Green in the deep blue: deep eutectic solvents as versatile systems for the processing of marine biomass

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\textbf{ABSTRACT}

Deep eutectic solvents (DES) consist of a mixture of compounds, which when combined, present a melting point lower than that of each individual compound. They are an emerging class of alternative solvents notable for their versatility and green credentials. Moreover, they can be synthesized with renewable, non-toxic and biocompatible components and can be reused over several cycles. Over the past decade, they have been increasingly used to extract biopolymers and small molecules from marine biomass and to synthesize biomaterials from marine-origin compounds. Herein, a general overview of the use of DES for processing marine biomass is provided. First, the relevant properties of DES for processing marine biomass, and use as extraction media and as functional adjuvants are described. Then, the current state of the art concerning the progress made on their use for processing crustacean and fish by-products and seaweeds for the extraction of polysaccharides, proteins, and pigments, as well as their use to create functional materials is explained. The issues concerning DES properties, extract purification, toxicity and biodegradation are also stated. Finally, the future perspectives of DES are discussed. Despite some presented challenges, DES are promising systems for the processing of marine biomass with high future potential.

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\section*{Introduction}

\textbf{Marine resources and DES}

Oceanic bioresources are abundant materials to produce highly valuable components such as proteins, polysaccharides, fatty acids, and oligo-elements, and may also provide the biomaterials of the future. Increasing competition for land usage for agriculture, for human habitations, and for the preservation of natural land-based ecosystems, turning toward the oceans for new renewable bioresources is an attractive proposition. In light of increased concern about over-exploitation of these living resources and the restrictions imposed on their harvesting, better usage is becoming a necessity. Complete utilization of biomass is an important topic in green chemistry, and the concept of multiple-product blue biorefineries is gaining traction (1). Almost all of the living organisms that humans take from the marine environment are transformed at least once before commercialization. Almost all of the living organisms that humans take from the marine environment are transformed at least once before arriving on the market. This transformation can be simply mechanical – gutting, skinning, and filleting; or chemical and deeply transformative – for example multiple-step extraction processes to produce biopolymers. These processes result in by-products, which may represent high proportions of the original matter, depending on the

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species up to 75% or more of landed weight (2) that can be used for the production of materials without putting further stress on the resource. Current applications for these by-products include high-volume use as agricultural/aquaculture feed and fertilizer (3, 4); or low-volume higher added-value pure products such as collagen, chitin, chitosan, and hydroxyapatite (5). A major hurdle for the latter is the complicated, costly extraction and purification processes required, as these complex biomolecules present a particular challenge for conventional solvents. Furthermore, these may include the use of volatile, hazardous, and toxic organic solvents, high energy consumption, as well as high water and solvent consummation. Long processing times with low efficiency and selectivity of the targeted products are also recurrent issues (6), and processing conditions must be optimized to account for the sensitivity of biomolecules to diverse physical (heat and light) and chemical stress. Holistic use of these resources must take into account sustainability, safety, and security of process and of the end product. As such there is a need for green solvents to process oceanic bioresources for the production of biomolecules, research is now focusing on suitable alternatives that fit both technico-economic and environmental stability requirements (7).

Deep eutectic solvents (DESs) are an emerging class of green alternatives to organic solvents and may offer viable alternatives for processing marine biomass. DESs are closely related to ionic liquids (ILs) (8) and share many of their advantages: negligible vapor pressure, high boiling points, large electrochemical window, and the possibility of recycling (9, 10). DESs cannot be considered traditional ILs because they are not entirely composed of ionic species, and can be obtained from non-ionic species (11). Further, ILs are difficult to produce, they may have mixed environmental credentials, while DESs are generally considered cheap and easy to produce, do not react with water, and many are readily biodegradable and non-toxic (12). DESs are particularly suitable for biomolecules solubilization, extraction, and functionalization (13). Indeed, an appropriately chosen DES can solubilize complex polysaccharides (14), or prevent denaturation of proteins or DNA during extraction for example (15).

DESs are combinations of two or more Lewis or Brønsted acids and bases that, when mixed together at precise ratios, constitute a liquid system with a melting point much lower (hence the often-used qualifier ‘deep’ [16]) than each composing species taken individually. Fundamentally, the formation of DESs depends on hydrogen bonds forming between one or more hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs). Ultimately, DESs are generally viscous liquids at room temperature due to the low lattice energy of the hydrogen bond networks.

DESs are a relatively young field of study, with the first studies published in 2001 (17) and the term being coined in 2003, by Abbott et al., who were instrumental in developing initial compounds (choline chloride: urea becoming an archetypal DES) and the theory involved in their chemistry (16, 18). Certain authors distinguish natural DESs (NADESs) from DESs when the individual species are primary metabolites, namely, amino acids, organic acids, sugars, or choline derivatives. The term was originally coined by Choi et al. in 2011 (19) who were exploring the reasons behind the abundance of a few very simple molecules in all microbial, mammalian, and plant cells. The authors postulated that the compounds would serve some basic function in living cells and organisms, constituting a liquid phase for solubilizing, storing, or transporting non-water-soluble metabolites in living cells and organisms. Considering the diversity of natural metabolites, NADES possess correspondingly diverse properties, usages, and limited toxicity (20), and careful study of these systems may be particularly useful for bio-inspired processing. DESs will be considered here to englobe NADES as a blanket term as all authors do not systematically refer to the systems by the same designation. When a reference used the specific term ‘NADES’ that particular designation will be respected and repeated here in the following text.

A special case concerns low transition temperature mixtures, originally coined by Francisco et al. in 2012 (21). When synthesizing and characterizing various candidate DESs by differential scanning calorimetry, the authors observed that no melting point was observed for some of the candidates (e.g. choline chloride/lactic acid 1:2 with a $T_g$ of $-77.73^\circ$C). As such, the mixtures are non-eutectic strictly sensu. However, they will also be considered here by the analogy of synthesis and mechanisms of formation, i.e. extensive hydrogen bonding occurring between two or more compounds in precise molecular ratios and resulting in viscous liquids and are synthesized in a similar manner to DESs. Indeed, some authors still refer to the mixtures as DESs (choline chloride/lactic acid is a recurrent example). Also included in this review are bio-based ILs (Bio-ILs), a term coined by Fukaya et al. in 2007 (22). Although these solvents are not eutectic mixtures of two organic compounds, they are room-temperature biological origin ILs and deserve mention as alternative and related solvents.
DESs are extremely versatile solvents used in extraction (23), analytical chemistry (24), as non-conventional reaction media for biocatalysis (25) and for specific applications in material synthesis (26), lubrication (27), cleaning agents (28), and many more (29). However, usage for the processing of marine biomass remains relatively limited despite growing interest (Figure 1(a)). Reports on processing of marine biomass using DESs are scarce. In total, 34 studies related to the use of these systems to process marine biomass were found during research for this review, which were then divided by types for the following sections (see Figure 1(b)). These could be broadly classed into two main goals: the extraction of a target compound or polymer or synthesis of a marine-based biomaterial. This represents only a tiny fraction of the total research effort devoted to these solvent systems since 2012 (6057 publications). The first use of DESs to process marine biomass was agarose, which was the first marine-origin polymer to make gels regarding the plasticizing effect of DESs in 2012 (30). The first reported use of DES systems to process molecules from marine biomass took place in 2013, with the dissolution of α-chitin by Sharma et al. (31) However, as evidenced by the acceleration of articles published since 2017, these versatile designer solvents are of increasing interest for the processing of marine bioresources. In this review, we provide an overview on the use of DESs for processing marine-origin biomass and discuss possible forward directions for research and industrial use.

**Relevant properties of DESs for processing marine biomass**

The properties of DESs depend on the individual species of which they are composed, and the ratio in which they form eutectic systems. Currently, the most common DESs are choline chloride-based, where the compound acts as an HBA (see examples in Figure 2). Choline chloride (ChCl) is a quaternary ammonium salt with choline cation and chloride anion, the choline cation is widely produced as an animal feed additive, and is considered an essential dietary nutrient (vitamin B4) for human health (32). ChCl-based DESs are generally acidic (33). Although most DESs are hydrophilic, recently hydrophobic DESs have been developed (34, 35). Terpenes such as menthol and thymol appear to be the best candidates for making sustainable and low-cost hydrophobic solvents (36), although many others exist (37).

DESs synthesis is relatively simple and is performed using one of the three following techniques: (I) powder components are mixed at an elevated temperature until melting and homogenizing; (II) components are freeze-dried under vacuum, mixed, and heated; (III) aqueous solutions of components are mixed and excess water evaporated in a rotary evaporator.

Concerning the processing of marine biomass, DESs have two major functions; solvents for targeted extraction and functional adjuvants in polymeric materials derived from marine extracts.

**DESs as extraction media**

The diverse nature of DESs enables the targeting of a highly diverse range of marine compounds ranging from polysaccharides to minerals. Generally, a variety of DESs is used to screen the best performing system for the appropriate raw material and target compound. This involves varying both HBA, with various quaternary ammonium compounds the most prevalent (ChCl, betaine hydrochloride) and HBD (carboxylic acids, simple polyols, or alkali), but also mixture ratios. DESs are very viscous and optimizing this parameter has been a priority since initial studies (16). Viscosity inhibits mobility of free species within the DES network, and thus mass transfer when DESs are used as extraction media (34). To improve viscosity, water may be included to a degree in the DESs to a very significant effect (20, 38). However, above specific thresholds, it affects

![Figure 1. Interest in DESs and related green solvents breakdown of domains of application: (a) DESs and related bio-ionic liquid processing of marine biomass from 2012 until June 2021; and (b) focus of research in articles (N = 34).](image-url)
interspecific hydrogen bonding within the DESs (39, 40). Temperature also regulates the cohesive forces in a DES, and when increased, it can cause a decrease in the internal resistance of molecules, thus decreasing overall viscosity of the liquid. Excessive temperatures, however, can induce undesirable changes in the DES or its components, for example, esterification reactions occurring between the HBA and HBD (41), or the decarboxylation of malonic acid above 60°C (42). This can also result in the modification of the target compound, for example, the acylation of chitin through interaction with carboxylic acid HBDs (43, 44).

Physical pretreatment affects extraction efficiency, and many techniques, such as microwave-assisted extraction, pulsed electric fields and high-voltage electric discharges, heating, ultrasound, and enzyme-assisted extractions are all strategies that have been used to increase extraction efficacy (45, 46). Optimizing these different pretreatments results in greater yields for an extractive process, and they are often used concurrently with DES systems.

**Figure 2.** DESs and NADES as solvents for the extraction of natural compounds and/or processing of marine biomass.

As extraction media, one of the challenges for broader application of DESs is isolation of target compounds and/or reaction products. Indeed, the DES, or a component thereof, may form tenacious hydrogen bonds with target molecules (47), making them difficult to remove. Washing, centrifugation, and filtration can be used for extracts/reaction products that are non-soluble or following a precipitation step but this is not always possible. However, when the target is precipitated or filtered DESs may be subsequently reused up to several cycles with little loss of efficacy (44, 48, 49).

Although significant progress has been made at a lab scale, the implementation of DESs in the industry has not
been established to date and few pilot-scale studies have been undertaken or published. Recent studies have highlighted the viability of DES processes for high-value, low-volume products from lignocellulosic biomass (50). However, DES extracts are currently commercialized as cosmetic ingredients without purification: notably isoquercetin from tree leaves (51), green tea catechins (52), and more (53–56). Furthermore, DES extracts have the potential to be used in pharmaceuticals (34, 57) or incorporated into feed or feed additives (58). The properties of the DESs may positively influence the effect of the extract: for example, increasing cancer cell line cytotoxicity or antimicrobial activity (59). DESs have also been shown to lock molecules in a specific conformation, even in greatly diluted solutions. For example, DES-curcumin systems were shown to potentiate or negate, the photo-dependent antibacterial activity of curcumin (60). As such, DESs show high potential for drug delivery systems (61).

**DE斯s as functional adjuvants in marine biomaterials**

Another promising avenue for DESs is as multifunctional adjuvants to polymeric-based marine materials. DESs have been used as plasticizers (62) for marine biomaterials as well as compatibilizers in biocomposites (63). At an intermediate level, DES processing can facilitate the chemical modification of a polymer, for example, making it more amenable to functionalization (64). Incorporating DESs in polymeric materials modifies the biocomposite’s mechanical and physical characteristics including water vapor permeability, tension at breaking point, and optical transparency (65).

**Toxicity/biodegradability**

DE斯s display different behavior/bioactivity than individual compounds or simple mixtures of the compounds at the same concentration and the system acquires new characteristics including toxicity. As such, inherent toxicity and biodegradability for DESs and NADES must be evaluated case-by-case, notably in soils and aquatic environments that may come in contact with effluents. Mixture toxicity modeling may be a useful tool to evaluate the environmental and toxicity risk of DES systems (66). In some studies, DESs prepared with ChCl paired with organic acids shows increased toxicity toward bacteria when compared with respective starting materials and can be considered ‘moderately toxic,’ depending on the acid HBD (67, 68). Wen et al. showed that DESs also had concentration-dependent inhibitory effects on freshwater hydra (Hydra sinensis) and garlic (Allium sativum), as well as bacteria, although the DESs they studied were predominantly less toxic than their individual components (69). Further study by Juneidi et al. (70) on fungi (Aspergillus niger) and common carp (Cyprinus carpio) showed one DES to reach ‘slight toxicity’ (American Fish and Wildlife Service denomination for Aquatic LC50 between 10 and 100 mg·L−1) for one compound studied – and all DESs were less toxic than their individual compounds. In another study, DESs containing urea HBD donors caused high earthworm mortality, and ChCl-based DESs caused reproductive harm, although the latter effects were not observed with betaine-hydrochloride as the HBA (71). Concerning atmospheric pollution, the low vapor pressure that characterizes most DESs greatly reduces the risk of emission to the atmosphere (72).

In human cell lines, DESs show low to moderate cytotoxic effects according to their composition (10). DESs containing organic acid HBD donors present higher cytotoxicity, for example, ChCl:oxalic acid DES showed higher cytotoxic effects than individual compounds and is comparable to organic solvents (12). They also had cytotoxic effects on selected normal and tumor cell lines, whereas all tested NADES did not (68). Further cell line studies reinforced these findings, along with computational modeling (conductor-like screening model for real solvent or COSMO-RS) that highlighted the differences in toxicity were highly dependent on the system composition (73). Cytotoxic effects of mixes of terpene-based DESs unobserved in individual components have also been demonstrated (59).

Biodegradability studies of DESs show some contrasting results (Table 1). Studies of ChCl and N,N-diethylammonium chloride (EAC)-based DESs were all ‘readily biodegradable’ according to OCED terminology, with several systems rapidly attaining the 60% threshold (12, 70). However, in a different study, Wen et al. showed that some DESs were not readily biodegradable, with just two out of eight DESs tested readily decomposed under testing conditions (69), although containing many of the same DESs examined in the other studies. This may be due to differences in the microorganisms obtained from wastewater treatment plants or the effect of different molecular ratios.

**DE斯s for processing crustacean by-products**

Crustacean shells are highly abundant by-products of the food industry. This low-value biomass is of particular interest for the development of specific biorefineries as it contains numerous potentially valuable products such as polysaccharides (chitin), minerals, proteins, and pigments like astaxanthin (74). The versatility of DESs is particularly suited for this type of transformation, with the possibility of developing several products, providing an economically viable model according to techno-economic...
evaluation modeling (50). Indeed, DESs have already been used with success for the extraction of the most valuable and bioactive compounds of this product, namely chitin and chitosan (Table 2) and astaxanthin (Table 3).

Chitin

Chitin is an essential component of crustacean shells and the second most abundant biopolymer on the planet. The presence of nitrogen in its chemical structure is an important distinguishing factor from other polysaccharides (75). As a raw material, chitin may have applications in tissue engineering (76, 77), nanocomposites (78–80), water treatment, and pollutant removal (81, 82), among many others (83).

In crustacean shells, chitin is linked to proteins and minerals. Conventional chitin extraction has two principal steps: demineralization and protein removal (79, 84); and if required, chitin dissolution. DES/NADES can do all three simultaneously, disrupting its natural hydrogen-bonding networks. Chitin is the most commonly targeted marine-origin polymer for extraction by DESs (85).

In 2013, Sharma et al. were the first to successfully solubilize crab-shell-derived alpha-chitin using DES-assisted by conventional heating and ultrasound-assisted heating. The authors achieved 9% (w/w) of chitin using a ChCl:thiourea system at 1:2 upon heating for 6 h at 100°C. Other DESs, ChCl:urea (1:2), ChCl:thiourea (1:2), choline bromide:urea (1:2), chlorocholine chloride:urea (1:2), and BETaine 90 hydrochloride:urea (1:4) were also able to solubilize chitin, although were very viscous at concentrations higher than 4% (w/w) (31).

Since the initial findings show the solubility of chitin, DESs have been used to extract chitin from several different sources and the processes optimized for solvent recycling (23, 86). In 2017, H. Lian’s group used mixtures of ChCl:T, ChCl:U, ChCl:glycerol, and ChCl:malonic acid to extract chitin from lobster shells. The lobster shells were put in the DES system under heating, water was added until precipitation (of chitin) occurred, and the mixture was centrifuged and washed until pH neutral was attained. Using this procedure, the ChCl:malonic acid system, after 2 h at 100°C had a better yield than conventional chemical processing, reaching yields of 20.63 ± 3.30% versus 16.53 ± 2.35% (dry shell weight), respectively. Moreover, the chitin obtained with DESs did not present significantly different ash and protein profiles than the chitin obtained with the classical procedure (86). Further work (87) showed that using temperatures of only 50°C was enough to obtain the highest yields with ChCl:malonic acid, and that it was possible to modulate chitin molecular weight with temperature and choice of DES. Furthermore, with the addition of a drying step using ethanol (1/10 DES:EtOH ratio) it is possible to regenerate calcium salts of the acid HBD. When using ChCl:levulinic acid, it was also possible to produce levulinic acid calcium salt, another high-value-added product used as a calcium supplement (87) or intermediate in levulinic biofuels (88). This multiple-product process highlights the interest of DESs in shell biorefinery processes.

Saravana et al. (23) used DESs to extract chitin from shrimp shells using a similar methodology. Fourteen DESs were screened, with the best results coming from the same system as above, ChCl:malonic acid at 80°C for 2 h under constant stirring. Moreover, the authors obtained yields double that of those found in the literature using classical methods: 26% as opposed to 13% (23, 89). Chitin films were cast using a 5% N,N-dimethylacetamide/lithium chloride solution with the extracted material, and showed superior performances across on the board as compared to films using commercial chitin standard: higher tensile strength, elongation at break, and Young’s modulus.

It has also been shown that the reuse of solvents is possible. Improving on the above results, Feng et al. (44) tested 11 systems with different enantiomers of HBD and found the best results with ChCl:DL-malonic acid for 3 h at 150°C, obtaining 98.6% purity O-malate chitin with 0.46 degrees of substitution (DS). In considerations for scale-up, it was demonstrated that pure ChCl:malonic acid could be used at 10 wt% water content with no loss of performance. The system could be used 5 times without significant loss of purity, but DS decreased from 0.46 to 0.18 (44). Bradic et al. used ChCl:lactic acid aqueous solution to isolate chitin in a

| Table 1. DES biodegradability according to OECD 301 D. |
|-----------------|-----------------|----------------|
| DES            | Ratio | Biodegradation (28 days) | Source |
| ChCl:glycerol  | 1:2   | 91% (70)                 |
| ChCl:urea      | 1:2   | 85%                      |
| ChCl:ethylene glycol | 1:2   | 77%                      |
| EAC:glycerol   | 1:2   | 75%                      |
| EAC:ethylene glycol | 1:2   | 70%                      |
| EACZnCl₂       | 1:2   | 68%                      |
| EACmalonic acid | 1:1   | 61%                      |
| EACZnN         | 1:1   | 80%                      |
| ChCl:urea      | 1:1   | ~80% (69)                |
| ChCl:acetamide | 1:1   | ~78%                     |
| ChCl:glycerol  | 1:1   | ~40%                     |
| ChCl:ethylene Glycol | 1:1   | ~30%                     |
| ChAcurea       | 1:1   | ~42%                     |
| ChAcacetamide  | 1:1   | ~40%                     |
| ChAcglycerol   | 1:1   | ~35%                     |
| ChAcethylene glycol | 1:1   | ~30%                     |
| ChCl:glucose   | 1:2   | 80% (12)                 |
| ChCl:glycerol  | 2:1   | 96%                      |
| ChCl:oxalic acid | 1:1   | 68%                      |

Note: ChCl: choline chloride; EAC: N,N-diethyl ethanol ammonium chloride; ZnN: zinc nitrate hexahydrate; ChAc: choline acetate.
### Table 2. DES for processing of chitin and chitosan.

| HBA            | HBD            | Ratio | Species/raw material/target | Protocol | Results | Source |
|----------------|----------------|-------|-----------------------------|----------|---------|--------|
| ChCl           | Urea           | 1:2   | α-chitin/ purification      | ChCl was added to DES and heated to 100°C for different times under additional ultrasonication or microwave irradiation | Yields 9% weight (v/v) | (21)   |
| ChCl           | Thiourea       | 1:2   |                              |          |         |        |
| ChBr           | Urea           | 1:2   |                              |          |         |        |
| ChCl           | Urea           | 1:2   |                              |          |         |        |
| Bet 90 HCl     | Urea           | 1:4   |                              |          |         |        |
| ChCl           | Glycerol       | 1:2   |                              |          |         |        |
| ChCl           | Ethylene Glycol| 1:2   |                              |          |         |        |
| ChCl           | Thiourea       | 1:1   | Lobster/α-chitin            | Lobster shell was added to DES at 100°C for 2 h | ChCl:malonic acid system, after 2 h at 100°C had a better yield than conventional chemical processing: 20.63 ± 3.30% dry shell weight versus 16.53 ± 2.35% dry shell weight | (86)   |
| ChCl           | Urea           | 1:2   | Lobster/α-chitin            | Water added until precipitation | Best yield/purity with ChCl:malonic acid: 19.5% with 93% purity | (87)   |
| ChCl           | Thiourea       | 1:1   | Lobster/α-chitin            | Centrifugation and washing until pH neutral | ChCl:levulinic acid produced levulinic acid calcium salt, a secondary valuable product |        |
| Malonic acid   |                | 1:2   |                              |          |         |        |
| ChCl           | Malonic acid   | 1:2   | Lobster/α-chitin and calcium| Drying of the supernatant DES mixture, addition to ethanol (1/10 DES:EtOH ratio) to the regenerate calcium salts | Best yield/purity with ChCl:malonic acid: 19.5% with 93% purity | (87)   |
| Malonic acid   | Lactic acid    | 1:1   |                              |          |         |        |
| Malonic acid   | Malonic acid   | 1:2   |                              |          |         |        |
| Lactic acid    | 1,4-Butanediol|       |                              |          |         |        |
| Ethylene Glycol| Urea           |       |                              |          |         |        |
| Urea           | 1,6 Hexanediol |       |                              |          |         |        |
| Glycerol       | Malonic acid   | 1:2   |                              |          |         |        |
| Malonic acid   | Citric acid    | 1:2   |                              |          |         |        |
| Malic acid     | Propylene glycol|       |                              |          |         |        |
| Tartaric acid  | Maleic anhydride|       |                              |          |         |        |
| Thiourea       |                |       |                              |          |         |        |
| ChCl           | Lactic acid    | 1:2   | Marsupenaeus japonicas/ α-chitin | Shrimp shell and DES were mixed at 1.25 (w/w) and stirred at 300 rpm for 2 h at 80°C. Centrifugation (4000 rpm) and precipitate were recovered. Washing until neutral pH. Oven-dried (40°C) then subjected to decolorization by using 10% (w/v) H2O2 at 80°C | Yield of 19.41 wt% ± 1.35 wt% chitin Properties of chitin films similar to standard films | (23)   |
| ChCl           | D-Malic acid   | 1:2   | Solenocera crassicornis/ α-chitin | 1:2 Shrimp shell/DES 3 h at 150°C constant stirring | Chitin yield 13.2 ± 1.1% with optimized conditions O-malate chitin with 98.6% purity and a DS of 0.46 System reusable 5 times without significant loss of purity, but DS decreased from 0.46 to 0.18 Yield of α-chitin: 20 wt% optimized conditions: <90% total chitin of raw material with ChCl:lactic acid Purity >98% DES recyclable with a method | (44)   |
| ChCl           | L-Malic acid   | 1:2   |                              |          |         |        |
| DL-Malic acid  | Citric acid    | 1:2   |                              |          |         |        |
| L-Lactic acid  | Urea           | 1:2   |                              |          |         |        |
| Urea           | 1,6 Hexanediol |       |                              |          |         |        |
| Glycerol       | Malonic acid   | 1:2   |                              |          |         |        |
| Malonic acid   | Citric acid    | 1:2   |                              |          |         |        |
| Malic acid     | Propylene glycol|       |                              |          |         |        |
| Tartaric acid  | Maleic anhydride|       |                              |          |         |        |
| Thiourea       |                |       |                              |          |         |        |
| ChCl           | Lactic acid    | 1:2   | Pandalus borealis/ α-chitin  | Dissolution of chitin in DES at 70°C for 4 h Addition of 100 mL deH2O To recycle DES obtained filtrate subjected to vacuum distillation | Yield of α-chitin: 20 wt% optimized conditions: <90% total chitin of raw material with ChCl:lactic acid Purity >98% DES recyclable with a method | (49)   |
| ChCl           | Malonic acid   | 1:2   |                              |          |         |        |
| Urea           | Citric acid    | 1:2   |                              |          |         |        |
| ChCl           | Oxalic acid    | 1:2   | Crab chitin/ α-chitin/chitin nanocrystals | Chitin powder was treated in DESs under mixing at 100°C for 1 h, centrifuged, and sonicated at an output power of 1000 W and 2/2 s on/off pulses for 30 min | O-acetylated nanocrystals Yield 87.5 wt% with ChCl:lactic acid Characteristic dimensions of individual chitin nanocrystals according to DES: 42–49 nm diameter; average lengths of 257–670 nm | (90)   |
| ChCl           | Lactic acid    | 1:2   |                              |          |         |        |
| Oxalic acid    | Dihydrate      | 1:2   |                              |          |         |        |
| Lactic acid    | Malonic acid   | 1:2   |                              |          |         |        |
| Malonic acid   | Citric acid    | 1:2   |                              |          |         |        |
| Citric acid    | monohydrate    |       |                              |          |         |        |
| DL-malic acid  |                |       |                              |          |         |        |
| Betaine chloride| Ferric chloride|       |                              |          |         |        |
| hexahydrate    |                |       |                              |          |         |        |
| ChCl           | Urea           | 1:2   | Shrimp shells/ α-chitin/chitin nanocrystals | 100°C for 1 h with chitin-to-DES mass ratio of 1:20 | Diameter of 10 nm and length of 268 nm, and crystallinity of 89.2% Efficient emulsion stabilizers: stable o/w emulsions at oil content of 50% with CNC dosage of 1 mg/g y-mono-phase films modeled as a super-cell, in contrast to the idea of a simple physical mixture of α and β phases | (91)   |
| ChCl           | Lactic acid    | 1:1   | Shrimp shells/ α-chitin/chitin films | Demineralized chitin was slowly added to DES under stirring at 100°C to obtain a 2% w/w solution. Solution immersed in deH2O, then carefully washed | Drying Reduction of NaOH concentration necessary for chitin decacyatation by 25%, and temperature by 20%. Best results with B:glycerol and ChCl:actic acid | (92)   |
| Oxalic acid    | Urea           | 1:2   |                              |          |         |        |
| Betaine        | Glycerol       | 1:2   |                              |          |         |        |
| Glycerol       |                | 1:4   | Chitin                      | Solvent mixed with chitin in a solid/liquid ratio of 1:50 (w/v) at 80°C for 24 h. | DES divided into three groups: (i) negligible decacyatation (choline) | (95)   |
| KHCO3          | Glycerol       | 1:4   |                              |          |         |        |
| K2CO3          | Ethylene glycol| 1:4   |                              |          |         |        |

(Continued)
Table 2. Continued.

| HBA          | HBD          | Ratio | Species/raw material/target | Protocol | Results | Source |
|--------------|--------------|-------|-----------------------------|----------|---------|--------|
| Choline      | Acetic acid  | 1:2   | Reaction quenched with distilled water/washed multiple times | Drying at 35°C | dihydrogen citrate; glycerol | (96) |
| dihydrogen   | Oxalic acid  | 1:4   | Dissolution of chitosan in DES at 10, 20, 33, 50, 67, 75 and 82 wt% (chitosan/DES) | Addition of dH2O to DES–chitosan concentration of 2% 20 µm films/drying at 25°C for 6 days | Improved mechanical properties until 67 wt% DES | (64) |
| citrate      | Malonic acid | 1:2   | Dissolution of chitosan in DES at 50, 67, 75 and 82 wt% (chitosan/DES) | Addition of dH2O to DES–chitosan concentration of 2% 20 µm films/drying at 25°C for 6 days | Chitosan chain swelling. | (96) |
| ChCl         | Lactic acid  | 1:2   | Dissolution of chitosan in DES at 10, 20, 33, 50, 67, 75 and 82 wt% (chitosan/DES) | Addition of dH2O to DES–chitosan concentration of 2% 20 µm films/drying at 25°C for 6 days | Improved tensile strength (ChCl:citric acid), water vapor impermeability (ChCl: citric acid) | (97) |
| ChCl         | Malonic acid | 1:1   | Dissolution of chitosan in 0.1% acetic acid solution. Additon of DES | Hot pressing | Higher thermal degradation stability than reference | (99) |
| Lactic acid  | 1:1          |       |                             | Heating 80°C for 30 min | Satisfactory transparency for all | (100) |
| Citric acid  | 1:1          |       |                             | Addition of 3% acetic acid 25/75 (with chitosan DES/with acetic acid) | Improved elasticity (ChCl:malonic acid), tensile strength (ChCl:citric acid), water vapor impermeability (ChCl: citric acid) | (97) |
| Glycerol     | 1:2          |       |                             | Heating 80°C for 30 min | Satisfactory transparency for all | (96) |
| ChCl         | Urea         | 1:2   | Dissolution of CMC and chitosan in 0.1% acetic acid solution. Additon of DES | Casting of films in 50 mL Petri dish | Lower viscosity and freer movement of the substrates to the enzymes (thus higher enzyme activity), pH relatively close to physiological conditions, at pH 8.4 | (96) |
| ChCl         | Urea:glycerol| 1:2:2 | Dissolution of 300 mg of chitosan in 0.5 mL of distilled water containing 2 mL ternary DES, addition of 100 mg lipase | Precipitation of polymer with 0.1 NaOH | Successful synthesis of TMC: quaternization of 12.5%–15.69% | (100) |
| ChCl:glycerol| 1:1:1        |       |                             | Centrifugation, neutralization in 0.1 HCl | Low water uptake | (96) |
| ChCl:glycerol| 1:2:1        |       |                             | Centrifugation and freeze-drying of precipitate | High proton conductivity | (96) |
| ChCl:glycerol| 1:2:2        |       |                             |                             |                             | (96) |

Notes: DES: deep eutectic solvent; HBD: hydrogen bond donor; HBA: hydrogen bond acceptor; ChCl: choline chloride; NC: not communicated. Best performing DES are in bold print.

Shrimp shells were dissolved in ChCl:lactic acid at 70°C for 4 h, before the addition of water to lower viscosity and precipitate water-insoluble fractions (including chitin). This was followed by filtration of the precipitate, drying of the DESs in vacuum, and repeating the process. Very high purity chitin (99%) was achieved in this manner, with yields of 20% of dry shrimp shells. On repeating the procedure, yields remained unchanged, although purity of the isolated chitin was lower (49).

DEs can also be used to produce chitin nanocrystals with controlled molecular weight (49, 86). This requires the removal of amorphous zones of raw chitin. This has recently been achieved using an ultrasound-assisted process and DES. ChCl-based-DESs were used with five different organic acids: oxalic acid, lactic acid, malonic acid, citric acid, and DL-malic acid as HBD, in molecular ratios of 1:2. Briefly, chitin powder was treated in DESs under mixing at 100°C for 1 h, quenched with distilled water, centrifuged, and sonicated to produce chitin nanocrystals. Each system produced characteristic crystal sizes, diameters of individual chitin nanocrystals ranged from 42 to 49 nm on average, with average lengths of 257–670 nm. As in Feng (44), all chitin nanocrystals were found to be O-acylated. The ChCl:lactic acid system was considered the best performing with a shorter reaction time, the highest mass yield of chitin nanocrystals (up to 87.5 wt%), and production of stable aqueous suspension at 0.5% weight (90). Follow-up work showed that using a ferric chloride hexahydrate and betaine chloride (molar ratio of 1:1) was also effective as a hydrolytic media for production of chitin nanocrystals with a high yield (up to 88.5 wt%). Optimal synthesis conditions were also found at 100°C for 1 h with a chitin-to-DES mass ratio of 1:20 under ultrasound, resulting in chitin nanocrystals with an average diameter of 10 nm and length of 268 nm (97). Furthermore, ChCl:urea has been shown to allow fine-tuning of the chitin crystallization process in order to produce films with β-dihydrated- or γ-chitin structures (92).

Recently, choline-acetate bio-IL was used to solubilize α-chitin and create porous materials suitable for tissue...
### Table 3. DES for extraction of astaxanthin.

| HBA              | Species/raw material | Protocol | Results | Source |
|------------------|----------------------|----------|---------|--------|
| ChCl Ethylene glycol | Shrimp by- products/ astaxanthin | Shrimp shell powder (0.1 g) was mixed with 1.0 mL of DES and water 10 wt % Ultrasound-assisted extraction 200 µL of the extract mixed with an equal volume of chromatography mobile phase (dichloromethane/ methanol/acetonitrile/water) 200 W max | 146 µg/g for shells and 218 µg/g of heads Better yield than ethanol extraction ChCl:1,2-Butanediol gave the best performance | (31) |
| Glycerol 1,2-Butanediol | Portunus trituberculatus /shell | 1 g natural product powder added to 40 mL acetone with 1.25 mg/mL DES-19 65 W ultrasonication for 90 min | Amount of astaxanthin extracted using DES was 155% of the IL additive yield | (105) |
| 1,2-Butanediol 1,4 | Cancer pagurus/H pluvialis/Mussels | 0.25 g of crab shell residue to 1 g of NDES Gentle stirring at 60°C for 2 h | NADES and AXT-rich have antiproliferative activity and inhibit Gram-positive and Gram-negative bacteria growth | (59) |
| Perillyl-alcohol Camphor | Myristic acid | DES mixed with shrimp shells at a 1:10 w/w ratio | 68.98 ± 1.22 mg astaxanthin/g shrimp waste | Improved qualities of chitosan films: antioxidant effect, higher tensile strength, higher Young’s modulus, lower tension at breaking point. | (98) |
| Menthol Perillyl alcohol | Myrist acid | Optimal conditions used were ChCl:L; ultrasonic power 40%; 45 min reaction time | | |
| Menthol Camphor | Glycerol | Chitosan chains swelled with the addition of DES, along with an increase in the mobility of charge carriers. ChCl-based DES (with malonic acid, lactic acid, citric acid, and glycerol) may be used compared to conventional techniques, with the best results achieved using Bet:glycerol and ChCl:lactic acid (ChCl:urea resulted in excessive secondary reaction products). In 18 h and while using 30 wt% NaOH at 80°C, a DDA of 80% was reached with homogeneous chitin deacetylation, as compared to 40% NaOH and temperatures over 100°C in conventional methods. Continuing this work, the authors studied chitin deacetylation using acidic and alkaline DES alone. This was supported by quantum chemical calculations evaluating feasibility of chitin deacetylation with DESs by studying the mechanism. The authors were unsuccessful in obtaining chitosan stricto sensu (more than 50% DDA chitin). ChCl:malonic acid was found to give the best results, giving 40% DDA chitin after 24 h at 120°C (95). DESs have high potential for use in the development of chitosan-based biomaterials affecting transparency, conductivity, mechanical properties, permeability, water solubility, and more (85). Sokolova’s group showed films cast with ChCl:malonic acid improved elongation at break up to 67 wt%, a lowered glass transition temperature, as well as complete dissolution in distilled water (96). The ionic conductivity of chitosan films can be modified with the presence of ChCl:lactic acid, with conductivity increasing with DES content (50–82 wt%), reaching maximum values at maximum tested content. Chitosan chains swelled with the addition of DES, along with an increase in the mobility of charge carriers (64). ChCl-based DES (with malonic acid, lactic acid, citric acid, and glycerol) may be used | |
| Menthol Eucalyptol | Oxalic acid | Optimal conditions used were ChCl:L; ultrasonic power 40%; 45 min reaction time | | |
| Menthol Eucalyptol | Lactic acid | | | |
| Menthol Eucalyptol | Tartaric acid | | | |
| Menthol Eucalyptol | Malic acid | | | |

**Chitosan**

Chitosan is the main derivative of chitin, the result of a process of deacetylation. This process can be carried out in both acidic and alkaline conditions, although because glycosidic bonds are vulnerable to acidic hydrolysis, the latter is most often preferred. Chitosan has the major advantage of being soluble in lightly acidic aqueous solutions and is thus more amenable to modification and use than its parent. Deacetylation changes the properties of the polymer and as such its solubility in various DES systems: for example, chitosan is not soluble in DES ChCl:thiourea, whereas chitin is soluble (31).

Chitin that is processed with DESs does not require the same conditions for deacetylation as chitin obtained by traditional processes. This is illustrated in the work of Vicente et al. (94) who used acidic, neutral, and alkaline DES systems (ChCl:lactic acid; betaine;glycerol; and ChCl:urea respectively) to dissolve chitin for subsequent deacetylation with a NaOH solution. All three trialed were successful in substantially reducing chitosan preparation times as compared to conventional techniques, with the best results achieved using Bet:glycerol and ChCl:lactic acid (ChCl:urea resulted in excessive secondary reaction products). In 18 h and while using 30 wt% NaOH at 80°C, a DDA of 80% was reached with homogeneous chitin deacetylation, as compared to 40% NaOH and temperatures over 100°C in conventional methods. Continuing this work, the authors studied chitin deacetylation using acidic and alkaline DES alone. This was supported by quantum chemical calculations evaluating feasibility of chitin deacetylation with DESs by studying the mechanism. The authors were unsuccessful in obtaining chitosan stricto sensu (more than 50% DDA chitin). ChCl:malonic acid was found to give the best results, giving 40% DDA chitin after 24 h at 120°C (95). DESs have high potential for use in the development of chitosan-based biomaterials affecting transparency, conductivity, mechanical properties, permeability, water solubility, and more (85). Sokolova’s group showed films cast with ChCl:malonic acid improved elongation at break up to 67 wt%, a lowered glass transition temperature, as well as complete dissolution in distilled water (96). The ionic conductivity of chitosan films can be modified with the presence of ChCl:lactic acid, with conductivity increasing with DES content (50–82 wt%), reaching maximum values at maximum tested content. Chitosan chains swelled with the addition of DES, along with an increase in the mobility of charge carriers (64). ChCl-based DES (with malonic acid, lactic acid, citric acid, and glycerol) may be used
as plasticizers of chitosan. Chitosan films were prepared with each, with satisfactory transparency for all, and improvement on specific aspects according to the chosen DES such as elasticity for ChCl:malic acid, and tensile strength and water vapor impermeability for ChCl: citric acid. In this case, the most viscous native DES showed the best overall performance when combined with chitosan, resulting in a homogenous surface and compact structure, and showing promise for applications in food packaging for example (97). In an original approach, Chandra Roy et al. combined an Astaxanthin-DES extract with commercial chitosan to create biodegradable active packaging materials, with the inclusion of the extract providing both plasticizing and antioxidant effects (98).

DESs have also been used to design hybrid chitosan-based membranes. Inclusion of ChCl:urea in hybrid chitosan-carboxymethylcellulose (CMC) membranes was shown to improve proton conductivity, temperature stability, and reduced membrane swelling in humid conditions (99). The material could find use in fuel cells for example.

Finally, DESs can be used as media for the enzymatic modification of chitosan. One example is N,N,N-trimethyl chitosan (TMC), a quaternized hydrophilic derivative of chitosan with applications in the pharmaceutical industry owing to solubility over a wide pH range. However, conventional synthesis of TMC results in unwanted o-methylation and scission of the source chitosan. These limitations were successfully addressed using ternary DES, lipases from Burkholderia cepacia and Candida rugosa as biocatalysts, and dimethyl carbonate as a methylating agent. The ternary DES was composed of ChCl as HBA and urea and glycerol as HBDs, in a ratio of 1:2:1. The use of this three-part mixture enabled lower viscosity and freer movement of the substrates to the enzymes (thus higher enzyme activity), and with a pH relatively close to physiological conditions, at pH 8.4 (100).

### Astaxanthin

Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is a xanthophyll carotenoid found in crustaceans shells, algae, yeasts, and bacteria. Its favorable effects on a metabolism have resulted in well-developed usage as a nutritional supplement (101). It is added to cosmetic products for photo-protective, antioxidant, and anti-inflammatory effects (102). Furthermore, it is widely supplemented in aquaculture to provide the pink coloration characteristic of salmonid fish and crustaceans (103). DESs have been used as media for the extraction of the astaxanthin from crustaceans, but also a range of other marine sources (Table 3).

The first study by Zhang et al. (104) used DESs and ultrasound-assisted extraction to optimize extraction of astaxanthin from shrimp shells and heads. By using a mixture of ChCl:1,2-butanediol in a 1:5 molecular ratio, the authors obtained yields higher than using ethanol-based extraction (142 μg/g vs 102 μg/g of dry shell). Further work by the same group Lee and Row (105) screened 19 different DESs as adjuvants for optimal extraction of astaxanthin from seaweeds Laminaria japonica and Undaria pinnatifida, and the brown crab Por
tunus trituberculatus. The two species of brown seaweeds were not used in further experiments with DESs because quantities were not high enough as compared to the crabs in preliminary trials (11.82 and 21.39 μg/g respectively). The best performing DES was methyl triphenyl phosphonium bromide and 1,2-butanediol (1:4) mixed at 130°C. The optimal conditions for the extraction of astaxanthin were 1 g solid sample, 40 mL acetone, and 0.25 mg/mL methyl triphenyl phosphonium bromide and 1,2-butanediol as an additive with 65 W ultra-sonication for 90 min. This resulted in yields of 73.49 μg/g astaxanthin from the crab by-products, 155% superior to yields obtained using traditional ILs in the same study.

More recently, terpene-based NADES were used to extract astaxanthin from brown crabs (Cancer pagurus), shrimp shells (Penaeus vannamei), mussels (Mytilus galloprovincialis), and microalgae (Hametococcus) (106). Of the 5 different NADES, a mixture of methanol:myristic acid in an 8:1 ratio was the most efficient solvent in extracting astaxanthin from crab shells, being able to match the yield obtained by Soxhlet extraction with acetone, after 2 h of extraction at 60°C. In the same study and with these optimized parameters, the solvent was able to extract three times as much astaxanthin from shrimp shells as compared to Soxhlet. However, the astaxanthin was not separated from the solvent after extraction, although each Astaxanthin-rich-NADES was largely non-cytotoxic against the cell lines studied. The antiproliferative and antimicrobial effects of the NADES and astaxanthin-rich NADES were evaluated and shown to inhibit colorectal cancer cells as well as Gram-positive and Gram-negative bacteria growth (106). As mentioned in the previous section, NADES were used to extract astaxanthin from shrimp shells for the following use as plasticizers in chitosan-based materials. After an initial screening of NADES optimized ultrasound-assisted extraction of astaxanthin was performed using ChCl:lactic acid, with the best results found at 30 minutes of treatment. The Astaxanthin-DES chitosan film showed improved properties, notably antioxidant activity, over chitosan films alone (98).
**DESs for processing fish and fish by-products**

Fish are an important food source for much of the world’s population, with nutritional profiles (amino acid and fatty acid content) different from terrestrial species. For the most part, fish is used for food and only subjected to basic mechanical/physical processing before sale: bleeding, gutting, beheading, filleting, skinning, trimming; and subsequent freezing or drying. The yield of such processing is species-specific and is often in the range of 30–50%, resulting in high amounts of by-products that may be valorized (2, 107). Potential high-value by-products attainable by further processing include collagen, chondroitin sulfate, and hydroxyapatite, which have all been successfully extracted with DESs (Table 4). DESs can also serve as solvents in the synthesis of novel biocomposites using these raw materials.

**Compounds extracted from fish by-products**

**Collagen**

Collagen is a protein polymer, one of the main components of animal connective tissue where it plays a structural role. It is made up of three polypeptide strands (alpha chains) organized into fibrils (108). For human use, it is a popular food additive, nutritional supplement (109), cosmetic ingredient (110), and has potential for use in wound healing (111) and biocomposites (112).

The use of DESs to extract collagen from fish biomass is still little explored, although the only published research reported achieving almost complete extraction of collagen peptides at 100% purity from cod skin. The authors used six kinds of ChCl-based DESs, with ChCl: oxalic acid (1:1) providing the best results. Powdered cod skins were added to 1:10 (w/v) to the DESs and then the mixture was stirred at 45°C. After heating, the extract was centrifuged and collagen was precipitated by the addition of ethanol, centrifuged, and finally dried. For higher molecular weight collagen, a shorter extraction time was necessary than for lower molecular weight collagen (113). The high purity and selectivity of the process warrant further exploration, perhaps with other matrices: i.e. cartilage or connective tissue.

**Chondroitin sulfate**

Chondroitin sulfate is a polysaccharide of the glycosaminoglycan family, made up of repeating disaccharide units of glucuronic acid and N-acetylgalactosamine linked by β-(1→3) glycosidic bonds and sulfated in different carbon positions. It is an essential component of an extracellular matrix of connective tissues, which plays a central role in diverse biological processes (114). It is commonly extracted from both terrestrial and marine sources, composition and concentration depending on organism and tissue. Uses of chondroitin sulfate are predominantly centered around medical/nutraceutical use as treatment for osteoarthritis (115) although new uses are emerging for tissue engineering (116). Classically, shark and ray fins have been the most commonly used to produce these molecules, although stock management is problematic for the long-term sustainability of these sources (117).

The NADES methanol:borneol, methanol:camphor, and trehalose:glycerol were tested for the extraction of chondroitin sulfate and hyaluronic acid from fish bones and eyeballs. Methanol:borneol was notably reported to have been used successfully to obtain 37.92 µg/g of dry codfish bones’ chondroitin sulfate disaccharides (118).

DESs have been used in polymerization to obtain materials with hierarchical and regular structures. ChCl: urea and ChCl:ethylene glycol DESs were used as scaffolds to create mesoporous chondroitin sulfate–silica composites. The material showed a good sorptive capacity of lead ions, much superior to average features found for others based on polysaccharides, sol-gels, or their composites (26, 119), with possible applications in analytical separation, remediation, purification, and extraction.

**Hydroxyapatite**

Hydroxyapatite \((\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2)\), a mineral form of calcium apatite, is a major component of fish scales and bones (120, 121). Hydroxyapatite is one of the most studied biomaterials in the medical field for both its proven biocompatibility and for being the main constituent of the mineral part of bone and teeth. As such, it is a popular choice for biocompatible materials for use in dentistry (122) and medical fields (123).

ChCl-based DESs with glycerol, citric acid, and acetic acid have been used to extract hydroxyapatite from the scales of the freshwater fish *Aristichthys nobilis*. After drying, grinding, and sieving, the scales were immersed in DES systems at raised temperature, centrifuged and the obtained hydroxyapatite was purified with a 5% NaOH solution to remove remaining proteins. Best extraction conditions used ChCl:glycerol at an extraction temperature of 70°C, a solid to liquid ratio of 1:15 (g/g), and a 2.5 h extraction time, resulting in a hydroxyapatite extraction yield of 47.67 ± 1.81%. Moreover, the extracted hydroxyapatite was deemed biocompatible, showing little hemolysis activity (124). Similarly, freshwater carp (*Carassius sp.*) scales have also been used to extract hydroxyapatite using DES. Of the five DESs tested, ChCl-1,4 butanediol was the best performing, with optimized conditions close to those cited in...
the previous study: a solid:liquid ratio of 1:15 (g/g), extraction temperature of 65°C, and 2 h extraction time, also using a NaOH washing step after extraction. Extraction yield reached 40.58% ± 0.14%, with heterogeneous morphology and good absorption capacities (125).

Other uses of DESs for processing fish-derived compounds

DNA extraction and stability

DNA from fish testes is an abundant co-product of fish aquaculture. The material is a promising nanotechnology biomaterial, combining self-assembly with programmability and a multitude of possibilities for chemical manipulation.

DEs have been shown to be suitable media for the extraction and storage of DNA from powdered salmon testes. Indeed, DNA is soluble and chemically and structurally stable in DESs consisting of mixtures containing glycerol and ethylene glycol. Furthermore, the storage medium is recyclable over three consecutive reuses (48). Authors were able to solubilize up to 5.5% (w/w) DNA in a ChCl:ethylene glycol system, which was found to be stable for six months at room temperature. Follow-up research showed that ChCl:ethylene glycol could be a suitable system for the functionalization of DNAFe3O4 nanoparticles and protonated layered dittamate sheets (H2·Ti2O5·H2O) to yield a hybrid material with magnetic and antibacterial properties (126).

DNA (Salmon testes) was solubilized in two bio-ILs namely choline-pyruvate and choline-glycolate up to 2.0 and 8.0 wt%, respectively. The solubilized DNA was found to maintain its chemical and structural stability for up to one year when stored at room temperature (25°C). Excessive H-bonding along with electrostatic interactions between the DNA and the bio-ILs, as observed in the isothermal titration calorimetry studies, was found to be the major reason behind the high concentration of DNA dissolution in choline-glycolate and its long-term stability.

Table 4. DES used to extract products from fish by-products.

| HBA       | HBD             | Ratio | Species/raw material/ target | Protocol | Results | Source |
|-----------|-----------------|-------|-----------------------------|----------|---------|--------|
| ChCl      | Urea            | 1:1   | Gadus morhua/skin/ collagen | High molecular weight collagen peptides | DES: Cod skins (80:1, mL/g) at 65°C for 2 h | 61.90% w/w collagen in cod skin ChCl:oxalic acid > ChCl:HAC > ChCl:lactic acid > ChCl:glycerol | (113) |
|           | Ethylene glycol |      |                             | Low molecular weight collagen peptides | DES: Cod skins (120:1, mL/g) at 65°C for 6 h | 100% purity hydrolyzed collagen peptides | |
|           | Glycerol        |      |                             | Note: HBD: hydrogen bond donor; HBA: hydrogen bond acceptor; ChCl: choline chloride; NC: not communicated. Best performing DES are in bold print. | | |
|           | Lactic acid     |      |                             | Note: | | |
|           | Acetic acid     |      |                             | Note: | | |
|           | Oxalic acid     |      |                             | Note: | | |
| Methanol  | Borneol         | NC    | Gadus morhua/bones/chondroitin sulfate | Drying, grinding, and sieving of scales | Extraction temperature of 70°C Solid:liquid ratio of 1:15 (g/g) 2.5 h extraction time | 37.92 µg/g dry codfish bones chondroitin sulfate disaccharide (ΔHexAβ1-3GalNAc4S) was extracted using Methanol:borneol | (118) |
| Trehalose | Camphor         |      |                             | Drying, grinding, and sieving of scales | | |
| ChCl      | Glycerol        | 1:2   | Aristichthys nobilii/scales/ hydroxyapatite | | | |
|           | Citric acid     | 2:1   |                             | Solid:liquid ratio of 1:15, 65°C; 2 h extraction | | |
|           | Acetic acid     | 1:2   |                             | | | |
| ChCl      | Glycerol        | 1:2   | Cassias sp. | Drying, grinding, and sieving of scales | | |
|           | Triethylene glycol |     |                             | Solid:liquid ratio 1:15, 65°C; 2 h extraction | | |
|           | Ethylene glycol | 1:2   |                             | | | |
|           | Glycol          | 1:4   |                             | | | |
|           | Butanediol      | 1:2   |                             | | | |
| ChCl      | Levulinic acid  | 1:2   | Salmo sp./Testes/DNA | Dry DNA powder added to DES solutions at constant stirring, at fixed ratios under a nitrogen atmosphere | Concentration of 5.5% (w/w) with ChCl: ethylene glycol after 2 h/2.5% (w/w) with ChCl:glycerol | (48) |
|           | Glycerol        |      |                             | | | |
|           | Ethylene glycol |      |                             | | | |
|           | Glycol          |      |                             | | | |
|           | Sorbitol        |      |                             | | | |
|           | Resorcinol      |      |                             | | | |

Notes: HBD: hydrogen bond donor; HBA: hydrogen bond acceptor; ChCl: choline chloride; NC: not communicated. Best performing DES are in bold print.

DEs and related solvents were tested primarily as plasticizers for algal polysaccharides, and for algal films as well as to a lesser extent as extraction media (Table 5). Seaweed polysaccharides are industrially refined, particularly the agars and carrageenans in red seaweeds (Rhodophyta), and alginites of brown seaweeds (Phaeophyceae) (127). Classic extraction steps include drying of biomass (if not processed fresh); acid or alkaline
chemical hydrolysis; filtering; solubilizing and final purification of the polymers. Processing often involves large amounts of water and requires concentration and separation of the polysaccharides through one of several techniques: evaporation, addition of ethanol or other organic solvents to the extraction media, and/or freezing and thawing (128). The spent polysaccharide extraction media is often sold as fertilizer (129, 130). However, seaweeds contain many other valuable compounds, and it is possible to extract several high-value compounds while producing seaweed hydrocolloids (131–133). DESs and bio-ILs are now emerging as alternative media for extraction, purification, and analysis of molecules produced by seaweeds (134).

**Proteins**

Although macroalgae contain relatively high amounts of proteins, extraction/purification of these molecules using DESs is little explored. Using an aquatic biphasic system with DESs and ammonium sulfate precipitation, Xu et al. (135) were able to purify R-phycoerythrin from *Porphyra yezoensis*. After an initial freeze-thaw crude protein extraction step, ChCl:urea with K$_2$HPO$_4$ was used to purify the resulting extract. The authors were thus able to purify up to 66.9% of the R-phycoerythrin from the initial extract, at drug-grade purity.

**Agar/agarose**

Agar is a commercially important product extracted from red seaweeds. It is a mixture of polysaccharides: linear agarose (about 70%, repeating unit – agarobiose) and agarpectin (D-galactose and L-galactose with acidic side-groups). With these types of polymers, DESs and bio-ILs are now emerging as alternative media for extraction, purification, and analysis of molecules produced by seaweeds.

Early work showed that agar and agarose are soluble in DES, Shamsuri and Daik (30) showed that ChCl:urea could be used as a plasticizer for casted agarose films. Concentrations of 30–70 wt% were tested, with 60 wt% ChCl:urea agarose displaying the highest tensile extension and tensile strain at break, albeit with relatively low elasticity. In 2014, Sousa et al. used ChCl:urea and ChCl:glycerol for the casting of agar films. ChCl:glycerol incorporation hindered film-forming, but ChCl:urea improved mechanical properties and elasticity as compared to aqueous agar films (136). Inclusion of urea only was also performed, and was unsuccessful, resulting in material inhomogeneity, indicating the ChCl was necessary (137).

DESs have also been used as compatibilizers for agar-plastic composites. Shamsuri et al. used ChCl:glycerol in high-density polyethylene–agar composites, with agar as the filler and HDPE as the matrix. ChCl:glycerol alone was insufficient to compatibilize the two polymers, the addition of a surfactant being necessary to homogenize the system. The resulting bio-composites displayed increased impact strength and tensile extension with inclusion of the DES surfactant (138). Agar–polyvinyl alcohol fibers were electrospun using ChCl:urea as an alternative to water. Agar and polyvinyl alcohol were dissolved in the DESs (20–30 min) in different ratios at 120–130°C before electrospinning. Each composition of agar/PVA-in-DES and combination of electrospinning conditions yielded a unique electrospun material, with smoothness and fiber size generally in the sub-micron range. The nanofibers with the best handling properties were obtained from the 50/50 and 30/70 agar/PVA solutions (63).

Bio-ILs were used to extract agarose from *Gracilaria dura* through precipitation (139). Dry *Gracilaria dura* was subjected to an initial alkali treatment, yielding alkali-treated seaweed – raw agar. This product was then subjected to a second alkali treatment. The extracts thus obtained were further treated with 2–10 wt% bio-ILs at 80°C, with agarose separated by centrifugation. Optimal results were obtained with a choline laurate usage level at 4.0% (w/w) with a yield of 14.0 ± 0.5% $W_{\text{agarose}}/W_{\text{dry } G. \text{ dura}}$. Moreover, the DESs could be recovered and purified through forward osmosis, then reused with only a slight loss of efficacy over three cycles. The extracted agarose had similar characteristics to commercial agarose, with a slightly higher sulfate content typical to *Gracilaria* species (140) although it was still suitable for gel electrophoresis, a common application of agarose. This precipitation method is less energy-intensive than typical freeze–thaw purification, and the powder obtained does not require milling (141).

**Carrageenan**

Carrageenan is a generic name for a family of high-molecular-weight polysaccharides that are also extracted from red seaweeds. The main repeating units of carrageenans are galactose units and 3,6-anhydrogalactose, both sulfated and unsulfated. The different types are conventionally identified by a Greek prefix (142), the most commercially important carrageenans being iota ($\iota$), kappa ($\kappa$), and lambda ($\lambda$)-carrageenans. So far, only $\kappa$-carrageenan has been the object of successfully published extraction with DESs, traditional extraction involving heating an alkaline solution. Complexation of ChCl with urea, ethylene glycol, and glycerol along with their hydrated counterparts (with 10% water) was used for the selective extraction of $\kappa$-carrageenan from
### Table 5. DESs used in the processing of algal polymers.

| HBA       | HBD      | Species/raw material                  | Protocol                                                                                     | Results                                                                                           | Source |
|-----------|----------|---------------------------------------|-----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|--------|
| ChCl      | Urea     | Agarose film                          | ChCl:urea ionic liquid mixed slowly with distilled water until dissolution.                  | Higher tensile extension and tensile strain at break compared to the pristine agarose film.       | (30)   |
|           |          |                                       | Addition of agarose and heating to 95°C until a transparent solution was observed.          | Reduced the glass transition temperature ($T_g$) of agarose films.                                   |        |
|           |          |                                       | Gelation at 25°C The gel was rigorously dried overnight at 70°C to obtain a freestanding film containing 30–70 wt% of ChCl:U. | Higher transparency.                                                                               |        |
|           |          |                                       |                                                                                              | Improved mechanical properties and elasticity.                                                    | (136)  |
| ChCl      | Urea     | Agar films                            | Solubilization of agar at 2–6 wt% in DES Compression molding                               | Most efficient compatibilization of the HDPE/agar system was found for the sample containing 0.2 wt% surfactant and 11 wt% DES. | (138)  |
| ChCl      | Glycerol | Agar-high density polyester biocomposite | Mixing a temperature of 150°C with the addition of HDPE followed by agar and DES + surfactant for a total of 12 min. Obtained biocomposites were converted into 1-mm sheets using a compression molding machine. At 150°C Final biocomposites were conditioned at 80°C | Higher tensile extension and tensile strain at break compared to the pristine agarose film. Higher transparency Reduced the glass transition temperature ($T_g$) of agarose films Improved mechanical properties and elasticity |        |
| ChCl      | Urea     | Agar–poly(vinyl alcohol)               | Agar and PVA blends (100/0, 50/50, 30/70, 20/80, 0/100 agar/PVA) were mixed with the DES and heated between 120 and 130°C under vigorous stirring for 20 min after which, the temperature was increased to 130°C until a homogeneous solution was obtained (20 min) | Best microfibers were obtained from the 50/50 and 30/70 agar/PVA solutions, consistent and mechanically resistant when manipulated by hand. | (63)   |
| ChCl      | Glycerol | Kappaphycus alvarezii/κ-carrageenan    | 500 mg powdered dry seaweed added to 10 g of DES, with 10% water, heated at 85/95°C for 1 h. Centrifugation, washing with isopropyl alcohol, and drying under vacuum. | 14.0 ± 0.5% w/w yield Reuse of bio-IL medium 3 times with a slight loss of efficacy Extracted agarose comparable to a commercial version Reduced sulfate content as compared to non-ionic surfactant. | (139)  |
| ChCl      | Urea     | Saccharina japonica/de-oiled S. japonica/alginate and fucoidan | 150°C, 19.85 bar, and 70% water content, and an U/S ratio of 36.81 mL/g Alginate precipitated by the addition of CaCl$_2$ Fucoidan precipitated through the addition of ethanol and subjected to two washing steps | High yield of alginate (28.12%) and fucoidan (14.93%) | (148)  |
| ChCl      | 1,2-Propanediol | Sargassum hornerii/polysaccharides | Solid–liquid ratio of 1:30 (g/mL), water content of 30% (v/v), and extraction temperature of 70°C | 116.33 mg/g Fucus vesiculosus polysaccharides Optimal conditions: DES water content of 32%, extraction time of 35 min, extraction temperature of 168°C, and a solid–liquid ratio of 39 mg/mL Antiradical and antiproliferative effects | (152)  |
|           | Glycerol |                                       |                                                                                              | Significant inhibition effects on DPPH and ABTS radicals.                                           | (149)  |

Notes: DES: deep eutectic solvent; HBD: hydrogen bond donor; HBA: hydrogen bond acceptor; ChCl: choline chloride; NC: not communicated.
Kappaphycus alvarezi. The dry seaweed was powdered and 500 mg was added to 10 g of DES, with and without 10% water, and heated at 85/95°C for 1 h. The κ-carrageenan was separated from the system through centrifugation and subsequently washed with isopropyl alcohol and dried under vacuum. Yields from 30.93 ± 0.90% to 60.25 ± 1.10% were on par or better than conventional alkali extraction for ChCl:ethylene glycol and ChCl:glycerol, with the inclusion of 10% water increasing efficiency. However, the purification process was incomplete, as traces of the DESs were found in the samples. Furthermore, gels obtained with DESs were slightly stiffer than κ-carrageenan from the alkali method, although more viscoelastic (resistant to breakage) (47). Overall, the process requires less steps, is shorter, and does not involve the high temperatures and pressure of the conventional method used by the authors.

Brown algae polysaccharides

Saccharina japonica is a species of brown seaweed extensively cultivated in East Asia for food and alginate, a widely used phycocolloid (143), parenthetically as a source of fucoidan, a type of bioactive (144) sulfated polysaccharide used in nutraceuticals (145) and cosmetics (146). The two polysaccharides from previously de-oiled S. japonica samples (147) were extracted using DESs combined with subcritical water extraction (148). Using a Box–Behnken response surface model optimization, optimal conditions were determined to be 150°C, 19.85 bar, and 70% water content, and a liquid/solid ratio of 36.81 mL/g to obtain a high yield of alginate (28.12% dw) and fucoidan (14.93% dw). Following exposure to the heightened temperature and pressure, crude alginate was separated by adding a CaCl2 solution and centrifugation. Crude fucoidan was obtained by precipitation with ethanol in the resulting supernatant, centrifuging, and two steps of washing – ethanol then acetone. Overall, the process was similar to conventional techniques, the exception being the replacement of sodium carbonate with DES, increased yield, and modification of rheological properties which need to balance against the increased cost of the raw materials.

ChCl:1,2-propanediol was used to extract polysaccharides from another species of brown seaweed, Sargassum horneri. Through single-factor experiments, optimal extraction conditions were a molar ratio of 1:2 with 30% water content (v/v), algae–liquid ratio of 1:30 (g/mL), and 70°C extraction temperature. As compared to traditional hot-water extraction, the extracted polysaccharide contained less DES. The extracted polysaccharide had significant antioxidant activity as evaluated by DPPH and ABTS radical assays (149). This shows potential use of the seaweed for example in cosmetic products and can encourage use of the sometimes invasive species (150).

Microwave-assisted extraction was evaluated for DESs extraction of polysaccharides from Fucus vesiculosus, a species well known for antioxidant activities (151). Nine different DESs were screened, with the best results obtained using ChCl:1,4-butanediol at a 1:5 molar ratio. The authors optimized extraction conditions with Box–Behnken design and response surface methodology to obtain maximum yields of 116.33 mg/g dw, with DES water content of 32%, 35 min extraction time, 168°C extraction temperature, and a solid–liquid ratio of 39 mg/mL. The extracted polysaccharides showed anti-DPPH and ABTS radical-scavenging and antiproliferative activities (152).

Seaweed-derived materials: other uses of DES

DESs were utilized because they can simultaneously act as solvents, soft templates, and catalysts for the growth of graphene nanosheets. ChCl:FeCl3 was employed as a template as well as a catalyst for the production of graphene nanosheets from Sargassum tenerimum. Seaweed granules and DESs were pyrolyzed at 700–900°C under a 95% N2 and 5% H2 atmosphere and gave Fe3O4/Fe-GN with a high surface area (220 m2 g−1) and high electric conductivity (2384.6 mS m−1) (153). The functionalized graphene nanosheets were used to reduce concentration of fluoride to 0.36–1.69 mg L−1 (75–87% removal efficiency) from contaminated groundwater (154).

Similarly, Yadav et al. used a ‘water-in-deep eutectic solvents’ system as a platform for low-temperature conversion of alginic acid to a multifunctional alginate–silica composites for the sorption of metal cations. The material showed a good sorptive capacity of Pb(II) ions, much superior to average features found for others based on polysaccharides, sol-gels, or their composites (26, 119).
Conclusions and perspectives

DESs are still limited in use in the processing of marine biomass. Until now, efforts have principally focused on one type of marine by-product: crustacean shells, for two major polymers: chitin and chitosan. However, initial steps in the processing of major categories of marine resource side-streams have been undertaken: fish, crustaceans, and seaweeds have all been successfully processed using these systems, and proof of concept solidly established. As evidenced here, DESs also afford new possibilities for the extraction of diverse polysaccharides of algal origin, carotenoids, and proteins. They have an advantageous influence on downstream processing (e.g. facilitating chitin deacetylation or chitosan modification). As well as provide niche applications such as media for DNA storage, which may gain in importance with the rapid development of biotechnologies.

Although the advantages of using such systems have been demonstrated: faster processing, lower waste generation, no requirement of volatile solvents and associated risks, and increased overall safety; looking forward, physical characteristics and ultimate fate of DESs are still unanswered questions. Overall, DESs maintain a green image from their inherent renewability and recyclability, even if ecotoxicological data is somewhat lacking. Exposure toxicity is also an important avenue to be explored, if the compounds are to be used on a broader scale. Processing hurdles for the purification of some compounds, such as astaxanthin at the time of writing, still hinder broader use. Means of scaling up beyond the laboratory have been modeled but commercial processing must be undertaken to prove the value of these systems in real-world conditions. Biologically inspired NADES systems deserve further study and will facilitate both the acceptance and adoption of the principles behind eutectic systems. Given the many variables to improve and streamline, DESs are a promising avenue for research and industry in the years to come.

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