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The perspectives of natural deep eutectic solvents in agri-food sector

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ABSTRACT

The principles of 'green chemistry' are gaining importance in agri-food sector due to the need to reduce pollution from toxic chemicals, make industrial processes safer and more sustainable, and to offer 'clean-labeled products' required by the consumers. The application of natural deep eutectic solvents (NADES) – natural product-based green liquids is considered the promising alternative to conventional organic solvents. This review is intended to summarize and discuss recent advances related to physicochemical properties of NADES, their applications, compatibility with analytic techniques and toxicological profile, pointing out the challenges and necessary improvements for their wider utilization in agri-food sector. NADES allow extraction of wide range of food compounds and they are proven to be convenient for food-related applications. However, their potential for industrial scale-up utilization is not completely investigated. Examined NADES are readily biodegradable, but only preliminary studies on NADES toxicity which include limited number of NADES molecules are available. Apart from fundamental research dealing with NADES formation and the nature of the interactions and structure underpinning the liquid phase formation, the question of purity of NADES obtained by different synthetic methodologies need to be addressed in the future. Data on physicochemical properties of synthesized NADES are still needed as they are relevant for industrial applications.

KEYWORDS

NADES; green solvents;
agri-food sector;
perspectives; application

Introduction

Rationale behind the study

The principles of 'green chemistry' are gaining importance in agri-food sector due to the need to reduce pollution from toxic chemicals, make industrial processes safer and more sustainable, and to offer 'clean-labeled products' required by the consumers. In that sense, there has been a growing interest in natural additives and different phytochemicals serving not only as nutrients, but also as functional food ingredients, including antioxidants, antimicrobials, food aromas and colorants (Kallel et al. 2014).

In addition, the sustainability initiatives demand significant efforts to efficiently exploit food waste and by-products as a bioresource for our next generation of energy, chemicals, pharmaceuticals, cosmetics, foods and other high value-added products (European Parliament resolution (2011/2175(INI) 2012). Thus, the food industry faces challenges of reducing waste, better utilization of by-products and isolating bioactive compounds which can be used as nutraceuticals.

Green solvents

Supercritical fluids (e.g. carbon dioxide or water), ionic liquids and deep eutectic solvents as well as water itself, are

well known green solvents. Although nontoxic, abundant and inexpensive, water is a poor solvent for most organic compounds, but rather reactive to cause a chemical degradation and has a limitation of polarity, which is why the most of organic bioactive compounds are not extracted very well.

Carbon dioxide as a supercritical fluid is more suitable for lipophilic compounds, so its utilization is limited to decaffeinated foods (Cvjetko Bubalo et al. 2015). Synthetic ionic liquids have been developed in the past decades, which have the advantage that at room temperature are non-flammable, nonvolatile, and easy recyclable, but they are made from petroleum which affects their safety and suitability for food applications.

Natural deep eutectic solvents (NADES)

Natural deep eutectic solvents (NADES) are firstly described by Choi et al. (2011) as a third class of liquids present in living cells, different from water and lipids, that play an important role as an alternative medium for biosynthesis, transport and storage of compounds with intermediate polarity. Later, these novel, advanced class of green solvents have been considered as fourth generation of ionic liquids (Radošević et al. 2015). Some investigations suggest that NADES have a pivotal role in maintaining metabolism in

plants in the absence of water during drought or cold conditions (Choi et al. 2011). Compared with other similar systems, NADES are rather based on biological than chemical concept, since ionic liquids or deep eutectic solvents might exist in nature with specific physiological functions. From a chemistry viewpoint, NADES might be classified as deep eutectic solvents (DES) or as low transition temperature mixtures (LTTM), being also ionic liquids (IL). NADES have been attracting great attention of the scientific community, not only due to their favorable physicochemical qualities (e.g. liquid state within wide temperature range, insignificant volatility, chemical and thermal stability, non-flammability, nontoxicity of constituting ingredients), but also due to their sustainable 'green' properties. Components of NADES are abundantly present in nature, readily available, and biorenewable (Dai et al. 2013c; Pena-Pereira, Kloskowski, and Namieśnik 2015), and since the NADES are generally composed of nontoxic substances occurring naturally in foods, they may be directly incorporated in food formulations without additional purification steps, being a major advantage over conventional solvents (Savi et al. 2019). Majority of NADES combinations are regarded as highly biodegradable and of low toxicity (Paiva et al. 2014; Radošević et al. 2016b; Wen et al. 2015). Simple and ease preparation of NADES with high purity and without waste generation (Pena-Pereira et al. 2015), meets the 12 principles of green chemistry (Anastas and Eghbali 2010).

In a number of experiments extraction yields by NADES were higher than that with conventional organic solvents (Dai et al. 2013c; de los Ángeles Fernández et al. 2018a; Jeong et al. 2017; Liu et al. 2017). Apart from their extraction ability, increased stability of natural compounds during extraction and storage (such as phenolic compounds, β -carotene and α -tocopherol) is another advantage of using NADES over traditional solvents (Dai, Verpoorte, and Choi 2014; de los Ángeles Fernández et al. 2018a; Milano et al. 2017; Xin et al. 2017; Zahrina et al. 2018). Furthermore, NADES were also suggested as solvents for protein (e.g. lysozyme, amylase, photosynthetic enzymes) and DNA stabilization (Choi et al. 2011; Dai et al. 2014; Milano et al. 2017; Xin et al. 2017). Consequently, studies on their potential application as drug delivery systems for poorly soluble bioactive compounds have been intensified (Faggian et al. 2016; Mano et al. 2015; Sut et al. 2017). Therefore, in recent years they have been suggested as an alternative to traditional volatile organic solvents in chemical processes and applications such as: solvents for carbon dioxide capture (García et al. 2015; Mulia et al. 2016b), solvents for biodiesel production and processing lignocellulose (Tang et al. 2017; Taslim et al. 2016), as electrolytes (Gomez, Spisso, and Silva 2017), in biocatalysis and electrochemical detection of phenolics (Gomez et al. 2016; Yang et al. 2017), as biodegradable reaction media (Azizi, Soleymani, and Mahmoudi 2017), as super absorbent for selective oil removal and as soil washing agents (Laitinen et al. 2017; Mukhopadhyay et al. 2016).

Referring to all mentioned above, NADES have a great potential for food applications. However, in spite of these

findings, major industries and scientific organizations are still reluctant to fully embrace NADES concept. Thus, the aim of this review is to analyze and critically discuss the issues relevant to potential applicability of NADES as extraction and separation solvents for agri-food components and contaminants, in order to better understand opportunities and challenges for their implementation as an 'green' alternative to organic solvents.

Preparation of NADES

Composition of NADES

NADES are composed of broad range of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) mixed together. The usual HBA are nontoxic quaternary ammonium salts, or amino acids (e.g. alanine, proline, glycine, betain) while HBD are organic acids (e.g. oxalic acid, lactic acid, malic acid, etc.) or carbohydrates (e.g. glucose, fructose, maltose, etc.). Alcohol, amine, aldehyde, ketone and carboxylic groups behave dually: as hydrogen-bond donor and acceptors (de los Ángeles Fernández et al. 2018a). The number of compounds and arrangements of molecules which may constitute NADES is estimated to ascend to 10^6 , so they are designated as tailored-made solvents due to their high versatility and virtually unlimited number of combinations (Silva et al. 2018). The nature of the interactions that take place depends on the type of solid materials forming liquids. Charge delocalization occurring via hydrogen bonding between HBA and HBD and Van der Waals interactions interfere with the ability of the initial compounds to crystallize, so they are the main cause of a significant melting point depression of NADES (de los Ángeles Fernández et al. 2018a). As far as the strength of hydrogen bonds is concerned, it fully correlates with the temperature of phase transition, viscosity, stability and dissolving properties of a given eutectic solvent (Jablonský et al. 2018). Spectroscopy (NMR and IR), mass spectrometry and recent quantum chemical and molecular dynamics simulations have provided support for monitoring such interactions (Aissaoui, Benguerba, and AlNashef 2017). By analyzing the pairwise interactions of the constituent components of the choline chloride-urea mixture, Ashworth et al. (2016) found that interaction of the choline cation with chloride or urea generated a wide range of low energy structures. Referring to the same authors, unlike the traditional solvents, an 'alphabet soup' of many different types of H-bond ($\text{OH}\cdots\text{O}=\text{C}$, $\text{NH}\cdots\text{O}=\text{C}$, $\text{OH}\cdots\text{Cl}$, $\text{NH}\cdots\text{Cl}$, $\text{OH}\cdots\text{NH}$, $\text{CH}\cdots\text{Cl}$, $\text{CH}\cdots\text{O}=\text{C}$, $\text{NH}\cdots\text{OH}$ and $\text{NH}\cdots\text{NH}$) can be formed within the NADES.

Referring to Jeliński and Cysewski (2018), the quantitative compositions of NADES can be obtained from spectroscopic measurements by computational models such as COnductor-like Screening MOdel for Realistic Solvents – COSMO-RS, but unfortunately, such data are usually unavailable, and the resulting uncertainties make it difficult to construct a NADES model.

Despite the enormous interest in the nature of interactions in NADES, fundamental questions about NADES formation have not yet been answered:

- if it is a coordination complex with a well-defined stoichiometry or a mixture in a composition range,
- what is the nature of the interactions and structure underpinning the liquid phase formation,
- how the solid-liquid phase diagram explains the phase relationships in NADES,
- what is the role of water in NADES formation and stability,
- what is the structure and dynamics of NADES at the molecular level (Florindo et al. 2019).

As there is insufficient basic knowledge concerning NADES, trial and error presents a strategy for discovering particular molar ratios of HBA and HBD capable of forming eutectic liquids.

For example, in order to prepare NADES to serve as nontoxic cryoprotective agent, Castro et al. (2018) selected trehalose (Treh) and glycerol (Gly) as starting compounds since both of the pure individual components have already been reported as cryoprotective agent. After mixing Gly and Treh in different molar ratios in order to obtain a eutectic mixture, they managed to obtain a transparent viscous liquid at room temperature using a molar ratio of 1:30 (Treh:Gly). The other selected molar ratios yielded a white solid mixture and/or liquid mixture with some crystals and were thus discarded.

Preparation methods of NADES

NADES are prepared by employing several physical methods with some modifications:

- i. evaporation, when the components are dissolved in water and solvent evaporated by rotaevaporation, while the obtained liquid is placed in a desiccator with silica gel until the constant weight is reached (Dai et al. 2013b);
- ii. heating and stirring, when the components are placed in a bottle with a stirring bar and cap and heated in a water bath with agitation until a clear liquid is obtained (Abbott et al. 2004; Dai et al. 2013b; Florindo et al. 2014),
- iii. freeze-drying, when the aqueous solutions of NADES and of the individual counterparts of NADES are freeze-dried for water sublimation to obtain NADES in its pure state (Gutiérrez et al. 2009),
- iv. grinding when the component mixture is grinded in a mortar with a pestle at room temperature until a homogeneous liquid is formed (Florindo et al. 2014);
- v. ultrasound-assisted heating, when the component mixture is exposed to ultrasound until a homogeneous liquid is formed (Bajkacz and Adamek 2018).

- vi. microwaving, when the component mixture is microwave irradiated at low power during a few seconds (Gomez et al. 2018).

The preparation of NADES by heating and stirring is the most common method in the literature (Florindo et al. 2014). This method is not only economical, but also enables easy control of the temperature and formation of the NADES, being of high importance in the case of using thermally unstable components (e.g. sugars). However, Florindo et al. (2014) found that method inappropriate for the preparation of five cholinium chloride:carboxylic acids NADES due to the generation of impurities ranging from 5% to 30%. As it was concluded by the authors, the impurities were the consequence of the HCl formation, which led to an ester bond formation between the cholinium cation and the acid. In comparison to the heating method, the authors gave the advantage to the grinding method as it resulted in NADES of high purity. In order to study the effect of the formation of the ester on the NADES properties, they compared the density and viscosity of cholinium chloride:gluratic acid liquids obtained by both methodologies. A marked difference was found in viscosity with an average deviation of 6.25% whereas in the case of density, the difference was smaller with an average absolute deviation of 0.15%.

Due to the simplicity of preparation, purity assessment of the obtained NADES is often missing. Furthermore, data on comparison on the efficiency and the impact of different synthetic methodologies on the physico-chemical properties are still scarce. Even when the same preparation method is applied, the difference in reported physicochemical properties of particular NADES can be observed (Lapeña et al. 2019).

One of the main features of NADES is their possibility to be used as extracting solvents for a wide range of molecules. Their uses in extraction depend on their physicochemical properties, such as viscosity, density, miscibility and polarity.

Physicochemical properties of NADES

The physicochemical properties of the NADES (viscosity, conductivity, density and polarity) affect their end-use (Choi et al. 2011; Dai et al. 2013b). They are dependent on the chemical nature of its components and on their intermolecular interactions (Liu et al. 2018). The presence of certain amounts of water, which also participate in hydrogen bonding, significantly affects arrangement of NADES molecules in this liquid crystal by enhancing their mobility while still retaining their unique features (Dai et al. 2013b, 2015). The presence of water lowers the melting point of some NADES and changes their physicochemical (viscosity, polarity, density and conductivity) and solvation properties (Aroso et al. 2017; Cardellini et al. 2014; Craveiro et al. 2016; Dai et al. 2013b, 2015; Xin et al. 2017). Relying on the results of FT-IR and ¹H NMR experiments, Dai et al. (2015) demonstrated that dilution with water caused the interactions HBD-HBA weaken gradually and even disappeared

completely at around 50% (v/v) water addition. Referring to the same authors, a small amount of water could reduce the viscosity of NADES to the range of water and increase the conductivity by up to 100 times for some NADES.

Literature abounds with the data on different physicochemical and thermodynamic properties of glyceline, representing one of the most promising and explored NADES formed by choline chloride (HBA) and glycerol (HBD) in molar ratio 1:2 (Lapeña et al. 2019). A series of NADES with reported physicochemical properties is presented in Table 1.

Polarity of NADES

Polarity is one of the most important properties of NADES, in terms of their extraction ability and their miscibility with other solvents. Most of NADES reported in the literature are hydrophilic, while hydrophobic NADES were reported in the literature for the first time in 2015 (van Osch et al. 2015; Ribeiro et al. 2015) and their utilization is yet to be explored.

Hydrophilic NADES

Majority of reported NADES are hydrophilic or highly hydrophilic in which, referring to Dai et al. (2013b), the polarity range varied from 44.81 kcal mol⁻¹ (higher than water) to 51.89 kcal mol⁻¹ (comparable to methanol), with the possibility to be modified through changing the amount of water present in the solvent. Among the hydrophilic NADES, organic acid based are the most polar (44.81 kcal mol⁻¹), followed by amino acids and pure sugar based NADES with a polarity similar to water (48.21 kcal mol⁻¹). Both sugar and polyalcohol based NADES are the least polar, with a polarity close to that of MeOH (51.89 kcal mol⁻¹). Hydrophilic NADES usually exhibit high solubilization capacity for polar metabolites due to their inherent high polarity, but they could be applied to some non-water-soluble metabolites (Dai et al. 2013b).

Hydrophobic NADES

In comparison to hydrophilic NADES, hydrophobic NADES were shown to possess lower viscosity as a result of eliminating the Coulombic charge interactions (Ribeiro et al. 2015). Florindo et al. (2018) prepared three NADESs by exclusively combining fatty acids, such as octanoic acid (C8), nonanoic acid (C9), decanoic acid (C10), and dodecanoic acid (C12), which were proven to act simultaneously as hydrogen bond donors and acceptors and to be stable when in contact with water environments as determined by NMR spectroscopy. In addition, these new solvents presented the lowest viscosities ever obtained (2–14 mPa·s) for this class of solvents and densities lower than water, regardless of the water content.

Van Osch et al. (2019) suggested four criteria to assess the sustainability of hydrophobic NADES from a chemical engineering point of view: a viscosity smaller than 100 mPa·s, a density difference between NADES and water

of at least 50 kg·m⁻³ upon mixing of the NADES and water, low transfer of the NADES to the water phase and minor to no pH change. The following NADES satisfied these criteria: thymol and coumarin (2:1), thymol and menthol (1:1), thymol and coumarin (1:1), thymol and menthol (1:2) and 1-tetradecanol and menthol (1:2) (Van Osch et al. 2019).

Rheological behavior of NADES

Shear flow behavior of NADES

Flowability of NADES under varied shear rates depend on the apparent viscosity indicating their expected field of application and uses (Elhamarnah et al. 2019). The flow behavior of NADES at room temperature has advantages over traditional solvents, so that they can be more easily used and operated in different industrial conditions (Troter et al. 2016; Paiva, Matias, and Duarte 2018). The rheological behavior of different mixtures of NADES at room temperature is showing a universal trend depending on the detailed microstructure of the HBD and HBA. In the literature there is no consensus about shear flow behavior of NADES. Some of the authors reported NADES as non-Newtonian liquids, exhibiting sheer-thickening or sheer-thinning behavior depending on the imposed shear rate (Altamash et al. 2017; 2018). However, NADES may behave as Newtonian fluids as well, independently of shear rate (Elhamarnah et al. 2019; R  ther et al. 2013; Cao et al. 2016; Aroso et al. 2017).

Troter et al. (2016) reported a shear-thinning behavior of citric acid:glucose and choline chloride: citric acid at 1:1 molar ratios in the temperature range of 293.15–363.15 K at atmospheric pressure within the shear rate of 0.1–1000 s⁻¹. Sheer thinning behavior of choline chloride:lactic acid, choline chloride: citric acid, choline chloride: malic acid, choline chloride:lactic acid and choline chloride: fructose NADES was also observed by Altamash et al. (2017) at 25 °C under a shear rate domain of 0.01–1000 s⁻¹ at atmospheric pressure. Shear-thinning behavior of NADES obtained by mixing betaine and alanine with lactic and malic acid in 1:1 molar ration was observed as well (Altamash et al. 2018).

The majority of (hydrophilic) NADES are characterized with high viscosity higher than that of pure organic solvents, determined to be in the range 200–500 mm² s⁻¹ at 40 °C, being one of their major disadvantages that may limit their extraction capacity and applicability such as time-consuming solvent transfer operations, slow mass transfer in dissolutions or extractions, preheating before processing and more energy for pumping (Dai et al. 2013b, 2015, 2016; Yan et al. 2017; Altamash et al. 2018). The high viscosity and reduced mobility of NADES at room temperature is associated with the extensive and complex hydrogen bonding between the HBD and HBA (Yan et al. 2017; Altamash et al. 2018). Altamash et al. (2018) found that NADES prepared with malic acid were characterized with high viscosity and behaved as semi-solid material at room temperature, while those prepared with lactic acid were liquid exhibiting lower viscosity. The NADES viscosities may vary greatly with the nature of the constituting components (HBD and HBA) and the temperature. For industrial application of NADES, low

Table 1. NADES with investigated physicochemical properties.

Composition	Molar ratio	Water content (%)	T _m (°C)	T _g (°C)	T _{decom} (°C)	Viscosity	Density (g mL ⁻¹)	Reference	
Malic acid:choline chloride:water	1:1:2	11.62	nr ^a	-71.32	201	44.59 (mm ² s ⁻¹) (40 °C)	1.2303 (40 °C)	Dai et al. (2013c)	
Glycerol:choline chloride:water	2:1:1	5.26	nr	-101.59	187	51.3 (mm ² s ⁻¹) (40 °C)	1.1742 (40 °C)		
Malic acid:β-alanine:water	1:1:3	19.48	nr	-70.88	164	174.6 (mm ² s ⁻¹) (40 °C)	1.352 (40 °C)		
Proline:malic acid:water	1:1:3	17.81	nr	-61.29	156	251.0 (mm ² s ⁻¹) (40 °C)	1.3184 (40 °C)		
Fructose:choline chloride:water	2:5:5	7.84	nr	-84.58	160	280.8 (mm ² s ⁻¹) (40 °C)	1.2078 (40 °C)		
Xylose:choline chloride:water	1:2:2	7.74	nr	-81.8	178	308.3 (mm ² s ⁻¹) (40 °C)	1.2095 (40 °C)		
Sucrose:choline chloride:water	1:4:4	7.40	nr	-82.96	>200	581.0 (mm ² s ⁻¹) (40 °C)	1.2269 (40 °C)		
Fructose:glucose:sucrose:water	1:1:1:11	22.0	nr	-50.77	138	720.0 (mm ² s ⁻¹) (40 °C)	1.3657 (40 °C)		
Glucose:choline chloride:water	2:5:5	7.84	nr	-83.86	170	397.4 (mm ² s ⁻¹) (40 °C)	1.2069 (40 °C)		
1,2-Propanediol:choline chloride:water	1:1:1	7.70	nr	-109.55	162	33.00 (mm ² s ⁻¹) (40 °C)	1.0833 (40 °C)		
Lactic acid:glucose:water	5:1:3	7.89	nr	-77.06	135	37.00 (mm ² s ⁻¹) (40 °C)	1.2947 (40 °C)		
Sorbitol:choline chloride:water	2:5:6	9.23	nr	-89.62	<200	138.4 (mm ² s ⁻¹) (40 °C)	1.1854 (40 °C)		
Xylitol:choline chloride:water	1:2:3	11.17	nr	-93.33	<200	86.10 (mm ² s ⁻¹) (40 °C)	1.17841 (40 °C)		
Benzoic acid:betain	1.5:1	nr	53	nr	>200	Relationship curves between viscosity and temperatures	1.18 (80 °C)		Cardellini et al. (2014)
2-Hydroxy-benzoic (salicylic) acid:betain	1.5:1	nr	63	nr	>200		nr		
4-Chloro-benzoic acid:betain	1.5:1	nr	28	nr	>200		1.27 (65 °C)		
2-Chloro-benzoic acid:betain	1.5:1	nr	39	nr	>200		1.29 (65 °C)		
3-Chloro-benzoic acid:betain	1.5:1	nr	43	nr	>200		nr		
2-Furoic acid:betain	2:1	nr	11	nr	>200		1.27 (30 °C)		
Phenylacetic acid:betain	2:1	nr	-7	nr	>200		1.16 (30 °C)		
D-(+)-Mandelic acid:betain	1:1	nr	13	nr	>200		1.22 (30 °C)		
Glycolic acid:betain	2:1	nr	-36	nr	>200		1.27 (25 °C)		
Oxalic acid:betain	2:1	nr	33	nr	>100		1.27 (50 °C)		
Citric acid:betain	1.5:1	nr	48	nr	>200		nr		
Choline chloride: D-(+)-glucose	1:1	5.5	nr	-28.4	nr	nr	1.27 (23 °C)	Craveiro et al. (2016)	
Choline chloride: Citric acid	1:1	0.2	76	-21.4	nr	nr	1.30 (23 °C)		
Choline chloride: D-(+)-sucrose	4:1	0.2	79.2	-42.0	nr	nr	1.22 (23 °C)		
Choline chloride: D-(+)-sucrose	1:1	0.2	nr	-15.8	nr	nr	1.35 (23 °C)		
Choline chloride: L-(+)-tartaric acid	2:1	1.9	nr	-41.6	nr	nr	1.26 (23 °C)		
Choline chloride: D-(+)-xylose	2:1	3.8	78.5	-46.4	nr	nr	1.23 (23 °C)		
Choline chloride: D-(+)-xylose	3:1	0.2	78.3	-51.2	nr	nr	1.22 (23 °C)		
Citric acid: D-(+)-sucrose	1:1	1.2	nr	-14.0	nr	nr	1.43 (23 °C)		
Citric acid: D-(+)-glucose	1:1	0.5	nr	9.8/48.7	nr	nr	1.45 (23 °C)		
D-(+)-glucose: L-(+)-tartaric acid	1:1	0.4	nr	-18.3	nr	nr	1.45 (23 °C)		
Choline chloride: D-(+)-xylose	1:1	0	DSC thermograms			-24.71 (Pa s) ^b	nr		Aroso et al. (2017)
		1				-24.04 (Pa s)	nr		
		3				-20.4 (Pa s)	nr		
		5				-17.55 (Pa s)	nr		
Choline chloride: D-(+)-glucose	1:1	0				-30.45 (Pa s)	nr		
		1				-25.80 (Pa s)	nr		
		3				-21.01 (Pa s)	nr		
		5				-18.53 (Pa s)	nr		
Choline chloride: D-(+)-sucrose	1:1	0				-30.87 (Pa s)	nr		
		1				-24.99 (Pa s)	nr		
		3				-10.92 (Pa s)	nr		
		5				-8.08 (Pa s)	nr		
Choline chloride: Citric acid	1:1	0				-30.53 (Pa s)	nr		
		1				-29.59 (Pa s)	nr		
		3				-28.21 (Pa s)	nr		

that the total vapor pressures of six hydrophobic NADES and the partial pressures of their constituents were very low in comparison to vapor pressures of commonly used volatile organic solvents using new and validated HS-GC-MS method. In this study it was shown that the total vapor pressure was dominated by the constituent with the highest vapor pressure. In line with that, the total vapor pressure of investigated NADES were strongly depended on the vapor pressures of the constituents used for their synthesis following the order menthol > thymol > decanoic acid > lidocaine. However, referring to the same authors, their total vapor pressures (55 Pa at 373 K) are not as low as those of typical low-volatile ILs (~1 Pa at 393 K), because the NADES constituents can be evaporated separately (and thus easier), while the ILs need to be evaporated as ion pairs (in order to keep electroneutrality).

Water activity of NADES

Water activity (a_w) is a quality and safety parameter relevant for most applications related to food processing and storage as it is a determinant for the growth of microorganisms as well as degradation reactions of a chemical, enzymatic, and physical nature (Renshaw et al. 2019). Gómez et al. (2019) measured a_w value of a range of NADES prepared with citric acid (CA), malic acid (MA), glucose (Glu), fructose (Fru), urea (U), β -alanine (BA) and choline chloride (CC). The water activity of NADESs decreased in the following sequence: MA:BA > CA:BA > MA:Fru \cong MA:Glu > U:Fru \cong U:Glu > CA:CC \cong CA:Fru > MA:CC > CA:Glu > Glu:CC \cong Fru:CC in the range between 0.653 and 0.177. The same authors demonstrated that the incorporation of water to NADES causes a gradual increase in the water activity. In their case, all samples reached a water activity higher than 0.900 when 60% of water was incorporated.

Applications of NADES in agri-food sector

Utilization of NADES in assurance of biorefinery concept

Agricultural residues are the excesses of production that are often discharged as wastes and only recently has residue utilization received broad emphasis as a component of waste management policy (Lucarini et al. 2018). It has been estimated that a third of all the food produced in the world is not consumed, which makes a total of about 1.3 billion tons of waste a year (Lucarini et al. 2018).

A biorefinery concept provides comprehensive, efficient, and flexible conversion of biomass feedstocks through a combination of physical, chemical, biochemical, and thermochemical processes into multiple products. As a broad technological definition, biorefinery is intended to convert all kinds of biomass (e.g. organic residues, energy crops, aquatic biomass) into a wide range of bio-based products, such as food and feed, chemicals, fuels, power, and heat (Lucarini et al. 2018).

The number of research articles employing NADES in the advancement of the existing biorefinery approaches

seems to be a promising strategy for clean fractionation or pretreatment of the lignocellulosic biomass residues (Kumar, Parikh, and Pravakar 2016). Kumar, Parikh & Pravakar (2016) evaluated the delignification of rice straw using lactic acid and choline chloride mixtures, and managed to solubilize 57% (w/v) of the biomass bound lignin from rice straw using 1:5 M ratio of NADES reagent.

The applicability of the green solvents in cellulosic ethanol production process and the effect of a group of acidic and neutral green reagents on cellulose degrading enzyme, Cellic Ctec 2 and biocompatibility with β -glycoside producing ethanol fermenting yeast strain *Clavispora NRRL Y-50464* for consolidated bioprocessing was explored by Kumar et al. (2016). Using choline chloride/glycerol treated rice straw, the authors managed to obtain 226.7 g L⁻¹ of reducing sugars with a saccharification efficiency of 87.1% at 20% solids loading and 12 FPU Cellic Ctec2. Further, Kumar et al. (2018) conducted comprehensive evaluation of an integrated process for cellulosic ethanol production from lactic acid + choline chloride + water mixture based NADES pretreated rice straw; solvent recovery and its reusability; extraction of value added products, lignin and xylan; enzymatic saccharification and fermentation. The authors demonstrated that pretreatment at 10% (w/v) solids loading followed by enzymatic hydrolysis at 25% (w/v) solids loading is the most effective integrated biorefinery process using NADES pretreated rice straw.

In an integrated biorefinery approach, maximum valorization of the lignocellulosic biomass is preferred and extraction and recovery of value added products is highly recommended by applying cheap, fast and environmentally safe procedures.

Sustainable extractions by NADES

Since the appearance of NADES, most of the applications relevant to the food sector have been devoted to the extraction of secondary metabolites from natural sources (de los Ángeles Fernández et al. 2018b).

Numerous studies have been undertaken concerning NADES utilization for the extraction of phenolic compounds, alkaloids, saponines, anthraquinones, essential oils, terpenoids, proteins, tanshinones, carbohydrates, polyunsaturated fatty acids and photosynthetic pigments (Bajkacz and Adamek 2017, 2018; Dai et al. 2013a, 2014, 2016; de los Ángeles Fernández et al. 2018a). The developed methods were found to be 'greener', more efficient and faster than conventional extraction methods (Cicci, Sed, and Bravi 2017; Dai et al. 2013a, 2014, 2016; Duan et al. 2016; Lores et al. 2017; van den Bruinhorst et al. 2016; Wang et al. 2016).

Since hydrophilic NADES have similar polarity to that of water and to those of the most polar organic solvents (MeOH, EtOH, etc.), the majority of applications in the literature are related to the extraction of phenolic compounds, a huge family of natural compounds comprising of simple, low molecular weight, single aromatic ring compounds and the large complex tannins and derivative polyphenol

structures, such as esters, glycosides, amides, etc. These applications were reviewed by Ramón et al. (2017) who found out that those inherent properties of plant material in terms of structure and composition combined with the diversity of phenolic compounds result in a high difficulty to both predict the right material–solvent system and find the best methodology to extract those molecules.

In line with that, Bajkacz and Adamek (2018) conducted comprehensive research on 17 types of NADES based on choline chloride, acetylcholine chloride, choline tartrate, betaine, and carnitine with different compositions in order to tailor a solvent with highest extraction efficiency for targeted flavonoids. A response surface methodology was used for multivariate optimization of some extraction parameters. Efficient recovery of extracted flavonoids was achieved using a 30% water solution of acetylcholine chloride/lactic acid ratio (2:1) as an extraction solvent. These NADES were further utilized for the extraction of target flavonoids from fruit (e.g. cranberry, fruits of *Lycium barbarum* L., grape, plum, and orange peel), vegetables (onion and broccoli), and spices (mustard, rosemary, and black pepper). The proposed extraction method using NADES improved the extraction efficiency in comparison to water and methanol (Bajkacz and Adamek 2018).

Following the same approach, tailor-made NADES suitable for the extraction of phenolic acids, anthocyanins, isoflavone, flavonols, flavanol and stilbenes were developed (Bajkacz and Adamek 2018; Dai et al. 2016; Liu et al. 2017; Zahrina et al. 2018). Using NADES as solvents, phenolic compounds were successfully extracted from fruits, medicinal plants and food by-products (Ramón et al. 2017).

Regarding natural aroma compounds, González et al. (2017) tested 14 different NADES to examine the solubility of vanillin and its extractability from vanilla pods. All tested NADES had higher extraction capacity for vanillin than ethanol, commonly used for the extraction of flavoring compounds. It appeared that the NADES with highest solubility were not necessarily the best extraction solvents for vanillin. Plant matrix effects seem to play a role in the extraction yields. Vanilla extracts in NADES have a potential to be directly used in food products especially in confectionary products or beverages, though a toxicological assessments still needs to be done (González et al. 2017).

Referring to Yang et al. (2017), vanillin is one of the most commonly used flavors. As the extraction from vanilla pods cannot provide sufficient quantities to meet the market demand, various biotechnological production methods have been developed. Yang et al. (2017) reported NADES as cosolvents in biocatalysis during the conversion of isoeugenol to vanillin catalyzed by *Lysinibacillus fusiformis*. A majority of tested DES or NADES were able to improve the production yields, with the best yield obtained by using a choline chloride-lactose and choline chloride-raffinose NADES (132% and 131% relative to the yield obtained in the NADES-free solution, respectively) (Yang et al. 2017).

Most natural compounds discussed above have a moderately high solubility in water; however, hydrophobic NADES

discovered recently are promising solvents when dealing with the extraction of less polar target biocompounds.

Biological activity and stability of target compounds in NADES extracts

Bioactive compounds exert positive effects on human health, but their effectiveness depends on factors that are still under investigation; such as the bioavailability, bioaccessibility antioxidant activity, anti-inflammatory properties etc. The investigation of effectiveness is extremely challenging, especially when dealing with human organisms and measuring the long-term physiological effect (Murador et al. 2019).

The effectiveness of NADES extracts is usually tested by antioxidant *in vitro* assays (e.g. DPPH, Oxygen radical absorbance capacity assay, Reducing power etc.) probably due to their relative simplicity. Apart from that, antimicrobial activity of NADES extracts is relevant for their potential food applications. Only recently, *in vitro* test using cell cultures were employed and there are only few reports using *in vivo* test on rats to test the biological activity of NADES extracts (Table 2).

Bakirtzi, Triantafyllidou, and Makris (2016) compared antioxidant activity of five medicinal plants using one conventional and four NADES as extraction solvents. Differences in the reduction power activity was only plant depended; while antiradical activity of NADES extracts (four out of 5 plants) were higher than those obtained with conventional solvents.

Similar observations were reported by Rajan, Prabhavathy, and Ramesh (2015), who found higher antioxidant and antimicrobial activity of NADES extracts of ginger than those obtained with conventional solvents. Moreover, Nam et al. (2015) demonstrated that certain NADES components (e.g. L-proline) contributed to the overall antioxidant activity of extracts, implying the existence of a synergic effect of the solvent and natural compound dissolved in it.

In vitro assay using MCF-7 and HeLa tumor cells were applied for the first time by Radošević et al. (2016b) to demonstrate that NADES enhanced the biological activities of extracts from grape skin, which contained phenolic compounds. They demonstrated that four out of five NADES extracts exhibited higher cytotoxic potential against the cancer cells than the conventional methanol extract, with the ChCl:malic acid (1:1) extract exhibiting the highest inhibition (Table 2). Referring to the authors, the increased anti-proliferative effect of the ChCl:malic acid extract compared to the other extracts could be partly explained by the effect of the NADES composition itself, since some organic acids, including malic acid, reveal many beneficial pharmacological effects, such as anti-inflammatory activity and antioxidant capacity.

Durand et al. (2017) compared the activity of several antioxidants dissolved in NADES (1,2-Propanediol/ChCl/Water) with their activity when dissolved in organic solvents (either DMSO or ethanol) applying the ROS (reactive oxygen species) inhibiting capacity using the fibroblast cell line. The best positive effects of the NADES formulation were

Table 2. NADES as a solvent for biological active compounds.

Biological activity	<i>In vitro</i> assay (test model)	Plant/compound	NADES	Reference
Antioxidant activity	DPPH assay	Ginger rhizome (<i>Zingiber officinale</i> Roscoe)	Sucrose: citric acid (1:1), L-proline: lactic acid (1:1), L-proline: oxalic acid (1:1), Trehalose: citric acid (1:1)	Rajan et al. (2015)
	DPPH assay	Dried flowers (<i>Sophora japonica</i> L.)	L-proline: glycerol (2:5)	Nam et al. (2015)
	Oxygen radical absorbance capacity assay	Grapes skin of the Croatian native red grape cultivar, <i>Vitis vinifera</i> cv. Plavac mali	Choline chloride: glucose (2:1), Choline chloride: fructose (1.9:1), Choline chloride: xylose (2:1), Choline chloride: glycerol (1:2), Choline chloride: malic acid (1:1)	Radošević et al. (2016b)
	CAT assay, Model Cell System (genetically modified fibroblast cell line), Confocal Microscopy (fibroblast cell line), Evaluation of mitochondrial targeting activity with MitoSOX Red Probe (fibroblast cell line)	Bis-EHBm, α -tocopherol, α -Tocopherol acetate, Decyl rosmarinate, Capsiate, Totarol, Hydroxytyrosol, Hydroxytyrosol acetate, CR-6, CR-6 palmitate, Sinapine	1,2-propanediol: choline chloride: water (1:1:1)	Durand et al. (2017)
	Reducing power assay DPPH assay	Dittany (<i>Origanum dictamnus</i>), Fennel (<i>Foeniculum vulgare</i>), Marjoram (<i>Origanum majorana</i>), Sage (<i>Salvia officinalis</i>) Mint (<i>Mentha spicata</i>)	Lactic acid: choline chloride (3:1), Lactic acid: sodium acetate (3:1), Lactic acid: ammonium acetate (3:1), Lactic acid: glycine: water (3:1:3)	Bakirtzi et al. (2016)
Antimicrobial activity	Paper disc diffusion method (<i>Bacillus cereus</i> , <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Salmonella typhi</i> , <i>Vibrio cholera</i> , <i>Staphylococcus aureus</i> , <i>Streptococcus viridans</i>)	Ginger rhizome (<i>Zingiber officinale</i> Roscoe)	Sucrose: citric acid (1:1), L-proline: lactic acid (1:1), L-proline: oxalic acid (1:1), Trehalose: citric acid (1:1)	Rajan et al. (2015)
Brown adipocyte activation	Cell viability assay using PrestoBlue (Differentiated T37i brown adipocytes)	Salsalate	1,2-propanediol: choline chloride: water (1:1:1)	Rozema et al. (2015)
Matrix metalloproteinase-9 (aMMP-9) inhibition activity	Zymography MTT cytotoxic assay (TNF- α activated human leukemia cell line (THP-1))	Resveratrol	1,2-propanediol: choline chloride: water (1:1:1)	Shamseddin et al. (2017)
Cytotoxic activity	WST-1 assay Morphological assessment by light microscopy (MCF-7 and HeLa cells)	Grapes skin of the Croatian native red grape cultivar, <i>Vitis vinifera</i> cv. Plavac mali	Choline chloride: glucose (2:1), Choline chloride: fructose (1.9:1), Choline chloride: xylose (2:1), Choline chloride: glycerol (1:2), Choline chloride: malic acid (1:1)	Radošević et al. (2016b)
	Propidium iodide fluorescence assay (fibroblast cell line)	Bis-EHBm, α -tocopherol, α -Tocopherol acetate, Decyl rosmarinate, Capsiate, Totarol, Hydroxytyrosol, Hydroxytyrosol acetate, CR-6, CR-6 palmitate, Sinapine	1,2-propanediol: choline chloride: water (1:1:1)	Durand et al. (2017)
Bioavailability	<i>In vivo</i> tests with rats	Rutin	proline: glutamic acid (2:1)	Faggian et al. (2016)
		Berberin	proline: malic acid: lactic acid: water (1:0.2:0.3:0.5), proline: malic acid (1:2) proline: urea (2:1)	Sut et al. (2017)

obtained with the antioxidants that exhibited a lower ROS inhibiting effect at short term, so they concluded that the formulation of antioxidants in NADES could greatly improve their activity for ROS inhibition, probably by enhancing their transport through membranes of the fibroblasts used in the bioassay.

Additionally, formulation of resveratrol with NADES increased its matrix metalloproteinase-9 (MMP-9) inhibition activity (Shamseddin et al. 2017), while dissolution of salsalate in NADES allowed *in vitro* evaluation of its bioactivity (Rozema et al. 2015).

Applying the *in vivo* model with rats, Faggian et al. (2016) managed to achieve a 100% improvement in the absorption of rutin with NADES (proline: glutamic acid (2:1)) in comparison with the water suspension. This NADES was able to dissolve a comparable amount of rutin as ethanol and twenty times higher than water. The authors also observed that the oral administration of rutin with NADES improved bioavailability of this polyphenol compared to the water suspension. This effect may be related to the fact that the NADES formulation allows the administration of rutin as a solution being more available for the

absorption by the gastrointestinal tract. The authors conclude that the NADES can be administered orally at moderate doses without major health hazards.

Following the same approach (*in vivo* model with rats), the same research group (Sut et al. 2017) managed to improve bioavailability of berberin by dissolving it in three NADES (proline:malic acid:lactic acid:water (1:0.2:0.3:0.5), proline:malic acid (1:2) and proline:urea (2:1)). Plasma levels of berberine following the administration of three NADES were significantly higher than the plasma level observed with the water suspension. The authors concluded that the increase in bioavailability is mainly related to the solubilization properties of the applied eutectic mixtures. NADES exhibit stabilizing effect on biological active compounds, which favors their application in cosmetic and pharmaceutical formulations. An example was reported by Dai, Verpoorte, and Choi (2014), who reported enhanced stability of carthamin (C-glucosyl quinochalcone), a red pigment in safflower, and a safflower extract in sugar based NADES in comparison to their stability in water or 40% ethanol under different light and temperature conditions, and storage duration.

Similar findings were reported for anthocyanins from purple and orange petals of *Catharanthus roseus* in lactic acid–glucose (LGH) (Dai et al. 2016). The improved stability of hydrophobic phenols among eight investigated phenolic compounds from food by-products extracted with lactic acid–glucose (LGH) was observed by de los Angeles Fernández et al. (2018b). Jeong et al. (2017) determined significantly lower decline in the content of tea catechins extracted with NADES composed of betaine, glycerol, and D-(+)-glucose, 4:20:1 (BGG-4) after 21 days of storage in comparison with their content in the extracts obtained with conventional solvents. The stabilizing ability of NADES is explained by the existence of intermolecular interactions, mainly hydrogen bonding between solutes and NADES that stabilize solutes molecules and lead to reduction in solutes movement and consequently protect solutes from oxidative degradation (Dai et al. 2016; de los Angeles Fernández et al. 2018a).

Sustainable separations with NADES

Sustainability demands separation processes to be conducted with preferably no to little CO₂ emissions. Solvent-based separation processes can reduce the required energy input for separation, improve biocompatibility, and be used when distillation is technically not feasible because of the delicate nature of (bio)molecules to be separated (Schoor et al. 2019).

Ng et al. (2015) achieved optimal conditions for liquid-liquid extraction of tocopherols from palm oil by using choline chloride-malic acid as extraction solvent. Moreover, this study showed that intermolecular interaction between the selected NADES and tocopherols, allowed selective separation of individual tocopherols in palm oil, where products with fractions rich in tocotrienols and low in tocopherols (particularly α -tocopherol) were favorable. Compared with organic solvents, tocopherol concentration in the NADES-mediated product

increased 2.5 fold and the tocopherol profile was significantly improved by enhancing tocotrienols fraction in the products from 80.8 to 99.8%.

The application of betaine NADES for the palm oil deacidification was demonstrated to be successful alternative to the process traditionally performed by steam stripping, which causes the loss of the majority of palm oil's natural antioxidants due to high temperature (Zahrina et al. 2018). The betaine monohydrate-glycerol NADES in a molar ratio of 1:8 was demonstrated to have an efficiency of palmitic acid extraction of 34.14%, and the amount of antioxidants can be preserved in the refined palm oil up to 99%.

Recently, polar NADES have been applied to form aqueous biphasic systems (ABS) for the extraction of proteins (Zeng et al. 2014; Xu et al. 2015; Li et al. 2016; Zhang et al. 2016). The ABS are a type of biphasic system constituted by two immiscible aqueous-rich phases, characterized by its high biocompatibility that can be used for liquid-liquid extraction processes. However, despite the promising results obtained in the extraction of proteins, the NADES stability in the aqueous media were not addressed in these papers. Referring to Farias et al. (2017) who tested the liquid-liquid equilibria of aqueous two-phase systems composed by NADES + K₂HPO₄ + water at 298.15 K, the hydrogen bonds of NADES are disrupted in aqueous solutions, leading to a differential partition of the HBA and HBD between the two phases. In order to create ABS in which the NADES integrity can be maintained, ABS composed of poly(propylene)glycol and mixtures of cholinium chloride, as HBA, and glucose, as HBD, were investigated by Farias et al. (2017). Their results suggest that a combination of factors, such as the hydrophobicity/hydrophilicity of the HBD, the nature of the ABS components, as well as the tie-line length, allows the preparation of systems in which the HBA:HBD stoichiometry used in NADES preparation is maintained in the phases in equilibrium, thus behaving as *de facto* ternary systems.

Hydrophobic NADES consisting of decanoic acid and various quaternary ammonium salts were shown to be efficient for the recovery of volatile fatty acids from diluted aqueous solutions (van Osch et al. 2015), although a few years later van Osch et al. (2019) questioned the use of quaternary ammonium salts from an environmental point of view, and instead suggested the use of the natural components such as terpenes. Ribeiro et al. (2015) reported that hydrophobic eutectic solvents can be prepared from mixtures of DL-menthol with a range of carboxylic acids and used as extractive solvents for caffeine, tryptophan, isophthalic acid, and vanillin from water. Most of reported NADES in literature are hydrophilic, but these hydrophobic examples offer highly interesting opportunities for future research on recovery of bio-based chemicals from dilute aqueous streams.

Novel assisting technologies in NADES extractions

NADES extractions are often combined with ultrasound (UAE) and microwave-assisted (MAE) technologies (Table 3). Both UAE and MAE provide shorter extraction

Table 3. Extraction techniques used to improve extraction efficiency.

Food matrix	Target compounds	NADES	Type of extraction	Reference
Grape skin	polyphenols	choline chloride: glucose (2:1), choline chloride: fructose (1,9:1), choline chloride: xylose (2:1), choline chloride: glycerol (1:2), choline chloride: malic acid (1:1)	UAE	Radošević et al. (2016b)
Tea leaves	alkaloids, flavonoid, and catechins	choline chloride:ethyl glycol (1:2 – 1:4), choline chloride:1,2-butanediol (1:3 – 1:4), choline chloride:1,3-butanediol (1:2 – 1:4), choline chloride:1,4-butanediol (1:2 – 1:4), choline chloride:2,3-butanediol (1:3 – 1:4), choline chloride:1,6-hexanediol (1:2 – 1:4), choline chloride:malonic acid (1:1), choline chloride:lactic acid (1:1), choline chloride:malic acid (1:1), choline chloride:acetamine (1:1), choline chloride:methylurea (1:1), choline chloride:urea (1:2)	MCE	Wang et al. (2017a)
Safflower, <i>Carthamus tinctorius</i> L.	polyphenols	lactic acid:glucose (5:1), proline – malic acid:choline chloride (1:1:1), choline chloride :sucrose– (1:1, 4:1), choline chloride:glucose– (1:1), choline chloride: sorbitol (3:1, 5:2), choline chloride:1,2-propanediol (2:1), fructose:glucose:sucrose (1:1:1)	mechanical agitation	Dai et al. (2013a)
Soy products	isoflavones	choline chloride:D(+)-glucose (2:1), choline chloride: L(+)-tartaric acid (1:1), choline chloride: citric acid (1:1), choline chloride: citric acid (2:1), choline chloride: citric acid (1:2), choline chloride: saccharose (2:1), choline chloride: glycerin (1:1), choline chloride: glycerin (2:1), choline chloride: D(+)-xylose (1:1), urea: choline chloride (1:1), urea: citric acid (2:1), urea: L(+)-tartaric acid (2:1), glycerin: D(+)-glucose (2:1), glycerin: L(+)-tartaric acid (2:1), glycerin: citric acid (2:1), choline chloride: citric acid: glycerin (1:1:1), choline chloride: citric acid: glycerin (2:2:1)	UAE	Bajkacz and Adamek (2017)
<i>Catharanthus roseus</i>	anthocyanins	choline chloride: 1,2-propanediol (2:1), lactic acid–glucose (5:1), proline–malic acid, malic acid–choline chloride (1:1), glucose–choline chloride (1:1), glucose:fructose:sucrose (1:1:1)	mechanical agitation and UAE	Dai et al. (2016)
Wine lees	anthocyanins	choline chloride: glucose (1:1), choline chloride: fructose (1,9:1), choline chloride: xylose (2:1), choline chloride: glycerol (1:2), choline chloride: malic acid (1:1)	UAE	Bosiljkov et al. (2017)
Safflower, <i>Carthamus tinctorius</i> L.	polyphenols, pigments- carthamin	glucose:choline chloride (2:5), sucrose:choline chloride (1:1), proline:malic acid (1:1), lactic acid:glucose (5:1), xylitol-choline chloride (2:5)	agitation with heating	Dai et al. (2014)
Fig (<i>Ficus carica</i> L.)	polyphenols and furanocoumarins	choline chloride:glycerol (1:1), choline chloride:D-(+)-galactose (1:1), choline chloride:L-proline (2:1), choline chloride:DL-malic acid (1:1), choline chloride:xylitol (5:2), choline chloride:D(-)fructose (1:1), choline chloride: sucrose (1:1), choline chloride: citric acid (2:1), choline chloride: D-(+)-glucose (1:1)	MAE, UAE	Wang et al. (2017b)
<i>Dioscorea opposita</i>	polysaharides	choline chloride-ethylene glycol (1:1, 1:2), choline chloride-glycerol (1:1, 3:2), choline chloride-1,4-butanediol (1:1), and choline chloride-1,6- hexanediol (1:1)	UAE	Zhang and Wang (2017)
Medicinal plants	Polyphenols	choline chloride:lactic acid (1:1), lactic acid:sodium acetate (1:1), lactic acid: ammonium acetate (1:1) and lactic acid:glycine (1:1)	UAE	Bakirtzi et al. (2016)
Palm oil	Palmitic acid	betaine monohydrate:glycerol (1:2, 1:3, 1:4, 1:6, 1:8), betaine monohydrate: propylene glycol (1:3, 1:4, 1:6, 1:8), betaine monohydrate:propylene glycol:glycerol (1:1:1, 1:1:2), betaine monohydrate: propylene glycol :propionic acid (1:2:2), betaine monohydrate:glycerol:propionic acid (1:1:1)	agitation with heating	Zahrina et al. (2018)
<i>Radix Scutellariae</i>	flavonoids	choline chloride:1,4-butanediol (1:2), choline chloride:glycerol (1:2), choline chloride:ethylene glycol (1:2), choline chloride: citric acid (1:2), choline chloride: malic	MAE	Wei et al. (2015b)

(continued)

Table 3. Continued.

Food matrix	Target compounds	NADES	Type of extraction	Reference
Onion, olive, tomato and pear by-products	polyphenols	acid (1:2), choline chloride:lactic acid (1:2, 3:1), choline chloride:glucose (1:2), choline chloride:sorbitol (1:2), choline chloride:sucrose (1:2), choline chloride:maltose (1:2), citric acid:sucrose (1:2), citric acid:glucose (1:2), lactic acid:sucrose (1:2), lactic acid :glucose (5:1), citric acid:glucose (1:1), fructose and citric acid (1:1)	agitation with heating, UAE	de los Ángeles Fernández et al. (2018b)
<i>Panax ginseng</i>	saponines, ginsenosides	choline chloride:glycerol 1:1, choline chloride:xylitol (5:2), choline chloride:D-(+)-glucose (1:1), L-proline:D-(+)-glucose (5:3), citric acid:D-(+)-glucose (1:1), citric acid:adonitol (1:1), betaine:DL-malic acid (1:1)	stirring, heating, and stirring and heating, UAE	Jeong et al. (2015)
Rice straw	lignin	lactic acid:betaine (2:1, 5:1), lactic acid-choline chloride (2:1, 5:1, 9:1)	agitation with heating	Kumar et al. (2016)
Green tea	catechins	betaine:sucrose (4:1), betaine: D-sorbitol (2:1), betaine:maltose (4:1), betaine:glucose (4:1), betaine:maltitol (4:1), betaine:xylitol (4:1), betaine:urea (1:2), betaine:glycerol (1:1), betaine:citric acid (1:1), citric acid:Suc (1:1) citric acid:sorbytol (1:1), citric acid:maltose (2:1), citric acid:glucose (1:1), citric acid:maltitol (2:1), citric acid:xylitol (1:1), citric acid:fructose (1:1), citric acid:glycerol (1:2), glycerol:sucrose (3:1), glycerol:sorbytol (2:1), glycerol:maltose (3:1), glycerol:glucose (3:1), glycerol:maltitol (3:1), glycerol:xylitol (2:1), glycerol:fructose (3:1), glycerol:galactose (3:1), glycerol:urea (1:1), betaine:glycerol:maltitol (4:4:1), betaine:glycerol:maltose (4:4:1), betaine:glycerol:urea (1:1:2), betaine:glycerol:citric acid (1:1:1), betaine:glycerol:glucose (4:4:1), citric acid:glycerol:maltitol (2:4:1), citric acid:glycerol:maltose (2:4:1), citric acid:glycerol:glucose (1:2:1), urea:glycerol:maltose (3:3:1), urea:glycerol:maltitol (3:3:1), urea:glycerol:glucose (2:2:1)	agitation with heating, UAE	Jeong et al. (2017)
Microalgal biomass	pigments (chlorophylls and carotenoids), proteins, lipides and carbohydrates	1,2-propanediol:choline chloride, water (1:1:1)	Mechanical agitation, beads-assisted extraction, UAE	Cicci et al. (2017)
Wheat and derived products	ochratoxin A	choline chloride:glycerol (1:2) and choline chloride:urea (1:2)	extraction with agitation	Piemontese et al. (2017)
Water	Orchanochlorine pesticides	choline chloride:phenol (1:2), choline chloride:acetic acid (1:2), choline chloride:urea (1:2) and choline chloride:glycerol (1:2)	dispersive solid-phase extraction	Yousefi et al. (2017)
Grape, apple, and orange	pesticides	choline chloride:4-chlorophenol (1:1), Choline chloride:4-chlorophenol (2:1), Choline chloride:4-chlorophenol (2:1)	liquid-liquid microextraction	Farajzadeh et al. (2016)
fish and algae	biological active elements, Cu, Fe, Ni, and Zn	Choline chloride:oxalic acid (2:1, 1:1, 1: 1.5, 1:2 and 1:2.5)	MAE	Ghanemi et al. (2014)
Tea, pharmaceutical supplements	biological active element, cobalt	choline chloride:phenol (1:2), choline chloride:phenol (1:3), choline chloride:phenol (1:4), tetrabutylammonium chloride: decanoic acid (1:2), trioctylammonium chloride-decanoic acid (1:2)	ultrasound-assisted liquid phase microextraction	Arain et al. (2016)
Rice	Cadmium	glycerol:proline (3:1), glycerol:L-alanine (3:1), glycerol:glycine (3:1), glycerol:L-threonine (3:1), glycerol: L-histidine (3:1), Choline chloride: DL-malic acid:water (1:1:2), chloride: L-(+)-tartaric acid:water (1:1:2), chloride: citric acid:water (1:1:2), choline chloride: xylitol:water (5:2:5), , choline chloride: sorbitol:water (5:2:5), choline chloride:D-(+)-xylose:water (3:1:3), choline chloride: glucose:water (5:2:5), choline chloride: fructose:water (5:2:5), choline chloride: sorbose:water (5:2:5), choline chloride: fructose:water (5:2:5), choline	UAE	Huang et al. (2018)

(continued)

Table 3. Continued.

Food matrix	Target compounds	NADES	Type of extraction	Reference
<i>Dioscorea opposita</i> Thunb	polysaccharides	chloride:mannose:water (5:2:5), choline chloride: D-(+)-Galactose:water (5:2:5), choline chloride:sucrose:water (4:1:4), choline chloride:L-(+)-Arabinose:water (5:2:5), choline chloride: L-(+)-Rhamnose:water (2:1:2), choline chloride: L-(+)-trehalose:water (4:1:4), chloride:ethylene glycol (1:1), choline chloride-glycerol (1:1), choline chloride-1,4-butanediol (1:4), and choline chloride-1,6-hexanediol (CCH)	agitation with heating, UAE	Zhang and Wang (2017)
<i>Anredera cordifolia</i>	flavonoid vitexin	betaine:1,4-butanediol (1:3)	Mechanical agitation	Mulia, Muhammad, and Krisanti (2016a) Wang et al. (2016)
<i>Salvia miltiorrhiza</i>	cryptotanshinone tanshinone I and tanshinone II A	choline chloride:ethyl glycol (1:2), choline chloride: glycol (1:2), choline chloride:1,2-butanediol (1:2), choline chloride:1,3-butanediol (1:2), choline chloride:1,4-butanediol (1:2), choline chloride:2,3-butanediol (1:2), choline chloride:1,6-hexanediol (1:2)	ball mill-assisted extraction	
<i>Berberidis Radix, Epimedii Folium, Notoginseng Radix et Rhizoma, Rhei Rhizoma et Radix, and Salviae Miltiorrhizae Radix et Rhizoma</i>	alkaloids, flavonoids, saponins, anthraquinones, and phenolic acids	choline chloride:D-glucose (1:1), choline chloride:maltose (2:1), choline chloride:sucrose (4:1), choline chloride:xylitol (5:2), choline chloride:D-sorbitol (3:1), choline chloride:ethylene glycol (1:2), choline chloride: glycerol (1:2), choline chloride: citric acid (2:1), choline chloride:laevulinic acid (1:2), choline chloride:oxalic acid (1:1), choline chloride:lactic acid (1:1), choline chloride:DL-malic acid (1:1), choline chloride:malonate (1:1), choline chloride:urea (1:2), choline chloride:1-methylurea (1:1), choline chloride:N,N'-dimethylurea (1:1), choline chloride:acetamide (1:1), betaine:D-glucose (1:1), betaine:maltose (5:2), betaine:sucrose (2:1), betaine:xylitol (1:1), betaine:D-sorbitol (1:1.2), betaine:ethylene glycol (1:2), betaine:glycerol (1:2), betaine: citric acid (2:1), betaine:laevulinic acid (1:2), betaine:lactic acid (1:1), betaine:DL-malic acid (1:1), betaine:urea (1:1), betaine:1-methylurea (1:10), L-proline: D-glucose (1:1), L-proline:sucrose (2:1), L-proline:D-sorbitol (1:2), L-proline:glycerol (2:5), L-proline: citric acid (1:1), L-proline:laevulinic acid (1:2), L-proline:oxalic acid (1:1), L-proline:lactic acid (1:1), L-proline:DL-malic acid (1:1), L-proline:malonate (1:1), L-proline:urea (1:1), L-proline:1-methylurea (1:1), L-proline:acetamide (1:1)	UAE	Duan et al. (2016)
<i>Chamaecyparis obtusa</i>	Terpenoids	choline chloride:with ethylene glycol (1:2), choline chloride:with ethylene glycol (1:3), choline chloride:with ethylene glycol (1:4), choline chloride:with ethylene glycol (1:5)	heating reflux extraction, UAE	Tang et al. (2014)
<i>Zingiber officinale</i>	6-gingerol, gingerols, shogoals	sucrose: citric acid (1:1, 2:1, 1:2), L-proline:lactic acid (1:1, 2:1, 1:2), L-proline:oxalic acid (1:1, 2:1, 1:2), trehalose:lactic acid (1:1, 2:1, 1:2),	heating reflux extraction, UAE	Rajan et al. (2015)
<i>Garcinia mangostana</i>	mainly α -mangostin	choline chloride:1,2-propanediol (1:1), choline chloride:1,2-propanediol (1:2), choline chloride:1,2-propanediol (1:3), choline chloride: citric acid (1:1), choline chloride: citric acid (2:1), choline chloride: glycerol (1:1), choline chloride: glycerol (3:2), choline chloride: glucose (1:1), choline chloride: glucose (5:2)	mechanical agitation	Mulia et al. (2015)
Palm oils	tocolos	choline chloride:acetic acid (1:2), choline chloride:malonic acid (1:2), choline chloride: citric acid (3:2)	mechanical agitation	Ng et al. (2015)
Sesame oils	Sesamol	choline chloride/ethylene glycol	UAE microextraction	Liu et al. (2017)

time, increased yields, and often improved quality of the extract (Cui et al. 2015; Lores et al. 2017; Wei et al. 2015a, 2015b), thus they are categorized under 'Green Extraction' techniques. Penetration of NADES into the matrix can be improved by the heat generated during UAE and MAE. The heat reduces solvent viscosity, so that solvent penetration is enabled, resulting in increased extraction yield. The optimization of all extraction parameters is essential to effectively extract the compounds of interest. Such experiments often use response surface methodology (RSM) to determine the optimal extraction conditions with a minimum of experiments (Bi, Tian, and Row 2013; Bosiljkov et al. 2017; Wang et al. 2017b; Wei et al. 2015b; Zhang and Wang 2017).

Since 2002, different Green Metrics Technologies, both qualitative and semi-quantitative have been proposed to evaluate the greenness of extraction techniques. The 'Green Certificate' methodology, which is based on the application of weighted penalty points and the use of a color code, presents a quantitative method which was proposed by Gałuszka et al. (2012). According to the 'Green Certificate', methods are classified according to the eco-scale as: a completely eco-friendly (100 points); excellent green (>75 points); acceptable green (>50 points); and inadequate green (<50 points). This metric system considers the scale of method applications (micro-, meso- and macroscale) and parameters such as reagent toxicity and volume, energy consumption and the amount of wastes generated in the extraction step (Armenta, Garrigues, and de la Guardia 2015).

Ultrasound-assisted extraction

The main driving force in UAE, acoustic cavitation causes disruption of plant cell walls, increase in contact surface, and better penetration of solvent which enhances the mass transport and extraction of active compounds (Tiwari 2015; Tomšik et al. 2016). For the isolation of isoflavones from soy products Bajkacz and Adamek (2017) tested seventeen different NADES formulation, after which choline chloride: citric acid was used to further study. The effect of extraction time, temperature and ultrasonic power at five levels was tested and the following parameters were selected as optimal: extraction time of 60 min, extraction temperature of 60 °C and ultrasonic power of 616 W. Zhang and Wang (2017) found the optimal deep eutectic solvent-based ultrasound-assisted extraction conditions for extraction of polysaccharides from Chinese yam. It was found that NADES composed of choline chloride and 1,4-butanediol and conditions such as water content of 32.89%, extraction temperature of 94 °C, and the extraction time of 44.74 min gave the highest extraction yield obtained at optimal UAE conditions, higher than that obtained with hot water extraction. Following the principles of the 'Green Certificate', a green and efficient NADES based-ultrasound mediated extraction of phenolic compounds from *Larrea cuneifolia* was developed and optimized by Espino et al. (2018). The optimized method utilizing NADES (lactic acid:dextrose = 5:1 with 15% of H₂O (v/v)), plant-solvent ratio of 75 mg mL⁻¹ and

ultrasound time of 42 min presented a highly satisfactory performance, obtaining 95.99 'green' points, which is comparable with the ultrasound mediated water extraction (96.5 'green' points). For water-soluble compounds such as caffeic and ferulic acid, the method showed an extraction yield better than H₂O, while in the case of quercetin and luteolin, poorly water-soluble compounds extraction ability of the proposed method was comparable to methanol extraction.

Microwave-assisted extraction

In MAE, microwave energy is transformed into heat by ionic conduction and dipole rotation mechanisms, in both the solvent and the sample, which can improve the availability of active compounds by interrupting the binding of active compounds to the plant matrix (Destandau, Michel, and Elfakir 2013; Peng et al. 2016). In order to isolate polyphenols and furanocoumarins from fig leaves, the most suitable NADES composition was chosen, followed by the investigation of different MAE parameters (extraction temperature, liquid-solid ratio and extraction time). Extraction yields at optimal MAE parameters obtained by mathematical optimization (temperature 50.58 °C, liquid/solid ratio 24.39 ml g⁻¹, extraction time 43.50 min) were higher than that obtained by UAE (Wang et al. 2017b). Wei et al. (2015a) found the optimal conditions for the MAE assisted simultaneous extraction of four target flavonoids from *Radix Scutellariae* to be: extraction temperature 60.4 °C, solvent to solid ratio 20:1 mL g⁻¹ and extraction time 11.75 min.

In the majority of mentioned studies, an extraction temperature of 50 °C or lower was shown to be optimal. In addition, it was demonstrated that UAE was characterized by longer extraction time in comparison to MAE.

Mechanochemical extraction

However, heat is not always favorable, especially when thermo sensitive compounds are extracted or a long extraction time is applied. To prevent heat-induced decomposition of the isolated compounds, Wang et al. (2016) suggested the utilization of a ball mill for cell disruption to increase the contact between the cell matrix and the viscous NADES resulting in the increase of the extraction yield. Later, Wang et al. (2017a) introduced the concept of mechanochemical extraction, which is characterized by a disruption of biomolecular lipid layers and cell walls in a multidirectional, simultaneous mechanical in-liquid smashing, which results in the release of bioactive compounds such as alkaloids, flavonoids, and catechins (Wang et al. 2017a). This kind of extractions (ball mill and mechanochemical extraction) demonstrated excellent extraction efficiency compared to the conventional solvents within a very short extraction time. Therefore, mechanochemical extraction may be recognized as a novel technique, providing shorter extraction time, simple manipulation and increased yields of high quality extracts.

Negative pressure cavitation extraction

Qi et al. (2015) were the first to study negative pressure cavitation extraction (NPCE) combined with NADES. Continuous air flow introduced into the solvent-solid system, produces an intense cavitation effect and a vigorous stirring effect, facilitating the mass transfer between the mixture of substrate and solvent. Extraction efficiency with NADES was better, compared with a similar method using 80% methanol or UAE (Qi et al. 2015).

Recovery of target compounds from NADES

The design of green and sustainable extraction methods demands solvents providing efficient, safe, sustainable, and a cost effective alternative to conventional ones. That should also include energy reduction in the recovery step of the ingredients from the extract. Ready-to-use food grade extracts, which may be directly applied in various food products, are desirable as they avoid additional recovery steps, being time and cost consuming (Chemat, Vian, and Cravotto 2012). However, there is a lack of research data about direct application of NADES extracts in food formulations. Jeong et al. (2017) suggested that NADES isolated catechins were ready for use without catechin isolation or recovery, since they are stable in NADES consisting of betaine, glycerol, and glucose. In the case when the recovery of pure active compounds is preferable, several methods are available, such as liquid–liquid extraction (LLE) using another solvent, solid phase extraction (SPE) using adsorbents and resins or molecular sieves to bind the solutes. Precipitation by addition of antisolvents (mainly water) can also be applied. Additionally, supercritical carbon dioxide extraction (only for products that have a sufficiently high solubility in CO₂) and crystallization by cooling or adding antisolvents can be used to separate solution and target molecules (Dai et al. 2013c).

Concerning recovery of polyphenolic compounds from NADES extract, the most common method is solid phase extraction using various resins (García et al. 2016). In order to recover the polyphenols from NADES extract, Wang et al. (2017b) used macroporous resin D101 for the recovery of five target polyphenols (e.g. caffeoylmalic acid, psoralicid-glucoside, rutin, psoralen and bergapten). Peng et al. (2016) used a macroporous resin NKA-9 for adsorption of phenolic acids (e.g. chlorogenic acid, caffeic acid, 3,5-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid) from *Lonicerae japonicae* flos after which they were recovered with ethanol solution. According to Wei et al. (2015a), an ME-2 resin effectively adsorbs four target flavonoids, while the polar ingredients of NADES could be eluted with deionized water. Furthermore, all applied types of solid phases demonstrated high efficiency, allowing recovery yields of above 75%, depending on the chemical nature of target compounds. Apart from using resins, polyphenols, such as rutin can be successfully recovered with water as antisolvent with a recovery of 75%. The recovery was increased up to 92% with simple application of solid phase extraction on a reversed-phase (C18) cartridge (Nam

et al. 2015). Huang et al. (2017) applied water as antisolvent, achieving rutin recovery of 95.1%. In addition, the authors described the recovery of rutin and the recyclability of the NADES after rutin recovery by evaporation of water by heating for 2 h at 90 °C under vacuum. The obtained regenerated NADES was successfully reused for rutin extraction (Huang et al. 2017). This simple and green purification of an active compound and the solvent renders this extraction process highly attractive. Ng et al. (2015) used liquid-liquid extraction with water-hexane mixture to separate bioactive compounds from NADES by gravitational settlement. After separation, the residual solvents were evaporated from NADES followed by drying and reused in another set of extraction trials. Moreover, solid phase extraction using HLB cartridges was employed for the recovery of saponins resulting in isolation efficiency higher than 80%. In addition, after recovery of isolated compounds, recycling of the NADES solvent was accomplished by simple freeze-drying of the washed solutions from the SPE.

NADES application to increase food safety

NADES technology has already proven a great applicability potential in food safety sector. NADES were applied in several studies for food analysis, concerning heavy metal and pesticide trace determinations. Since cadmium accumulation in rice is a global issue, Huang et al. (2018) investigated the utilization of choline chloride- and glycerol-based NADES as washing agents for the removal of cadmium from rice flour to avoid the use of environmentally harmful reagents. Rice contaminated with cadmium was washed with 2.0 mL of the washing solution heated to 60 °C and shaken at 100 rpm for 1 h. During this period, ultrasound assisted extraction was performed every 15 min for 5 min. The concentrations of Cd in washed rice flour were determined without chemical modifier using a graphite furnace atomic absorption spectrophotometer, where NADES choline chloride:xylitol:water (5:2:5) was observed to have high Cd removal efficiency (90.4%). The proposed method had no negative effects on the main chemical components or structure of the treated rice flour. Moreover, NADESs are easy to dissolve in water and can be separated from rice flour, making them as convenient washing agents with a wide application in cereal-based industry.

NADES application in food analysis

As solvents, NADES have been used in various food analysis applications. Karimi et al. (2015) applied choline chloride:urea as an effective solvent for the extraction of heavy metals from sesame oil, soybean oil, olive oil, sunflower oil and corn oil. They developed a very sensitive and simple microextraction method using lead and cadmium as the model elements for contaminated samples. Ghanemi et al. (2014) reported an efficient sample preparation method for the analysis of Cu, Fe, Ni, and Zn by an ICP-OES method in marine samples (fish and algae). This method is based on the use of NADES in combination with microwave radiation

for the sample preparation. Microwave conditions were optimized in terms of nitric acid concentration, temperature, microwave power, time and pressure, resulting in several advantages over the conventional method. Microwave digestion using NADES for the preparation and analysis of Cu, Fe, Ni, and Zn provided simplicity of the experimental procedure, the use of low-cost materials, relatively high speed of sample preparation (20 s), low concentration of nitric acid used, lower pressure (1 bar) and lower temperature (150 °C). Therefore, this method was successfully applied in the digestion of different marine samples (muscle and liver tissues from a marine fish, and macroalgae) possessing a broad range of metal concentrations, suggesting that broad range of metals in varied biological matrices can be determined by this method. When NADES were used for the extraction of cobalt from tea samples, only a few steps were needed with a low toxicity extraction solvent (Arain, Yilmaz, and Soylak 2016). Yousefi, Shemirani, and Ghorbanian (2017) described a fast and easy method for the ultra-trace analysis of organochlorine pesticides, using a NADES based magnetic bulky gels. In these gels, deep eutectic solvents (choline chloride:urea) serve as carriers for multi-walled carbon nanotube nanocomposite based nanofluids. These gels were used in developing dispersive solid-phase extraction (dSPE) method, which was reported to be capable of shortening the sample preparation time in comparison to the traditional solid phase extraction. Coupling this method with GC micro-electron capture detector and using large-volume injection technique resulted in low limit of detection values. The high sensitivity, good linearity and good repeatability obtained by the proposed method, make it suitable for application to the trace analysis of water samples.

Farajzadeh, Mogaddam, and Aghanassab (2016) reported a dispersive liquid-liquid microextraction procedure using choline chloride based NADES for the extraction of some pesticide residues in fruit juices and vegetables followed by gas chromatography analysis using flame ionization detection. Furthermore, NADES are suggested to be used as environmentally friendly solvents for biopesticides (Mouden et al. 2017).

Piemontese et al. (2017) used choline chloride:glycerol and choline chloride:urea solvents for the extraction of ochratoxin A which commonly contaminates food commodities including grains, nuts, spices, coffee beans, olives and grapes. Furthermore, choline chloride based NADES were effective in the extraction and solubilization of ochratoxin A, giving a recovery of up to 89% for spiked samples of durum wheat, bread crumbs and biscuits. Recovery and repeatability proved to be within the required specifications set by the current European Commission (2006) regulation (No. 1881/2006). Hence, the use of conventional, hazardous and volatile organic solvents typical of the standard and official methods was successfully substituted with NADES.

Lores et al. (2017) proposed a fast and miniaturized procedure based on the use of NADES in combination with ultrasound-assisted extraction for gluten determination by a commercial enzyme-linked immunosorbent assay.

Gomez et al. (2016) developed a simple and sensitive electrochemical method for quercetin determination from complex plant matrices based on NADES electrolyte combined with unmodified screen-printed electrodes.

NADES were also successfully employed in the extraction of sesamol from sesam oils (Liu et al. 2017). Authors emphasized that the NADES enriched phase was directly injected into a reverse-phase HPLC system for analysis without phase inversion, which could simplify the sample pretreatment process indicating NADES as an ideal, promising green solvent.

NADES based on xylitol, citric acid, and malic acid were used as solvents in ultrasound-assisted extraction of plant samples prior to elemental analysis by inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma-optical emission spectrometry (ICP-OES) for the determination of As, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, and Zn in the extracts (Santana et al. 2019). The extraction recoveries ranged from 80% to 120%, with some analytes presenting poor recoveries. Referring to the authors, UAE using NADES is a promising technique for the elemental extraction of plant samples.

NADES toxicity and biodegradability

NADES are considered as environmentally safe and nontoxic green solvents, since they are derived from natural primary metabolites. However, an important issue for a wide and commercial application is their toxicological impact on different living organisms, and, if any to understand their mode of action. Moreover, one should know their biodegradation and products thereof. Investigations on the toxicity were conducted by using different test models that are listed in Table 4.

In vitro cytotoxicity tests

These tests are considered as a starting point in a toxicity assessment. Furthermore, they allow screening and ranking of chemicals toxicity and provide a clarification of toxic effect of chemicals on basic cell function, and also could play significant role in determination of toxic concentration (Eisenbrand et al. 2002). Cytotoxicity can be considered in two ways, as being a risk factor in toxicity, and as being of interest in cancer treatment (as discussed in the section 'Volatility of NADES'). Radošević et al. (2015) investigated the effect of three choline chloride based NADES (with glucose, glycerol and oxalic acid as hydrogen bond donors) and their individual components, on viability and morphology of channel catfish ovary (CCO) cells and MCF-7 human tumor cells after 72 h of exposure. In both cell lines ChCl:glucose and ChCl:glycerol, as well as their individual components (choline chloride, glycerol and glucose) demonstrated low cytotoxicity (EC_{50} values > 10 mM). However, ChCl:oxalic acid affected viability and morphology of both cell lines by reaching EC_{50} values of 1.64 and 4.19 mM for CCO and MCF-7 cells, respectively. In addition, oxalic acid also exerted cytotoxic effect on both cell lines. Interestingly,

Table 4. Toxicological investigations related to NADES solvents.

Toxicology assessment	Assay	Test model	NADES	Reference
Cytotoxicity	WST-1 assay	CCO fish cell line, MCF-7 human tumor cell line	ChCl:glucose (1:2), ChCl:oxalic acid (1:1), ChCl:glycerol (2:1), ChCl, glycerol, glucose, oxalic acid	Radošević et al. (2015)
	WST-1 assay	CCO fish cell line	ChCl:malic acid (1:1), ChCl:malic acid (1.5:1), ChCl:citric acid (2:1), ChCl:lactic acid (1:1), ChCl:fructose (3:2), ChCl:xylose (2:1), ChCl:mannose (5:2), Choline alaninate, Choline arginate, Choline asparinate, Choline glycinate, Choline lactate, Choline malate, Choline citrate, Choline oxalate	Radošević et al. (2016a)
	MTS assay	L929 fibroblast-like cells	ChCl:D(+) Glucose (1:1), ChCl:citric acid (1:1), ChCl:citric acid (2:1), ChCl:sucrose (4:1), ChCl:sucrose (1:1), ChCl:L(+) tartaric acid (2:1), ChCl:D-xylose (2:1), ChCl:D-xylose (3:1), citric acid:sucrose (1:1), citric acid:D(+) glucose (1:1), D(+) glucose: L(+) tartaric acid (1:1)	Paiva et al. (2014)
	MTT viability assay, Computational methodology for COSMO-RS	Human cervical cancer cell line (HelaS3), human ovarian cancer cell line (CaOV3), mouse skin cancer cell line (B16F10), human breast cancer cell line (MCF-7)	ChCl:Fructose:Water (5:2:5), ChCl:Glucose:Water (5:2:5), ChCl:Sucrose:water (4:1:4), ChCl:Glycerol:Water (1:2:1), ChCl:Malonic acid (1:1).	Hayyan et al. (2016)
	MTT viability assay, Membrane permeability assay, Oxidative stress assay	Human cervical cancer cell line (HelaS3), human prostate cancer cell line (PC3), human gastric cancer cell line (AGS), human skin malignant melanoma cell line (A375), human breast cancer cell line (MCF-7), human hepatocyte cell line (WRL-68)	ChCl:Fructose (2:1), ChCl:Glucose (2:1)	Mbous et al. (2017)
Inhibition of Acetylcholinesterase enzyme (AChE)	Ellman's method	AChE type VI-S (from the electric eel)	Choline:Glycine (1:1), Choline:Alanine (1:1), Choline:Valine (1:1), Choline:Leucine (1:1), Choline:Isoleucine (1:1), Choline:Serine (1:1), Choline:Threonine (1:1), Choline:Methionine (1:1), Choline:Aspartic acid (1:1), Choline:Glutamine (1:1), Choline:Asparagine (1:1), Choline:Glutamine (1:1), Choline:Lysin (1:1), Choline:Histidine (1:1), Choline:Arginine (1:1), Choline:Proline (1:1), Choline:Pheylalanine (1:1), Choline:Tryptophan (1:1), ChCl	Hou et al. (2013)
Catalase inhibition assay	Method by Goth	Serum catalasa	Choline:Glycine (1:1), Choline:Alanine (1:1), Choline:Valine (1:1), Choline:Leucine (1:1), Choline:Isoleucine (1:1), Choline:Serine (1:1), Choline:Threonine (1:1), Choline:Methionine (1:1), Choline:Aspartic acid (1:1), Choline:Glutamine (1:1), Choline:Asparagine (1:1), Choline:Glutamine (1:1), Choline:Lysin (1:1), Choline:Histidine (1:1), Choline:Arginine (1:1), Choline:Proline (1:1), Choline:Pheylalanine (1:1), Choline:Tryptophan (1:1), ChCl	Hou et al. (2013)
Bacterial growth inhibition	Paper disc diffusion method, WST-1 assay	<i>Staphylococcus aureus</i> , <i>Listeria monocytogenes</i> , <i>Escherichia coli</i> , <i>Salmonella enteritidis</i>	ChCl:urea (1:2), ChCl:acetamide (1:2), ChCl:ethylene glycol (1:2), ChCl:glycerol (1:2), ChCl:1, 4-	Zhao et al. (2015)

(continued)

Table 4. Continued.

Toxicology assessment	Assay	Test model	NADES	Reference
	Tube dilution method	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Salmonella enteritidis</i> , <i>Listeria monocytogenes</i>	butanediol (1:4), ChCl:triethylene glycol (1:4), ChCl:xylitol (1:1), ChCl:D-sorbitol (1:1), ChCl:p-toluenesulfonic acid (1:1), ChCl:oxalic acid (1:1), ChCl:levulinic acid (1:2), ChCl:malonic acid (1:1), ChCl:malic acid (1:1), ChCl:citric acid (1:1), ChCl:tartaric acid (2:1), ChCl:xylose:water (1:1:1), ChCl:sucrose:water (5:2:5), ChCl:fructose:water (5:2:5), ChCl:glucose:water (5:2:5), ChCl:maltose:water (5:2:5) Choline:Glycine (1:1), Choline:Alanine (1:1), Choline:Valine (1:1), Choline:Leucine (1:1), Choline:Isoleucine (1:1), Choline:Serine (1:1), Choline:Threonine (1:1), Choline:Methionine (1:1), Choline:Aspartic acid (1:1), Choline:Glutamine (1:1), Choline:Asparagine (1:1), Choline:Glutamine (1:1), Choline:Lysin (1:1), Choline:Histidine (1:1), Choline:Arginine (1:1), Choline:Proline (1:1), Choline:Pheylalanine (1:1), Choline:Tryptophan (1:1), ChCl	Hou et al. (2013)
	Broth dilution methods	<i>Escherichia coli</i>	ChCl:urea (1:1), ChCl:acetamide (1:1), ChCl:glycerol (1:1), ChCl:ethylene glycol (1:1), Choline acetate:urea (1:1), Choline acetate:acetamide (1:1), Choline acetate:glycerol (1:1), Choline acetate:ethylene glycol (1:1)	Wen et al. (2015)
	Filter paper diffusion assay	<i>Staphylococcus aureus</i> , <i>Listeria monocytogenes</i> , <i>Escherichia coli</i> , <i>Salmonella enteritidis</i>	ChCl:1,2-Propanediol (1:1), ChCl:Glycerol (1:1), ChCl:Glucose (2:5), ChCl:Sucrose (1:1), ChCl:Xylitol (1:2), ChCl:Sorbitol (2:5), Glycerol:L-Proline (3:1), Glycerol:L-Alanine (3:1), Glycerol:Glycine (3:1), Glycerol:L-Histidine (3:1), Glycerol:L-Threonine (3:1), Glycerol:L-Lysine (4.5:1), Glycerol:L-Arginine (4.5:1)	Huang et al. (2017)
	Filter paper diffusion assay	<i>Bacillus subtilis</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i>	ChCl:glycerin (3:1), ChCl:ethylene glycol (3:1), ChCl:triethylene glycol (3:1), ChCl:urea (3:1), ChCl, glycerin, ethylene glycol, triethylene glycol, urea	Hayyan et al. (2013)
Toxicity towards yeast cells	FTIR – based biological bioassay	yeast strain <i>Saccharomyces cerevisiae</i> (LCF 520)	Benzoic acid:betain (1.5:1), 2-Hydroxy-benzoic (salicylic) acid:betain (1.5:1), 4-Chloro-benzoic acid:betain (1.5:1), 2-Chloro-benzoic acid:betain (1.5:1), 3-Chloro-benzoic acid:betain (1.5:1), 2-Furoic acid:betain (2:1), Phenylacetic acid:betain (2:1), D-(+)-Mandelic acid:betain (1:1), Glycolic acid:betain (2:1), Oxalic acid:betain (2:1), Citric acid:betain (1.5:1)	Cardellini et al. (2014)
Phytotoxicity	Toxicity test on garlics (measurement of the root lengths and observation of the root tip cells abnormality)	Garlic cloves (<i>Allium sativum</i>)	ChCl:urea (1:1), ChCl:acetamide (1:1), ChCl:glycerol (1:1), ChCl:ethylene glycol (1:1), Choline acetate:urea (1:1), Choline acetate:acetamide (1:1), Choline acetate:glycerol (1:1), Choline acetate:ethylene glycol (1:1)	Wen et al. (2015)

(continued)

Table 4. Continued.

Toxicology assessment	Assay	Test model	NADES	Reference
	Toxicity test on wheat seeds (monitoring of germination inhibition, shoot height and root length inhibition), Lipid peroxidation assay on leaves, Determination of chlorophyll content in leaves, Antioxidant enzyme activities assays on leaves	Wheat seeds (<i>Triticum aestivum</i>)	ChCl:glucose (1:2), ChCl:oxalic acid (1:1), ChCl:glycerol (2:1), ChCl, glycerol, glucose, oxalic acid	Radošević et al. (2015)
Toxicity towards aquatic organisms	Toxicity test on hydras (mesurment of survival time and monitoring of morphological changes)	Hydra (<i>Hydra sinensis</i>)	ChCl:urea (1:1, 1:2 or 2:1), ChCl:acetamide (1:1, 1:2 or 2:1), ChCl:glycerol (1:1, 1:2 or 2:1), ChCl:ethylene glycol (1:1, 1:2 or 2:1), salt ChCl, Choline acetate:urea (1:1, 1:2 or 2:1), Choline acetate:acetamide (1:1, 1:2 or 2:1), Choline acetate:glycerol (1:1, 1:2 or 2:1), Choline acetate:ethylene glycol (1:1, 1:2 or 2:1), salt Choline acetate	Wen et al. (2015)
	Brine shrimp assay (Hatching of brine shrimp eggs)	Brine shrimp larvae or hatches (<i>Artemia salina leach</i>)	ChCl:glycerin (3:1), ChCl:ethylene glycol (3:1), ChCl:triethylene glycol (3:1), ChCl:urea (3:1), ChCl, glycerin, ethylene glycol, triethylene glycol, urea	Hayyan et al. (2013)
Toxicity towards mice and rats	<i>In vivo</i> assessment (Investigation of biochemical and histological parameters related to the liver and kidney function, 15 days after administration)	Imprinting Control Region (ICR) mice	ChCl:Fructose (2:1), ChCl:Glucose (2:1)	Mbous et al. (2017)
	<i>In vivo</i> assessment	Wistar rats	Betaine:glycerol, 1:2 mole ratio +10% (v/v) of water, Proline:glutamic acid (2:1)	Benlebna et al. (2018) Faggian et al. (2016)
Biodegradability	Closed bottle tests		ChCl:urea (1:1), ChCl:acetamide (1:1), ChCl:glycerol (1:1), ChCl:ethylene glycol (1:1), Choline acetate:urea (1:1), Choline acetate:acetamide (1:1), Choline acetate:glycerol (1:1), Choline acetate:ethylene glycol (1:1)	Wen et al. (2015)
	Closed bottle tests		ChCl:glucose (1:2), ChCl:oxalic acid (1:1), ChCl:glycerol (2:1), ChCl, glycerol, glucose, oxalic acid	Radošević et al. (2015)
	Closed bottle test; CO ₂ headspace tests		Choline:Glycine (1:1), Choline:Alanine (1:1), Choline:Valine (1:1), Choline:Leucine (1:1), Choline:Isoleucine (1:1), Choline:Serine (1:1), Choline:Threonine (1:1), Choline:Methionine (1:1), Choline:Aspartic acid (1:1), Choline:Glutamine (1:1), Choline:Asparagine (1:1), Choline:Glutamine (1:1), Choline:Lysin (1:1), Choline:Histidine (1:1), Choline:Arginine (1:1), Choline:Proline (1:1), Choline:Pheylalanine (1:1), Choline:Tryptophan (1:1), ChCl	Hou et al. (2013)
	Closed bottle test		ChCl:1,2-Propanediol (1:1), ChCl:Glycerol (1:1), ChCl:Glucose (2:5), ChCl:Sucrose (1:1), ChCl:Xylitol (1:2), ChCl:Sorbitol (2:5), Glycerol:L-Proline (3:1), Glycerol:L-Alanine (3:1), Glycerol:Glycine (3:1), Glycerol:L-Histidine (3:1), Glycerol:L-Threonine (3:1), Glycerol:L-Lysine (4.5:1), Glycerol:L-Arginine (4.5:1)	Huang et al. (2017)

(continued)

Table 4. Continued.

Toxicology assessment	Assay	Test model	NADES	Reference
	Closed bottle test		ChCl:urea (1:2), ChCl:acetamide (1:2), ChCl:ethylene glycol (1:2), ChCl:glycerol (1:2), ChCl:1, 4- butanediol (1:4), ChCl:triethylene glycol (1:4), ChCl:xylitol (1:1), ChCl:D-sorbitol (1:1), ChCl:p- toluenesulfonic acid (1:1), ChCl:oxalic acid (1:1), ChCl:levulinic acid (1:2), ChCl:malonic acid (1:1), ChCl:malic acid (1:1), ChCl:citric acid (1:1), ChCl:tartaric acid (2:1), ChCl:xylose:water (1:1:1), ChCl:sucrose:water (5:2:5), ChCl:fructose:water (5:2:5), ChCl:glucose:water (5:2:5), ChCl:maltose:water (5:2:5)	Zhao et al. (2015)

CCO cells were more susceptible to ChCl:oxalic acid and oxalic acid than MCF-7 cells. According to Radošević et al. (2015) observed cytotoxic effect of ChCl:oxalic acid and oxalic acid was result of the formation of calcium oxalate crystals in cells, exhibiting significant detrimental effect on cells. The observed inhibition of cell proliferation could be related to the changes in pH of the culture medium after addition of ChCl:oxalic acid or oxalic acid, since authors determined significant decrease in cell culture medium pH values after the addition of mentioned compounds. In their further investigations, Radošević et al. (2016b) reported low inhibition activity ($EC_{50} > 2000 \text{ mg L}^{-1}$) of five choline chloride-based NADES containing glucose, fructose, xylose, glycerol and malic acid towards viability of two human cancer cell lines, MCF-7 cell line derived from breast adenocarcinoma and HeLa cell line derived from cervical adenocarcinoma. In line with their previous research, Radošević et al. (2016a) found low cytotoxic effect of several ChCl-based NADES ($EC_{50} > 2000 \text{ mg mL}^{-1}$) and choline-based NADES ($EC_{50} > 2000 \text{ mg mL}^{-1}$), except for choline:oxalic acid (that reached $EC_{50} = 1738 \text{ mg mL}^{-1}$) on CCO cells. These authors observed slightly higher toxicity of ChCl:malonic acid NADES; treatment of CCO cells with ChCl:malonic acid at different molar ratio resulted in significant differences in measured response, indicating that molar ratio had an impact on toxicity. Valuable information about relation between NADES chemical nature and their cytotoxicity was provided by Paiva et al. (2014), who examined impact of 11 different NADES on viability of L929 fibroblast-like cells at the NADES concentration of 25 mg mL^{-1} . In this study, NADES containing tartaric acid demonstrated the highest cytotoxicity. These results together with the results of other studies (Radošević et al. 2015; Hayyan et al. 2016) lead few authors to hypothesize that the nature of hydrogen bond donors (HBD) has great impact on NADES cytotoxicity. Very interesting results were obtained for citric acid-based NADES, where citric acid:sucrose (1:1) exhibited the lowest inhibition activity (about 100% of cell viability was reached), while citric acid:glucose (1:1) was among the most potent NADES (about 10% of viability). In addition, choline chloride: citric acid at the molar rate of 1:1

showed few times lower cytotoxic effect than that of molar rate of 2:1, while different molar rate in the case of choline chloride:sucrose did not caused statistically significant differences in inhibition activity. These results indicate that toxic effect of NADES is probably a consequence of NADES unique properties that could be tailored by right combination of NADES component as well as by their molar ratio. Additionally, NADES viscosity could also play significant role in cytotoxic effects, which was investigated by Hayyan et al. (2016) who reported the influence of viscosity and water addition of choline chloride based-NADES cytotoxicity on three human cancer cell lines (HeLaS3, CaOV3; MCF-7) and one mouse cancer cell line (B16F10), after 48 h of exposure. It was found that NADES composed of choline chloride and malonic acid (1:1) without addition of water, demonstrated the higher inhibition activity on viability of all four investigated cell lines, by reaching IC_{50} values in range of 15 to 35 mM, that were several-fold lower than IC_{50} values of other investigated NADES. Also, ChCl:glycerol demonstrated slightly higher activity towards mice cancer cells in comparison to investigated human cancer cells. Although cytotoxicity of other investigated NADES was mainly in correlation with their viscosity, Hayyan et al. (2016) pointed out connection between NADES toxicity and cellular requirements of cancer cells, and highlighted the application of COSMO-RS software for the analysis of the cytotoxic mechanism of NADESs. Simulation of the interactions between NADESs and phospholipids from cellular membranes suggested that NADES strongly interacted with cell surfaces, while their cytotoxicity might have been determined by their accumulation and aggregation. Software simulation indicated that NADES could lead to disintegration of cell membrane and consequently cell damage, which was also proposed by other researchers (Hayyan et al. 2016; Mbous et al. 2017; Paiva et al. 2014; Radošević et al. 2015, 2016a). Mbous et al. (2017) assessed cytotoxicity of choline chloride:fructose (2:1) and choline chloride:glucose (2:1) by applying MTT viability assay on five cancer (HelaS3, PC3, A375;AGS, MCF-7) and one non-cancer (WRL-68) human cell lines after 48 h of exposure. According to the obtained EC_{50} values (98–516 mM),

investigated NADES were classified as a harmful material. Additionally, significant differences in activity were observed between investigated NADES. Moreover, susceptibility of applied cell lines varied significantly, with AGS cells being the most susceptible to NADES cytotoxicity, whilst HeLaS3 the least. Interestingly, NADES exhibited significant cytotoxicity towards applied non-cancer cell line, such as human hepatocyte cell line (WRL-68) ($EC_{50} = 112$ and 185 mM, for ChCl:fructose and ChCl:glucose, respectively) indicating potential toxic effect on health cells. Authors also observed that examined NADES increased membrane permeability and elevated oxidative stress in applied cells after 24 h of exposure, but to a lesser extent in comparison to DES.

Inhibition potency towards enzymes

Regarding NADES inhibition potency towards enzymes, Hou et al. (2013) reported the inhibition activity of numerous choline:amino acids NADES towards acetylcholinesterase (EC_{50} values in range 2400 – 3800 μ M), a key enzyme in the termination of impulse cholinergic neurotransmission distributed in nearly all higher organisms, using well known Ellman's method (Nadpal et al. 2018). The reported inhibition potency of examined NADES toward activity of serum catalase, by reaching EC_{50} values in range 1.95 – 2.41 M, indicated that NADES exhibited the greater affinity for inhibition of acetylcholinesterase in comparison to catalase (Hou et al. 2013).

Invertebrates, plants, fish, algae and bacteria are widely utilized test organisms for bioassays applied in ecotoxicological assessment (Hassan et al. 2016).

Antimicrobial activity

NADES antimicrobial activity was investigated in few studies by using several different bacterial strains. Wen et al. (2015) estimated the impact of NADES on the growth of *E. coli* by inoculating the *E. coli* strain into a DES-containing Mueller–Hinton broth and comparing it with the same bacterium grown in the DES-free medium. The obtained results indicated that the examined choline chloride and choline acetate-based NADES were nontoxic at lower concentrations (<75 mM), while exhibited antibacterial activity at higher concentrations. Additionally, authors observed that investigated NADESs were more toxic than the solutions of their individual components, as well as that toxicity was related not only to HBD, but also to the salt. Moreover, Zhao et al. (2015) demonstrated that among numerous examined NADES, only those based on organic acids exhibited antibacterial activity. The observed toxicity could be a consequence of pH changes, since authors observed that the pH of organic acid based NADES was far below the optimal for bacterial growth. Gram negative bacteria were more sensitive in comparison to Gram positives, possibly due to the interaction of NADES components with the polysaccharide or peptide chains of the cell wall through hydrogen bonding or electrostatic interactions, resulting in damage of cell walls

(Hou et al. 2013; Zhao et al. 2015). Hayyan et al. (2013) and Huang et al. (2017) by performing the qualitative evaluation of antimicrobial activity of choline chloride ChCl- and glycerol-based NADES towards two Gram positive and two Gram negative bacteria strains did not observed any inhibition activity. However, these results could be also in agreement with the results of the previous studies showing no or low toxicity of low NADES concentration to bacteria.

By evaluating the toxic impact of glycolic acid/trimethylglycine NADES via an FTIR-bioassay on *Saccharomyces cerevisiae* cells, Cardellini et al. (2014) observed that this eutectic mixture acted as a dehydrating agent on yeast cells.

Phytotoxicity

NADES phytotoxicity was assessed in two studies. Wen et al. (2015) investigated toxic effect of ChCl and choline acetate (ChAc)- based NADES and their individual components on the root growth of *Allium sativum* (garlic), at the concentration of 0.01 M, after 7 days of exposure. It was found that the extent of observed deleterious effect was dependent on the chemical nature of examined compounds. Namely, salt ChCl demonstrated significant inhibitory activity on the root length growth, as well as the HBD ($G > EG > A$) and two NADES (ChCl:A and ChCl:EG). Phytotoxicity was lower when these compounds were incorporated in NADES, while ChCl based-NADES were more toxic than ChAc-based, accordingly to the higher toxicity of ChCl salt. The electron micrographs of the root tip cell revealed that all NADES or their components damaged root cells to certain extent. Similarly, Radošević et al. (2015) observed the low germination inhibition of ChCl-based NADES on *Triticum aestivum* (wheat) ($EC_{50} > 5000$ mg mL^{-1}), whereas after germination, NADES inhibited root growth (EC_{50} in the range 409 – 3249 mg mL^{-1}) to higher extent than shoot growth (EC_{50} in the range 490 – 3698 mg mL^{-1}), that could be a consequence of direct root contact with NADES in a medium. Significant differences in activity between the tested NADES were noticed with ChCl:oxalic acid being the most toxic, followed by ChCl:glucose and ChCl:glycerol. In other studies, the observed toxicity of oxalic acid-derived NADES were probably caused by changes in the pH of a medium after its addition, since significantly lower pH values of ChCl:oxalic acid solution were recorded than for the others. ChCl:glucose exhibited only 2 to 3 times lower inhibition activity than that of ChCl:oxalic acid that could be the result of its greater uptake by the root. In addition, it seems that tested NADES caused the growth inhibition of wheat in early stage of the development by causing oxidative stress as a consequence of their inhibitory activity towards antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) (Radošević et al. 2015).

Toxicity towards aquatic organisms

Certain toxic effects on aquatic organisms were reported in two studies. Hayyan et al. (2013) found toxicity of ChCl-

based NADES towards *Artemia salina leach* (brine shrimp larvae or hatches). Although authors discussed and compared toxic effect of NADES individual components and solution of NADES component in the same concentration as of NADES, the exact applied concentration is not available for the comparison. However, authors pointed out that viscosity and lack of oxygen could also be related to the higher toxicity of NADES in comparison to that observed for solutions of salt and HBD. On the contrary, Wen et al. (2015) investigated the toxic effect of ChCl and ChAc-based NADES towards *Hydra sinensis* (hydras), at the concentration of 10 mM. Furthermore, these authors compared the toxicity of salts, as well as ChCl and ChAc-based NADES with four HBDs at three molar ratios (1:1, 1:2 and 2:1). The obtained result revealed deleterious effect of pure salts, while HBD did not show significant activity. The incorporation of these salts in NADES predominantly lowered their toxic impact. Although ChCl salt demonstrated the higher toxic impact in comparison to ChAc, the differences in activity between ChCl and ChAc-based NADES were noticeable only in the case of using acetamid as HBD, whereas NADES with other HBD did not demonstrated significant salt-dependent variation in activity. Regarding molar ratio, it seems that molar ratio had the significant effect to certain extent, but not for all investigated NADES.

In general, heretofore studies have indicated that NADES toxicity is predominantly related to the chemical nature of NADES, being a function of the hydrogen bonding. Several studies demonstrated that the presence of organic acids in NADES increased the deleterious effects (Hayyan et al. 2013; 2016; Paiva et al. 2014; Radošević et al. 2016a, 2016b). By comparing the toxicity of ChCl- and ChAc based NADES, Wen et al. (2015) concluded that the nature of the salt also had the influence on NADES toxicity. Moreover, the molar ratio affected the toxicity of certain NADES (e.g. ChAc:urea, ChCl: citric acid, ChCl: malic acid) (Paiva et al. 2014; Radošević et al. 2016a; Wen et al. 2015). Mbous et al. (2017) suggested that the presence of water in NADES decrease their toxic effect. Both factors, the molar ratio and water content, are associated with NADES viscosity, so these results are in line with the reported relationship between higher viscosity and higher cell lethality (Hayyan et al. 2016). However, these presumptions need further experimental investigations. Hayyan et al. (2013) confirmed the higher toxicity of tested NADES on brine shrimp than that of their individual components, while Radošević et al. (2015) found similar observation in the case of ChCl:oxalic acid NADES on two cell lines. On the contrary, Wen et al. (2015) observed that investigated NADES exhibited lower phytotoxicity in comparison to their individual components. Therefore, it is inconclusive whether toxic effect is caused by NADES, since observed and compared toxicity could be the result of changes in optimal experimental conditions for applied test organisms, such as changes in pH value in medium after NADES addition (Mbous et al. 2017; Radošević et al. 2015; Zhao et al. 2015), the lack of oxygen and difficulty in movement of aquatic organisms caused by high viscosity (Hayyan et al. 2013; Radošević et al. 2016a),

as well as NADES dehydrating ability (Cardellini et al. 2014). Performed *in vitro* studies on cell cultures demonstrated the variation in cell sensitivity that could be associated with the application of different experimental conditions as well as by the sensitivity of used cell types. Intrinsic cell sensitivity presents only one factor for potential toxicity. The important factors for the toxicity estimation include: chemical kinetics (absorption, biotransformation, distribution and excretion) of investigated compounds that have direct influence on the concentration to which cells are exposed *in vivo* (Eisenbrand et al. 2002). However, these information for NADES still remain to be explored.

Animal toxicity

Acute toxicity assessment was performed in one study by using mice as test animals (Mbous et al. 2017) at three applied doses: high-dose (20 g kg⁻¹), medium-dose (10 g kg⁻¹) low-dose (5 g kg⁻¹) and vehicle group (H₂O) as a control. According to obtained results of biochemical analysis of animal's blood, investigated NADESs exhibited hepatotoxic effect. In addition, LD₅₀ values were determined (LD₅₀ = 1.84 and 1.24 g mL⁻¹, for ChCl:fructose and ChCl:glucose, respectively). However, the way of administration of these very viscous NADES remain unclear, as well as the observed possible hepatotoxicity that could be attributed to their viscosity. There are only few reports which applied *in vivo* rat model methods to test the NADES toxicity. It was proven that proline:glutamic acid (2:1) (Faggian et al. 2016), proline:malic acid:lactic acid:water (1:0.2:0.3:0.5), proline:malic acid (1:2) and proline:urea (2:1) NADES (Sut et al. 2017) can be administered orally at moderate doses without major health hazards on rats. However, Benlebna et al. (2018) demonstrated some adverse effects of the oral administration of a high dose of the extract from green coffee beans in NADES extract (betaine:glycerol, 1:2 mole ratio +10% (v/v) of water) in rats. The gavage of this extract induced mortality in two rats out of six, dietary restriction, excessive water consumption, weight loss, adipose tissue loss, hepatomegaly, plasma oxidative stress, and increased blood lipid levels. The authors associated these effects with the large quantity of NADES extracts provided to rats.

Biodegradation of NADES

Biodegradation of NADES plays an important role in their overall ecotoxicological impact. A non-diluted NADES will degrade very slowly, but if sufficient amount of water is added to NADES prior to their disposal, the biodegradation after the dilution goes easily. The majority of investigated compounds were classified as readily biodegradable compounds (Hou et al. 2013; Huang et al. 2017; Radošević et al. 2015; Wen et al. 2015; Zhao et al. 2015). Wen et al. (2015) found that not all NADES are highly biodegradable. It was demonstrated that NADES biodegradation is associated with their chemical nature (Hou et al. 2013; Huang et al. 2017; Radošević et al. 2015; Wen et al. 2015; Zhao et al. 2015). Dai et al. (2015) confirmed that dilution of NADES with

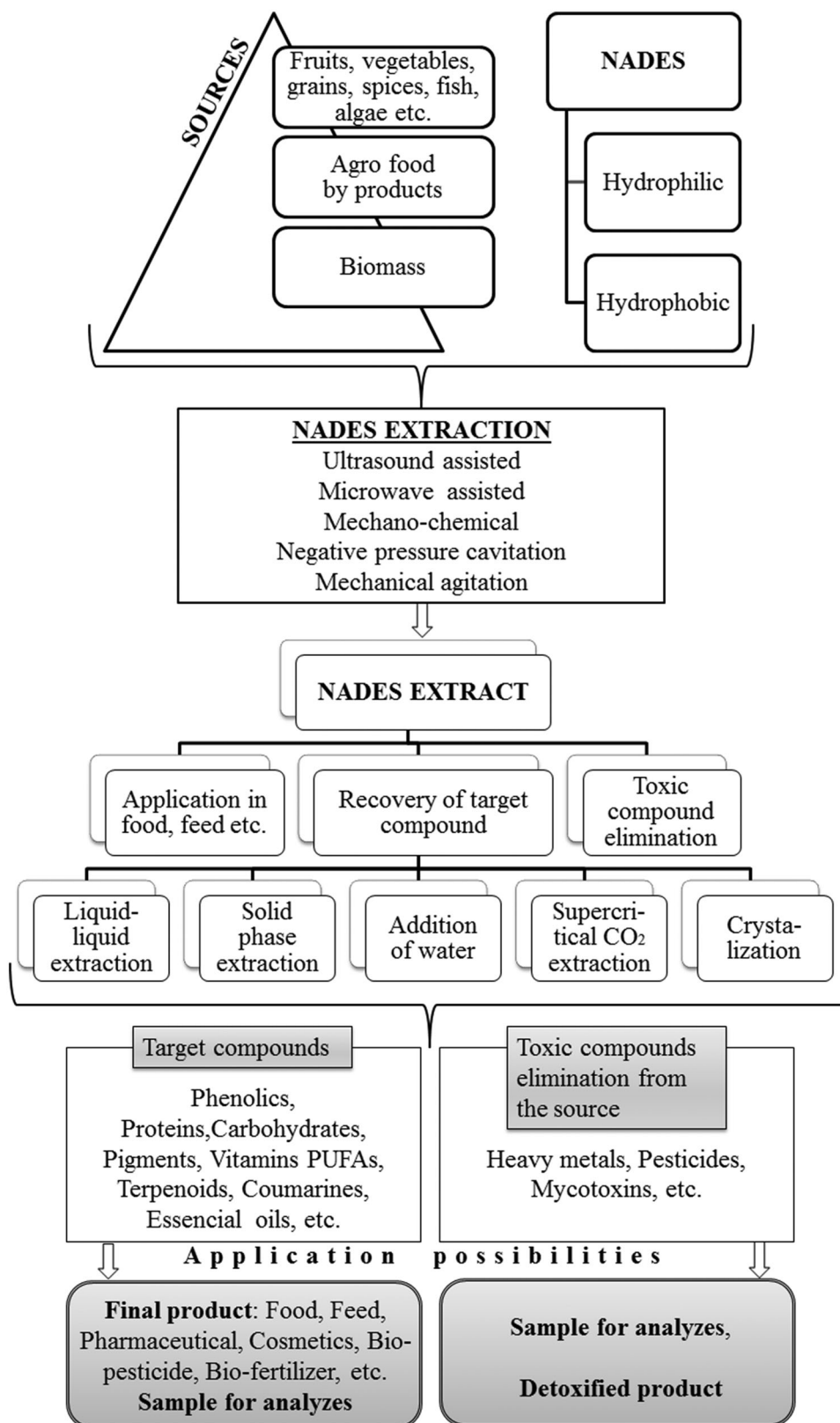


Figure 1. The perspectives of natural deep eutectic solvents in agri-food sector.

water weaken their interactions, which completely disappeared upon NADES dilution of 50% v/v. Consequently, NADES dilution had the significant impact on the observed ratio of biodegradation that could partially explain the differences in observed biodegradability.

Taking all above mentioned facts into account, the examined NADES are mainly considered as low toxic and readily biodegradable solvents. One cannot extrapolate non toxicity of the individual ingredients to nontoxicity of the NADES. The evaluation of toxicity and biodegradability of each

NADES needs to be assessed. So far, only preliminary information on toxicological profiles of predominantly choline NADES have been reported, probably as this compound is the only for which a maximum safe daily dose has been reported. For the most other common NADES constituents such as sugars, organic acids and amino acids no toxicity profiles are known. Consequently, further investigations are required on potential toxicity of NADES in relation to their safety for humans.

Application of NADES extracts from Agri-industrial sources

As discussed above, bioactive components from agri-industrial sources can be recovered using NADES-mediated extraction technologies which follow the principles of green chemistry (Figure 1). Relying on the findings of Faggian et al. (2016) and Sut et al. (2017), the ability of NADES to improve bioavailability opens interesting possibilities for their use as vehicles of bioactive compounds for pharmaceutical and nutraceutical applications. Antimicrobial activity of NADES extracts provides the opportunity for the development of pharmaceutical formulations, such as a topical formulation for dermal candidiasis (Espino et al. 2018). Apart from food/feed, pharmaceutical and nutraceutical applications, NADES extracts from agro-industrial sources can be considered for the production of green agrochemicals (fertilizers and diverse pesticides) to address the global challenges for sustainable food production (Mouden et al. 2017).

Conclusion and future perspectives

Over the last few years, the investigation of NADES utilization as extraction solvents for phytochemicals and other food components has been expanding rapidly. They present the green alternatives to conventional organic solvents, with unique physicochemical properties that offer possibility to design the solvents for specific purposes. Also, it was demonstrated that these solvents in most cases provide higher extraction efficiency in comparison to the conventional organic solvents. NADES extraction is frequently combined with microwave- and ultrasound-assisted techniques, innovative extraction technologies in terms of reduction energy and required time, as well as in terms of enhancing extraction yields. Apart from that, NADES showed several advantages over the organic solvents, such as enhanced biological activity of bioactive compounds and their higher stability in NADES solvents under different storage conditions. Nevertheless, there are several challenges in the development of sustainable analytical methodologies and the application of new green solvents, such as deficient sensitivity, inadequate accuracy and precision, increased implementation costs, as well as commercial availability of solvents and their price. Since NADES are nonvolatile, there is still a limited number of techniques to concentrate and isolate bioactive compounds or food components from NADES. Such studies will be important for developing new applications.

On the other hand, several studies demonstrated that NADES do not interfere with the analytical determination of solutes, offering the possibilities for solute analytical characterization without time- and cost-consuming purification steps. Although NADES are frequently used as solvents in food research at lab scale, NADES scale-up application in industry is hardly explored. In addition, the possibilities for applications of NADES in products for human consumption still need to be developed, including the assessment of their rheological behavior, as well as their toxicological and biological impact. To eventually exploit the obviously high potential of NADES in food analysis, and food processing, different applications need urgently to be explored. Apart from fundamental research dealing with NADES formation and the nature of the interactions and structure underpinning the liquid phase formation, the question of purity of NADES obtained by different synthetic methodologies needs to be addressed in the future.

Disclosure statement

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References

- Abbott, A. P., D. Boothby, G. Capper, D. L. Davies, and R. K. Rasheed. 2004. Deep eutectic solvents formed between choline chloride and carboxylic acids: Versatile alternatives to ionic liquids. *Journal of the American Chemical Society* 9:9142–7. doi: [10.1021/ja048266j](https://doi.org/10.1021/ja048266j).
- Aissaoui, T., Y. Benguerba, and I. M. AlNashef. 2017. Theoretical investigation on the microstructure of triethylene glycol based deep eutectic solvents: COSMO-RS and TURBOMOLE prediction. *Journal of Molecular Structure* 1141:451–6. doi: [10.1016/j.molstruc.2017.04.009](https://doi.org/10.1016/j.molstruc.2017.04.009).
- Altamash, T., M. S. Nasser, Y. Elhamarnah, M. Magzoub, R. Ullah, B. Anaya, S. Aparicio, and M. Atilhan. 2017. Gas solubility and rheological behavior of natural deep eutectic solvents (NADES) via combined experimental and molecular simulation techniques. *Chemistryselect* 2:7278–95. doi: [10.1002/slct.201701223](https://doi.org/10.1002/slct.201701223).
- Altamash, T., M. S. Nasser, Y. Elhamarnah, M. Magzoub, R. Ullah, H. Qiblawey, S. Aparicio, and M. Atilhan. 2018. Gas solubility and rheological behavior study of betaine and alanine based natural deep eutectic solvents (NADES). *Journal of Molecular Liquids* 256:286–95. doi: [10.1016/j.molliq.2018.02.049](https://doi.org/10.1016/j.molliq.2018.02.049).
- Anastas, P., and N. Eghbali. 2010. Green chemistry: Principles and practice. *Chemical Society Reviews* 39 (1):301–12. doi: [10.1039/B918763B](https://doi.org/10.1039/B918763B).
- Araín, M. B., E. Yilmaz, and M. Soylak. 2016. Deep eutectic solvent based ultrasonic assisted liquid phase microextraction for the FAAS determination of cobalt. *Journal of Molecular Liquids* 224:538–43. doi: [10.1016/j.molliq.2016.10.005](https://doi.org/10.1016/j.molliq.2016.10.005).
- Armenta, S., S. Garrigues, and M. de la Guardia. 2015. The role of green extraction techniques in green analytical chemistry. *TrAC Trends in Analytical Chemistry* 71:2–8. doi: [10.1016/j.trac.2014.12.011](https://doi.org/10.1016/j.trac.2014.12.011).

- Aroso, I. M., A. Paiva, R. L. Reis, A. Rita, and C. Duarte. 2017. Natural deep eutectic solvents from choline chloride and betaine – Physicochemical properties. *Journal of Molecular Liquids* 241: 654–61. doi: [10.1016/j.molliq.2017.06.051](https://doi.org/10.1016/j.molliq.2017.06.051).
- Ashworth, C. R., R. P. Matthews, T. Welton, and P. A. Hunt. 2016. Doubly ionic hydrogen bond interactions within the choline chloride-urea deep eutectic solvent. *Physical Chemistry Chemical Physics* 18 (27):18145–60. doi: [10.1039/C6CP02815B](https://doi.org/10.1039/C6CP02815B).
- Azizi, N., T. Soleymani, and M. Mahmoudi. 2017. Multicomponent domino reactions in deep eutectic solvent: An efficient strategy to synthesize multisubstituted cyclohexa-1, 3-dienamines. *Journal of Molecular Liquids* 246:221–4. doi: [10.1016/j.molliq.2017.09.049](https://doi.org/10.1016/j.molliq.2017.09.049).
- Bajkacz, S., and J. Adamek. 2018. Development of a method based on natural deep eutectic solvents for extraction of flavonoids from food samples. *Food Analytical Methods* 11 (5):1330–4. doi: [10.1007/s12161-017-1118-5](https://doi.org/10.1007/s12161-017-1118-5).
- Bajkacz, S., and J. Adamek. 2017. Evaluation of new natural deep eutectic solvents for the extraction of isoflavones from soy products. *Talanta* 168:329–35. doi: [10.1016/j.talanta.2017.02.065](https://doi.org/10.1016/j.talanta.2017.02.065).
- Bakirtzi, C., K. Triantafyllidou, and D. P. Makris. 2016. Novel lactic acid-based natural deep eutectic solvents: Efficiency in the ultrasound-assisted extraction of antioxidant polyphenols from common native Greek medicinal plants. *Journal of Applied Research on Medicinal and Aromatic Plants* 3 (3):120–7. doi: [10.1016/j.jarmap.2016.03.003](https://doi.org/10.1016/j.jarmap.2016.03.003).
- Benlebna, M., M. Ruesgas-Ramón, B. Bonafos, G. Fouret, F. Casas, C. Coudray, E. Durand, M. Cruz Figueroa-Espinoza, and C. Feillet-Coudray. 2018. Toxicity of natural deep eutectic solvent betaine:glycerol in rats. *Journal of Agricultural and Food Chemistry* 66 (24): 6205–12. doi: [10.1021/acs.jafc.8b01746](https://doi.org/10.1021/acs.jafc.8b01746).
- Bi, W., M. Tian, and K. H. Row. 2013. Evaluation of alcohol-based deep eutectic solvent in extraction and determination of flavonoids with response surface methodology optimization. *Journal of Chromatography A* 1285:22–30. doi: [10.1016/j.chroma.2013.02.041](https://doi.org/10.1016/j.chroma.2013.02.041).
- Bosiljkov, T., F. Dujmić, M. Cvjetko Bubalo, J. Hribar, R. Vidrih, M. Brnčić, E. Zlatić, I. Radojčić Redovniković, and S. Jokić. 2017. Natural deep eutectic solvents and ultrasound-assisted extraction: Green approaches for extraction of wine lees anthocyanins. *Food and Bioprocess Technology* 102:195–203. doi: [10.1016/j.fbp.2016.12.005](https://doi.org/10.1016/j.fbp.2016.12.005).
- Castro, V. I. B., R. Craveiro, J. M. Silva, R. L. Reis, A. Paiva, and C. A. R. Duarte. 2018. Natural deep eutectic systems as alternative non-toxic cryoprotective agents. *Cryobiology* 83:15–26. doi: [10.1016/j.cryobiol.2018.06.010](https://doi.org/10.1016/j.cryobiol.2018.06.010).
- Cao, X., Z. Jiang, W. Cui, Y. Wang, and P. Yang. 2016. Rheological properties of municipal sewage sludge: Dependency on solid concentration and temperature. *Procedia Environmental Sciences* 31:113–21. doi: [10.1016/j.proenv.2016.02.016](https://doi.org/10.1016/j.proenv.2016.02.016).
- Cardellini, F., M. Tiecco, R. Germani, G. Cardinali, L. Corte, L. Roscini, and N. Sperti. 2014. Novel zwitterionic deep eutectic solvents from trimethylglycine and carboxylic acids: Characterization of their properties and their toxicity. *RSC Advances* 4 (99): 55990–6002. doi: [10.1039/C4RA10628H](https://doi.org/10.1039/C4RA10628H).
- Chemat, F., M. A. Vian, and G. Cravotto. 2012. Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences* 13 (7):8615–27. doi: [10.3390/ijms13078615](https://doi.org/10.3390/ijms13078615).
- Choi, Y. H., J. van Spronsen, Y. Dai, M. Verberne, F. Hollmann, I. W. C. E. Arends, G.-J. Witkamp, and R. Verpoorte. 2011. Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology? *Plant Physiology* 156 (4):1701–5. doi: [10.1104/pp.111.178426](https://doi.org/10.1104/pp.111.178426).
- Cicci, A., G. Sed, and M. Bravi. 2017. Potential of choline chloride-based natural deep eutectic solvents (NADES) in the extraction of microalgal metabolites. *Chemical Engineering Transactions* 57:61–6.
- Craveiro, R., I. Aroso, V. Flammia, T. Carvalho, M. T. Viciosa, M. Dionísio, S. Barreiros, R. L. Reis, A. R. C. Duarte, and A. Paiva. 2016. Properties and thermal behavior of natural deep eutectic solvents. *Journal of Molecular Liquids* 215:534–40. doi: [10.1016/j.molliq.2016.01.038](https://doi.org/10.1016/j.molliq.2016.01.038).
- Cui, Q., X. Peng, X.-H. Yao, Z.-F. Wei, M. Luo, W. Wang, C.-J. Zhao, Y.-J. Fu, and Y.-G. Zu. 2015. Deep eutectic solvent-based microwave-assisted extraction of genistin, genistein and apigenin from pigeon pea roots. *Separation and Purification Technology* 150:63–72. doi: [10.1016/j.seppur.2015.06.026](https://doi.org/10.1016/j.seppur.2015.06.026).
- Cunha, S. C., and J. O. Fernandes. 2018. Extraction techniques with deep eutectic solvents. *TrAC Trends in Analytical Chemistry* 105: 225–39. doi: [10.1016/j.trac.2018.05.001](https://doi.org/10.1016/j.trac.2018.05.001).
- Cvjetko Bubalo, M., S. Vidović, I. Radojčić Redovniković, and S. Jokić. 2015. Green solvents for green technologies. *Journal of Chemical Technology & Biotechnology* 90:1631–9. doi: [10.1002/jctb.4668](https://doi.org/10.1002/jctb.4668).
- Dai, Y., E. Rozema, R. Verpoorte, and Y. H. Choi. 2016. Application of natural deep eutectic solvents to the extraction of anthocyanins from *Catharanthus roseus* with high extractability and stability replacing conventional organic solvents. *Journal of Chromatography A* 1434:50–6. doi: [10.1016/j.chroma.2016.01.037](https://doi.org/10.1016/j.chroma.2016.01.037).
- Dai, Y., G.-J. Witkamp, R. Verpoorte, and Y. H. Choi. 2013a. Natural deep eutectic solvents as a new extraction media for phenolic metabolites in *Carthamus tinctorius* L. *Analytical Chemistry* 85 (13): 6272–8. doi: [10.1021/ac400432p](https://doi.org/10.1021/ac400432p).
- Dai, Y., G.-J. Witkamp, R. Verpoorte, and Y. H. Choi. 2015. Tailoring properties of natural deep eutectic solvents with water to facilitate their applications. *Food Chemistry* 187:14–9. doi: [10.1016/j.foodchem.2015.03.123](https://doi.org/10.1016/j.foodchem.2015.03.123).
- Dai, Y., J. van Spronsen, G.-J. Witkamp, R. Verpoorte, and Y. H. Choi. 2013b. Natural deep eutectic solvents as new potential media for green technology. *Analytica Chimica Acta* 766:61–8. doi: [10.1016/j.aca.2012.12.019](https://doi.org/10.1016/j.aca.2012.12.019).
- Dai, Y., J. van Spronsen, G.-J. Witkamp, R. Verpoorte, and Y. H. Choi. 2013c. Ionic liquids and deep eutectic solvents in natural products research: Mixtures of solids as extraction solvents. *Journal of Natural Products* 76 (11):2162–73. doi: [10.1021/np400051w](https://doi.org/10.1021/np400051w).
- Dai, Y., R. Verpoorte, and Y. H. Choi. 2014. Natural deep eutectic solvents providing enhanced stability of natural colorants from safflower (*Carthamus tinctorius*). *Food Chemistry* 159:116–21. doi: [10.1016/j.foodchem.2014.02.155](https://doi.org/10.1016/j.foodchem.2014.02.155).
- de los Angeles Fernández, M., J. Boiteux, M. Espino, F. J. V. Gomez, and M. F. Silva. 2018a. Natural deep eutectic solvents-mediated extractions: The way forward for sustainable analytical developments. *Analytica Chimica Acta* 1038:1–10. doi: [10.1016/j.aca.2018.07.059](https://doi.org/10.1016/j.aca.2018.07.059).
- de los Angeles Fernández, M., M. Espino, F. J. V. Gomez, and M. F. Silva. 2018b. Novel approaches mediated by tailor-made green solvents for the extraction of phenolic compounds from agro-food industrial by-products. *Food Chemistry* 239:671–8. doi: [10.1016/j.foodchem.2017.06.150](https://doi.org/10.1016/j.foodchem.2017.06.150).
- Destandau, E., T. Michel, and C. Elfakir. 2013. Microwave-assisted extraction. In *Natural product extraction: Principles and applications*, edited by M. A. Rostagno and J. M. Prado, 113–56. Cambridge, UK: The Royal Society of Chemistry.
- Dietz, C. H. J. T., J. T. Creemers, M. A. Meuleman, C. Held, G. Sadowski, M. van Sint Annaland, F. Gallucci, and M. C. Kroon. 2019. Determination of the total vapor pressure of hydrophobic deep eutectic solvents: Experiments and perturbed-chain statistical associating fluid theory modeling. *ACS Sustainable Chemistry & Engineering* 7:4047–57. doi: [10.1021/acssuschemeng.8b05449](https://doi.org/10.1021/acssuschemeng.8b05449).
- Duan, L., L.-L. Dou, L. Guo, P. Li, and E.-H. Liu. 2016. Comprehensive evaluation of deep eutectic solvents in extraction of bioactive natural products. *ACS Sustainable Chemistry & Engineering* 4 (4):2405–11. doi: [10.1021/acssuschemeng.6b00091](https://doi.org/10.1021/acssuschemeng.6b00091).
- Durand, E., J. Lecomte, R. Upasani, B. Chabi, C. Bayrasy, B. Baréa, E. Jublanc, M. J. Clarke, D. J. Moore, J. Crowther, et al. 2017. Evaluation of the ROS inhibiting activity and mitochondrial targeting of phenolic compounds in fibroblast cells model system and enhancement of efficiency by natural deep eutectic solvent (NADES) formulation. *Pharmaceutical Research* 34 (5):1134–46. doi: [10.1007/s11095-017-2124-4](https://doi.org/10.1007/s11095-017-2124-4).
- Eisenbrand, G., B. Pool-Zobel, V. Baker, M. Balls, B. J. Blaauboer, A. Boobis, A. Carere, S. Kevekordes, J.-C. Lhuguenot, R. Pieters, and J.

- Kleiner. 2002. Methods of *in vitro* toxicology. *Food and Chemical Toxicology* 40 (2–3):193–236.
- Elhamarnah, Y. A., M. Nasser, H. Qiblawey, A. Benamor, M. Atilhan, and S. Aparicio. 2019. A comprehensive review on the rheological behavior of imidazolium based ionic liquids and natural deep eutectic solvents. *Journal of Molecular Liquids* 277:932–58. doi: 10.1016/j.molliq.2019.01.002.
- Espino, M., M. de los Angeles Fernández, F. J. V. Gomez, J. Boiteux, and M. Fernanda Silva. 2018. Green analytical chemistry metrics: Towards a sustainable phenolics extraction from medicinal plants. *Microchemical Journal* 141:438–43. doi: 10.1016/j.microc.2018.06.007.
- European Commission. 2006. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. *Official Journal of European Union* 364: 5–24 and Successive Amendments. <http://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:32006R1881>.
- European Parliament resolution (2011/2175(INI)). 2012. European Parliament resolution of 19 January 2012 on how to avoid food wastage: Strategies for a more efficient food chain in the EU (2011/2175(INI)). *Official Journal of European Union* 227:25–32. <https://publications.europa.eu/en/publication-detail/-/publication/b2a48b16-fe76-11e2-a352-01aa75ed71a1>.
- Faggian, M., S. Sut, B. Perissutti, V. Baldan, I. Grabnar, and S. Dall'Acqua. 2016. Natural deep eutectic solvents (NADES) as a tool for bioavailability improvement: Pharmacokinetics of rutin dissolved in proline/glycine after oral administration in rats: Possible application in nutraceuticals. *Molecules* 21 (11):1531–42. doi: 10.3390/molecules21111531.
- Farajzadeh, M. A., M. R. A. Mogaddam, and M. Aghanassab. 2016. Deep eutectic solvent-based dispersive liquid–liquid microextraction. *Analytical Methods* 8 (12):2576–83. doi: 10.1039/C5AY03189C.
- Farias, F. O., F. H. B. L. Sosa, I. Mafra, J. A. P. Coutinho, and M. R. Mafra. 2017. Study of the pseudo-ternary aqueous two-phase systems of deep eutectic solvent (choline chloride:sugars) + K₂HPO₄+ water. *Fluid Phase Equilibria* 448:143–51. doi: 10.1016/j.fluid.2017.05.018.
- Florindo, C., F. Lima, B. D. Ribeiro, and I. M. Marrucho. 2019. Deep eutectic solvents: Overcoming XXI century challenges. *Current Opinion in Green and Sustainable Chemistry* 18:31–6. doi: 10.1016/j.cogsc.2018.12.003.
- Florindo, C., F. S. Oliveira, L. P. N. Rebelo, A. M. Fernandes, and I. M. Marrucho. 2014. Insights into the synthesis and properties of deep eutectic solvents based on cholinium chloride and carboxylic acids. *ACS Sustainable Chemistry & Engineering* 2 (10):2416–25. doi: 10.1021/sc500439w.
- Florindo, C., L. Romero, I. Rintoul, L. C. Branco, and I. M. Marrucho. 2018. From phase change materials to green solvents: Hydrophobic low viscous fatty acid-based deep eutectic solvents. *ACS Sustainable Chemistry & Engineering* 6:3888–95. doi: 10.1021/acssuschemeng.7b04235.
- Gałaszka, A., Z. M. Migaszewski, P. Konieczka, and J. Namieśnik. 2012. Analytical Eco-Scale for assessing the greenness of analytical procedures. *TrAC Trends in Analytical Chemistry* 37:61–72. doi: 10.1016/j.trac.2012.03.013.
- García, A., E. Rodríguez-Juan, G. Rodríguez-Gutiérrez, J. J. Rios, and J. Fernández-Bolaños. 2016. Extraction of phenolic compounds from virgin olive oil by deep eutectic solvents (DESs). *Food Chemistry* 197:554–61. doi: 10.1016/j.foodchem.2015.10.131.
- García, G., S. Aparicio, R. Ullah, and M. Atilhan. 2015. Deep eutectic solvents: Physicochemical properties and gas separation applications. *Energy & Fuels* 29:2616–44. doi: 10.1021/ef5028873.
- Ghanemi, K., M.-A. Navidi, M. Fallah-Mehrdardi, and A. Dadolahi-Sohrab. 2014. Ultra-fast microwave-assisted digestion in choline chloride–oxalic acid deep eutectic solvent for determining Cu, Fe, Ni and Zn in marine biological samples. *Analytical Methods* 6 (6): 1774–81. doi: 10.1039/C3AY41843J.
- Gómez, A. V., C. C. Tadini, A. Biswas, M. Buttrum, S. Kim, V. M. Boddu, and H. N. Cheng. 2019. Microwave-assisted extraction of soluble sugars from banana puree with natural deep eutectic solvents (NADES). *LWT* 107:79–88. doi: 10.1016/j.lwt.2019.02.052.
- Gomez, F. J. V., A. Spisso, and M. F. Silva. 2017. Pencil graphite electrodes for improved electrochemical detection of oleuropein by the combination of natural deep eutectic solvents and graphene oxide. *Electrophoresis* 38 (21):2704–11. doi: 10.1002/elps.201700173.
- Gomez, F. J. V., M. Espino, M. A. Fernández, and M. F. Silva. 2018. A greener approach to prepare natural deep eutectic solvents. *Chemistryselect* 3:6122–5. doi: 10.1002/slct.201800713.
- Gomez, F. J. V., M. Espino, M. de los Angeles Fernandez, J. Raba, and M. F. Silva. 2016. Enhanced electrochemical detection of quercetin by natural deep eutectic solvents. *Analytica Chimica Acta* 936:91–6. doi: 10.1016/j.aca.2016.07.022.
- González, C. G., N. R. Mustafa, E. G. Wilson, R. Verpoorte, and Y. H. Choi. 2017. Application of natural deep eutectic solvents for the “green” extraction of vanillin from vanilla pods. *Flavour and Fragrance Journal* 33:1–6. doi: 10.1002/ffj.3425.
- Gutiérrez, M. C., M. L. Ferrer, C. R. Mateo, and F. del Monte. 2009. Freeze-drying of aqueous solutions of deep eutectic solvents: A suitable approach to deep eutectic suspensions of self-assembled structures. *Langmuir* 25 (10):5509–15. doi: 10.1021/la900552b.
- Hassan, S. H. A., S. W. van Ginkel, M. A. M. Hussein, R. Abskharon, and S. E. Oh. 2016. Toxicity assessment using different bioassays and microbial biosensors. *Environment International* 92–93:106–18. doi: 10.1016/j.envint.2016.03.003.
- Hayyan, M., A. Hashim, A. Hayyan, M. A. Al-Saadi, I. M. Alnashef, M. E. S. Mirghani, and O. K. Saheed. 2013. Are deep eutectic solvents benign or toxic? *Chemosphere* 90 (7):2193–5. doi: 10.1016/j.chemosphere.2012.11.004.
- Hayyan, M., Y. P. Mbous, C. Y. Looi, W. F. Wong, A. Hayyan, Z. Salleh, and O. Mohd-Ali. 2016. Natural deep eutectic solvents: Cytotoxic profile. *SpringerPlus* 5 (1):913–25. doi: 10.1186/s40064-016-2575-9.
- Hou, X.-D., Q.-P. Liu, T. J. Smith, N. Li, and M.-H. Zong. 2013. Evaluation of toxicity and biodegradability of cholinium amino acids ionic liquids. *PLoS One* 8 (3):e59145. doi: 10.1371/journal.pone.0059145.
- Huang, Y., F. Feng, J. Jiang, Y. Qiao, T. Wu, J. Voglmeir, and Z.-G. Chen. 2017. Green and efficient extraction of rutin from tartary buckwheat hull by using natural deep eutectic solvents. *Food Chemistry* 221:1400–5. doi: 10.1016/j.foodchem.2016.11.013.
- Huang, Y., F. Feng, Z.-G. Chen, T. Wu, and Z.-H. Wang. 2018. Green and efficient removal of cadmium from rice flour using natural deep eutectic solvents. *Food Chemistry* 244:260–5. doi: 10.1016/j.foodchem.2017.10.060.
- Jablonský, M., A. Škulcová, A. Malvis, and J. Šima. 2018. Extraction of value-added components from food industry based and agroforest biowastes by deep eutectic solvents. *Journal of Biotechnology* 282: 46–66. doi: 10.1016/j.jbiotec.2018.06.349.
- Jeliński, T., and P. Cysewski. 2018. Application of a computational model of natural deep eutectic solvents utilizing the COSMO-RS approach for screening of solvents with high solubility of rutin. *Journal of Molecular Modeling* 24 (7):180–97. doi: 10.1007/s00894-018-3700-1.
- Jeong, K. M., J. Ko, J. Zhao, Y. Jin, D. E. Yoo, S. Y. Han, and J. Lee. 2017. Multi-functioning deep eutectic solvents as extraction and storage media for bioactive natural products that are readily applicable to cosmetic products. *Journal of Cleaner Production* 151:87–95. doi: 10.1016/j.jclepro.2017.03.038.
- Jeong, K. M., M. S. Lee, M. W. Nam, J. Zhao, Y. Jin, D.-K. Lee, S. W. Kwon, J. H. Jeong, and J. Lee. 2015. Tailoring and recycling of deep eutectic solvents as sustainable and efficient extraction media. *Journal of Chromatography A* 1424:10–7. doi: 10.1016/j.chroma.2015.10.083.
- Kallel, F., D. Driss, F. Chaari, L. Belghith, F. Bouaziz, R. Ghorbel, and S. E. Chaabouni. 2014. Garlic (*Allium sativum* L.) husk waste as a potential source of phenolic compounds: Influence of extracting solvents on its antimicrobial and antioxidant properties. *Industrial Crops and Products* 62:34–41. doi: 10.1016/j.indcrop.2014.07.047.

- Karimi, M., S. Dadfarnia, A. Mohammad, H. Shabani, F. Tamaddon, and D. Azadi. 2015. Deep eutectic liquid organic salt as a new solvent for liquid-phase microextraction and its application in ligandless extraction and pre-concentration of lead and cadmium in edible oils. *Talanta* 144:648–54. doi: [10.1016/j.talanta.2015.07.021](https://doi.org/10.1016/j.talanta.2015.07.021).
- Kumar, A. K., B. S. Parikh, and M. Pravakar. 2016. Natural deep eutectic solvent mediated pretreatment of rice straw: Bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue. *Environmental Science and Pollution Research* 23 (10):9265–75. doi: [10.1007/s11356-015-4780-4](https://doi.org/10.1007/s11356-015-4780-4).
- Kumar, A. K., B. S. Parikh, E. Shah, L. Z. Liu, and M. A. Cotta. 2016. Cellulosic ethanol production from green solvent-pretreated rice straw. *Biocatalysis and Agricultural Biotechnology* 7:14–23. doi: [10.1016/j.bcab.2016.04.008](https://doi.org/10.1016/j.bcab.2016.04.008).
- Kumar, A. K., S. Sharma, E. Shah, and A. Patel. 2018. Technical assessment of natural deep eutectic solvent (NADES) mediated biorefinery process: A case study. *Journal of Molecular Liquids* 260:313–22. doi: [10.1016/j.molliq.2018.03.107](https://doi.org/10.1016/j.molliq.2018.03.107).
- Laitinen, O., T. Suopaja, O. Monika, and H. Liimatainen. 2017. Hydrophobic, superabsorbing aerogels from choline chloride-based deep eutectic solvent pretreated and silylated cellulose nanofibrils for selective oil removal. *ACS Applied Materials & Interfaces* 9: 25029–37. doi: [10.1021/acsami.7b06304](https://doi.org/10.1021/acsami.7b06304).
- Lapeña, D., L. Lomba, M. Arta, C. Lafuente, and B. Giner. 2019. The NADES glycine as a potential green solvent: A comprehensive study of its thermophysical properties and effect of water inclusion. *Journal of Chemical Thermodynamics* 128:164–72. doi: [10.1016/j.jct.2018.07.031](https://doi.org/10.1016/j.jct.2018.07.031).
- Li, N., Wang, Y. K. Xu, Y. Huang, Q. Wen, and X. Ding. 2016. Development of green betaine-based deep eutectic solvent aqueous two-phase system for the extraction of protein. *Talanta* 152:23–32. doi: [10.1016/j.talanta.2016.01.042](https://doi.org/10.1016/j.talanta.2016.01.042).
- Liu, W., K. Zhang, Y. Qin, and J. Yu. 2017. A simple and green ultrasonic-assisted liquid-liquid microextraction technique based on deep eutectic solvents for the HPLC analysis of sesamol in sesame oils. *Analytical Methods* 9 (28):4184–9. doi: [10.1039/C7AY01033H](https://doi.org/10.1039/C7AY01033H).
- Liu, Y., J. B. Friessen, J. B. McAlpine, D. C. Lankin, S.-N. Chen, and G. F. Pauli. 2018. Natural deep eutectic solvents: Properties, applications, and perspectives. *Journal of Natural Products* 81 (3):679–90. doi: [10.1021/acs.jnatprod.7b00945](https://doi.org/10.1021/acs.jnatprod.7b00945).
- Lores, H., V. Romero, I. Costas, C. Bendicho, and I. Lavilla. 2017. Natural deep eutectic solvents in combination with ultrasonic energy as a green approach for solubilisation of proteins: Application to gluten determination by immunoassay. *Talanta* 162:453–9. doi: [10.1016/j.talanta.2016.10.078](https://doi.org/10.1016/j.talanta.2016.10.078).
- Lucarini, M., A. Durazzo, A. Romani, M. Campo, G. Lombardi-Boccia, and F. Cecchini. 2018. Bio-based compounds from grape seeds: A biorefinery approach. *Molecules* 23 (8):1888–900. doi: [10.3390/molecules23081888](https://doi.org/10.3390/molecules23081888).
- Mano, F., I. M. Aroso, S. Barreiros, P. Borges, R. L. Reis, A. R. C. Duarte, and A. Paiva. 2015. Production of poly(vinyl alcohol) (PVA) fibers with encapsulated natural deep eutectic solvent (NADES) using electrospinning. *ACS Sustainable Chemistry & Engineering* 3 (10):2504–9. doi: [10.1021/acssuschemeng.5b00613](https://doi.org/10.1021/acssuschemeng.5b00613).
- Mbous, Y. P., M. Hayyan, W. F. Wong, C. Y. Looi, and M. A. Hashim. 2017. Unraveling the cytotoxicity and metabolic pathways of binary natural deep eutectic solvent systems. *Scientific Reports* 7 (1): 41257–71. doi: [10.1038/srep41257](https://doi.org/10.1038/srep41257).
- Milano, F., L. Giotta, M. R. Guascito, A. Agostiano, S. Splendorio, L. Valli, and V. Capriati. 2017. Functional enzymes in nonaqueous environment: The case of photosynthetic reaction centers in deep eutectic solvents. *ACS Sustainable Chemistry & Engineering* 5: 7768–76. doi: [10.1021/acssuschemeng.7b01270](https://doi.org/10.1021/acssuschemeng.7b01270).
- Mouden, S., P. G. L. Klinkhamer, Y. H. Choi, and K. A. Leiss. 2017. Towards eco-friendly crop protection: Natural deep eutectic solvents and defensive secondary metabolites. *Phytochemistry Reviews* 16 (5): 935–51. doi: [10.1007/s11101-017-9502-8](https://doi.org/10.1007/s11101-017-9502-8).
- Mukhopadhyay, S., S. Mukherjee, A. Hayyan, M. Hayyan, M. A. Hashim, and B. Sen Gupta. 2016. Enhanced removal of lead from contaminated soil by polyol-based deep eutectic solvents and saponin. *Journal of Contaminant Hydrology* 194:17–23. doi: [10.1016/j.jconhyd.2016.09.007](https://doi.org/10.1016/j.jconhyd.2016.09.007).
- Mulia, K., E. Krisanti, F. Terahadi, and S. Putri. 2015. Selected natural deep eutectic solvents for the extraction of α -Mangostin from mangosteen (*Garcinia mangostana* L.) pericarp. *International Journal of Technology* 6 (7):1211–20. doi: [10.14716/ijtech.v6i7.1984](https://doi.org/10.14716/ijtech.v6i7.1984).
- Mulia, K., F. Muhammad, and E. Krisanti. 2016a. Extraction of vitexin from binahong (*Anredera cordifolia* (Ten.) steenis leaves using betaine-1,4 butanediol natural deep eutectic solvent (NADES). The International Conference on Chemistry, Chemical Process and Engineering, Yogyakarta, Indonesia, 15–16 November.
- Mulia, K., S. Putri, E. Krisanti, and Nasruddin. 2016b. Natural deep eutectic solvents (NADES) as green solvents for carbon dioxide capture. The International Conference on Chemistry, Chemical Process and Engineering, Yogyakarta, Indonesia, 15–16 November.
- Murador, D. C., L. M. de Souza Mesquita, N. Vannuchi, A. R. C. Braga, and V. V. de Rosso. 2019. Bioavailability and biological effects of bioactive compounds extracted with natural deep eutectic solvents and ionic liquids: Advantages over conventional organic solvents. *Current Opinion in Food Science* 26:25–34. doi: [10.1016/j.cofs.2019.03.002](https://doi.org/10.1016/j.cofs.2019.03.002).
- Nadpal, J. D., M. M. Lesjak, Z. O. Mrkonjić, T. M. Majkić, D. D. Četojević-Simin, M. M. Mimica-Dukić, and I. N. Beara. 2018. Phytochemical composition and *in vitro* functional properties of three wild rose hips and their traditional preserves. *Food Chemistry* 241:290–300. doi: [10.1016/j.foodchem.2017.08.111](https://doi.org/10.1016/j.foodchem.2017.08.111).
- Nam, M. W., J. Zhao, M. Sang Lee, J. Hoon Jeong, and J. Lee. 2015. Enhanced extraction of bioactive natural products using tailor-made deep eutectic solvents: Application to flavonoid extraction from *Flos sophorae*. *Green Chemistry* 17 (3):1718–27. doi: [10.1039/C4GC01556H](https://doi.org/10.1039/C4GC01556H).
- Ng, M. H., Y. M. Choo, M. A. Hashim, and N. S. Jayakumar. 2015. Performance of choline-based deep eutectic solvents in the extraction of tocots from crude palm oil. *Journal of the American Oil Chemists' Society* 92:1709–16. doi: [10.1007/s11746-015-2720-6](https://doi.org/10.1007/s11746-015-2720-6).
- Paiva, A., A. A. Matias, and A. R. C. Duarte. 2018. How do we drive deep eutectic systems towards an industrial reality? *Current Opinion in Green and Sustainable Chemistry* 11:81–5. doi: [10.1016/j.cogsc.2018.05.010](https://doi.org/10.1016/j.cogsc.2018.05.010).
- Paiva, A., Craveiro, R. I. Aroso, M. Martins, R. L. Reis, A. Rita, and C. Duarte. 2014. Natural deep eutectic solvents – Solvents for the 21st century. *ACS Sustainable Chemistry & Engineering* 4:1063–71. doi: [10.1021/sc500096j](https://doi.org/10.1021/sc500096j).
- Pena-Pereira, F., A. Kloskowski, and J. Namieśnik. 2015. Perspectives on the replacement of harmful organic solvents in analytical methodologies: A generation of eco-friendly alternatives. *Green Chemistry* 17 (7):3687–705. doi: [10.1039/C5GC00611B](https://doi.org/10.1039/C5GC00611B).
- Peng, X., M.-H. Duan, X.-H. Yao, Y.-H. Zhang, C.-J. Zhao, Y.-G. Zu, and Y.-J. Fu. 2016. Green extraction of five target phenolic acids from *Lonicera japonica* flos with deep eutectic solvent. *Separation and Purification Technology* 157:249–57. doi: [10.1016/j.seppur.2015.10.065](https://doi.org/10.1016/j.seppur.2015.10.065).
- Piemontese, L., F. Perna, A. Logrieco, V. Capriati, and M. Solfrizzo. 2017. Deep eutectic solvents as novel and effective extraction media for quantitative determination of Ochratoxin A in wheat and derived products. *Molecules* 22 (1):121–30. doi: [10.3390/molecules22010121](https://doi.org/10.3390/molecules22010121).
- Qi, X.-L., X. Peng, Y.-Y. Huang, L. Li, Z.-F. Wei, Y.-G. Zu, and Y.-J. Fu. 2015. Green and efficient extraction of bioactive flavonoids from *Equisetum palustre* L. by deep eutectic solvents-based negative pressure cavitation method combined with macroporous resin enrichment. *Industrial Crops and Products* 70:142–8. doi: [10.1016/j.indcrop.2015.03.026](https://doi.org/10.1016/j.indcrop.2015.03.026).
- Radošević, K., J. Železnjak, M. Cvjetko Bubalo, I. Radojčić Redovniković, I. Slivac, and V. Gaurina Srček. 2016a. Comparative *in vitro* study of cholinium-based ionic liquids and deep eutectic solvents toward fish cell line. *Ecotoxicology and Environmental Safety* 131:30–6. doi: [10.1016/j.ecoenv.2016.05.005](https://doi.org/10.1016/j.ecoenv.2016.05.005).
- Radošević, K., M. C. Bubalo, V. G. Srček, D. Grgas, T. L. Dragičević, and I. R. Redovniković. 2015. Evaluation of toxicity and

- biodegradability of choline chloride based deep eutectic solvents. *Ecotoxicology and Environmental Safety* 112:46–53. doi: [10.1016/j.ecoenv.2014.09.034](https://doi.org/10.1016/j.ecoenv.2014.09.034).
- Radošević, K., N. Ćurko, V. Gaurina Srček, M. Cvjetko Bubalo, M. Tomašević, K. Kovačević Ganić, and I. Radojčić Redovniković. 2016b. Natural deep eutectic solvents as beneficial extractants for enhancement of plant extracts bioactivity. *LWT - Food Science and Technology* 73:45–51. doi: [10.1016/j.lwt.2016.05.037](https://doi.org/10.1016/j.lwt.2016.05.037).
- Rajan, M., A. Prabhavathy, and U. Ramesh. 2015. Natural deep eutectic solvent extraction media for *Zingiber officinale* Roscoe: The study of chemical compositions, antioxidants and antimicrobial activities. *The Natural Products Journal* 5 (1):3–13. doi: [10.2174/221031550501150414094719](https://doi.org/10.2174/221031550501150414094719).
- Ramón, M. R., M. C. Figueroa-Espinoza, and E. Durand. 2017. Application of deep eutectic solvents (DES) for phenolic compounds extraction: Overview, challenges, and opportunities. *Journal of Agricultural and Food Chemistry* 65:3591–601. doi: [10.1021/acs.jafc.7b01054](https://doi.org/10.1021/acs.jafc.7b01054).
- Renshaw, R. C., G. A. Dimitrakis, J. P. Robinson, and S. W. Kingman. 2019. The relationship of dielectric response and water activity in food. *Journal of Food Engineering* 244:80–90. doi: [10.1016/j.jfoodeng.2018.08.037](https://doi.org/10.1016/j.jfoodeng.2018.08.037).
- Ribeiro, B. D., C. Florindo, L. C. Iff, M. A. Z. Coelho, and I. M. Marrucho. 2015. Menthol-based eutectic mixtures: Hydrophobic low viscosity solvents. *ACS Sustainable Chemistry & Engineering* 3: 2469–77. doi: [10.1021/acssuschemeng.5b00532](https://doi.org/10.1021/acssuschemeng.5b00532).
- Rozema, E., A. D. van Dam, H. C. M. Sips, R. Verpoorte, O. C. Meijer, S. Kooijman, and Y. H. Choi. 2015. Extending pharmacological dose-response curves for salsalate with natural deep eutectic solvents. *RSC Advances* 5:61398–401. doi: [10.1039/C5RA10196D](https://doi.org/10.1039/C5RA10196D).
- Rüther, T., K. R. Harris, M. D. Horne, M. Kanakubo, T. Rodopoulos, J. P. Veder, and L. A. Woolf. 2013. Transport, electrochemical and thermophysical properties of two Ndonor-functionalised ionic liquids. *Chemistry - A European Journal* 19 (52):17733–44. doi: [10.1002/chem.201302258](https://doi.org/10.1002/chem.201302258).
- Santana, A. P. R., D. F. Andrade, J. A. Mora-Vargas, C. D. B. Amaral, A. Oliveira, and M. H. Gonzalez. 2019. Natural deep eutectic solvents for sample preparation prior to elemental analysis by plasma-based techniques. *Talanta* 199:361–9. doi: [10.1016/j.talanta.2019.02.083](https://doi.org/10.1016/j.talanta.2019.02.083).
- Savi, L. K., D. Carpiné, N. Waszczynskij, R. H. Ribani, and C. W. Isidoro Haminiuk. 2019. Influence of temperature, water content and type of organic acid on the formation, stability and properties of functional natural deep eutectic solvents. *Fluid Phase Equilibria* 488:40–7. doi: [10.1016/j.fluid.2019.01.025](https://doi.org/10.1016/j.fluid.2019.01.025).
- Schuur, B., T. Brouwer, D. Smink, and L. M. J. Sprakel. 2019. Green solvents for sustainable separation processes. *Current Opinion in Green and Sustainable Chemistry* 18:57–65. doi: [10.1016/j.cogsc.2018.12.009](https://doi.org/10.1016/j.cogsc.2018.12.009).
- Shamseddin, A., C. Crauste, E. Durand, P. Villeneuve, G. Dubois, T. Durand, J. Vercauteren, and F. Veas. 2017. Resveratrol formulated with a natural deep eutectic solvent inhibits active matrix metalloprotease-9 in hormetic conditions. *European Journal of Lipid Science and Technology* 119 (11):1700171. doi: [10.1002/ejlt.201700171](https://doi.org/10.1002/ejlt.201700171).
- Silva, J. M., R. L. Reis, A. Paiva, and A. R. C. Duarte. 2018. Design of functional therapeutic deep eutectic solvents based on choline chloride and ascorbic acid. *ACS Sustainable Chemistry & Engineering* 6 (8):10355–63. doi: [10.1021/acssuschemeng.8b01687](https://doi.org/10.1021/acssuschemeng.8b01687).
- Sut, S., M. Faggian, V. Baldan, G. Poloniato, I. Castagliuolo, I. Grabnar, B. Perissutti, P. Brun, F. Maggi, D. Voinovich, et al. 2017. Natural deep eutectic solvents (NADES) to enhance berberine absorption: An *in vivo* pharmacokinetic study. *Molecules* 22 (11): 1921–32. doi: [10.3390/molecules22111921](https://doi.org/10.3390/molecules22111921).
- Tang, B., W. Bi, H. Zhang, and K. H. Row. 2014. Deep eutectic solvent-based HS-SME coupled with GC for the analysis of bioactive terpenoids in *Chamaecyparis obtusa* leaves. *Chromatographia* 77 (3–4):373–7. doi: [10.1007/s10337-013-2607-3](https://doi.org/10.1007/s10337-013-2607-3).
- Tang, X., M. Zuo, Z. Li, H. Liu, C. Xiong, and X. Zeng. 2017. Green processing of lignocellulosic biomass and its derivatives in deep eutectic solvents. *Chemistry and Sustainability* 10:2696–706. doi: [10.1002/cssc.201700457](https://doi.org/10.1002/cssc.201700457).
- Taslim, I. L., R. Manurung, A. Winarta, and A. D. Ramadhani. 2016. Biodiesel production from ethanolysis of DPO using deep eutectic solvent (DES) based choline chloride – Ethylene glycol as co-solvent. The International Conference on Chemistry, Chemical Process and Engineering, Yogyakarta, Indonesia, 15–16 November.
- Tiwari, B. K. 2015. Ultrasound: A clean, green extraction technology. *TRAC - Trends in Analytical Chemistry* 71:100–9. doi: [10.1016/j.trac.2015.04.013](https://doi.org/10.1016/j.trac.2015.04.013).
- Tomšik, A., B. Pavlič, J. Vladić, M. Ramić, J. Brindza, and S. Vidović. 2016. Optimization of ultrasound-assisted extraction of bioactive compounds from wild garlic (*Allium ursinum* L.). *Ultrasonics Sonochemistry* 29:502–11. doi: [10.1016/j.ulsonch.2015.11.005](https://doi.org/10.1016/j.ulsonch.2015.11.005).
- Troter, D., M. Zlatkovic, D. Djokic-Stojanovic, S. Konstantinovic, and Z. Todorovic. 2016. Citric acid-based deep eutectic solvents: Physical properties and their use as cosolvents in sulphuric acid-catalysed ethanolysis of oleic acid. *Advanced Technologies* 5 (1):53–65. doi: [10.5937/savteh1601053T](https://doi.org/10.5937/savteh1601053T).
- van den Bruinhorst, A., P. Kouris, J. Timmer, M. H. J. M. de Croon, and M. C. Kroon. 2016. Exploring orange peel treatment with deep eutectic solvents and diluted organic acids. *Natural Products Chemistry and Research* 4:1–5.
- van Osch, D. J. G. P., C. H. J. T. Dietz, J. van Spronsen, M. C. Kroon, F. Gallucci, M. van Sint Annaland, and R. Tuinier. 2019. A search for natural hydrophobic deep eutectic solvents based on natural components. *ACS Sustainable Chemistry & Engineering* 7:2933–42. doi: [10.1021/acssuschemeng.8b03520](https://doi.org/10.1021/acssuschemeng.8b03520).
- van Osch, D. J. G. P., L. F. Zubeir, A. van den Bruinhorst, M. A. Rocha, and M. C. Kroon. 2015. Hydrophobic deep eutectic solvents as water-immiscible extractants. *Green Chemistry* 17 (9):4518–21. doi: [10.1039/C5GC01451D](https://doi.org/10.1039/C5GC01451D).
- Wang, M., J. Wang, Y. Zhang, Q. Xia, W. Bi, X. Yang, and Y. Chen. 2016. Fast environment-friendly ball mill-assisted deep eutectic solvent-based extraction of natural products. *Journal of Chromatography A* 1443:262–6. doi: [10.1016/j.chroma.2016.03.061](https://doi.org/10.1016/j.chroma.2016.03.061).
- Wang, M., J. Wang, Y. Zhou, M. Zhang, Q. Xia, W. Bi, and D. D. Y. Chen. 2017a. Ecofriendly mechanochemical extraction of bioactive compounds from plants with deep eutectic solvents. *ACS Sustainable Chemistry & Engineering* 5 (7):6297–303. doi: [10.1021/acssuschemeng.7b01378](https://doi.org/10.1021/acssuschemeng.7b01378).
- Wang, T., J. Jiao, Q.-Y. Gai, P. Wang, N. Guo, L.-L. Niu, and Y.-J. Fu. 2017b. Enhanced and green extraction polyphenols and furanocoumarins from fig (*Ficus carica* L.) leaves using deep eutectic solvents. *Journal of Pharmaceutical and Biomedical Analysis* 145:339–45. doi: [10.1016/j.jpba.2017.07.002](https://doi.org/10.1016/j.jpba.2017.07.002).
- Wei, Z., X. Qi, T. Li, M. Luo, W. Wang, Y. Zu, and Y. Fu. 2015a. Application of natural deep eutectic solvents for extraction and determination of phenolics in *Cajanus cajan* leaves by ultra performance liquid chromatography. *Separation and Purification Technology* 149:237–44. doi: [10.1016/j.seppur.2015.05.015](https://doi.org/10.1016/j.seppur.2015.05.015).
- Wei, Z.-F., X.-Q. Wang, X. Peng, W. Wang, C.-J. Zhao, Y.-G. Zu, and Y.-J. Fu. 2015b. Fast and green extraction and separation of main bioactive flavonoids from *Radix Scutellariae*. *Industrial Crops and Products* 63:175–81. doi: [10.1016/j.indcrop.2014.10.013](https://doi.org/10.1016/j.indcrop.2014.10.013).
- Wen, Q., J. Chen, Y. Tang, J. Wang, and Z. Yang. 2015. Assessing the toxicity and biodegradability of deep eutectic solvents. *Chemosphere* 132:63–9. doi: [10.1016/j.chemosphere.2015.02.061](https://doi.org/10.1016/j.chemosphere.2015.02.061).
- Xin, R., S. Qi, C. Zeng, F. I. Khan, B. Yang, and Y. Wang. 2017. A functional natural deep eutectic solvent based on trehalose: Structural and physicochemical properties. *Food Chemistry* 217: 560–7. doi: [10.1016/j.foodchem.2016.09.012](https://doi.org/10.1016/j.foodchem.2016.09.012).
- Xu, K., Y. Wang, Y. Huang, N. Li, and Q. Wen. 2015. A green deep eutectic solvent-based aqueous two-phase system for protein extracting. *Analytica Chimica Acta* 864:9–20. doi: [10.1016/j.aca.2015.01.026](https://doi.org/10.1016/j.aca.2015.01.026).
- Yan, Y. C., W. Rashmi, M. Khalid, K. Shahbaz, T. C. S. M. Gupta, and N. Mase. 2017. Potential application of deep eutectic solvents in heat transfer application. *Journal of Engineering Science and Technology* 12:1–14.

- Yang, T.-X., L.-Q. Zhao, J. Wang, G. Song, H. Liu, H. Cheng, and Z. Yang. 2017. Improving whole-cell biocatalysis by addition of deep eutectic solvents and natural deep eutectic solvents. *ACS Sustainable Chemistry & Engineering* 5:5713–22. doi: [10.1021/acssuschemeng.7b00285](https://doi.org/10.1021/acssuschemeng.7b00285).
- Yousefi, S. M., F. Shemirani, and S. A. Ghorbanian. 2017. Deep eutectic solvent magnetic bucky gels in developing dispersive solid phase extraction: Application for ultra trace analysis of organochlorine pesticides by GC-micro ECD using a large-volume injection technique. *Talanta* 168:73–81. doi: [10.1016/j.talanta.2017.03.020](https://doi.org/10.1016/j.talanta.2017.03.020).
- Zahrina, I., M. Nasikin, E. Krisanti, and K. Mulia. 2018. Deacidification of palm oil using betaine monohydrate-based natural deep eutectic solvents. *Food Chemistry* 240:490–5. doi: [10.1016/j.foodchem.2017.07.132](https://doi.org/10.1016/j.foodchem.2017.07.132).
- Zeng, Q., Y. Wang, Y. Huang, X. Ding, J. Chen, and K. Xu. 2014. Deep eutectic solvents as novel extraction media for protein partitioning. *The Analyst* 139 (10):2565–73. doi: [10.1039/c3an02235h](https://doi.org/10.1039/c3an02235h).
- Zhang, H., Y. Wang, K. Xu, N. Li, Q. Wen, Q. Yang, and Y. Zhou. 2016. Ternary and binary deep eutectic solvents as a novel extraction media for protein partitioning. *Analytical Methods* 8 (46): 8196–207. doi: [10.1039/C6AY01860B](https://doi.org/10.1039/C6AY01860B).
- Zhang, L., and M. Wang. 2017. Optimization of deep eutectic solvent-based ultrasound-assisted extraction of polysaccharides from *Dioscorea opposita* Thunb. *International Journal of Biological Macromolecules* 95:675–81. doi: [10.1016/j.ijbiomac.2016.11.096](https://doi.org/10.1016/j.ijbiomac.2016.11.096).
- Zhao, B.-Y., P. Xu, F.-X. Yang, H. Wu, M.-H. Zong, and W.-Y. Lou. 2015. Biocompatible deep eutectic solvents based on choline chloride: Characterization and application to the extraction of rutin from *Sophora japonica*. *ACS Sustainable Chemistry & Engineering* 3 (11): 2746–55. doi: [10.1021/acssuschemeng.5b00619](https://doi.org/10.1021/acssuschemeng.5b00619).