Natural Deep Eutectic Solvents for Sustainable Extraction Techniques

Subjects: Chemistry, Analytical
Contributor: Ciro Cannavacciuolo, Stefania Pagliari, Jessica Frigerio, Chiara Maria Giustra, Massimo Labra, Luca Campone

The analysis of foods is a comprehensive process of extraction, identification, and quantification of several classes of compounds from natural matrices. The detection and quantification of primary metabolites (sugars, amino acids, vitamins, and lipids), contaminants (toxins, heavy metals, and allergens), and secondary metabolites (polyphenolics, flavonoids, terpenes, and alkaloids) is a crucial practice for ensuring the safety and quality of foods and related functional products. Due to the variable structure of food analytes, a gap in a universal method suitable for the extraction and analysis of all compounds is lacking. Moreover, conventional extractants are usually made of organic solvents and common extraction techniques usually require a long extraction time to exhaust the matrix. The actual discussions about climatic changes provide a growing awareness of the scientific and industrial community to reduce the environmental impact by using sustainable processes. In general, the main principles of “green chemistry” are based on the design of processes aimed to reduce energy consumption and the use of eco-friendly solvents with less toxicity to the environment and human health.

Keywords: NADES ; green extraction ; food analysis

1. Physicochemical Properties of Natural Deep Eutectic Solvents (NADESs) for the Extraction Process

The intermolecular interactions, mainly hydrogen bond interactions, between the hydrogen bond acceptor (HBA) and the hydrogen bond donor (HBD) are responsible for the physicochemical properties of the deep eutectic solvents (DES) [1][2]. By changing either the HBA/HBD ratio or one of the components, the NADES can be specifically tailored for different applications. This is a major advantage in terms of achieving desirable properties and improving extraction efficiency [3][4]. Viscosity, polarity, density, and pH condition are the main physicochemical properties affecting the extraction of natural compounds from food matrices. The addition of water as a third component of the system can modulate the conditions of the NADESs for the extraction of several compounds and chemicals for food analysis applications.

Generally, the high viscosity of HDA and HDB mixtures cause a low mass transfer phenomenon affecting the extraction efficiency. Temperature can drastically reduce the viscosity but a mediation with the thermolability of natural compounds must be considered. Added water, reported in a range of 10% to 80% [5], disrupts the hydrogen bond interaction between HAD and HDB modulating the viscosity of the system. Data reported by Zhekenov et al., 2019 suggest a maximum limit of 50% molar fraction of water beyond the system act as a solution [6].

Polarity is the key property to solubilizing metabolites in a solid-liquid extraction. Fixing the choline chloride (ChCl) as HBA, the use of different HBD can affect the polarity of the system. Craveiro et al., 2016 reported the use of sugars (glucose, sucrose, xylose) and organic acids (citric acid, tartaric acid) generating different polarity systems. In particular, NADES composed of ChCl and organic acids resulted more polar than those combined with sugars [7]. The water addition can also affect the polarity of the NADES [8][9]. HBD used for the preparation of NADES act both as hydrophilic and lipophilic components. Components with electronegative groups can form dipole−dipole interactions with polar solvents explaining the hydrophilic properties [10]. On the other hand, natural lipophilic compounds commonly used in NADES are characterized by a polar moiety forming dipole-dipole interactions with polar solvents and a hydrophobic moiety with a tendency to aggregate in aqueous solution to minimize the area of contact between water and nonpolar molecules. Several NADES systems are described by the hydrophilicity/lipophilicity balance phenomenon. For instance, fatty acid- and terpenes-based natural compounds acting as HBD have been reported for NADES [11][12][13].

Complete profiling of complex food matrices by using organic solvents allows the extraction of hydrophilic components or lipophilic components separately while the ignored components that remain in the extraction residues are discarded from the analysis. The advantage to use hydrophilic or hydrophobic NADES is the efficient enhancement of bioactive components with various polarities. The density of hydrophilic or hydrophobic solvents can change allowing a double-phase separation. The two-phase system formed with hydrophilic NADES and hydrophobic NADES can resolve the problem of extraction selectivity and could simultaneously extract the bioactive compounds with various polarities from plant materials. In 2015, Van Osch et al. developed a NADES two-phase system and evaluated the recovery of volatile fatty acids from dilute aqueous solutions [12]. A two-phase aqueous system (NADES–salt solution) has also been established to extract proteins [14][15][16][17] and non-polar anthraquinones [16]. Moreover, Jun Chao et al., 2018 developed a two-phase NADES system (ChCl-LA1/Ch-M/MCO) to extract bioactive metabolites with different polarities from Ginkgo biloba leaves [18].

2. Preparation of NADES

The NADESs can be produced from different natural compounds such as ChCl, sugars, amino acids, and polyols. These compounds are in solid form and only after mixing, in a specific combination and molar rates, do they turn into a liquid state [19]. Five methods are reported to prepare NADES: thermal mixing, vacuum evaporation, freeze-drying, ultrasonication, and microwave.

- Thermal mixing is a simpler and faster approach that involves mixing and stirring at a temperature of 50–80 °C of two components with water until a colorless liquid is formed [8][20][21].

- Vacuum evaporation is also similar; the components are dissolved in water and evaporated at about 50 °C in rotary evaporation, and finally, the resulting liquid is placed in a desiccator to reach a constant weight [8][21].

- Freeze-drying involves dissolving the components in water, then freezing and drying them, resulting in a viscous, transparent solution [21][22].

- Microwave-assisted synthesis exploits the production of microwaves that upon interaction with precursors generate collisions between molecules and between the hydrogen bond donor and hydrogen bond acceptor components due to dipole rotation resulting in dielectric heating that speeds up the synthesis time [23][24]. According to Popovic et al., 2022, microwaves could be one of the fastest methods for the preparation of some NADES, taking even less than a minute [20]. However, because of the possible overheating caused by the technique, it is advisable to divide the process into several cycles of a few seconds interspersed with cooling pauses [25]. The entire preparation is carried out in closed systems with controlled pressure and temperature.

- Ultrasound-assisted is a little-explored but effective way of preparing NADESs. The cavitation process promotes, through the release of heat and pressure exerted because of bubbles implosion, the interaction between the hydrogen bond acceptor (HBA) and the hydrogen bond donor (HBD) [21]. According to when described by Santana et al., 2019, the preparation of NADES by ultrasound can also be performed by heating the mixture around 50 °C [21]. This approach requires several minutes with intermediate times between microwaves and the remaining techniques described above.

The preparation of NADES (**Figure 1**) through the different methods generates clear solvents without the presence of precipitates at room temperature with the same properties [20][21]. The principal difference consists in the timing of the process. Ultrasound and microwave methods offer high speed and efficiency of preparation compared with thermal mixing, vacuum evaporation, and freeze-drying; however, the mixing and stirring method is still widely used because it is very simple, easy to perform and allows the production of high volumes of solvent.

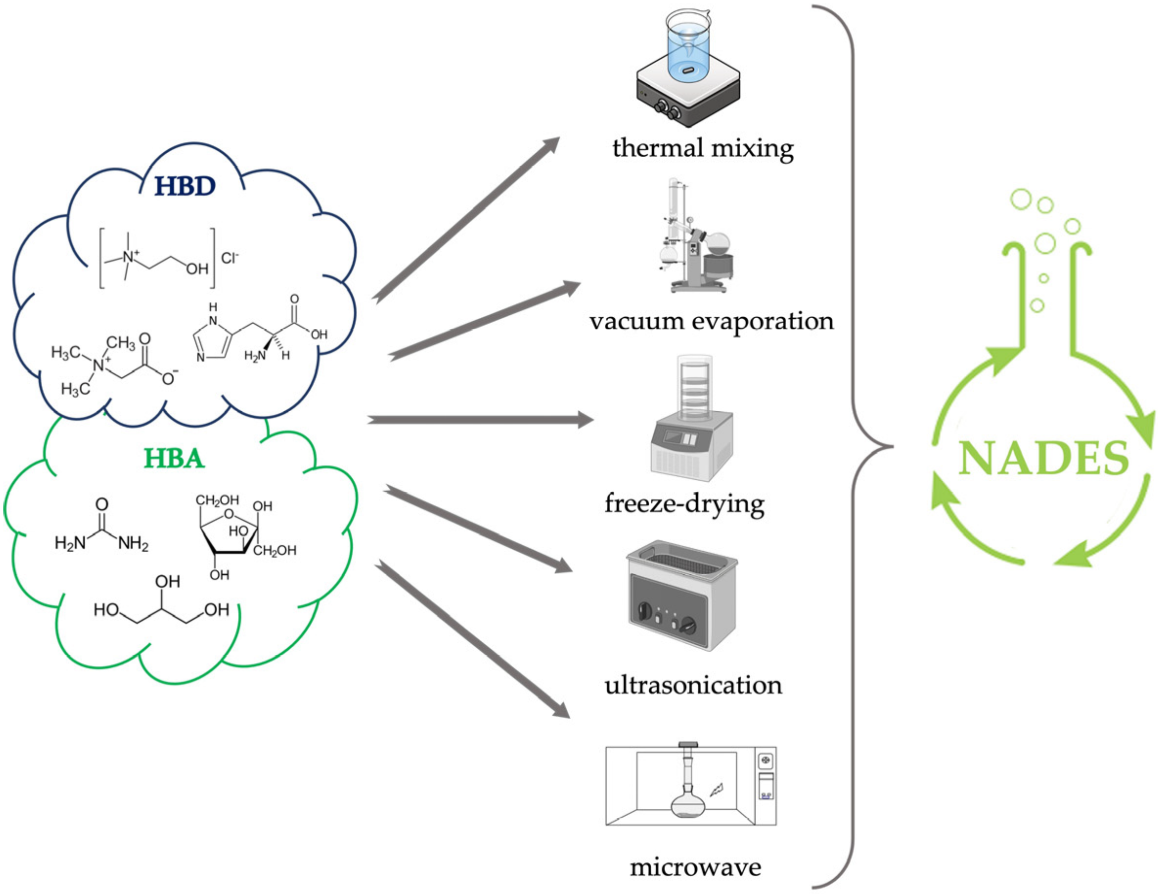

Figure 1. Graphical representation of NADES preparation techniques.

3. Use of NADES as Green Solvent in the Extraction Techniques

The use of NADES as extraction solvent is often combined with techniques such as ultrasound-assisted liquid extraction (USAE), microwave (MAE), or pressurized liquid extraction (PLE). This is mainly due to the properties of NADES used as alternative solvents to classical solvents for lower toxicity and higher extraction efficiency, making them suitable for green chemistry extraction techniques that aim to reduce cost, risk, extraction time and environmental impact [20][26]. Numerous studies employing such solvents on food matrices for the recovery of various bioactive compounds are reported in the literature. Mostly, Bajkacz et al., 2017 and Rashid et al., 2023 optimized a method for recovering phenols respectably from soybean and apple by-products by using different types of NADES and ultrasonic techniques. The results demonstrated how the combination of ultrasonic with NADES solvents can be an innovative method of phenol recovery due in part to the H-interactions formed between NADES and phenols; furthermore, the extraction efficiency was implemented by adding 30% water to the NADES mixture, which from the results proved to be the best condition [26][27]. Still, Loarce et al., 2011 have used NADES as modifiers for PHWE extraction, significantly improving anthocyanin recovery compared to water alone while keeping the process green and sustainable [28]. The process was optimized using water with 30% NADES composed of choline chloride and oxalic acid at a temperature of 60 °C as solvent. Another example is the study conducted by Fan and Li., 2022 that through the coupling of NADES and microwaves, finally these solvents were also used with microwave hydrodistillation for the extraction of essential oil from *A. sinensis*. The combination of NADES with microwaves that promote plant cell rupture compared to conventional methods allows implementation of the recovery of molecules by reducing while maintaining the process with low environmental impact and high efficiency [29].

4. Toxicity and Sustainability

The green chemistry approach requires minimizing the risks and hazards associated with the process and its impact on the environment and human health. It is precisely for this motive that numerous scholars have begun to move toward the use of safer and more environmentally friendly solvents. At this juncture are NADES, which are considered nontoxic, environmentally sustainable, and biodegradable, thanks mainly to the fact that they are made up of compounds of natural origin. Choline chloride (ChCl), for example, one of the main HBAs, is widely used for the preparation of NADES and used commercially on a large scale as an additive for chicken feed, in addition, NADES are also used for the solubilization of drugs for oral dosing in rats, and the synthesis of biodegradable polyesters with antibacterial properties [30]. The emergence of NADES is related to their very low toxicity, which allows them to be used more safely than the previous ionic solvents (ILs), which, on the other hand, can have similar toxicity to the organic compounds they replace [31].

The toxicity of NADES has long been evaluated by considering only the single toxicity data of all the components used in the preparation, which are reported to be safe [32]. However, more recent studies have indicated that these molecules when mixed may show higher toxicity than the single components, related to the structure of the deep solvents, due to synergistic effects between the individual elements. Hayyan et al., 2013 conducted some experiments to evaluate the toxicity and cytotoxicity of ChCl and phosphonium-based deep solvents [33]. The models used for toxicity evaluation were two strains of Gram+ bacteria *Bacillus subtilis* and *Streptococcus aureus* and two Gram- strains *Escherichia coli* and *Pseudomonas aeruginosa*, while cytotoxicity was considered on *Artemia salina*. The results show that phosphonium-based deep eutectic solvents have higher toxicity on all bacterial strains, highlighting a possible antibacterial effect, unlike choline chloride-based NADES. However, for both types of HBAs, the mixture of NADES was more toxic than the individual components, even for cytotoxicity, the ammonium-based deep eutectic solvents showed a greater effect on *Artemia salina* than the individual components. Justifications for this behavior may be due to several factors such as hydrogen bonding between HBD and the salt anion. It is known that the delocalization of charge that occurs during hydrogen bonding makes the mixture more toxic; in fact, substances with delocalized charges are more toxic than chemicals with localized charges. The lack of oxygen and high viscosity that impairs the movement of *A. salina* may also influence by modifying the toxicity of the mixture. Further experiments conducted by Hayyan et al., 2013 also hypothesize that NADES can interact with cell surfaces and that their accumulation and aggregation may cause increased cytotoxicity [33]. Still, Radosevic et al., 2015 evaluated different aspects of the toxicity of different deep eutectic solvents based on ChCl, in particular, they considered phytotoxicity on wheat, toxicity on fish and human cells, and biodegradability using wastewater microorganisms through closed bottle test [34]. The results show low to moderate cytotoxicity on cells with cell inhibition comparable to that of industrial solvents, inhibition in wheat germination was also not observed with oxidative stress manifestation only at high amount addition, all NADES tested were classified as biodegradable. NADES thus show a good correlation between biodegradability and toxicity with some advantages such as low vapor pressure and low flammability making them preferable to the organic solvents they are supposed to replace. These results, therefore, showed that natural deep eutectic solvents based on choline chloride have a potential green profile and a very good prospect for use; however, considering the different toxicity observed for some mixtures, especially those based on phosphonium, further studies are needed to understand and clear their impact on the environment and organisms [33].

References

1. Mbous, Y.P.; Hayyan, M.; Hayyan, A.; Wong, W.F.; Hashim, M.A.; Looi, C.Y. Applications of Deep Eutectic Solvents in Biotechnology and Bioengineering—Promises and Challenges. Biotechnol. Adv. 2017, 35, 105–134.
2. Liu, Y.; Friesen, J.B.; McAlpine, J.B.; Lankin, D.C.; Chen, S.N.; Pauli, G.F. Natural Deep Eutectic Solvents: Properties, Applications, and Perspectives. J. Nat. Prod. 2018, 81, 679–690.
3. Smith, E.L.; Abbott, A.P.; Ryder, K.S. Deep Eutectic Solvents (DESs) and Their Applications. Chem. Rev. 2014, 114, 11060–11082.
4. Nam, M.W.; Zhao, J.; Lee, M.S.; Jeong, J.H.; Lee, J. Enhanced Extraction of Bioactive Natural Products Using Tailor-Made Deep Eutectic Solvents: Application to Flavonoid Extraction from Flos Sophorae. Green Chem. 2015, 17, 1718–1727.
5. Rente, D.; Paiva, A.; Duarte, A.R. The Role of Hydrogen Bond Donor on the Extraction of Phenolic Compounds from Natural Matrices Using Deep Eutectic Systems. Molecules 2021, 26, 2336.
6. Zhekenov, T.; Toksanbayev, N.; Kazakbayeva, Z.; Shah, D.; Mjalli, F.S. Formation of Type III Deep Eutectic Solvents and Effect of Water on Their Intermolecular Interactions. Fluid Phase Equilib. 2017, 441, 43–48.
7. Craveiro, R.; Aroso, I.; Flammia, V.; Carvalho, T.; Viciosa, M.T.; Dionísio, M.; Barreiros, S.; Reis, R.L.; Duarte, A.R.C.; Paiva, A. Properties and Thermal Behavior of Natural Deep Eutectic Solvents. J. Mol. Liq. 2016, 215, 534–540.
8. Dai, Y.; van Spronsen, J.; Witkamp, G.J.; Verpoorte, R.; Choi, Y.H. Natural Deep Eutectic Solvents as New Potential Media for Green Technology. Anal. Chim. Acta 2013, 766, 61–68.
9. Huang, Y.; Feng, F.; Jiang, J.; Qiao, Y.; Wu, T.; Voglmeir, J.; Chen, Z.G. Green and Efficient Extraction of Rutin from Tartary Buckwheat Hull by Using Natural Deep Eutectic Solvents. Food Chem. 2017, 221, 1400–1405.
10. Abbott, A.P.; Boothby, D.; Capper, G.; Davies, D.L.; Rasheed, R.K. Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. J. Am. Chem. Soc. 2004, 126, 9142–9147.

11. Tuntarawongsa, S.; Phaechamud, T. Menthol, Borneol, Camphor and WS-3 Eutectic Mixture. Adv. Mater. Res. 2012, 506, 355–358.

12. Van Osch, D.J.G.P.; Zubeir, L.F.; Van Den Bruinhorst, A.; Rocha, M.A.A.; Kroon, M.C. Hydrophobic Deep Eutectic Solvents as Water-Immiscible Extractants. Green Chem. 2015, 17, 4518–4521.

13. Van Osch, D.J.G.P.; Dietz, C.H.J.T.; Van Spronsen, J.; Kroon, M.C.; Gallucci, F.; Van Sint Annaland, M.; Tuinier, R. A Search for Natural Hydrophobic Deep Eutectic Solvents Based on Natural Components. ACS Sustain. Chem. Eng. 2019, 7, 2933–2942.

14. Li, N.; Wang, Y.; Xu, K.; Huang, Y.; Wen, Q.; Ding, X. Development of Green Betaine-Based Deep Eutectic Solvent Aqueous Two-Phase System for the Extraction of Protein. Talanta 2016, 152, 23–32.

15. Xu, K.; Wang, Y.; Huang, Y.; Li, N.; Wen, Q. A Green Deep Eutectic Solvent-Based Aqueous Two-Phase System for Protein Extracting. Anal. Chim. Acta 2015, 864, 9–20.

16. Pang, J.; Sha, X.; Chao, Y.; Chen, G.; Han, C.; Zhu, W.; Li, H.; Zhang, Q. Green Aqueous Biphasic Systems Containing Deep Eutectic Solvents and Sodium Salts for the Extraction of Protein. RSC Adv. 2017, 7, 49361–49367.

17. Deng, W.W.; Zong, Y.; Xiao, Y.X. Hexafluoroisopropanol-Based Deep Eutectic Solvent/Salt Aqueous Two-Phase Systems for Extraction of Anthraquinones from Rhei Radix et Rhizoma Samples. ACS Sustain. Chem. Eng. 2017, 5, 4267–4275.

18. Cao, J.; Chen, L.; Li, M.; Cao, F.; Zhao, L.; Su, E. Two-Phase Systems Developed with Hydrophilic and Hydrophobic Deep Eutectic Solvents for Simultaneously Extracting Various Bioactive Compounds with Different Polarities. Green Chem. 2018, 20, 1879–1886.

19. Yang, Z. Natural Deep Eutectic Solvents and Their Applications in Biotechnology. Adv. Biochem. Eng. Biotechnol. 2019, 168, 31–59.

20. Popovic, B.M.; Micic, N.; Potkonjak, A.; Blagojevic, B.; Pavlovic, K.; Milanov, D.; Juric, T. Novel Extraction of Polyphenols from Sour Cherry Pomace Using Natural Deep Eutectic Solvents—Ultrafast Microwave-Assisted NADES Preparation and Extraction. Food Chem. 2022, 366, 130562.

21. Santana, A.P.R.; Mora-Vargas, J.A.; Guimarães, T.G.S.; Amaral, C.D.B.; Oliveira, A.; Gonzalez, M.H. Sustainable Synthesis of Natural Deep Eutectic Solvents (NADES) by Different Methods. J. Mol. Liq. 2019, 293, 111452.

22. Gutiérrez, M.C.; Ferrer, M.L.; Mateo, C.R.; Monte, F. Del Freeze-Drying of Aqueous Solutions of Deep Eutectic Solvents: A Suitable Approach to Deep Eutectic Suspensions of Self-Assembled Structures. Langmuir 2009, 25, 5509–5515.

23. Gomez, F.J.V.; Espino, M.; Fernández, M.A.; Silva, M.F. A Greener Approach to Prepare Natural Deep Eutectic Solvents. ChemistrySelect 2018, 3, 6122–6125.

24. Zhu, X.H.; Hang, Q.M. Microscopical and Physical Characterization of Microwave and Microwave-Hydrothermal Synthesis Products. Micron 2013, 44, 21–44.

25. Bajkacz, S.; Adamek, J. Development of a Method Based on Natural Deep Eutectic Solvents for Extraction of Flavonoids from Food Samples. Food Anal. Methods 2018, 11, 1330–1344.

26. Bajkacz, S.; Adamek, J. Evaluation of New Natural Deep Eutectic Solvents for the Extraction of Isoflavones from Soy Products. Talanta 2017, 168, 329–335.

27. Rashid, R.; Mohd Wani, S.; Manzoor, S.; Masoodi, F.A.; Masarat Dar, M. Green Extraction of Bioactive Compounds from Apple Pomace by Ultrasound Assisted Natural Deep Eutectic Solvent Extraction: Optimisation, Comparison and Bioactivity. Food Chem. 2023, 398, 133871.

28. Loarce, L.; Oliver-Simancas, R.; Marchante, L.; Díaz-Maroto, M.C.; Alañón, M.E. Modifiers Based on Natural Deep Eutectic Mixtures to Enhance Anthocyanins Isolation from Grape Pomace by Pressurized Hot Water Extraction. LWT 2021, 149, 111889.

29. Fan, Y.; Li, Q. An Efficient Extraction Method for Essential Oil from Angelica Sinensis Radix by Natural Deep Eutectic Solvents-Assisted Microwave Hydrodistillation. Sustain. Chem. Pharm. 2022, 29, 100792.

30. Morrison, H.G.; Sun, C.C.; Neervannan, S. Characterization of Thermal Behavior of Deep Eutectic Solvents and Their Potential as Drug Solubilization Vehicles. Int. J. Pharm. 2009, 378, 136–139.

31. De Morais, P.; Gonçalves, F.; Coutinho, J.A.P.; Ventura, S.P.M. Ecotoxicity of Cholinium-Based Deep Eutectic Solvents. ACS Sustain. Chem. Eng. 2015, 3, 3398–3404.

32. Radošević, K.; Ćurko, N.; Gaurina Srček, V.; Cvjetko Bubalo, M.; Tomašević, M.; Kovačević Ganić, K.; Radojčić Redovniković, I. Natural Deep Eutectic Solvents as Beneficial Extractants for Enhancement of Plant Extracts Bioactivity. LWT 2016, 73, 45–51.

33. Hayyan, M.; Hashim, M.A.; Hayyan, A.; Al-Saadi, M.A.; AlNashef, I.M.; Mirghani, M.E.S.; Saheed, O.K. Are Deep Eutectic Solvents Benign or Toxic? Chemosphere 2013, 90, 2193–2195.
34. Radošević, K.; Cvjetko Bubalo, M.; Gaurina Srček, V.; Grgas, D.; Landeka Dragičević, T.; Redovniković, R.I. Evaluation of Toxicity and Biodegradability of Choline Chloride Based Deep Eutectic Solvents. Ecotoxicol. Environ. Saf. 2015, 112, 46–53.