Analytical Technique	Extraction Efficiency	Comments	Ref.
Brown crab and shrimp shells and *H. pluvialis*	Astaxanthin	Menthol:myristic acid (8:1; 2.5 g; -)	SLE	HPLC-UV		- Comparison with Soxhlet extraction. - Cytotoxicity, antiproliferative and antimicrobial studies were carried out. - Other DESs were tested: perilyl alcohol: camphor, DL-menthol: perilyl alcohol, DL-menthol: camphor, DL-menthol: eucalyptol.	[10]
Citrus essential oil	Linalool	TBAC:analyte (20:1; -)	Heating and stirring	GC	99 [b]	- Other HBAs were also evaluated: seven quaternary ammonium salts. - Deterpenation was carried out. - A back extraction step was carried out. - COSMO-RS was applied.	[42]
Citrus essential oil	Linalool	ChCl:glycerol (1:2; -)	LLE	GC-(Q)-MS	-	- Deterpenation was carried out. - The addition of water was evaluated. - Different sample to solvent ratios were investigated (1:2, 1:1, and 2:1, 3:1, 4:1 and 5:1 wt). - COSMO-RS was applied.	[44]
Crude palm oil	Tocopherols and tocotrienols	ChCl:malonic acid (1:1; 30 g)	LLE	HPLC-PDA	$1.08 \cdot 10^{-2}$ [a]	- Other HBDs were evaluated: - DES was diluted in methanol. - Different ratios of sample/DES were evaluated. - Different behaviors were found for tocopherols and totrienols. - DES was recycled and reused.	[46]
Fish scales	Hydroxyapatite	ChCl:glycerol (1:2; 4.5 g;-)	SLE	FTIR, XRD, EDS, ICP-OES, SEM, TG and particle size distribution analysis	48	- Other HBDs were evaluated: citric and acetic acids.	[50]

Table 3. *Cont*.

Sample (Amount)	Bioactive Compound	DES (Molar Ratio; Amount; Water Content)	Extraction Procedure	Analytical Technique	Extraction Efficiency	Comments	Ref.
Olive oil	2 phenolic alcohols, 2 flavonoids and 6 phenolic metabolites	ChCl:xylitol:water (2:1:3; 14 g)	LLE	HPLC-DAD	-	- DES was removed using a resin prior to analysis.	[40]
Olive oil	4 phenolic acids and alcohols, 2 flavonoids, 6 phenolic metabolites and enolic acid	ChCl-based DESs (-; 14 g)	LLE	HPLC-DAD and HPLC-(Q-ToF)-MS	-	- Other HBDs were also evaluated: sugars, alcohols, carboxylic acids, and urea. - Different molar ratios were studied, including 1:1, 1:2, 1:5, 2:1, 4:1 and 1:1:1. - DES was removed prior to analysis. - Comparison with conventional solvent extraction.	[25]
Powdered and lyophilized milk	3 seleno amino acids	Lactic acid:glucose (5:1; 3.09 mL; 25% (*v*/*v*) water)	UAE	HPLC-ICP-MS	90–109 [b]	- Citric acid: glucose and fructose:citric acid were evaluated.	[49]
Quail egg	Immunoglobulins	BTBAC: glycerol (1:2; 500 µL)	UA-LLME-preparative HPLC	SEC-UV	85 [b]	- Other HBAs were evaluated: BTBAB, TBAB, BTMAC and ChCl. - Sample:solvent ratio (85% *v*/*v*).	[48]
Sesame oil	3 lignans	ChCl:*p*-cresol (1:2; 400 µL)	UA-LLME	HPLC-UV	97–120 [b]	- Other HBDs were evaluated: phenol, *o*-cresol, *m*-cresol, catechol and 4-methoxyphenol. - Comparison with polyol-based DESs. - Comparison with conventional LLE.	[45]

Table 3. *Cont.*

Sample (Amount)	Bioactive Compound	DES (Molar Ratio; Amount; Water Content)	Extraction Procedure	Analytical Technique	Extraction Efficiency		Comments	Ref.
Tea seed oil	31 phenolic compounds identified, 25 phenolic compounds quantified	ChCl:glycerol (1:2; 6 g)	LLE	UHPLC-(QqQ)-MS/MS; UHPLC-Q-ToF-MS/MS	8.4–$2.7 \cdot 10^{-5}$ [a]	- - - -	Other HBDs were also evaluated: xylitol (with water addition), malonic acid, EtGly and propylene glycol. Different molar ratios were investigated: 1:1, 1:2, 2:1 and 1:1:1. Comparison with conventional solvents extractions. DES was removed using a resin prior to analysis.	[41]
Wheat flour	Folic acid (vitamin B9)	TOMAC:isoamyl alcohol (1:4; 150 μL)	SLE, VA-DLLME	HPLC-UV	92–100 [b]	- - -	BTEAC-based DESs with thymol and octanoic acid as HBDs, at a molar ratio 1:2, were also investigated. Other molar ratios were evaluated: 1:1, 1:2, 1:3, 1:4 and 1:5. Methanol (250 μL) was used as disperser.	[47]

[a] yield g of analytes/g of matrix; [b] recovery (%). BTBAB: benzyl tributyl ammonium bromide; BTBAC: benzyl tributyl ammonium chloride; BTEAC: benzyltriethylammonium chloride; BTMAC: benzyl trimethyl ammonium chloride; ChCl: ChCl; COSMO-RS: conductor-like screening model for real solvents; DAD: diode array detector; DES: deep eutectic solvent; EtGly: ethylene glycol; GC: gas chromatography; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor; HPLC: high performance liquid chromatography; ICP: inductively coupled plasma; LLE: liquid–liquid extraction; MS: mass spectrometry; MS/MS: tandem mass spectrometry; PDA: photodiode array detector; Q: single quadrupole; QqQ: triple quadrupole; SEC: size exclusion chromatography; SLE: solid–liquid extraction; TBAB: tetrabutylammonium bromide; TBAC: tetrabutylammonium chloride; ToF: time of flight; TOMAC: trioctylmethylammonium chloride; UA-LLME: ultrasound-assisted; UHPLC: ultra-high performance liquid chromatography; UV: ultraviolet.; VA-DLLME: vortex assisted dispersive liquid–liquid microextraction.

In this kind of application, again ChCl was the preferred HBA combined with alcohols, organic acids, sugars or urea [40,41,43–46,50] although other quaternary ammonium salts combined with alcohols [42,47,48], lactic acid combined with glucose [49] or terpenes (menthol) together with myristic acid [10] have been also applied. In all cases, natural components were used, thus the application of NADESs has been the choice in the studies developed so far in this area. However, the selection of the final applied solvent was based, in most cases, on the results obtained from previous studies in which several HBDs or even HBAs and variated molar ratio combinations were explored to reach the best extraction performance. In this sense, it should be highlighted the work carried out by Wang et al. [41], in which liquid–liquid extraction (LLE) using NADESs was applied for profiling free and bound phenolic compounds in tea seed oil. The authors tested different binary and ternary DESs using ChCl as HBA and xylitol, malonic acid, glycerol, ethylene glycol and propylene glycol as HBDs and formed using variated molar ratio. Results showed that all DESs had potential for the extraction of free and bound phenolic acid and flavonoids except for some bound flavonoids at low levels of concentration. In general terms, malonic HBDs showed the lower extraction capacity respect to reference MeOH/water extraction solvents. The authors explained this behavior based on the capacity of organic acids to donate hydrogen bonds which can come up with a competition between malonic acid and phenolic compounds to interact with DESs molecules and the low pH of the organic acid that could favor the degradation of phenolic compounds. Sugar-based DESs showed good extraction capacity for phenolic acids, however, its high viscosity makes it unsuitable for the extraction performance unless water is added, which makes the procedure more complex. Finally, regarding alcohol based NADESs, those increase their extraction efficiency with polarity, being glycerol the most polar and the one with the highest extraction yields, surely due to the similarity with phenolic compounds. Therefore, ChCl:glycerol was applied as the most suitable extraction solvent. The procedure allowed the identification of 31 phenolic compounds and the quantification of 25 of them by application of UHPLC-(QqQ)-MS/MS and UHPLC-(QTOF)-MS/MS, respectively.

As previously indicated, apart from ChCl, other quaternary salts were also investigated for the extraction of terpenoids, vitamins or proteins. An interesting example is the strategy developed by Li et al. [42] to carry out the deterpenation (separation between terpenes and terpenoids) of citrus essential oil intensified by in situ formation of a DES between a quaternary ammonium salt and the compound of interest. With this aim, seven HBAs were thoroughly evaluated including ChCl, tetraethylammonium chloride (TEAC), tetrapropylammonium chloride (TPrAC), tetrapropylammonium bromide (TPrAB), tetrabutylammonium chloride (TBAC), tetrabutylammonium bromide (TBAB), tetrapentylammonium chloride (TPeAC), and tetrapentylammonium bromide (TPeAB). As indicated in Figure 2, in which the deterpenation mechanism based on the formation of a new DES composed of the ammonium salt and terpenoids is shown, terpenoid can act as weak HBDs in the presence of strong ammonium salt HBAs, whereas terpene is not able to develop interactions that involve the obtention of new DESs with the ammonium salt. This aspect allows the in-situ formation of a DESs between terpenoid and ammonium quaternary salts and, consequently, deterpenation process. Finally, the introduction of a strong HBD allows breaking the interaction previously established and, subsequently, the release of the terpenoid for its determination using a gas chromatographer.
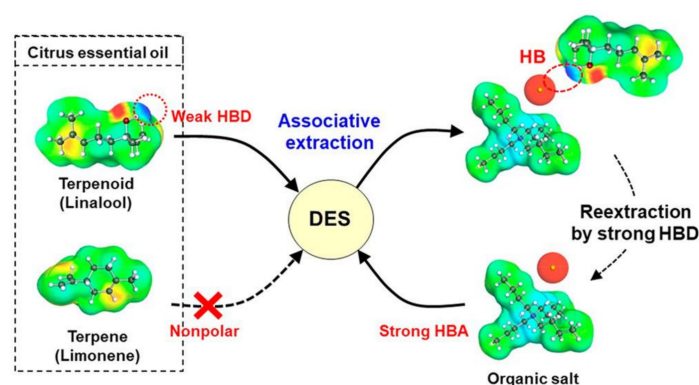
Figure 2. Citrus essential oil deterpenation approach intensified by in situ formation of a DES between a quaternary salt and terpenoids. Reprinted from Li, J.; Wang, J.; Wu, M.; Cheng, H.; Chen, L.; Qi, Z. Deep Deterpenation of Citrus Essential Oils Intensified by in Situ Formation of a Deep Eutectic Solvent in Associative Extraction. Industrial and Engineering Chemistry Research, 2020, 59 (19), 9223–9232. with permission from American Chemical Society [42]. Copyright 2020.

Besides, in order to simplify the HBAs comparison and selection, a quantum chemistry-based statistical thermodynamics approach using COSMOS-RS software was applied for calculating interactions among solvents and solutes. COSMOS-RS results indicated that Van der walls interactions were dominant for salt cations, increasing with the length of the alkyl chain, whereas hydrogen bonds were for salt ions of the HBAs, stronger for Cl^- than for Br^-. Thus, TPeAC should have been the best HBAs to reach the aim of this work. However, experimental studies of model citrus essential oil with the selected HBAs showed that solid–liquid phases were formed with almost all HBAs, while for TBAC two liquid phases resulted, which favors the separation of terpenoids and terpenes by the developed strategy. Therefore, TBAC was selected as the most suitable HBA. Terpeneless product evaluation with a linalool purity of 98.76% demonstrated the success and efficiency of the approach to carry out the deterpenation of citrus essential oil using NADESs.

As it was demonstrated by Li et al. [42], the application of computational calculation such as COSMOS-RS software provides numerous advantages to understand the interaction established between DESs and analytes and DES formation, which considerably simplifies the research study and reduces the experimental work and consequently the cost and time consumption associated.

Following the discussion about DESs applied in this area, it should be highlighted the work developed by Rodrigues et al. [10], in which a terpene-based NADES was applied for the extraction of astaxanthin from brown crab, shrimp shells and *H. pluvialis* prior to determination by HPLC-UV and the development of different bioactivity studies including cytotoxicity, antiproliferative and antimicrobial studies. Taking into account the advantages provided by these kinds of NADESs due to their higher volatility and the similarity with target compounds when terpenoids are the aim of the study, it is strangethat these kinds of DESs had not been more frequently applied. In this case, the authors investigated diverse combinations such as perilyl alcohol: camphor, DL-menthol: perilyl alcohol, DL-menthol: camphor, DL-menthol: eucalyptol and compared the extraction performance with conventional soxhlet method. The results indicated that menthol:myristic acid (8:1) were able to match or even increase until 657 times the astaxanthin yield obtained by a soxhlet extraction with acetone, which demonstrated the suitability of this NADES as an alternative solvent for the extraction of terpenoids from biomass.

Regarding extraction procedures, both SLE and LLE, depending on the nature of the sample, were carried out with or without assistance. Nevertheless, their combination with microextraction techniques has been also carried out in this case [45,47,48]. Remarkable is the work developed by Faraji et al. [47] in which a vortex assisted-dispersive liquid–liquid microextraction (VA-DLLME) using trioctylmethylammonium chloride (TOMAC):isoamyl alcohol in a molar ratio 1:4 was used as extractant for the evaluation of folic acid in wheat

flour. With this aim, 5 g of sample were initially submitted to a SLE by ammonium buffer. After that, the aqueous supernatant (1 mL) and together with 9 mL of ammonium buffer were located in a centrifuge tube and 150 μL of NADESs dissolved in 250 μL were rapidly injected. Afterwards, the mixture was vortexed during 1 min, centrifuged and the NADESs drop enriched with folic acid was diluted and injected in the HPLC-UV system for its analysis. The different parameters affecting the extraction performance such as pH, salt amount and DES volume were carefully evaluated, as well as the type of DESs. In this case, three different DESs, benzyltriethylammonium chloride (BTEAC):thymol, BTEAC:octanoic acid and TOMAC:isoamyl alcohol were tested in various molar ratios. Results showed that the ion-paring (electro-static interaction) between TOMAC and folic acid carboxylic groups were more efficient than the interactions established for the other DESs increasing the extraction efficiency. Additionally, as it was indicated by the authors, this hydrophobic DESs removes emulsifiers and reduces solvent consumption compared to hydrophilic DESs, leads to the extent of DESs efficiency in water samples without the use of emulsifier solvents and, additionally, it can eliminate ion-pairing agents needed for folic acid extraction. The combination of VA-DLLME with NADESs resulted to be a suitable approach for the evaluation of folic acid in wheat flour with LOQs in the range 1–3 mg/kg and recovery higher than 90%.

Finally, it should be highlighted that, as described in previous sections, the application of NADESs for the evaluation of the discussed matrices involves the re-extraction with organic solvents on many occasions. This fact is related to the limitation of DESs to be volatilized and their incompatibility with chromatographic and detection systems which complicate the possibilities of preconcentration and the reliable evaluation of the compounds of interest. In addition, the necessity of introducing a re-extraction process reduces the green nature of the procedures using toxic organic solvents and increase the complexity of the developed approaches. In this sense, further research is essential to develop new strategies that allow solving such limitations.

6. Conclusions and Future Outlook

In the last years, the use of eutectic solvents for extraction and isolation of bioactive compounds from natural raw materials, agro-industrial products and by-products has sharply increased due to the particular characteristics of these solvents and the advantages that their use provides in this field. The extraction capacity of DESs and NADESs depends on their components, which are mainly primary metabolites of cells, on their concentrations, and of course, on the extraction method used for their application. To help with the selection of DESs/NADESs, among the characteristics that must be taken into account are the viscosity and polarity of these solvents since the latter can influence the bioactivity of the bioactive component to be studied. These solvents have managed to extract a wide variety of bioactive compounds such as alkaloids, phenolic compounds, essential oils, carotenoids, and proteins, which confirms their great versatility and easy customization. Besides, they can be used with a plethora of natural products and by-products as we have described in the manuscript. In addition, they are made up of relatively low-cost components. Considering the wide variety of these solvents, possible uses, customization, and extraction capacity, selecting the "best solvent" continues to be a challenge to be studied further for each application.

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