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Can deep eutectic systems and algae deliver sustainable bioactives and nutrients? A systematic review

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The growing demand for sustainable extraction approaches has positioned deep eutectic systems (DESs) as promising, and often greener, alternatives to conventional solvents for valorizing algal and cyanobacterial biomass. This systematic review, supported by quantitative data integration and multivariate statistical analysis, analyzes peer-reviewed studies on the recovery of proteins, carbohydrates, lipids, fatty acids, phytosterols, polyphenols, and pigments from microalgae, macroalgae, and cyanobacteria, and highlights the main challenges in applying DESs to biomass processing. To ensure comparability, extraction conditions, DES composition, biomass origin, and assisted extraction techniques were systematically examined, with results normalized across studies. Hydrophilic DESs, typically based on choline chloride, sugars, or glycerol, generally show high efficiency for proteins and phycobiliproteins, whereas hydrophobic systems derived from fatty acids or terpenes favor the extraction of lipids and lipophilic pigments. However, water content, viscosity, and biomass-solvent interactions can significantly modulate these trends, and deviations are reported. Ultrasound-assisted extraction is among the most frequently employed techniques to enhance DES extraction. Principal component analysis revealed clear clustering of algal species and DES formulations according to compound class, confirming polarity-driven selectivity for specific macronutrients, pigments and phenolics. Beyond selective extraction, DESs and natural DESs (NADESs) support biomass pretreatment and stabilization, and can mitigate off-flavors and odors, thus reducing both energy and solvent consumption while aligning with circular-economy principles. Although further research is required to address scalability and standardization, DES-based algal processing holds strong potential as a practical and sustainable route to producing functional ingredients.

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Green foundation

1. Deep eutectic systems (DESs) are increasingly recognized as sustainable solvents capable of valorizing algal and cyanobacterial biomass, resources with strong potential as renewable and alternative food sources. This review presents a systematic meta-analysis of peer-reviewed studies, critically evaluating advances in DES-based extraction of bioactives and assessing their efficiency, environmental benefits, and limitations.
2. The integration of biodegradable, low-toxicity DESs with renewable algal feedstocks directly supports the transition toward greener bioprocessing, circular bioeconomy, and sustainable ingredient production. As both DESs and microalgae represent core elements of green innovation, their combination has wide relevance across food, pharmaceutical, and biorefinery sectors.
3. While DESs hold strong potential as green solvents, further progress is needed to optimize their composition and properties, reduce toxicity, and improve scalability. This review outlines key research frontiers in solvent design and process integration, providing a roadmap for advancing DES-algae systems toward practical, energy-efficient, and industry-relevant green technologies.

Introduction

Microalgae, macroalgae, and cyanobacteria: key characteristics and challenges in biomass valorization

Algae are broadly grouped into two categories: macroalgae, the macroscopic multicellular seaweeds, and microalgae, which are microscopic predominantly unicellular organisms. In parallel, cyanobacteria are frequently discussed in the same context. Although prokaryotic, they share many functional

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traits with microalgae, including oxygenic photosynthesis and the ability to generate biomass rich in bioactive compounds.

Microalgae and cyanobacteria play a major role in modern biotechnology. Estimates suggest they produce nearly half of the atmospheric oxygen, while their photoautotrophic growth fixes carbon dioxide and thus contributes to lowering the global carbon footprint.[1] Unlike higher plants, they lack roots, stems, and leaves, and can be cultivated on nonarable land and in wastewater, avoiding competition with food crops.[2,3] In addition, thanks to their high plasticity, they grow in fresh and saline waters, across polar to tropical climates, and over wide pH ranges. Their growth rates are typically five to ten times higher than those of conventional crops, making them highly attractive as a biomass resource.[1] Furthermore, cultivation is carried out in open systems such as ponds and raceways as well as in closed systems such as stirred tanks and photobioreactors.

The biomass of microalgae and cyanobacteria is rich in proteins, pigments, and lipids including omega-3 fatty acids. Consequently, they are increasingly incorporated into foods and nutritional products, and their bioactive metabolites are being explored for cosmetic and pharmaceutical applications due to reported antioxidant, anti-inflammatory, antimicrobial, and anticancer activities.[4] In agriculture, they are gaining traction as biostimulants and biofertilizers, helping increase yields while reducing pollution.[5] Much current research focuses on optimizing cultivation to improve productivity and the economics of industrial scale production.[6]

Macroalgae or seaweeds comprise multicellular red, brown, and green lineages that inhabit coastal and benthic marine environments.[3] They are recognized as valuable industrial feedstocks owing to their polysaccharide content, most notably alginate, agar, and carrageenan, which see widespread use in the food, pharmaceutical, and cosmetic sectors. In addition to polysaccharides, macroalgae are significant sources of iodine, vitamins, and antioxidants. Supply is derived both from wild harvesting and from aquaculture.[7]

The structural features, biochemical composition, and major application pathways of microalgae, macroalgae, and cyanobacteria have been comprehensively detailed in several recent reviews, which describe cellular organization and the distribution of key metabolites relevant to their valorization.[8–12] These studies outline how algae accumulate lipids, proteins, carbohydrates, polysaccharides, and pigments of industrial interest, underpinning their use in food and feed ingredients, nutraceuticals, cosmetics, biofertilizers and biostimulants, environmental remediation, and bioenergy production. Building on this established background, the present work focuses specifically on deep eutectic systems as extraction media for these compounds.

Despite taxonomic differences, all three groups possess substantial ecological and biotechnological potential. Their diversity, cultivation flexibility, and rich chemical profiles position them as key resources for sustainable technologies, especially in the context of climate change, food security, and the transition to renewables. Yet significant hurdles still limit broader industrial uptake. Cultivation remains a major bottleneck, as variable productivity, high costs, and inconsistent yields continue to impede commercialization. Downstream processing poses additional challenges. Conventional extraction methods rely on organic, often toxic solvents, typically requiring multistep, energy intensive operations with long processing times and high costs. These methods are frequently insufficiently selective for target fractions, can generate hazardous waste, and risk residual solvent contamination of final products, which compromises safety and market acceptance. Environmental and occupational health impacts add further concern, and overall biomass utilization often remains suboptimal.[13]

These constraints underscore the need, beyond cultivation optimization, for new, sustainable processing strategies. Solvents and extraction techniques aligned with green chemistry principles are required to enable efficient, selective, and safe isolation of bioactive molecules without introducing additional environmental or health risks. In this context, green solvents, particularly Deep Eutectic Systems (DESs), have attracted growing attention in recent years. This emerging class of solvents is increasingly viewed as a promising alternative to conventional approaches, with strong potential to support sustainable biomass valorization.

Deep eutectic systems (DESs)

DESs and their natural variants, known as natural deep eutectic systems (NADES), have emerged as promising greener alternatives to conventional organic solvents and ionic liquids for the extraction of bioactive compounds from various natural matrices.[14] DESs are formed by mixing hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs), resulting in eutectic mixtures with melting points significantly lower than those of the individual components. This melting-point depression results from strong hydrogen bonding and partial charge delocalization within the HBA–HBD complex, which disrupt the ability of the components to form an ordered crystalline lattice and thereby reduce the energy required for melting.[15] When the constituents of a DES are primary metabolites, such as amino acids, organic acids, and sugars, the resulting mixture is classified as a NADES.

These solvents are attractive because of their low volatility, nonflammability, potential biodegradability, and straightforward preparation, often achieved by simply mixing the components under mild heating without additional solvents.[16] Compared with many ionic liquids, which can be costly and synthetically demanding and may raise structure dependent toxicity concerns, DESs based on natural or food grade components are often considered biocompatible and suitable for food, pharmaceutical, and cosmetic applications. The compositional flexibility of DESs enables fine tuning of key physicochemical properties, including viscosity, polarity, and acidity, to match the requirements of specific extraction processes.[17]

DESs are also well aligned with several United Nations Sustainable Development Goals (SDGs), notably SDG 7 Affordable and Clean Energy, SDG 12 Responsible

Consumption and Production, and SDG 13 Climate Action, due to their role in enabling low-impact, energy-efficient processes and facilitating circular valorization of waste and biomass.[17,18]

One of the key advantages of DESs in extraction process is their selectivity. Tailored combinations of donors and acceptors allow specific hydrogen bonding and other intermolecular interactions with target solutes, so polarity and solvation can be tuned to favor particular classes of bioactives, such as lipids,[19] fatty acids,[19–25] proteins,[20–26] polyphenols,[21–27] and alkaloids.[22–28] Tuning can also reduce co-extraction of unwanted substances. For example, DES extracts of *Fucus vesiculosus* contained significantly lower levels of trace elements compared to conventional solvents, resulting in safer extracts.[14]

Beyond extraction, DESs have also been explored as pretreatment agents for rigid biomass, where they can disrupt cell structures and improve the recovery of intracellular compounds.[29–31] In addition to these roles, these mixtures have also shown significant potential in stabilizing media for a variety of molecules, including volatile compounds,[23] enzymes,[24] and phenolic compounds.[25] For example, Vladic *et al.* showed that a dill extract stabilized in a betaine-lactic acid NADES maintained its chemical profile and preserved bioactivity during storage.[26] This stabilizing effect is commonly attributed to the extensive hydrogen bonding network within DESs, which can protect labile compounds from degradation and enhance their chemical stability.[27]

DESs for algal bioactives: review and outlook

Given the importance and potential of micro- and macroalgae and cyanobacteria, together with the key attributes of DESs, a clear research space has emerged: selective, green extraction and stabilization of bioactive components from algae and cyanobacteria. Specifically, DES can be tailored by choosing the HBA/HBD constituents, their ratios, and the water content to solubilize target classes such as lipids, pigments, polysaccharides, and proteins. As a result, the use of DES to obtain products from algae and cyanobacteria has attracted noticeable scientific attention in recent years. Most studies to date address microalgae and cyanobacteria, while fewer use macroalgae as feedstock, as demonstrated in the studies summarized in Tables 1–3. The evidence base is also fragmented. Researchers employ varied DES types and formulations, different processing techniques such as stirred batch, ultrasound, microwave, and pressurized extraction, diverse material pretreatments, and broad operating windows for temperature, solid to liquid ratio, water content, and time (Tables 1–3). This heterogeneity limits comparability across studies and slows technology transfer.

This systematic and data-integrated review analyzes the field by linking DES properties with target fractions and process performance, mapping patterns and knowledge gaps, and defining key challenges for wider application of DESs in biomass valorization. We also discuss whether DES can enable efficient valorization of algal biomass, improve acceptance of algal products, and move extraction from laboratory demonstrations to economically and environmentally sustainable technologies. The scope covers DESs applied to proteins, lipids and fatty acids, phytosterols, polyphenols, polysaccharides, and lipophilic and hydrophilic pigments.

Literature selection and visualization methods

Research methodology is visualized in Fig. 1. Four databases were searched: PubMed, Google Scholar, Scopus, and Web of Science, using combinations of the following keywords: "*deep eutectic solvents*", "*natural deep eutectic solvents*", "*algae*", "*microalgae*", "*macroalgae*", "*cyanobacteria*", and "*extraction*". Boolean operators such as AND and OR were used to refine the queries (*e.g.*, "*deep eutectic solvents*" AND "*microalgae*" OR "*cyanobacteria*"), and searches included both "solvents" and/or "systems". For clarity and consistency, all eutectic formulations discussed in this review are referred to collectively as DESs. Although some publications use the term NADES, most studies do not provide sufficient information on component origin to apply this distinction reliably. Therefore, the broader term DES is used throughout the manuscript.

Only peer-reviewed articles published in English were included, with no restriction on publication year, as this is a relatively emerging research area. The initial search yielded 48 articles, of which 29 met the inclusion criteria. Excluded studies were either duplicates, non-peer-reviewed, or review articles.

From the selected publications, the following data were manually extracted: species of algae or cyanobacteria, country of cultivation, DES composition, molar ratio of DES components, control solvent (if any), extraction technique, and extraction conditions. Reported yields of extracted compounds were converted to milligrams per gram of dry biomass (mg per g DW) to enable cross-study visualization. However, this harmonization introduces uncertainty because the reviewed studies differed in the initial state of the biomass (fresh, wet paste, lyophilized powder), drying procedures, moisture content, and pretreatment intensity (*e.g.*, bead-beating, sonication, milling). As many articles do not report residual moisture or provide a clearly defined DW basis, these variations can influence calculated yields. Consequently, the quantitative comparisons in this review should be interpreted as trend-based and exploratory rather than as absolute, directly comparable extraction efficiencies.

When authors reported extraction results directly on a dry-weight basis (mg per g DW), these values were used as published. When yields were reported on a wet-weight basis and the moisture content or dry-matter fraction was available, values were converted to mg per g DW. In cases where the biomass was explicitly described as a dry microalgal powder (*e.g.*, lyophilized or spray-dried) but moisture was not reported, the biomass mass was treated as effectively equivalent to dry weight, consistent with the low residual moisture typical of such powders.[32] This approximation may introduce a minor error in absolute yields, but this is negligible compared with the much larger variability among species, solvents, and extraction conditions, and it does not affect the interpretation of relative trends.

Table 1 Maximum extracted concentrations of selected macronutrients and fatty acids from algae and cyanobacteria using DESs

Chemical class	Chemical compound	Max concentration (mg per g DW)	Species	Country of origin	DES systems applied	DES molar ratio	Biomass-to-solvent ratio	Extraction method	References
Proteins	Total protein content (TPro)	387.0	*Palmaria palmata*	Spain	Glycerol : Glucose, 25% water	2 : 1	1 : 10	PLE	Cokdinleyen *et al.*[44]
Total lipid and fatty acid content	Free fatty acids (FFA)	218.0	*Arthrospira platensis*	France	1,3-Propanediol : octanoic acid : decanoic acid	1 : 3 : 1	1 : 10	DAC	Wils *et al.*[51]
Total lipid and fatty acid content	Total lipid content (TLip)	183.0	Neochloris texensis	Turkey	choline chloride : urea : water	1 : 2 : 2	1 : 20	Ultrasonic bath	Ozel *et al.*[38]
Individual fatty acids	Arachidonic acid (C20:4n-6)	117.5	*Porphyridium cruentum*	France	1,3-Propanediol : octanoic acid : decanoic acid	1 : 3 : 1	1 : 10	DAC	Wils *et al.*[51]
Individual fatty acids	Decanoic acid (C10:0)	0.14	*Nannochloropsis gaditana*	Spain	Choline chloride : fructose : water	2 : 1 : 2	1 : 90	Magnetic stirring, water bath	Garcia Soto *et al.*[49]
Individual fatty acids	Dodecanoic acid (C12:0)	0.039	*Nannochloropsis gaditana*	Spain	Choline chloride : ethylene glycol : water	1 : 2 : 1	1 : 90	Magnetic stirring, water bath	Garcia Soto *et al.*[49]
Individual fatty acids	Eicosapentaenoic acid (EPA)	20.66	*Nannochloropsis gaditana*	Spain	Choline chloride : Ethylene glycol	1 : 2	1 : 90	Ultrasonic bath	Moreno Martínez *et al.*[19]
Individual fatty acids	Eicosatetraenoic acid (C20:4n-3)	0.85	*Nannochloropsis gaditana*	Spain	Choline chloride : ethylene glycol : water	1 : 2 : 1	1 : 90	Magnetic stirring, water bath	Garcia Soto *et al.*[49]
Individual fatty acids	Hexadecanoic acid (C16:0)	35.5	*Arthrospira platensis*	France	Nonanoic acid : decanoic acid : dodecanoic acid	3 : 2 : 1	1 : 20	UAE	Wils *et al.*[51]
Individual fatty acids	Hexadecenoic acid (C16:1)	25.89	*Nannochloropsis gaditana*	Spain	Choline chloride : Ethylene glycol	1 : 2	1 : 90	Ultrasonic bath	Moreno Martínez *et al.*[19]
Individual fatty acids	Myristic acid (C14:0)	32.46	*Arthrospira platensis*	France	Nonanoic acid : decanoic acid : dodecanoic acid	3 : 2 : 1	1 : 20	UAE	Wils *et al.*[51]
Individual fatty acids	Octadecadienoic acid (C18:2)	160.0	*Arthrospira platensis*	France	1,3-Propanediol : octanoic acid : decanoic acid	1 : 3 : 1	1 : 10	DAC	Wils *et al.*[51]
Individual fatty acids	Octadecanoic acid (C18:0)	32.44	*Arthrospira platensis*	France	Nonanoic acid : decanoic acid : dodecanoic acid	3 : 2 : 1	1 : 20	UAE	Wils *et al.*[51]
Individual fatty acids	Octadecatrienoic acid (C18:3)	35.0	*Arthrospira platensis*	France	1,3-Propanediol : octanoic acid : decanoic acid	1 : 3 : 1	1 : 10	DAC	Wils *et al.*[51]
Individual fatty acids	Octadecenoic acid (C18:1)	19.31	*Arthrospira platensis*	France	Nonanoic acid : decanoic acid : dodecanoic acid	3 : 2 : 1	1 : 20	UAE	Wils *et al.*[51]
Individual fatty acids	Octanoic acid (C8:0)	0.00552	*Nannochloropsis gaditana*	Spain	Choline chloride : fructose : water	2 : 1 : 2	1 : 90	Magnetic stirring, water bath	Garcia Soto *et al.*[49]
Individual fatty acids	Pentadecanoic acid (C15:0)	0.052	*Nannochloropsis gaditana*	Spain	Choline chloride : ethylene glycol : water	1 : 2 : 1	1 : 90	Magnetic stirring, water bath	Garcia Soto *et al.*[49]
Phytosterols	Phytosterols (PST)	3.16	*Codium tomentosum*	Portugal	Menthol : octanoic acid	1 : 1	1 : 10	Maceration	Resende *et al.*[52]
Carbohydrates	Total carbohydrate content (TCC)	58.34	*Porphyridium purpureum*		TMAC : glycerol	1 : 2	1 : 20	UAE	Li *et al.*[45]
Carbohydrates	Total sulphated polysaccharide content (TSPC)	131.0	*Palmaria palmata*		Glycerol : glucose, 50% water	2 : 1	1 : 10	PLE	Cokdinleyen *et al.*[44]

Table 2 Maximum extracted concentrations of pigments (carotenoids, chlorophylls, and phycobiliproteins) from algae and cyanobacteria using DESs

Chemical class	Chemical compound	Max concentration (mg per g DW)	Species	Country of origin	DES systems applied	DES molar ratio	Biomass-to-solvent ratio	Extraction method	References
Carotenoids and chlorophylls	Antheraxanthine	0.59	*Nannochloropsis oculata*	Greece	Betaine : 1,2 propanediol	2 : 5	1 : 10	UAE	Gkioni *et al.*[62]
Carotenoids and chlorophylls	Astaxanthin	20.71	*Haematococcus pluvialis*	Italy	Thymol : oleic acid	3 : 1	1 : 40	Water bath	Pitacco *et al.*[56]
Carotenoids and chlorophylls	Auroxanthine	0.18	*Nannochloropsis oculata*	Greece	Betaine : 1,2 propanediol	2 : 5	1 : 10	Grounding, UAE	Gkioni *et al.*[62]
Carotenoids and chlorophylls	Canthaxanthine	1.41	*Chromochloris zofingiensis*	China	Octanoic acid : decanoic acid	2.3 : 1	1 : 15	UAE	Yang *et al.*[57]
Carotenoids and chlorophylls	Total carotenoids	47.03	*Arthrospira platensis*	Portugal	Glucose : glycerol : water	1 : 2 : 4	1 : 70	Ultrasonic bath	Martins *et al.*[58]
Carotenoids and chlorophylls	Chlorophyll a	39.0	*Chlorella vulgaris*	Turkey	Choline chloride : acetic acid	1 : 2	1 : 20	Ultrasonic bath	Ozel *et al.*[38]
Carotenoids and chlorophylls	Chlorophyll b	23.4	*Chlorella vulgaris*	Turkey	Choline chloride : acetic acid	1 : 2	1 : 20	Ultrasonic bath	Ozel *et al.*[38]
Carotenoids and chlorophylls	Total chlorophylls	62.4	*Chlorella vulgaris*	Turkey	Choline chloride : acetic acid	1 : 2	1 : 20	Ultrasonic bath	Ozel *et al.*[38]
Carotenoids and chlorophylls	Fucoxanthin	22.03	*Tisochrysis lutea*	New Zeland	Thymol : dodecanoic acid	1.25 : 1	1 : 25	Magnetic stirring	Xu *et al.*[63]
Carotenoids and chlorophylls	Lutein	6.26	*Scenedesmus* sp.	China	Fenchyl alcohol : thymol	1 : 1	1 : 50	Water bath	Fan *et al.*[39]
Carotenoids and chlorophylls	Total pigments	165.19	*Arthrospira platensis*	Portugal	Glucose : glycerol : water	1 : 2 : 4	1 : 70	Ultrasonic bath	Martins *et al.*[58]
Carotenoids and chlorophylls	Violaxanthine	2.34	*Nannochloropsis oculata*	Greece	Betaine : 1,2 propanediol	2 : 5	1 : 10	UAE	Gkioni *et al.*[62]
Carotenoids and chlorophylls	β-Carotene	0.32	*Scenedesmus* sp.	China	Fenchyl alcohol : thymol	1 : 1	1 : 50	Water bath	Fan *et al.*[39]
Phycobiliproteins	Allophycocyanin	52.0	*Palmaria palmata*	Spain	Glycerol : glucose, 50% water	2 : 1	1 : 10	PLE	Cokdinleyen *et al.*[44]
Phycobiliproteins	B-Phycoerythrin	43.0	*Palmaria palmata*	Spain	Glycerol : glucose, 50% water	2 : 1	1 : 10	PLE	Cokdinleyen *et al.*[44]
Phycobiliproteins	C-Phycocyanin	90.85	*Arthrospira platensis*	France	Glycerol : glucose	2 : 1	1 : 5	Ultrasonic bath	Hilali *et al.*[59]
Phycobiliproteins	R-Phycocyanin	28.0	*Palmaria palmata*	Spain	Glycerol : glucose, 50% water	2 : 1	1 : 10	PLE	Cokdinleyen *et al.*[44]
Phycobiliproteins	R-Phycoerythrin	13.09	*Porphyra yezoensis*	China	Choline chloride : urea, K_2HPO_2	1 : 2	Not mentioned	DES-ATPS	Xu *et al.*[63]

Table 3 Maximum extracted concentrations of polyphenols and related compounds from algae and cyanobacteria using DESs

Chemical class	Chemical compound	Max concentration (mg per g DW)	Species/class	Country of origin	DES systems applied	DES molar ratio	Biomass-to-solvent ratio	Extraction method	References
Polyphenols and related compounds	Ascorbic acid	0.3	*Fucus vesiculosus*	Russia	Glucose : lactic acid : water	1 : 5 : 3	1 : 10	Ultrasonic bath	Obluchinskaya *et al.*[54]
Polyphenols and related compounds	Caffeic acid	0.2	*Chlorella vulgaris*	Portugal	Choline chloride : 1,3-propanediol, 30% water	1 : 4	1 : 20	Liquid nitrogen	Wan Mahmood *et al.*[21]
Polyphenols and related compounds	Ferulic acid	37.74	*Phaeophyceae*, *Rhodophyceae*, *Chlorophyceae*	USA	Choline chloride : urea	1 : 2	1 : 20	Ultrasonic bath	Hashemi *et al.*[66]
Polyphenols and related compounds	Gallic acid	3.2	*Chlorella vulgaris*	Portugal	Choline chloride : 1,3-propanediol, 30% water	1 : 4	1 : 20	Liquid nitrogen	Wan Mahmood *et al.*[21]
Polyphenols and related compounds	Luteolin-7-*O*-glucoside	0.9115	*Phaeophyceae*, *Rhodophyceae*, *Chlorophyceae*	USA	Choline chloride : lactic acid	1 : 2	1 : 20	Ultrasonic bath	Hashemi *et al.*[66]
Polyphenols and related compounds	Phlorotannins	71.6	*Fucus vesiculosus*	Russia	Choline chloride : lactic acid	1 : 3	1 : 10	Ultrasonic bath	Obluchinskaya *et al.*[54]
Polyphenols and related compounds	Total phenolic acids	119.67	*Phaeophyceae*, *Rhodophyceae*, *Chlorophyceae*	USA	Proline : lactic acid	1 : 1	1 : 20	Ultrasonic bath	Hashemi *et al.*[66]
Polyphenols and related compounds	Total phenolic compounds	127.09	*Phaeophyceae*, *Rhodophyceae*, *Chlorophyceae*	USA	Proline : lactic acid	1 : 1	1 : 20	Ultrasonic bath	Hashemi *et al.*[66]
Polyphenols and related compounds	*p*-Coumaric acid	0.19	*Chlorella vulgaris*	Portugal	Choline chloride : 1,4-butanediol, 30% water	1 : 4	1 : 20	Liquid nitrogen	Wan Mahmood *et al.*[21]

Fig. 1 Research methodology.

Data visualization *via* the Sankey diagram and pie charts, was performed to graphically represent the relationships between cultivation countries, algae and cyanobacteria species, and the chemical classes of extracted compounds. To explore interrelationships among extracted compounds and assess patterns in extraction performance across algal species and DES compositions, correlation analysis heat-mapping were applied to the compiled dataset. Correlation analysis was used to identify statistically significant associations (Spearman's $\rho > 0.5$, $p < 0.05$) between compound yields within and across chemical classes, while heat-mapping was applied to visualize dominant trends. The concentration values as mg per g DW were normalized (*z*-scored) to eliminate scale bias, and heat maps were generated separately for each major compound group (macronutrients, fatty acids and phytosterols, lipophilic pigments, hydrophilic pigments and phenolics) and both for: (i) corresponding micro-, macroalgal and cyanobacteria species; and (ii) DES/NADES formulations used in research articles. These visualizations supported the interpretation of the extracted literature data.

The literature on DES-based algal extraction is shown to be highly heterogeneous, with differences in analytical units, reporting formats, biomass pretreatments, solvent compositions, extraction times, and quantified outputs. As a result, effect size pooling, heterogeneity statistics, and publication bias assessment couldn't be meaningfully applied. For this reason, the study was framed as a systematic review with quantitative data integration. Instead of effect-size meta-analysis, we employed multivariate exploratory tools to identify patterns, solvent–compound relationships, and cross-study trends within this heterogeneous dataset.

Fig. 2 illustrates the chemical structures of the components most frequently used to formulate DESs in the studies reviewed. These compounds represent the diversity of molecules, from quaternary ammonium salts and amino acids to organic acids, polyols, sugars, and terpenoids, that enable eutectic formation.

Geographic, taxonomic, and target compound chemical profiling

To better understand the research focus and biomass selection in studies employing DES for algae and cyanobacteria extraction, the distribution of cultivation origins, target species, and

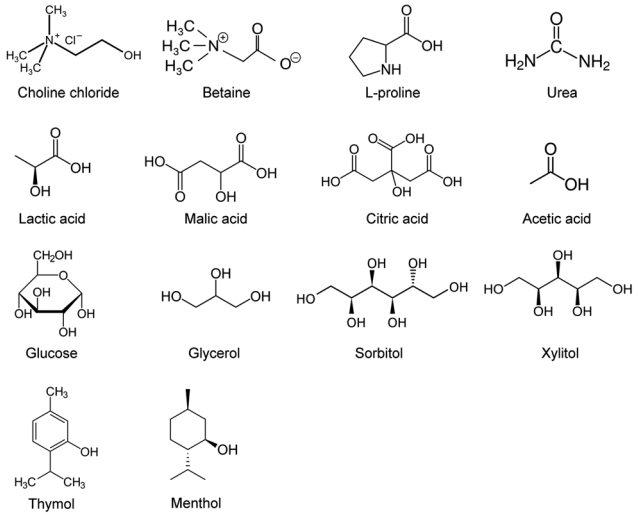

Fig. 2 Chemical structures of representative DES components commonly used in algal extraction.

extracted chemical compound classes were analyzed and shown in Fig. 3.

An analysis of the geographic origin of cultivated algae and cyanobacteria biomass revealed a clear dominance of European countries. As shown in Fig. 3a, 19 out of 29 studies (approximately 66%) sourced biomass from Europe. The most frequently represented countries were Portugal (7 studies) and France (6), followed by Spain and China (3 each). Several other countries were reported only once, including Russia, the USA, the Netherlands, Morocco, Greece, Japan, Turkey, Italy, and New Zealand. This geographic clustering suggests that DES-based extraction studies have primarily relied on biomass readily accessible within European research networks, likely supported by regional collaboration, funding schemes, and regulatory frameworks that actively promote sustainable biotechnologies.

In the global context, total algal production in 2022 reached 37.8 million tonnes, with Asia dominating by a wide margin.[28] Europe, by contrast, is a small producer, in 2019, EU aquaculture accounted for less than 1% of global production.[33] Nevertheless, algae represent an emerging and strategic sector within the European Blue Bioeconomy, generating an estimated turnover of over €350 million in 2018.[34] Although production volumes remain limited, Europe has positioned itself as a hub for pilot-scale cultivation and downstream innovation. The EU has recognized the potential of algae to contribute to food security, climate mitigation, and environmental protection, embedding this vision in key policies such as the Green Deal, the Farm to Fork Strategy, and the Sustainable Blue Economy Communication, with the 2022 EU Algae Initiative setting out concrete actions to support the

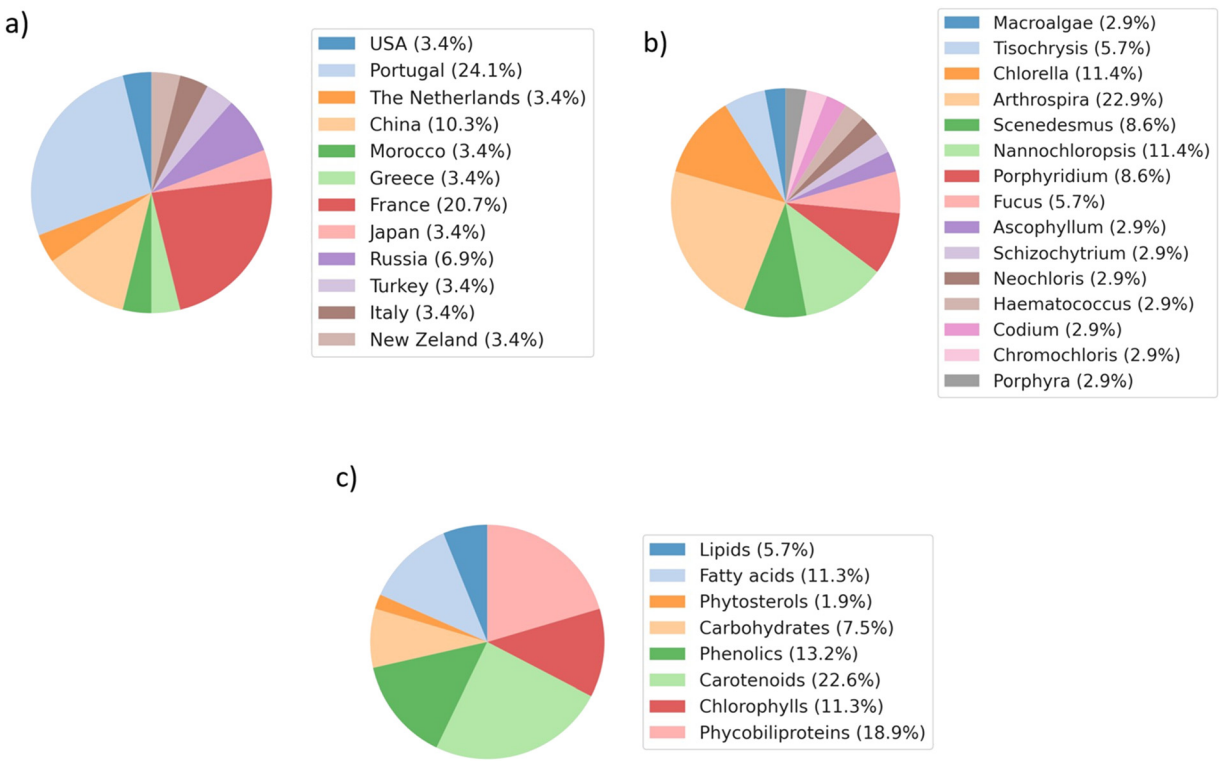

Fig. 3 Distribution of (a) cultivation countries, (b) algae and cyanobacteria species, and (c) extracted chemical compound classes in studies applying DES for biomass valorization. Each section of the pie charts represents the number of studies targeting the respective category, expressed as a percentage of the total.

sector.[35,36] According to Gallego *et al.*[37] 66 European producers currently market 146 different microalgal-derived products, while another 49 companies provide supporting services and technologies, such as production optimization and scale-up. The most commonly cultivated species is *Spirulina* (*Limnospira* spp.), followed by *Chlorella* spp. and *Nannochloropsis* spp., mainly for human consumption and cosmetic applications. Photobioreactors remain the dominant cultivation system in Europe. Therefore, the strong representation of European biomass in DES-related studies thus reflects not only local availability but also a broader strategic alignment between cultivation capacity and research on green extraction methods.

The species most commonly used in DES extraction research are also visualized in Fig. 3b. The cyanobacteria Spirulina (*Arthrospira platensis*) was the most frequently investigated, appearing in 8 out of 29 studies (28%), followed by *Chlorella vulgaris* (4 studies), *Nannochloropsis* (4) (*N. gaditana*, *N. oculata*, *N. oceanica*), *Scenedesmus* (3) (*S. incrassatulus* and *S. protuberans*), and *Porphyridium* (3) (*P. purpureum*, *P. cruentum*). Other species such as *Tisochrysis lutea*, *Haematococcus pluvialis*, *Chromochloris zofingiensis*, *Neochloris texensis*, *Palmaria palmata*, *Codium tomentosum*, *Porphyra yezoensis*, and macroalgae like *Fucus vesiculosus* and *Ascophyllum nodosum* were studied less frequently – 1 study each. This taxonomic diversity highlights the wide range of biochemical potentials under investigation for DES-based valorization, though a few species dominate current research trends, due to their robust cultivation profiles and market relevance.

The extracted chemical compounds were classified into eight main categories, as illustrated in Fig. 3c. Carotenoids were the most frequently targeted class (12 studies), where the authors determined: total carotenoid content, β-carotene, lutein, astaxanthin, fucoxanthin, violaxanthine, auroxanthine, antheraxanthine. Following were phycobiliproteins (10 studies), including pigments like C-phycocyanin, R-phycocyanin, allophycocyanin, R-phycoerythrin, and B-phycoerythrin, which are predominantly found in cyanobacteria and red algae. Phenolic compounds appeared in 7 studies, encompassing a wide array of target compound groups, such as total phenolic compounds, total phenolic acids, phlorotannins, as well as specific phenolic acids and other compounds, such as ferulic, gallic, caffeic, *p*-coumaric acids and luteolin-7-*O*-glucoside. Both the chlorophylls (including total chlorophylls, chlorophyll a, and chlorophyll b) and fatty acids were targeted each in 6 studies, with fatty acid profiling ranging from short-chain to long-chain polyunsaturated acids (C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:4, C20:5). Meanwhile, proteins and carbohydrates each accounted for 4 studies, typically evaluated as total protein content or total carbohydrates and sulfated polysaccharides in macroalgae. Total lipids and total phytosterols were targeted less frequently in 3 studies and 1 study, respectively. This distribution reflects the predominance of antioxidant pigments and high-value biomolecules in the current research, driven by their relevance for nutraceutical, cosmetic, and functional food applications.

To visualize the global distribution of algal and cyanobacterial biomass and its valorization through DES-based extraction, a Sankey diagram was created (Fig. 4). The diagram represents a flow from the cultivation country (left), through the species studied (middle), to the extracted chemical class (right). Again, several species such as *Arthrospira*, *Chlorella*, *Scenedesmus*, and *Nannochloropsis* were shown to be studied in multiple regions, indicating their global relevance and cultivation adaptability. By mapping the links between cultivation regions, algal species, and extracted compounds, the diagram highlights patterns that point both to the adaptability of certain strains and to the strong demand for specific bioactives. Such insights can guide the more targeted development of green extraction methods and biorefinery strategies.

Utilizing DESs as pretreatment agents

Before isolating target compounds from biomass, a critical first step is breaking the structural barriers that limit efficient recovery. This pretreatment step is especially demanding for matrices with rigid cell walls. Common approaches include mechanical size reduction and bead milling, ultrasound or microwave assistance, high pressure homogenization, thermal and hydrothermal treatments, acid or alkali digestion, organic solvents, and enzyme cocktails. Although effective, these methods can be energy intensive, slow, and costly, and they may be nonselective, generate problematic waste streams, leave solvent residues, or degrade heat and oxidation sensitive metabolites.

Beyond their role as extraction media, DESs have shown potential as pretreatment agents. By weakening cell walls and membranes, they increase access to intracellular compounds and can improve extraction yields and overall process efficiency. Their composition and water content can be tuned to balance effective disruption with preservation of labile metabolites. Asevedo *et al.*[29] reported that DES pretreatment improved the recovery of phenolic compounds and antioxidant activity from plant biomass, which they attributed to the ability of these solvents to soften rigid cellular architectures and facilitate release. Danilović *et al.*[30] evaluated the pretreatment of *Chlorella* sp. using choline chloride-based DESs to enhance lipid extraction and underscored the value of DESs as environmentally friendly and cost-efficient media for applications such as biodiesel production and animal feed. Their results showed that choline-chloride DESs form hydrogen bonds with cellulose microfibrils, disrupt the microalgal cell wall network and thus facilitate lipid release. In another study, Asevedo *et al.* examined choline chloride and urea (1 : 2) both as a pretreatment and as an extraction solvent for *Dunaliella salina*.[29] In a two-step process, DES was first used to disrupt the cell wall, which improved accessibility for a subsequent lipid extraction with an ethanol–hexane mixture and resulted in a lipid recovery of 74.99%. Choline chloride : urea enhanced solvent permeation through the cell wall, whereas more acidic systems such as choline chloride : oxalic acid altered permeability and degraded carotenoids. The one-pot method employed DES directly for the extraction of carotenoids from

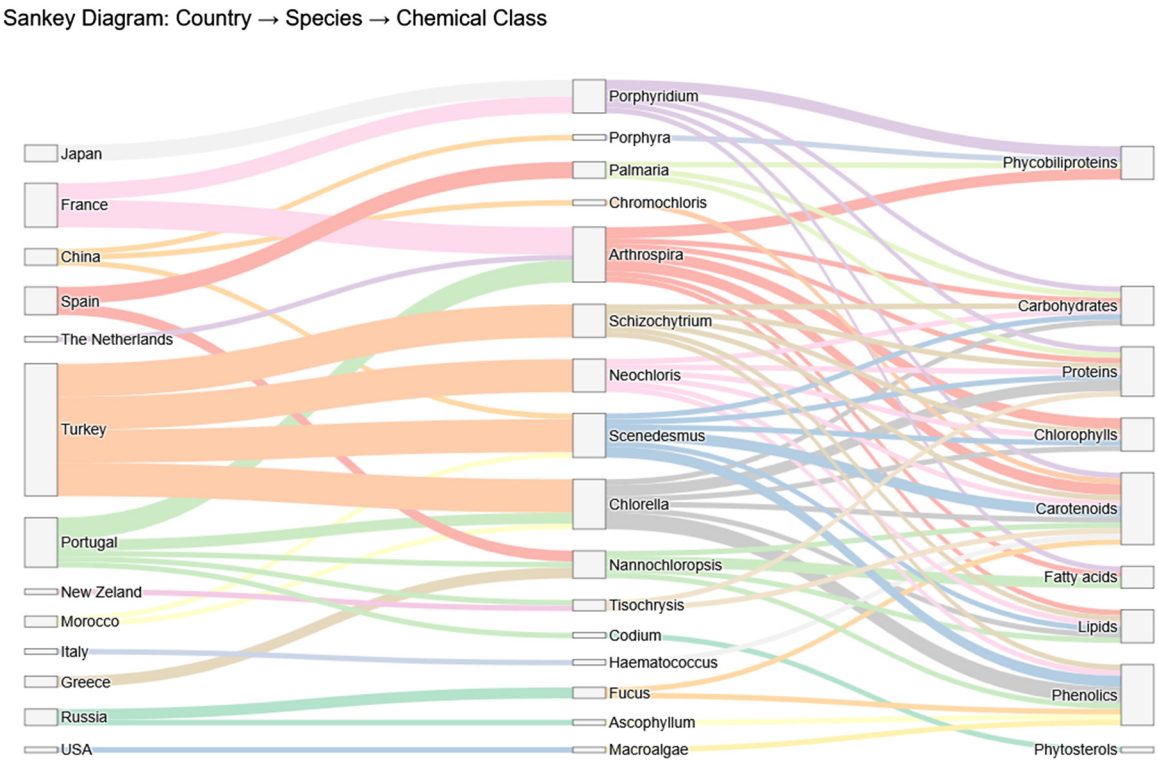

Fig. 4 Sankey diagram illustrates the relationship between the cultivation country, algae and cyanobacteria species, and the chemical class of compounds extracted using DES. The diagram highlights the diversity of biomass sources across different geographic regions and the distribution of target compound classes.

wet biomass, achieving a recovery of 84.06%. These results highlight the dual role of DESs in enhancing cell permeability and serving as efficient, green solvents for bioactive compound recovery. More recently, Nemani *et al.* applied DES-based aqueous two-phase systems (ATPS) to disrupt the tough cell wall of *H. pluvialis*, a freshwater green microalga known for its high astaxanthin content.[31] The use of choline chloride : urea (1 : 2) and choline chloride : glucose (2 : 1) within ATPS enabled effective cell wall disruption at 60 °C. This pretreatment facilitated nearly complete astaxanthin extraction (99.64%). The authors analysed DES-salt aqueous two-phase systems using binodal curves, tie-line lengths, partition coefficients and Gibbs free energy changes to show how DES composition and temperature control astaxanthin partitioning and cell disruption efficiency. Fan *et al.* demonstrated that a tailored DES outperformed conventional organic solvents in both the recovery and stabilization of lutein.[39] Using a combination of COnductor-like Screening MOdel for Real Solvents (COSMO-RS) screening, molecular dynamics (MD) simulations and symmetry-adapted perturbation theory (SAPT), the authors rationally designed a fenchyl alcohol : thymol NADES optimized for carotenoid extraction. Their computational analysis revealed that electrostatic and dispersion components of the hydrogen-bonding network, together with significant van der Waals contacts between the aromatic HBD (thymol) and the ε-ionone ring of lutein, are the dominant drivers of lutein solubilization and stabilization. COSMO-RS, which predicts solubility, activity coefficients and phase behavior from molecular surface charge density distributions, further showed that lutein is substantially more apolar than the individual DES components and disrupts the DES hydrogen-bonding network upon dissolution. This finding indicates that the extraction mechanism cannot be explained by the traditional "like-dissolves-like" principle alone. In addition to solute–solvent interactions, Fan *et al.* also noted that DES molecules can interact with and perturb cell membranes, thereby facilitating the release and solubilization of intracellular lutein.[39]

From a process perspective, DES pretreatment offers several practical advantages. Pretreatment and extraction can be carried out in the same solvent system, which reduces unit operations, lowers solvent and energy use, and decreases overall cost compared with running the steps separately. The properties of a DESs can be tuned to match the specific requirements and characteristics of the biomass by adjusting the HBA and HBD composition, water content, viscosity, polarity, and acidity. Also, the same medium is compatible with different processing techniques, such as ultrasound or microwave assistance, to shorten processing time. Importantly, the solvent environment that opens the cell wall can help preserve sensitive metabolites such as pigments, polyunsaturated lipids, and enzymes.

Extraction of bioactive and nutritive compounds

Macronutrients, such as proteins, lipids, and carbohydrates derived from algae are increasingly recognized for their nutri-

tional, functional, and industrial importance.[38] Microalgal proteins are rich in essential amino acids, making them valuable as sustainable alternatives to animal- or soy-based proteins in food, feed, and nutraceuticals. Lipids, especially those rich in polyunsaturated fatty acids (PUFAs) like omega-3, are vital for human health, supporting cardiovascular, neural, and anti-inflammatory functions. Carbohydrates from microalgae, including polysaccharides and oligosaccharides, contribute to bioenergy production and exhibit prebiotic, immunomodulatory, and antioxidant activities.[38] In addition to macromolecules, algae and cyanobacteria are also recognized for their diverse array of metabolites, including pigments, polyphenols, enzymes, fatty acids, phytosterols, vitamins, and other bioactive compounds. Among these, pigments have attracted particular attention due to their broad range of potential applications in the food and beverage industry, cosmetics and personal care, pharmaceuticals, nutraceuticals, and animal feed, which also translates into substantial commercial value. According to Market Research Future, the global algal pigments market was valued at USD 4.81 billion in 2024 and is projected to increase from USD 5.28 billion in 2025 to USD 12.2 billion by 2034, with an estimated compound annual growth rate (CAGR) of 9.80% over the period 2025–2034.[40] In parallel, interest in algal proteins as alternative protein sources that can contribute to food security is accelerating. The global algal proteins market was valued at USD 994.3 million in 2024 and is projected to reach USD 1.5 billion by 2030 (CAGR 7.1% for 2024–2030).[41] Owing to their balanced amino-acid profiles and rich micronutrient content compared with typical plant proteins, microalgae are increasingly framed as future superfoods.[42]

Given the methodological variability among studies, including differences in biomass pretreatment, moisture content, and analytical quantification, mg per g DW yield values of extracted bioactive and nutritive compounds provide a standardized yet imperfect basis for comparison. Thus, the multivariate analysis applied aims to identify qualitative patterns in solvent–compound selectivity rather than deliver precise quantitative benchmarking across studies.

Extraction of proteins and bioactive enzymes. Different DES compositions, water content, extraction methods, and biomass types play an important role in the efficiency of protein recovery. Craveiro *et al.*[43] investigated the effect of biomass concentration and DES formulation on protein extraction from *T. lutea*, *C. vulgaris*, and *Spirulina* sp. They tested three DES mixtures: polyethylene glycol : urea (2 : 1), choline chloride : polyethylene glycol (1 : 2), and choline chloride : urea (1 : 2). The study demonstrated that protein extraction efficiency varies significantly across algal species and DES formulations, emphasizing the need for species-specific solvent systems and process optimization. For *T. lutea*, the highest protein yield was achieved with polyethylene glycol : urea, reaching 81% of total protein in the biomass. However, the same solvent had lower performance in *C. vulgaris* (around 11%), likely due to its rigid cell wall, even after bead-beating pretreatment. *Spirulina* sp., a cyanobacterium with more accessible proteins, showed a yield of 220–240 mg per g DW, with significant improvement when biomass concentration was increased.

Data in Table 1 shows that the maximum protein concentration reported using DES-based extraction was 387 mg per g DW from *P. palmata* using a glycerol : glucose (2 : 1 molar ratio, with 25% water) and pressurized liquid extraction (PLE) method.[44] The high yield likely reflects the solvent's strong hydrogen-bonding capacity, a favorable viscosity-polarity balance that promotes penetration into the red algal cell wall, and the pressure-assisted disruption achieved during PLE.

Li *et al.*, for example, found that L-proline : glycerol (2 : 5) was most effective for extracting total proteins from *P. purpureum*, yielding 44.53 mg per g DW under mild ultrasound-assisted conditions.[45] Similarly, Ozel *et al.* used ultrasound-assisted extraction at 50 °C to extract proteins from four microalgal species.[38] Their results showed that the acidic DES choline chloride : acetic acid was more effective for *N. texensis* and *S. protuberans* (up to 29 mg per g DW), while choline chloride : urea worked better for *C. vulgaris* and *Schizochytrium* sp.

Taken together, these studies highlight that urea- and sugar-based DESs, particularly when combined with techniques such as ultrasound or PLE, can provide high protein recovery. Nonetheless, efficiency strongly depends on algal species, solvent composition, water content, and the extraction method applied.

While protein yield is an important parameter, it does not always fully reflect the quality of the extract. In biotechnological and industrial contexts, the functionality of proteins, especially enzymes, is just as critical as the total amount recovered. Enzymes represent a biologically active fraction of proteins, and their preservation during extraction determines whether the process merely isolates bulk protein or provides extracts with real catalytic and application potential. Among them, carbonic anhydrase (CA) is especially important because it helps convert carbon dioxide into bicarbonate and protons. In microalgae, this enzyme supports photosynthetic efficiency, pH regulation, and inorganic carbon assimilation, making it a key target for extraction.[43] The authors studied the extraction of CA as a marker of protein functionality to evaluate whether proteins extracted with DES solvents still maintain their biological activity. Since carbonic anhydrase is a well-known and naturally abundant enzyme in microalgae, it is a useful model to test the preservation of activity after extraction. Three DES formulations (polyethylene glycol : urea (2 : 1), choline chloride : polyethylene glycol (1 : 2), and choline chloride : urea (1 : 2)) were applied to *T. lutea*, *C. vulgaris*, and *Spirulina* sp. under mild conditions (room temperature, 4 hours, stirring) with biomass concentrations of 5 and 10 mg per g DES. After extraction, the samples were dialyzed and analyzed for enzyme activity using a nitrophenol-based esterase assay. Polyethylene glycol : urea and choline chloride : polyethylene glycol were more effective in preserving enzyme functionality, because they are significantly less viscous, contain more water, and form stabilizing hydrogen-bond networks with proteins. These properties can support improved enzyme mobility and reduced structural stress during extraction. In contrast, choline

chloride : urea showed limited performance, likely due to its high viscosity and lower hydration ability, which hinder mass transfer and restrict enzyme flexibility, resulting in much lower preserved activity. Among all species, *T. lutea* showed the highest CA activity when extracted with polyethylene glycol : urea, indicating that this solvent system effectively preserves protein functionality.

These findings demonstrate that DESs, particularly PEG- and glycerol-based systems, can be tailored both to enhance protein yields and to ensure the preservation of enzymatic activity. This dual benefit significantly broadens their potential applications. Instead of serving solely as protein extraction tools, DESs could provide enzyme-rich extracts directly applicable in biotechnological and environmental processes. For example, microalgal carbonic anhydrase obtained through DES extraction may be integrated into CO_2 capture systems as a sustainable alternative to synthetic catalysts, combining efficiency with environmental safety.

The heatmap in Fig. 5A shows that, based on the values reported in the reviewed studies, *Palmaria* displays the highest measured levels of total proteins (TP) and total sulphated polysaccharides (TSP). *Neochloris*, *Scenedesmus*, and *Schizochytrium* exhibit the strongest total lipid (TL) signals across the included datasets, with values substantially exceeding those reported for most other species. The reviewed articles also show that *Nannochloropsis* frequently presents elevated free fatty acids (FFA), while *Porphyridium* displays the second-highest FFA levels (after *Arthrospira*) together with moderate total carbohydrates (TC). *Arthrospira* is represented by a more even distribution of TP, TC, and FFA concentrations across publications. Chlorella shows moderate TP and TC values and comparatively higher TL levels, whereas *Tisochrysis* appears predominantly characterized by moderate TP, with no measurable carbohydrates, lipids, or FFAs in the reviewed datasets.

The heatmap reflects that, in the studies included in this review, *Palmaria* yielded the highest extracted TP and TSP concentrations, *Neochloris*, *Scenedesmus*, and *Schizochytrium* produced the strongest extracted TL signals, *Porphyridium* and *Nannochloropsis* showed elevated extracted FFA levels, and *Arthrospira* and *Chlorella* generated more balanced macronutrient profiles under the specific extraction conditions applied in those studies.

The heatmap in Fig. 5B reveals that the most efficient extraction systems for macronutrients are the glucose : glycerol-based DES/NADES, particularly Glu : Gly : W and Glu : Gly : Pro : W, which exhibit the highest intensities for total proteins (TP) and total sulphated polysaccharides (TSP). Glu : Gly : W is also unique in extracting measurable total carbohydrates (TC) and moderate total lipids (TL), giving it the broadest macronutrient profile among all tested solvents.

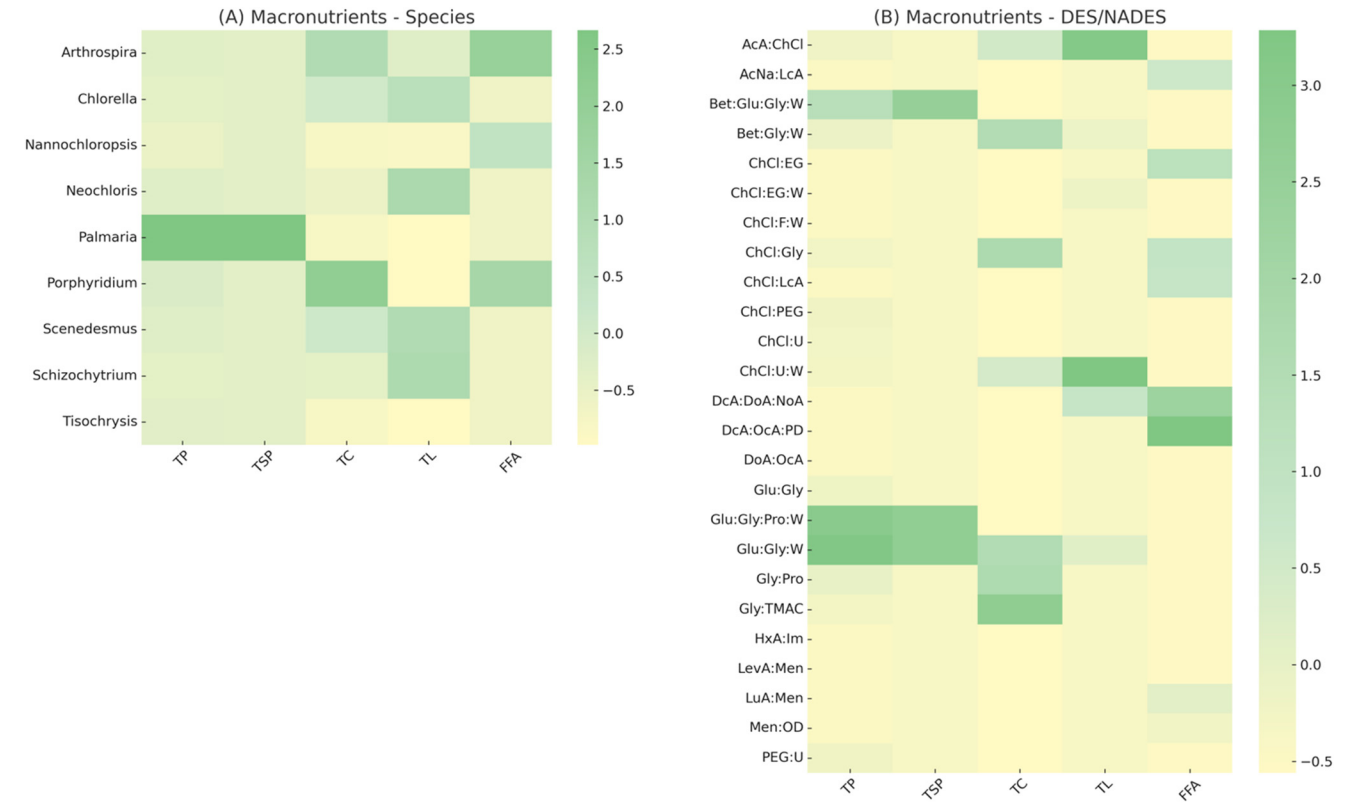

Fig. 5 Heatmap of macronutrient fractions extracted with from different species (A) using various DES/NADES formulations (B) reviewed in this study. Dark-green cells represent relatively higher concentrations, while yellowish tones indicate lower levels or absence. Abbreviations: TP – total proteins; TSP – total sulphated polysaccharides; TC – total carbohydrates; TL – total lipids; FFA – free fatty acids.

Choline-chloride DESs also show strong extraction potential but with more selective patterns. ChCl : U : W yields high TL, while ChCl : Gly and ChCl : PEG extract moderate TP and TC. ChCl : LcA, ChCl : U, and ChCl : Xyl produce noticeable FFA signals, indicating that acidic or polyol-containing DES combinations are particularly suited for recovering free fatty acids.

Hydrophobic DESs, including DcA : DoA : NoA and DcA : OcA : PD, display extremely high FFA values, confirming their strong affinity for nonpolar lipid fractions. AcNa : LcA and ChCl : EG also show elevated FFA extraction, though to a lesser extent.

In contrast, several solvents such as DoA : OcA, HxA : Im, LevA : Men, and LuA : Men exhibit minimal extraction across all macronutrient categories, reflecting limited solvating power for polar or high-molecular-weight biomolecules.

The heatmap demonstrates that Glu : Gly : W and Glu : Gly : Pro : W were the most effective all-round systems (TP, TSP, TC, TL), while hydrophobic DESs (*e.g.*, DcA : OcA : PD) specialized in FFA extraction in the studies analyzed. ChCl-based DESs showed intermediate extraction efficiencies, with specific systems favoring carbohydrates or lipids.

Extraction of lipids, fatty acids, and phytosterols. Lipids are among the most frequently studied fractions of microalgal biomass due to their dual importance in both energy and health-related applications. On the one hand, they represent a renewable source for biodiesel production, while on the other, their structural diversity makes them valuable for functional food and nutraceutical purposes. Traditional extraction methods rely significantly on organic solvents such as chloroform and methanol, raising concerns over environmental impact and product safety. DESs have therefore emerged as eco-friendly and selective alternatives, capable of efficiently extracting total lipids while reducing or even eliminating the need for toxic solvents. Lo *et al.* demonstrated a green and effective extraction strategy using a semi-hydrophobic eutectic solvent composed of imidazole and hexanoic acid (15 : 85) to recover lipids from *N. oceanica*.[46] Extraction from wet biomass at 50 °C for 2 hours yielded over 80% efficiency, comparable to traditional methods. While exact values were not provided in units adequate for comparison, this approach supports solvent design as a strategy to enhance solubility and avoid biomass drying steps.

Among all reviewed studies, Ozel *et al.*[38] achieved the highest yields of total lipid compounds, investigating four species and finding that the less polar DES combination choline chloride : urea : water (1 : 2 : 2) gave the best results for *N. texensis* with a yield of 183 mg per g DW, followed by *Schizochytrium* sp. with 176 mg per g DW using choline chloride : acetic acid : water (1 : 2 : 2). This performance may result from the reduced viscosity of the water-diluted urea DES, which improves mass transfer during extraction. Ultrasound-assisted extraction (UAE) further enhances solvent penetration, supporting the high lipid recovery reported. Although the relative performance of urea- and acid-based DESs varied among species, the hydrated choline chloride : urea system was particularly effective for *N. texensis* under the applied conditions. Beyond total lipids, attention has increasingly shifted toward the recovery of specific lipid fractions, especially polyunsaturated fatty acids (PUFAs), which hold exceptional nutritional and biomedical relevance. Fatty acids such as eicosapentaenoic acid and arachidonic acid are highly valued for their roles in cardiovascular protection, inflammation control.[47] In addition, these fatty acids are used as precursors for biofuel production.[48] Moreno Martínez *et al.*[19] and García-Soto *et al.*[49] evaluated multiple DESs for extracting fatty acids from *N. gaditana*, a known EPA-rich microalga. Using choline chloride : ethylene glycol (1 : 2) and ultrasound pretreatment, Moreno Martínez *et al.* reported the highest EPA yield at 20.66 mg per g DW, which is the maximum EPA value listed in Table 1.[19] Other FAMEs such as C16:1 (25.89 mg g^{-1}) and C18:0 (32.44 mg g^{-1}) were also efficiently extracted under similar conditions. In *A. platensis*, Wils *et al.* reported outstanding yields for saturated and unsaturated fatty acids using systems like 1,3-propanediol : octanoic acid : decanoic acid (1 : 3 : 1) and nonanoic acid : decanoic acid : dodecanoic acid (3 : 2 : 1).[50,51] Notable concentrations included: 160 mg per g DW for octadecadienoic acid (C18:2); 35 mg per g DW for octadecatrienoic acid (C18:3); 35.5 mg per g DW for hexadecanoic acid (C16:0); and 117.5 mg per g DW for arachidonic acid (C20:4) in *P. cruentum.* Advanced extraction methods like dual asymmetric centrifugation further enhanced recovery, with up to 218 mg per g DW of free fatty acids obtained from *Spirulina* using propanediol : octanoic acid : decanoic acid.[51] These results demonstrate that hydrophobic DES systems enriched in medium-chain fatty acids (*e.g.*, octanoic, nonanoic, decanoic, dodecanoic acids) are highly effective lipid solvents capable of extracting a broad spectrum of saturated and unsaturated fatty acids from both cyanobacteria and red microalgae. Their strong nonpolar character enhances solubilization of long-chain fatty acids such as C16:1, C18:0, C18:2, C18:3, and C20:4, while the incorporation of amphiphilic polyols, such as 1,3-propanediol, improves fluidity and mass transfer. These DES systems perform particularly well when combined with intensified extraction techniques such as dual asymmetric centrifugation, which further disrupts cell structures and significantly increases the recovery of free fatty acids.

Within the lipid fraction, phytosterols represent another high-value group of compounds. Structurally similar to cholesterol, phytosterols are recognized for their cholesterol-lowering, anti-inflammatory and anticancer properties. Resende *et al.* applied COSMO-RS computational screening to select DES candidates for extracting phytosterols from the green macroalga *Codium tomentosum*.[52] The most effective system was menthol : octanoic acid (1 : 1), which achieved a phytosterol yield of 3.16 mg per g DW under maceration at room temperature for 3 hours, a value that represents the highest phytosterol concentration (Table 1). Notably, this yield exceeded the performance of conventional Soxhlet extraction using dichloromethane (2.86 mg per g DW), proving the potential of hydrophobic DESs for selective extraction of lipophilic molecules like phytosterols. Furthermore, repeated cycles of extraction with the same solvent (without regeneration) led to

a 4.4-fold increase in overall phytosterol concentration, supporting the reusability and scalability of the method.

These findings illustrate that DESs can be tailored not only for bulk lipid extraction but also for the selective recovery of high-value fractions such as PUFAs and phytosterols. By combining environmental compatibility with high efficiency, DES-based methods could present a viable pathway toward sustainable utilization of algal lipids in both energy production and health-promoting applications.

The heatmap in Fig. 6A shows that, across the studies included in this review, *Nannochloropsis* generated the most intense and diverse extracted fatty-acid profile, with consistently strong signals reported for EPA, C16:1, C18:1, C16:0, and C18:2. *Porphyridium* is represented by exceptionally high extracted levels of arachidonic acid (ARA), appearing as a single dominant maximum not observed in the other datasets. *Arthrospira* displays moderate but broad signals for several fatty acids, including C18:1, C16:0, C18:3, and C18:2, indicating that the reviewed studies reported a more evenly distributed extraction pattern rather than one dominated by specific compounds. In contrast, *Codium* shows minimal extracted fatty acids but exhibits a pronounced phytosterol signal, indicating that, in the available publications, extraction efforts yielded sterol-rich rather than fatty-acid-rich profiles from this species.

The heatmap reflects species-specific extraction outcomes reported in the literature: *Nannochloropsis* with the strongest multi-fatty-acid yields, *Porphyridium* with the highest extracted ARA, *Arthrospira* with moderate mixed fatty-acid levels, and *Codium* with predominantly phytosterol-focused extraction results.

The Fig. 6B shows that choline chloride-based systems such as ChCl : EG, ChCl : Gly, and ChCl : LcA show the broadest extraction capability, with consistently high intensities for C16:1, EPA, C18:1, C16:0, and C18:2, reflecting their strong solvating power for both saturated and unsaturated medium-chain fatty acids. In contrast, more dilute or ternary systems such as ChCl : EG : W and ChCl : F : W display uniformly weak extraction across almost all analytes, indicating a strong dilution effect from water or weaker HBD–HBA interactions.

An interesting pattern is observed for DcA : OcA : PD, which selectively extracts extraordinarily high levels of ARA (C20:4), suggesting a strong affinity of this hydrophobic system for long-chain polyunsaturated fatty acids. Similarly, DcA : DoA : NoA provides high extraction of C18:3, C18:2, C18:0, and C14:0, highlighting the strong nonpolar character of fatty-acid-based DES systems.

Most DES combinations show very low phytosterol extraction (PS), except Men : OcA, which is the only system producing a PS signal, aligning with the expected sterol-solubilizing properties of menthol-based hydrophobic DES. The heatmap shows that choline chloride DESs favored medium-chain and omega-3 fatty acids, whereas fatty-acid-based DESs preferentially extracted long-chain PUFAs, and menthol-based DES

Fig. 6 Heatmap of fatty acids and phytosterols extracted with from different species (A) using various DES/NADES formulations (B) reviewed in this study. Dark-green cells represent relatively higher concentrations, while yellowish tones indicate lower levels or absence. Abbreviations: C16:1 – hexadecenoic acid; EPA (C20:5 n-3) – eicosapentaenoic acid; ARA (C20:4 n-6) – arachidonic acid; C18:1 – octadecenoic acid; C16:0 – hexadecanoic acid (palmitic acid); C20:4 – eicosatetraenoic acid; C18:3 n-3 (ALA) – octadecatrienoic acid (α-linolenic acid); C18:2 n-6 (LA) – octadecadienoic acid (linoleic acid); C18:0 – octadecanoic acid (stearic acid); C14:0 – myristic acid; C15:0 – pentadecanoic acid; C12:0 – dodecanoic acid (lauric acid); C10:0 – decanoic acid (capric acid); C8:0 – octanoic acid (caprylic acid); PS – phytosterols.

selectively targeted phytosterols, reflecting distinct polarity-driven extraction preferences.

Extraction of carbohydrates. Cokdinleyen *et al.*[44] confirmed that red macroalga *P. palmata* is a promising source of sulfated polysaccharides, which are bioactive compounds of interest for health and nutraceutical applications. The extraction efficiency depended strongly on the NADES composition and extraction conditions. In their two-step strategy, they first used UAE with a glycerol : glucose (2 : 1) with 50% water at 25 °C, achieving a carbohydrate yield of 54.3 μg per mg DW. A second step with PLE at 40 °C enhanced the yield to 131 μg per mg DW (Table 1), surpassing the first step and confirming that residual biomass still contains significant extractable carbohydrates. Although water-based PLE gave the highest yield (141.9 μg per mg DW), the NADES method provided balanced efficiency and better compound preservation, supporting its use in biorefineries.

In a similar context, Li *et al.*[45] explored five glycerol-based systems for extracting bioactives from *P. purpureum*, a red microalga. Using UAE at 25 °C for 10 min (biomass-to-solvent ratio of 1 : 20), they found that DES composed of tetramethylammonium chloride : glycerol (1 : 2) gave the highest total carbohydrate content (58.34 mg per g DW, Table 1). This yield is among the highest reported in the literature and is consistent with the values in Table 1, where *P. purpureum* and other red microalgae often outperform green species in carbohydrate recovery. These results underscore the synergistic effects between amino acid-based or quaternary ammonium salt-based DESs and mild ultrasound conditions, enabling better solubilization of water-soluble polysaccharides and other carbohydrates.

Ozel *et al.*[38] investigated carbohydrate extraction from four microalgae: *Schizochytrium* sp., *C. vulgaris*, *S. protuberans*, and *N. texensis*, using choline chloride : acetic acid (1 : 2) and choline chloride : urea (1 : 2) systems. The extraction was performed at 50 °C for 1 hour under UAE with a 20 : 1 solvent-to-biomass ratio. Results revealed that *S. protuberans* with choline chlorde : acetic acid yielded the highest amount (18.32 mg per g DW). The other species yielded lower amounts, especially *N. texensis* (2.55 mg per g DW), showing a clear species-specific and DES-specific pattern in extraction efficiency. These values align with the data in Table 1, where carbohydrate contents vary significantly depending on microalgal species and solvent type, with urea- and acid-based DESs generally performing better in green algae.

The heatmap in Fig. 6B shows that carbohydrate extraction is highly dependent on DES composition, with only a few systems demonstrating notable efficiency. Glu : Gly : W is the strongest performer, showing the highest TC intensity among all solvents, indicating that the presence of water in glucose : glycerol NADES substantially enhances carbohydrate solubilization. Glu : Gly : Pro : W also exhibits elevated TC values, though slightly lower than Glu : Gly : W, suggesting that proline contributes additional hydrogen-bonding capacity without compromising carbohydrate recovery.

Moderate carbohydrate extraction is observed for Gly : Pro, ChCl : Gly, and Bet : Gly : W, all of which contain glycerol-based hydrogen-bond donors. This highlights the importance of polyol-rich, highly hydrophilic DES systems in interacting with carbohydrate-rich fractions. A weaker but still measurable TC signal appears for ChCl : U : W, showing that hydration likewise improves the carbohydrate extraction ability of choline–urea systems.

In contrast, the majority of DESs, including hydrophobic combinations (DcA : DoA : NoA, DoA : OcA, DcA : OcA : PD), acidic DESs (ChCl : LcA, ChCl : MA, AcNa : LcA), and other polyol-based or menthol-based systems, show no carbohydrate extraction at all. This demonstrates that TC recovery requires highly polar, hydrogen-bond-rich, water-containing DES, whereas hydrophobic or weakly polar systems are ineffective.

Lipophilic pigments: carotenoids and chlorophylls. Lipophilic pigments, such as carotenoids (*e.g.*, astaxanthin, lutein, fucoxanthin, β-carotene) and chlorophylls (a and b) play essential roles in algal photosynthesis and photoprotection, while offering remarkable health-promoting properties. These compounds exhibit potent antioxidant, anti-inflammatory, anti-diabetic, and anticancer activities, making them highly valued in the nutraceutical, cosmetic, and pharmaceutical industries.[39,53] Carotenoids, such as lutein are crucial for eye and cognitive health, especially in early development, while astaxanthin and fucoxanthin have been linked to skin protection, metabolic regulation, and cardiovascular benefits.[39,54] Chlorophylls not only function as photosynthetic agents but also contribute to oxidative stress reduction and have potential anticancer effects.[38] Their amphiphilic structure and susceptibility to degradation under light, heat, or oxygen stress necessitate careful solvent selection for extraction and stabilization. In this context, the use of DES offers a sustainable, efficient, and stabilizing alternative to conventional solvents, enhancing the recovery and shelf-life of these valuable compounds.[55,56]

Among the most investigated carotenoids, astaxanthin has received significant attention due to its potent bioactivities. Fan *et al.*[39] reported obtaining 0.21 mg per g DW of astaxanthin from *Scenedesmus* sp. using fenchyl alcohol : thymol combination. As shown in Table 2, Pitacco *et al.*[56] reported even higher yields of up to 20.71 mg per g DW from *H. pluvialis* with thymol : oleic acid (3 : 1) at 60 °C. Yang *et al.*[57] extracted canthaxanthin from *Chromochloris zofingiensis*, with yields reaching 1.41 mg per g DW using octanoic : decanoic acid and a pre-ground biomass. Fucoxanthin recovery was demonstrated by Xu *et al.*[53] using a thymol : dodecanoic acid from *T. lutea*, with a maximum yield of 22.03 mg per g DW, the highest among all reported DES-based extractions (Table 2). Obluchinskaya *et al.*[54] reported a lower fucoxanthin yield (0.98 mg g^{-1}) from macroalga *Fucus vesiculosus*, reflecting the influence of biomass type and extraction parameters. Lutein extraction from *Scenedesmus* sp. using fenchyl alcohol : thymol by Fan *et al.*[39] reached 6.26 mg per g DW (Table 2), with minor co-extraction of β-carotene (0.32 mg g^{-1}) and astaxanthin (0.21 mg g^{-1}), highlighting the selectivity of the applied DES system.

The variability in carotenoid yields across studies can be explained by the strong molecular affinity between hydrophobic DES components and the structural features of caroten-

oids. Carotenoids contain long conjugated polyene chains and, in the case of xanthophylls such as astaxanthin, canthaxanthin and fucoxanthin, additional oxygenated functional groups. Hydrophobic DESs formulated with medium-chain fatty acids (octanoic, decanoic, dodecanoic, oleic acid) or aromatic components such as thymol have physicochemical properties that closely match carotenoid chemistry. Their apolar domains establish strong van der Waals interactions with the extended polyene backbone, which enhances solubilization and stabilizes the carotenoid within the solvent matrix. Aromatic HBDs like thymol also participate in π–π stacking and hydrophobic interactions with the carotenoid rings, further improving affinity and extraction efficiency.

Martins *et al.*[58] reported exceptionally high yields of chlorophyll a and b (56.44 mg per g DW) and total carotenoids (47.03 mg per g DW) from *Spirulina* with glucose : glycerol : water (1 : 2 : 4). Hilali *et al.*[59] also reported high total chlorophyll recovery (8.9 mg g^{-1}) with menthol : 1,2-octanediol, while Wils *et al.*[60] recorded up to 6.9 mg per g DW total chlorophylls and 2.2 mg per g DW carotenoids from *Spirulina* using glucose : glycerol : water.

Ozel *et al.*[38] found that choline chloride : acetic acid enabled maximum recovery of carotenoids (33.55 mg per g DW) and chlorophylls (60.4 mg per g DW) from *S. protuberans*, emphasizing the role of acidic DES in pigment solubilization. In contrast, Fernandes *et al.*[61] showed that ionic liquids were less efficient relative to ethanol control for both chlorophylls and carotenoids.

In terms of violaxanthin and antheraxanthin, Gkioni *et al.*[62] observed superior recovery using ethanol compared to betaine:1,2-propanediol from *N. oculata* (Table 2), suggesting a performance gap still exists between DES and conventional solvents for certain xanthophylls.

These results show that hydrophilic–amphiphilic DESs such as glucose : glycerol : water are able to solubilize chlorophylls and xanthophylls through extensive hydrogen bonding, while hydrophobic DESs based on menthol or medium-chain diols penetrate thylakoid membranes and interact strongly with pigment hydrophobic domains. The superior recovery of violaxanthin and antheraxanthin in ethanol highlights that, for some epoxy-containing xanthophylls, conventional solvents still provide a more compatible solvation environment than current DES formulations.

Despite that, the reviewed studies confirm the versatility and tunability of DES for pigment extraction. While solvent composition and extraction parameters significantly impact yield and selectivity, the combination of DES with techniques like ultrasound, PEF, or biomass pretreatment provides a sustainable alternative to organic solvents. Continued optimization is needed to close the performance gap for some pigments.

The heatmap in Fig. 7A shows that, across the studies included in this review, *Arthrospira* produced the strongest overall extracted pigment intensities, particularly for total chlorophylls (TChl), total carotenoids (TCar), and total pigments (Tpig). *Chlorella* and *Neochloris* also show prominent

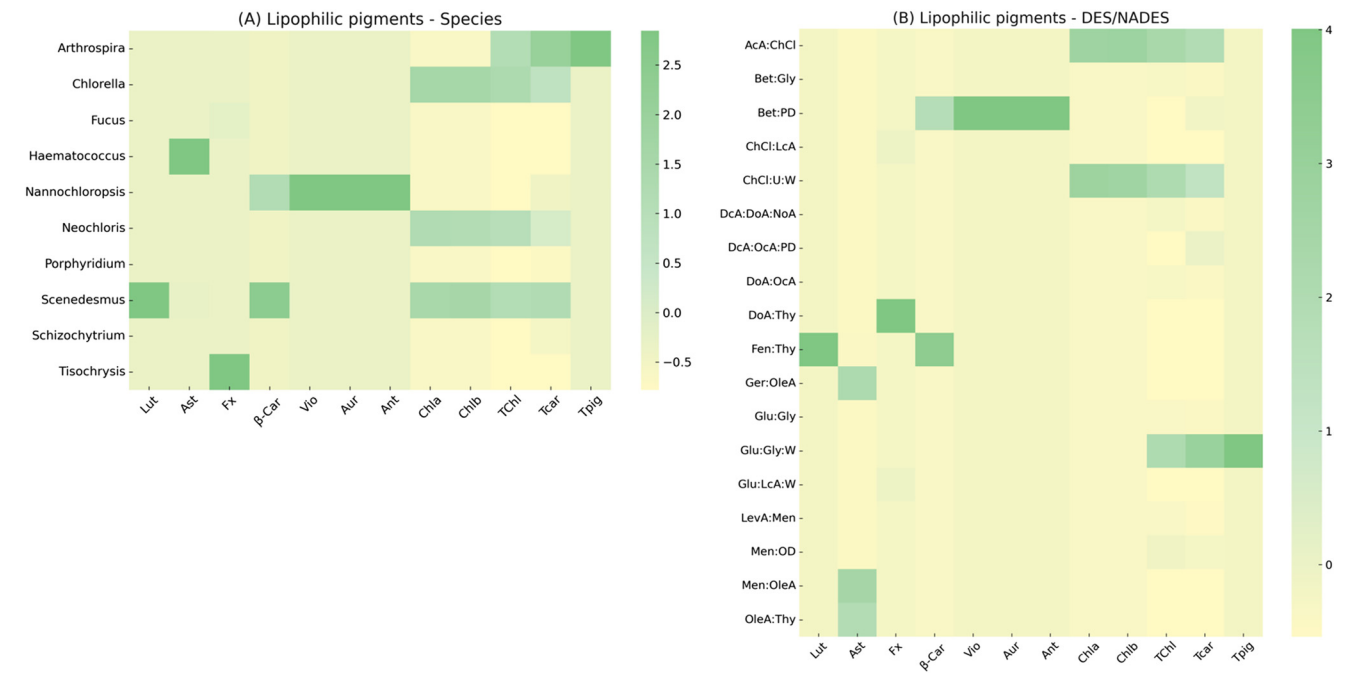

Fig. 7 Heatmap of lipophilic pigments extracted with from different species (A) using various DES/NADES formulations (B) reviewed in this study. Dark-green cells represent relatively higher concentrations, while yellowish tones indicate lower levels or absence. Abbreviations: Lut – lutein; Ast – astaxanthin; Fx – fucoxanthin; β-Car – β-carotene; Vio – violaxanthin; Aur – auroxanthin; Ant – antheraxanthin; Chl a – chlorophyll *a*; Chl b – chlorophyll *b*; TChl – total chlorophylls; TCar – total carotenoids; TPig – total pigments.

extracted chlorophyll profiles, with high reported levels of chlorophyll a (Chla) and chlorophyll b (Chlb). *Scenedesmus* displays a similar chlorophyll pattern, accompanied by low-to-moderate extracted lutein (Lut) and β-carotene.

Distinct single-pigment maxima are also evident in the dataset. *Haematococcus* shows a strong isolated signal for astaxanthin (Ast), while *Tisochrysis* and *Fucus* are characterized by high extracted fucoxanthin (Fx), with *Tisochrysis* exhibiting the highest Fx concentration reported. *Nannochloropsis* shows moderate extracted levels of β-carotene, violaxanthin (Vio), auroxanthin (Aur), and antheraxanthin (Ant), but negligible chlorophylls. In contrast, *Schizochytrium* and *Porphyridium* exhibit very low or absent pigment extraction.

The heatmap reflects extraction outcomes reported in the reviewed studies: chlorophyll-rich profiles for *Chlorella*, *Neochloris*, and *Scenedesmus*; strong fucoxanthin extraction for *Tisochrysis* and *Fucus*; and a distinct astaxanthin-focused extraction for *Haematococcus*.

The heatmap in Fig. 7B reveals that AcA : ChCl and ChCl : U : W show the strongest extraction of chlorophyll a (Chla), chlorophyll b (Chlb) and total chlorophylls (TChl), indicating that choline-chloride systems with acidic components or urea support efficient solubilization of chlorophylls. These same systems also produce notable extraction of total carotenoids (TCar), confirming their broader pigment compatibility.

Highly selective extraction behaviors are evident among hydrophobic DES combinations. DoA : Thy (dodecanoic acid: thymol) demonstrates a pronounced affinity for fucoxanthin (Fx). Similarly, Ger : OleA (geraniol : oleic acid) extracts substantial quantities of astaxanthin (Ast), forming the clearest astaxanthin-specific cluster among all DES tested. This indicates strong π–π and hydrophobic interactions capable of stabilizing ketocarotenoids.

Fen : Thy shows moderate extraction of lutein (Lut) and low levels of β-carotene, confirming its role as a mixed carotenoid-solubilizing system. Other fatty-acid-based DESs (DcA : DoA : NoA, DcA : OcA : PD, DoA : OcA) demonstrate negligible pigment extraction, revealing their limited solvating ability for most pigments under the studied conditions.

A unique pattern is observed for Glu : Gly : W, which produces extremely high signals for TChl, TCar, and especially total pigments (Tpig), the most intense value in the entire dataset. This suggests that glucose-based DES diluted with water forms a highly effective medium for general pigment solubilization.

The heatmap demonstrates that chlorophylls were best extracted by choline-chloride and glucose-based DES, fucoxanthin by DoA : Thy, astaxanthin by Ger : OleA, and lutein by Fen : Thy, highlighting a strong dependence of pigment recovery on DES polarity and hydrogen-bonding structure.

Hydrophilic pigments: phycobiliproteins. Phycobiliproteins, particularly C-phycocyanin (C-PC), R-phycocyanin (R-PC), allophycocyanin (APC), and phycoerythrins (PE), represent a group of highly valuable hydrophilic pigments found primarily in cyanobacteria, such as *Spirulina*, and red algae. These water-soluble, fluorescent proteins serve as light-harvesting antennas in photosynthesis and are increasingly popular because of their potent antioxidant, anti-inflammatory, immunomodulatory, and anticancer properties. Their applications span across food, cosmetics, diagnostics, and pharmaceuticals, with high-purity variants, such as phycoerythrins R-PE and B-PE commanding premium prices due to their intense fluorescence and therapeutic potential.[45,63,64] However, the efficient and sustainable extraction of these pigments remains challenging due to their intracellular localization, structural sensitivity, and the need to preserve bioactivity.

Recent studies have explored the use of DESs, particularly NADESs, as green alternatives for phycobiliprotein extraction. *A. platensis* has been the most widely studied biomass, with glycerol : glucose (2 : 1) and glucose : glycerol : water (1 : 2 : 4) systems demonstrating high C-PC yields of up to 90.85 mg g^{-1} and 61.72 mg per g DW under UAE at mild temperatures, as shown in Table 2.[58,59] Wils *et al.*[60] also showed that hydrophilic DES could extract up to 39.6 mg per g DW of C-PC, outperforming many conventional solvents. Biphasic DES systems further enhanced selectivity and purity, as demonstrated by Pereira *et al.*[65] who achieved a 99% extraction efficiency of C-PC using a biphasic system composed of decanoic acid : thymol (2 : 3) and a phosphate buffer solution (PBS). They suggested that the salting-out effect of PBS, together with the hydrophobicity of the synthesised DES, played a key role in C-PC partitioning. Red microalgae, such as *P. purpureum* and *P. palmata*, have also shown promise as sources of phycoerythrins. Li *et al.*[45] reported 7.32 mg per g DW of B-PE from *P. purpureum* using L-proline : glycerol under mild UAE conditions, while Van Gheluwe *et al.*[64] achieved 12.5 mg per g DW using glycerol-based DES also enhancing pigment stability.

Notably, Xu *et al.*[63] extracted R-phycoerythrin R-PE at 13.09 mg per g DW with a high purity of 3.825 using a choline chloride : urea-based aqueous two-phase system, further validating the potential of DES-based systems in high-purity pigment recovery. In that study, purity is defined as the absorbance ratio A565/A280, and the authors indicate that values ≥3.0 correspond to drug-grade; thus, 3.825 meets this criterion. Cokdinleyen *et al.*[44] achieved particularly high yields of B-PE (43 mg per g DW), APC (52 mg per g DW), and R-PC (28 mg per g DW) from *P. palmata* using PLE with glycerol : glucose (Table 2). The favourable performance of DES for phycobiliprotein extraction can be attributed to their hydrophilic nature, strong hydrogen-bonding capacity, and ability to stabilize protein–chromophore complexes. Glycerol : glucose systems provide an ideal polarity–viscosity balance for solubilizing C-PC and other PBPs while preserving structural integrity under mild UAE conditions. Biphasic DES systems further enhance purity through selective partitioning driven by salting-out and hydrophobic phase separation. Proline- and glycerol-based NADES outperform in stabilizing sensitive phycoerythrins, while ChCl : U ATPS enables drug-grade purity due to preferential PBP partitioning. High-pressure PLE improves extraction from tougher red algal matrices, resulting in some of the highest reported B-PE, APC and R-PC yields.

The heatmap in Fig. 8A shows that, across the studies included in this review, *Arthrospira* yields the most intense

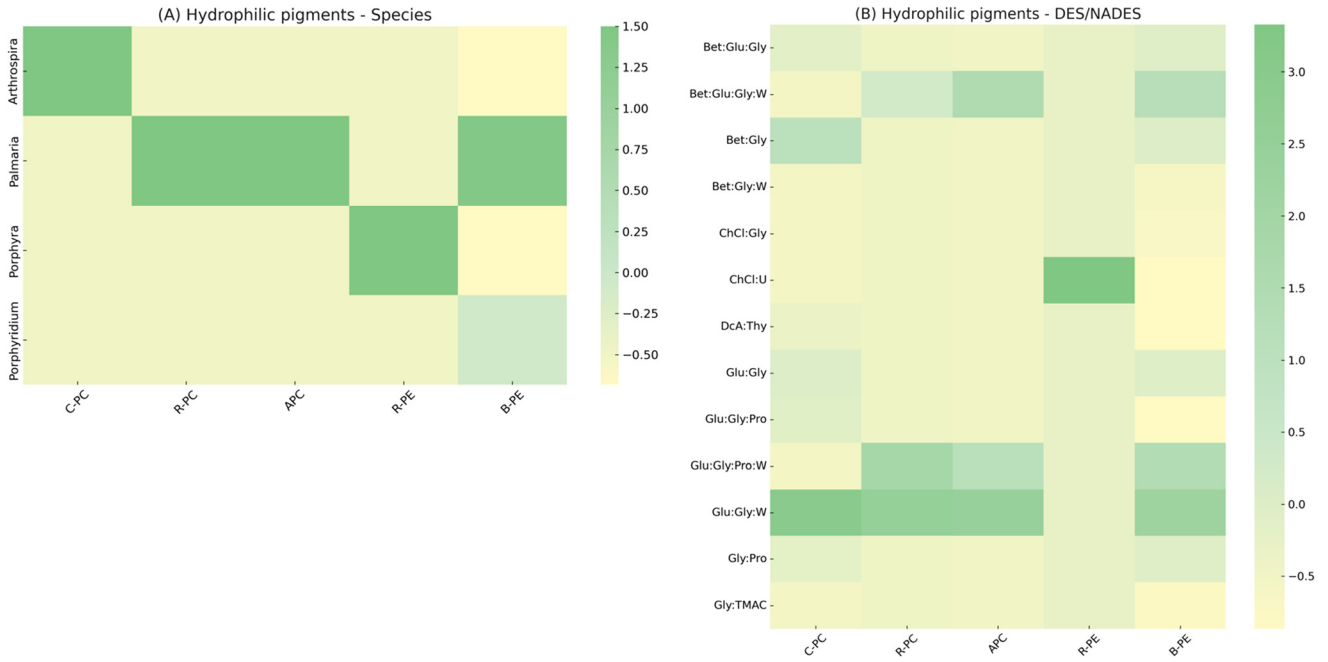

Fig. 8 Heatmap of hydrophilic pigments extracted with from different species (A) using various DES/NADES formulations (B) reviewed in this study. Dark-green cells represent relatively higher concentrations, while yellowish tones indicate lower levels or absence. Abbreviations: C-PC – C-phycocyanin; R-PC – R-phycocyanin; APC – allophycocyanin; R-PE – R-phycoerythrin; B-PE – B-phycoerythrin.

extracted signal for C-phycocyanin (C-PC), representing the highest single phycobiliprotein value in the dataset. *Palmaria* displays a distinctly different pattern, with high extracted concentrations of allophycocyanin (APC), R-phycocyanin (R-PC), and B-phycoerythrin (B-PE), resulting in the most diverse phycobiliprotein profile reported among the analyzed species.

Porphyra exhibits a selective moderate extraction of R-phycoerythrin (R-PE), with no measurable levels of other phycobiliproteins in the reviewed studies. *Porphyridium* shows a moderate extracted signal for B-phycoerythrin (B-PE) as its only detected phycobiliprotein.

The heatmap reflects distinct extraction outcomes within species: *Arthrospira* is represented by strong C-PC extraction, while *Palmaria*, *Porphyra*, and *Porphyridium* show profiles dominated by PE and/or APC in the reviewed publications.

The heatmap in Fig. 8B reveals that the most efficient and broad-spectrum system is Glu : Gly : W, which shows the highest intensities for C-phycocyanin (C-PC), R-phycocyanin (R-PC), allophycocyanin (APC) and B-phycoerythrin (B-PE). This makes it the only DES capable of extracting all major phycobiliprotein classes at high yield, indicating that glucose : glycerol DES diluted with water forms an exceptionally effective hydrophilic environment for solubilizing these water-soluble proteins.

Two other glucose–glycerol-based systems show notable but more selective extraction patterns. Glu : Gly : Pro : W displays strong recovery of R-PC, APC, and B-PE, while Glu : Gly and Glu : Gly : Pro moderately extract C-PC and B-PE, showing that the presence of proline or water adjusts pigment specificity.

The betaine : glucose : glycerol systems also exhibit distinct extraction behavior. Bet : Glu : Gly and Bet : Gly extract C-PC efficiently and show moderate activity toward B-PE, while their water-containing counterparts (Bet : Glu : Gly : W, Bet : Gly : W) demonstrate higher affinities for R-PC and APC, suggesting that hydration enhances the solubility of red-shifted phycobiliproteins.

Other DESs display more restricted extraction. DcA : Thy (hydrophobic) shows only low recovery of C-PC, and Gly : Pro moderately extracts C-PC and B-PE, while systems such as ChCl : Gly, Gly : TMAC, DoA : OcA, and fatty-acid-based mixtures show minimal or no phycobiliprotein extraction. ChCl : U stands out as the only DES extracting R-phycoerythrin (R-PE) specifically, though at moderate intensity.

The heatmap demonstrates that the strongest phycobiliprotein extraction is achieved by highly polar, hydrogen-bond-rich DESs, especially Glu : Gly : W, while fatty-acid-only hydrophobic DESs show limited recovery.

Polyphenols and related compounds. Polyphenols and related metabolites are widely distributed in marine and freshwater algae. Phenolic acids such as ferulic, caffeic, gallic, and *p*-coumaric acids occur broadly in algal biomass. According to Table 3, Hashemi *et al.*[66] reported the highest ferulic acid yield of 37.74 mg per g DW from mixed native algae representing *Phaeophyceae*, *Rhodophyceae*, and *Chlorophyceae* using choline chloride with urea at 1 : 2 under UAE. Wan Mahmood *et al.*[21] extracted 3.2 mg per g DW of gallic acid and 0.2 mg per g DW of caffeic acid from *C. vulgaris* using choline chloride : 1,3-propanediol (1 : 4) with 30% water, while *p*-coumaric acid reached 0.19 mg per g DW using a 1,4-butanediol-based system. These results indicate the effectiveness of polyol- and urea-based DES for extracting moderately polar phenolic acids. Phenolic acids

possess both polar functional groups (–OH, –COOH) and hydrophobic aromatic rings, making them well suited for extraction by DES with strong hydrogen-bonding capacities and medium polarity. Urea- and polyol-based DES form dense hydrogen-bond networks with phenolic hydroxyl and carboxyl groups, while added water reduces viscosity and improves mass transfer under ultrasound. Urea also disrupts phenolic–polysaccharide linkages in algal cell walls, enhancing release and resulting in the high yields observed across multiple studies.

Flavonoids, which underpin many antioxidant, anti-inflammatory, and antimicrobial effects, also respond strongly to solvent design and process choice (Table 3). Consistent with this selectivity toward polyphenol subclasses, Hashemi *et al.*[66] recovered 0.91 mg per g DW of luteolin-7-*O*-glucoside from native mixed algae using choline chloride with lactic acid at 1 : 2, pointing to the value of organic acid-based DES for glycosylated flavonoids. Furthermore, Hashemi *et al.*[66] reported a maximum of total phenolic content (TPC) 127.09 mg GAE per g DW using proline : lactic acid (1 : 1) on mixed native algae. In *Spirulina*, Martins *et al.*[58] obtained 36.50 mg GAE per g DW with a glucose : glycerol : water mixture (1 : 2 : 4) under ultrasonication, while Ozel *et al.*[38] reported 33.44 mg GAE per g DW in *S. protuberans* extract using choline chloride : acetic acid (1 : 2). Thus, organic acid-based DES such as ChCl : lactic acid show good affinity for glycosylated flavonoids likely due to their high polarity and strong H-bonding networks. Proline in systems like Pro : LcA contributes additional H-bond donor/acceptor sites and enhances solvent polarity, facilitating solvation of polar phenolics.

Within brown algae, phlorotannins merit separate attention because they are unique to this group and often drive bioactivity. Obluchinskaya *et al.*[54] reported 71.6 mg per g DW of phlorotannins from *Fucus vesiculosus* using choline chloride : lactic acid (1 : 3), an outcome that aligns with the strong affinity of lactic acid-based DES with moderate water content for hydrophilic phenolics in macroalgal tissues.

Furthermore, antioxidant activity is closely linked to phenolic content, Hashemi *et al.*[66] observed antioxidant activity measured *via* DPPH and ABTS correlating with high TPC values, while Fassi Fihri *et al.*[67] demonstrated that DES extracts could reach IC_{50} values of 3.98 mg mL^{-1} and antibacterial MICs of 0.25 mg mL^{-1}, highlighting the multifunctional application of DES-based phenolic extractions in health and biorefinery.

The heatmap in Fig. 9A shows that, across the studies included in this review, the macroalgae group exhibits the strongest overall extracted phenolic profile, with exceptionally high reported values for total phenolic acids (TPA), total phenolics (TPC), and ferulic acid (FA). *Fucus* also displays strong phenolic extraction, particularly for phlorotannins (PT), which represent the highest PT values reported among all species in the dataset.

Chlorella presents a moderate but comparatively diverse extraction pattern, with measurable levels of TPC, gallic acid (GA), and *p*-coumaric acid (*p*-CA), making it the only species

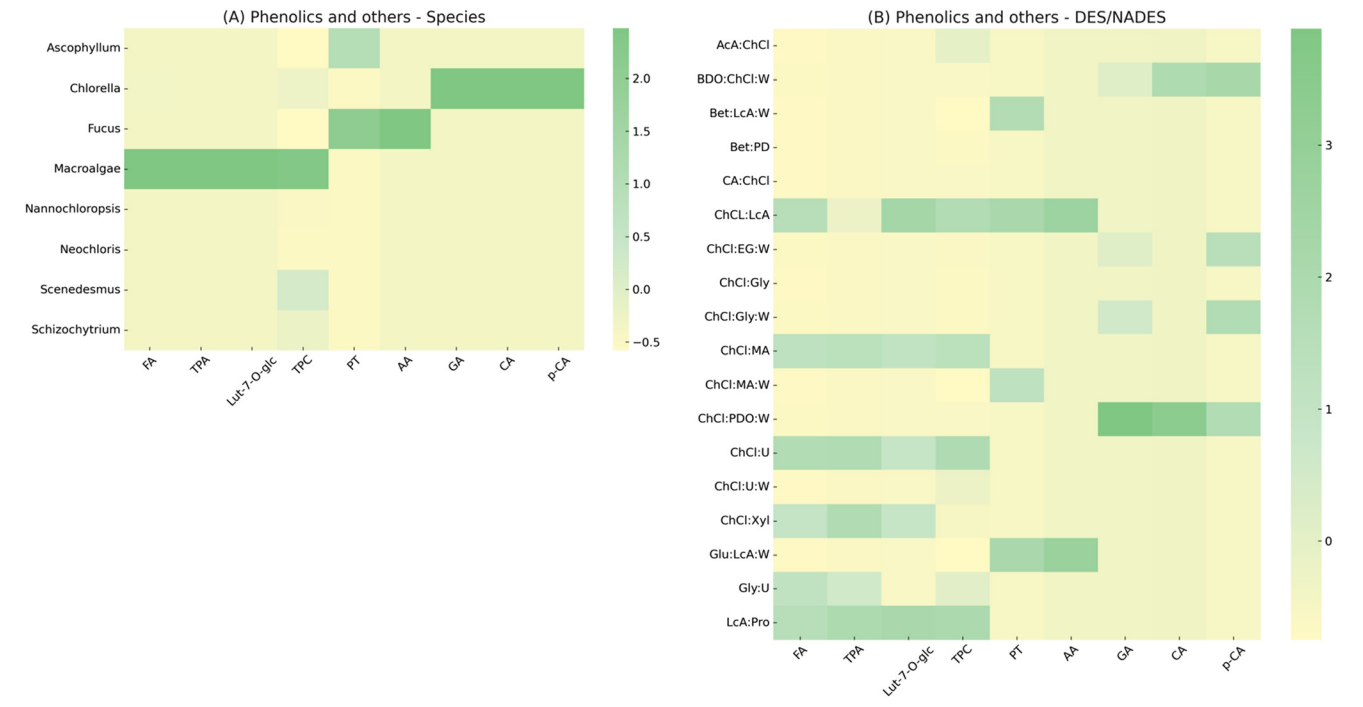

Fig. 9 Heatmap of phenolic and other compounds extracted with from different species (A) using various DES/NADES formulations (B) reviewed in this study. Dark-green cells represent relatively higher concentrations, while yellowish tones indicate lower levels or absence. Abbreviations: FA – ferulic acid; Lut-7-*O*-glc – luteolin-7-*O*-glucoside; GA – gallic acid; CA – caffeic acid; *p*-CA – *p*-coumaric acid; TPA – total phenolic acids; TPC – total phenolic content; PT – phlorotannins; AA – ascorbic acid.

with multiple individual phenolics detected in addition to TPC. *Scenedesmus* shows a single pronounced signal for TPC, indicating that although its extraction profile is less diverse, substantial total phenolic content was still obtained in the reviewed studies.

In contrast, *Ascophyllum*, *Nannochloropsis*, *Neochloris*, and *Schizochytrium* exhibit very limited phenolic extraction, with almost no detectable individual phenolics, except for the PT signal observed for *Ascophyllum*.

The heatmap reflects that the reviewed studies report the strongest phenolic extraction for brown macroalgae (macroalgae group, *Fucus*), whereas green microalgae yield more modest and selective phenolic profiles under the extraction conditions applied.

The heatmap in Fig. 9B demonstrates that the most efficient and broad-spectrum systems are ChCl : LcA, ChCl : MA, ChCl : U, ChCl : Xyl, and LcA : Pro, which all show intense signals for total phenolics (TPC) and total phenolic acids (TPA). Among these, LcA : Pro exhibits the highest TPC value, while ChCl : U and ChCl : MA display the strongest TPA intensities, indicating that choline-chloride systems paired with organic acids are highly effective in liberating phenolic fractions.

A clear and unique pattern is observed for ChCl : LcA, which is the only system showing strong extraction of phlorotannins (PT). Glu : LcA : W and ChCl : MA also produce substantial PT levels, although lower than ChCl : LcA, confirming that lactic-acid-based DESs favor phlorotannin solubilization.

Hydrated DES systems (ChCl : Gly : W, ChCl : EG : W, BDO : ChCl : W, ChCl : PDO : W) show increased extraction of specific minor phenolics such as gallic acid (GA) and caffeic acid (CA), highlighting the beneficial role of water in enhancing the extraction of small polar phenolics.

On the other hand, systems such as Bet : PD, CA : ChCl, ChCl : Gly, and hydrophobic mixtures (*e.g.*, DcA : Thy) show only low or negligible extraction of phenolic compounds.

The heatmap demonstrates that acidic choline-chloride DES (ChCl : LcA, ChCl : MA, ChCl : U) and lactic-acid-based NADES (LcA : Pro, Glu : LcA : W) were the most effective systems for extracting bulk phenolic fractions such as TPC, TPA, and PT. In contrast, several hydrated DES formulations (*e.g.*, BDO : ChCl : W, ChCl : PDO : W) show comparatively higher signals for small individual phenolics such as GA and CA, indicating that added water can enhance the recovery of low-molecular-weight polar compounds in specific solvent compositions.

Other metabolites and bioactives. In a study on the green extraction of bioactive compounds from *A. platensis*, Obluchinskaya *et al.*[54] investigated the efficacy of various NADES formulations in extracting ascorbic acid. Among the solvents tested, the betaine : glycerol (1 : 8) system demonstrated the highest ascorbic acid yield, reaching 3.37 mg per g DW. This value surpassed the one obtained using conventional ethanol extraction, which yielded 2.73 mg per g DW. The results underline the superior extraction capacity of the NADES system, attributed to its enhanced hydrogen-bonding interactions and polarity compatibility with ascorbic acid. These findings reinforce the potential of NADES as effective alternative to traditional solvents in extracting water-soluble vitamins from microalgal biomass.

Statistical distribution and correlation analysis of extracted compounds

To provide a comprehensive overview of compound variability across studies, the distribution of extracted chemical classes was evaluated using a box plot, while intercompound relationships were examined through Spearman's rank correlation (r_s). This approach enables the identification of general patterns, outliers and potential associations between different classes of bioactive compounds extracted using DES and NADES systems. Spearman's r_s was selected due to its robustness against non-normal data distribution and its suitability for detecting monotonic relationships in heterogeneous meta-analytical datasets.

The box-plot in Fig. 10 provides a comprehensive overview of the dispersion and central tendencies of compound concentrations reported across multiple studies employing DES- and NADES-based extraction from algae and cyanobacteria. Each box illustrates the interquartile range (IQR), median and standard error, with whiskers showing data dispersion and potential outliers. The box width is proportional to the number of studies reporting data for each compound, reflecting the research coverage and frequency of extraction in the literature. Considerable variability is observed in compound classes, reflecting both the physicochemical diversity of target metabolites and the heterogeneity of solvent systems and extraction conditions used.

Lipophilic metabolites, such as carotenoids, chlorophylls and total lipids display broad interquartile ranges and a high frequency of outliers, indicating that extraction efficiency is highly dependent on solvent hydrophobicity and hydrogen-bonding capacity. Such variability can be attributed to differences in DES composition, particularly the use of fatty acid-based or alcohol-based hydrogen bond donors, and to the different pretreatment methods applied to disrupt cell walls (*e.g.*, ultrasonication, microwave assistance or high-shear mixing).

Hydrophilic compounds, such as proteins, carbohydrates and phenolic acids tend to show narrower distributions, which implies more consistent recoveries across studies and greater robustness of polar formulations for these analytes. Polyphenolic compounds exhibit moderate dispersion, suggesting that DES acidity and water content strongly modulate their solubility and stability during extraction. The relatively high median values for total phenolics further confirm that choline chloride-based DESs containing organic acids (lactic, malic, or citric acid) or polyols (glycerol, glucose) provide optimal extraction environments for these molecules.

Total proteins and total lipids exhibit the highest median extraction yields, often exceeding 100 mg per g DW, and in some cases approaching or surpassing 300 mg per g DW, highlighting the ability of DESs to efficiently solubilize macro-

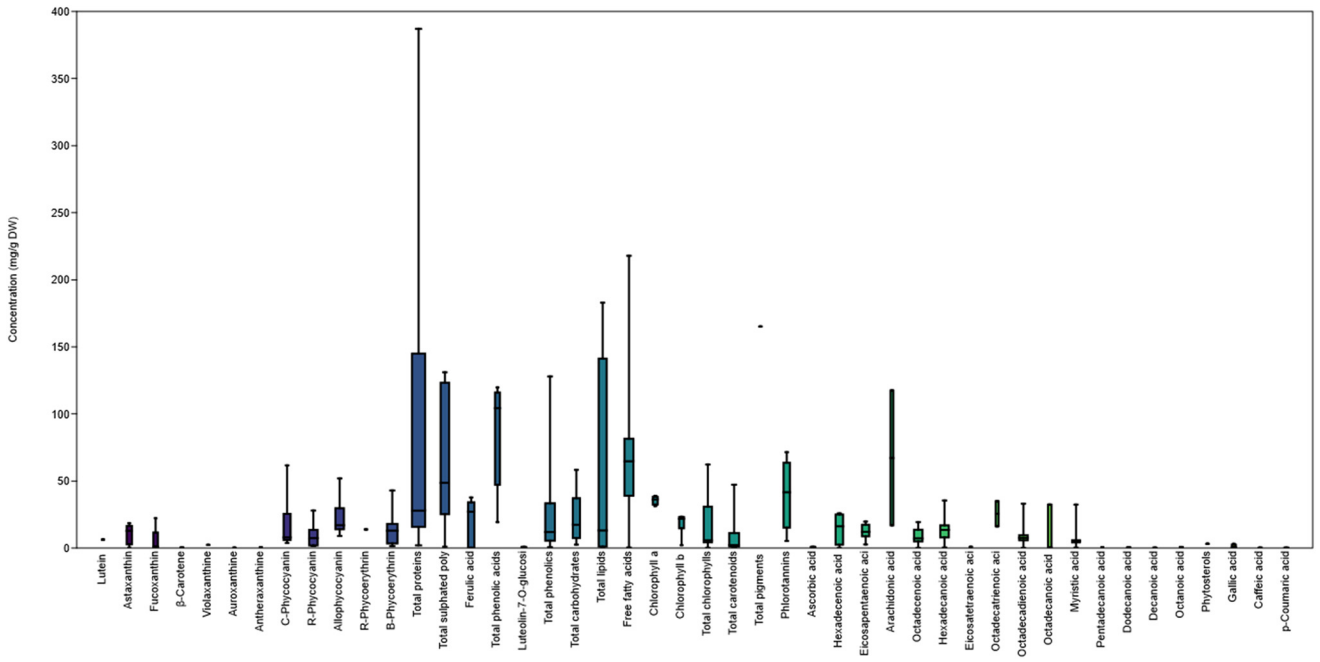

Fig. 10 Box plots representing the distribution of extracted bioactive compounds (mg per g DW) across all studies included in the review.

molecules with amphiphilic character. Total phenolics also show moderate-to-high medians (10–60 mg per g DW), consistent with the strong affinity of acidic and polyol-based DES for polar antioxidant compounds. The largest overall variability is observed for total proteins, with whiskers extending beyond 350 mg per g DW, reflecting both biological heterogeneity among species and methodological diversity in extraction conditions.

However, specific pigments (lutein, fucoxanthin, β-carotene) and minor phenolic acids (ferulic, caffeic, *p*-coumaric) are represented by narrower boxes, indicating fewer studies and lower extract concentrations (typically <10 mg per g DW). This limited data coverage suggests underexplored compound classes and potential gaps in the literature.

The Spearman's rank correlation analysis revealed several statistically significant ($p \leq 0.05$) associations among the analyzed compounds, indicating non-random co-extraction behaviors across the reviewed studies. Strong positive correlations ($r_s > 0.7$) were observed primarily among compounds belonging to similar polarity domains and biochemical families (Fig. 11). Lipophilic compounds, such as total lipids, free fatty acids, total carotenoids and chlorophyll a/b showed significant positive inter-correlations, suggesting that solvents optimized for hydrophobic interactions (particularly fatty-acid- or alcohol-based DESs) facilitate simultaneous extraction of pigments and lipid fractions.

Conversely, negative or near-zero correlations ($r_s < -0.3$) occurred between highly polar metabolites (proteins and polysaccharides) and nonpolar lipid or pigment classes, reflecting distinct solubility requirements and limited overlap of DES systems capable of efficiently recovering both hydrophilic and hydrophobic components. Within the phycobiliprotein group, moderate positive correlations between C-phycocyanin, R-phycocyanin and phycoerythrins indicate co-extraction under similar aqueous NADES compositions, consistent with their shared structural and polarity features. This separation suggests that aqueous or polar NADES (*e.g.*, choline chloride-glycerol or -glucose systems) effectively extract water-soluble proteins and pigments but perform poorly for lipophilic compounds.

Additionally, moderate positive correlations between certain fatty acids (octadecadienoic, eicosapentaenoic, and arachidonic acids) reflect their co-occurrence within similar lipid fractions and their co-extraction under hydrophobic DES systems. On the contrary, phenolic acids (ferulic, caffeic, *p*-coumaric and gallic acids) exhibited only sporadic or weak correlations with other compound classes, which might suggest that their extraction is highly sensitive to solvent acidity and water content rather than shared solubility patterns.

These results quantitatively prove the selective nature of DES-extraction technologies, where each solvent family favors a distinct group of compounds, while cross-class correlations remain limited due to differing solvation mechanisms.

DES in microalgae extraction: barriers and opportunities

While DESs clearly demonstrate significant potential for facilitating the conversion of algal and cyanobacterial biomass into sustainable food sources, their practical implementation is still constrained by a number of challenges and limitations. Addressing these barriers will be essential to fully realize the effective and sustainable valorization of this biomass.

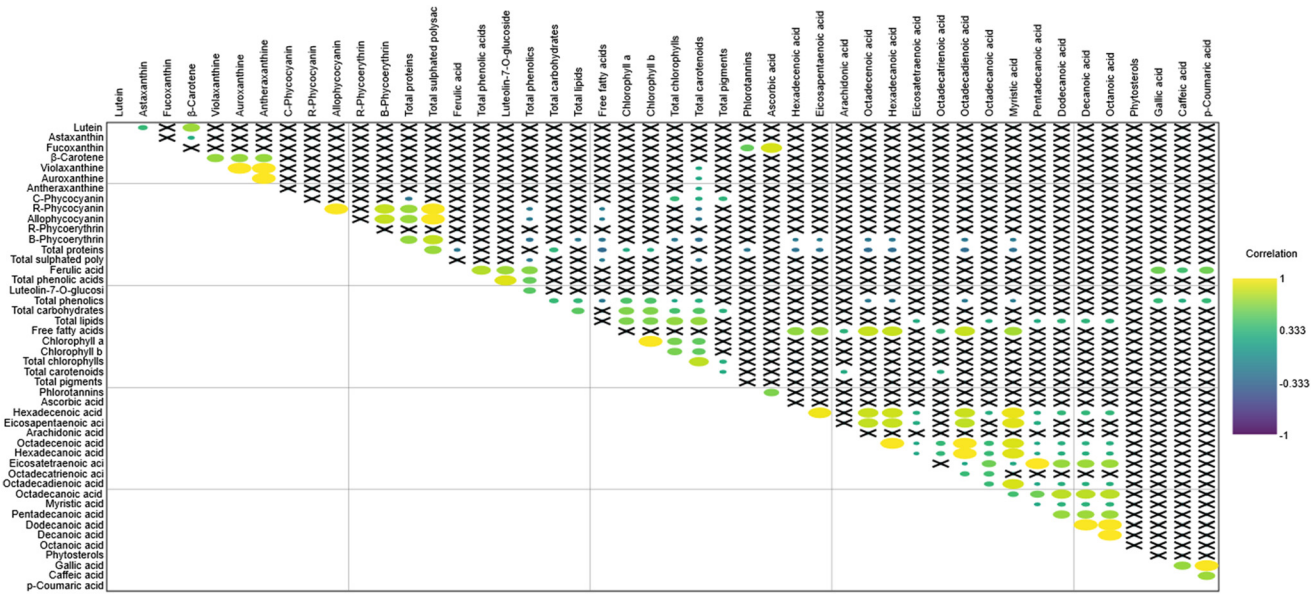

Fig. 11 Spearman's rank correlation (r_s) matrix illustrating statistically significant relationships ($p \leq 0.05$) among the concentrations of bioactive compounds extracted from algal and cyanobacterial biomass using DES and NADES systems. Non-significant correlations ($p > 0.05$) are marked with crosses (×). The color gradient represents correlation strength and direction, ranging from −1 (strong negative, purple) to +1 (strong positive, yellow-green).

One of the main drawbacks limiting the practical application of DESs is their high viscosity, which results from strong intermolecular interactions such as hydrogen bonding, van der Waals forces, and ionic interactions. These interactions can reduce molecular mobility, hinder mass transfer, and complicate downstream handling.[68] A widely applied strategy to mitigate high viscosity is the controlled addition of water. Hydration progressively alters the supramolecular organization of DESs. Gera *et al.*[69] showed that water addition causes a gradual depletion of urea from the interface of the choline chloride : urea (1 : 2) DES, whereas at water contents above 40 wt% the surface undergoes an abrupt reorganization in which choline ions are displaced into the bulk and water accumulates at the interface; at water fractions above 60 wt% the interfacial spectrum becomes indistinguishable from pure water. Consistently, Benítez-Correa *et al.*[70] observed that viscosity decreases sharply in ChCl-based DESs upon water addition, and FTIR spectra showed shifts of O–H stretching bands toward higher frequencies, indicating a weakening of HBA–HBD hydrogen bonding. However, excessive dilution (>50% v/v) disrupts the supramolecular structure required for DES functionality,[71] and even moderate hydration (≤40%) may reduce hydrogen-bond density and solubilization capacity.[64] On the other hand, water molecules may also form stabilizing hydrogen bonds with metabolites, thereby protecting sensitive compounds. Another challenge is the intrinsic hygroscopicity of DESs, which may introduce variability during handling and storage.[68]

In addition to structural changes, the dynamic behavior of DESs, including diffusion, relaxation times, and hydrogen-bond lifetimes, also influences extraction performance. Monteiro *et al.*[72] demonstrated that hydration of a betaine : glycerol DES progressively depletes Bet–Gly and Gly–Gly coordination shells, and that a major structural transition occurs at approximately 30 wt% water, where the second solvation shell collapses and new water-like hydration shells emerge. Similarly, Bhattacharjee *et al.*[73] reported that the hydrogen-bond network characteristic of their eutectic system is maintained up to roughly 40 wt% water, whereas higher hydration disrupts the native structure. These findings indicate that hydration induces concurrent structural reorganization and dynamic changes, both of which affect solute mobility and thereby contribute to mass-transfer behavior relevant for extraction.

Several approaches have been reported to mitigate the high viscosity of DESs. Increasing temperature is highly effective: Savi *et al.*[74] showed that the viscosity of citric-acid/sucrose DESs decreases steadily with increasing temperature and follows Arrhenius-type behavior. Compositional tuning provides another means of viscosity control. Mero *et al.*[75] demonstrated that increasing the fraction of the hydrogen-bond donor (from 1 : 2 to 1 : 4) decreases viscosity across choline- and betaine-based DES families, and that the identity of both HBA and HBD strongly influences baseline fluidity. The use of low-viscosity organic co-solvents offers additional flexibility. Duarte de Alencar *et al.*[76] reported that methanol, isopropanol, and DMSO substantially reduce DES viscosity, in many cases by several orders of magnitude, based on an extensive dataset of 1618 measurements. Viscosity can also be modulated through molecular-level design. Jančíková *et al.*[77] highlighted that variations in HBA and HBD identity, including acidity, modify polarity, hydrogen-bond strength, and nanostructure, thereby shaping fluidity. Complementary machine-learning analysis showed that viscosity is strongly governed by HBA/

HBD molar ratio and functional-group composition, confirming that fluidity can be engineered through rational structural modification.[78]

Besides modifying physicochemical properties, operational strategies can also help alleviate the practical consequences of high viscosity. Moderate temperature elevation and conventional mechanical agitation do not change the intrinsic viscosity of DESs but can reduce mass-transfer limitations by improving solvent–matrix contact and accelerating diffusive transport. In addition, assisted extraction methods such as UAE and PLE can compensate for the limited diffusivity of viscous DESs by enhancing cell disruption, increasing solvent penetration, and boosting mass-transfer rates.[44]

The suitability of extraction methods depends strongly on the physicochemical behavior of DESs and the structural properties of algal and cyanobacterial biomass. Stirred-batch extraction is simple and widely used but can suffer from slow mass transfer when DES viscosity is high. UAE helps overcome this limitation, acoustic cavitation disrupts cells and enhances solvent penetration,[79] and UAE–DES extraction has been shown to outperform DES-based batch methods in yield, energy use, and processing time.[80] Microwave-assissted extraction (MAE) provides rapid energy transfer and can accelerate extraction, although careful control is needed to avoid degradation of thermolabile compounds such as carotenoids.[81,82] PLE further improves mass transfer by using elevated temperature and pressure to reduce viscosity and increase solubility, enabling efficient extraction in short times with low solvent volumes.[83] Overall, UAE and PLE are well suited for viscous DESs, whereas MAE offers rapid extraction but requires attention to thermal sensitivity. Batch extraction remains useful for initial screening but is generally less efficient for rigid biomass.

A further technical limitation of DESs arises in analytical processes, such as gas chromatography (GC), particularly when the solvent remains present in the final extract. Certain DESs, especially those formulated with fatty acids or terpenes, are poorly compatible with GC due to their low volatility and high abundance in the extract. Namely, DES constituents can dominate the chromatogram, producing broad solvent bands that overlap target analytes causing baseline drift, coelution, and unreliable quantification.[84] In addition, incomplete evaporation of DESs (that are low volatile systems) in the injector may lead to column contamination. Furthermore, the high viscosity of DESs complicates precise and reproducible injection at the microliter scale, while their elevated boiling points can hinder solvent evaporation, allowing volatile analytes to elute prematurely. Some DES constituents may even thermally degrade before reaching the column, creating additional background noise. Collectively, these effects compromise resolution and detection accuracy.

Proposed solutions include selective mass spectrometers, or the introduction of glass wool or packing material in the injector liner to stabilize evaporation. Alternatively, careful selection of DES components that do not interfere with analytes under investigation can minimize chromatographic overlap.[85] Nonetheless, due to the dominance of solvent peaks, back-extraction into a volatile organic solvent prior to GC injection is often recommended, or, when dealing with DESs based on fatty acids, replacing GC with LC-based analytical methods.

Toxicity represents another barrier to widespread adoption, especially of DESs. Factors influencing cytotoxicity include charge delocalization through hydrogen bonding, synergistic effects during DES formation, molar ratio, pH, viscosity, and interactions with cellular intermediates.[68] For instance, DESs based on organic acids such as citric acid and malonic acid have been shown to increase cytotoxicity and should therefore be applied with caution and subjected to comprehensive toxicity evaluation. Nonetheless, NADES generally display lower acute toxicity profiles compared to conventional DESs.[86] Still, regulatory acceptance remains limited. For instance, choline chloride, one of the most common HBAs, is banned in cosmetic formulations in the EU.[64] This has stimulated interest in safer alternatives such as betaine, sugars, and glycerol.

Furthermore, their sustainability has been questioned. Whether they represent genuinely green solvents or simply a research trend can only be established through rigorous ecological and economic assessment. Recent quantitative sustainability assessments reinforce these concerns. A cradle-to-gate life-cycle assessment (LCA) demonstrated that most of its global warming potential, eutrophication, acidification, and toxicity burdens arise not from DES formation itself, but from the energy-intensive upstream synthesis of choline chloride and urea. As a result, the DES for example exhibited lower impacts than dichloromethane and ethyl acetate, but higher impacts than methanol and ethanol, contradicting the common assumption that DESs are intrinsically sustainable.[87] However, substituting constituents with natural metabolites does not guarantee improved environmental profiles. In the same study, citric-acid-based DES displayed the highest impacts across all LCA categories, driven by water consumption and CO_2 emissions during citric acid fermentation. These findings show that sustainability is not an inherent property of DESs as many common precursors (*e.g.*, choline chloride, malonic acid) are fossil-derived, energy-intensive, or poorly biodegradable, and toxicity and biodegradability vary widely among formulations.[88] Overall, DES sustainability is highly formulation-dependent, and their "greenness" must be evaluated case by case rather than presumed based on low volatility or natural origin.

DESs, particularly NADESs, which are generally recognized as safe, may remain in the final extract intended for food, cosmetic, or pharmaceutical applications, a feature that represents one of their main advantages. Yet, when their removal is necessary, the process may be technically demanding and costly, depending on solvent composition and extract properties.

There is no universal DES that can be consistently effective for the extraction of all types of biomass. Although predictions can be made based on DES characteristics, such as polarity, viscosity, and other physicochemical properties, as well as on

the nature of the target compounds, extraction still requires optimization. This is due to the structural diversity of different biomass types and the complexity of their component mixtures.

When these issues are addressed, DESs can be powerful tools for processing algal and cyanobacterial biomass. Algae and cyanobacteria are compelling feedstocks for sustainable production of foods and bioactive compounds. They reach high productivity, do not compete for arable land, can grow in seawater or wastewater, and contain abundant proteins, lipids, pigments, and other metabolites. Combined with optimized DES-based pretreatment, extraction, and stabilization, these advantages can be translated into highly efficient processes.

DESs can also help overcome a practical barrier to wider use of algal products: consumer resistance due to undesirable taste and odor. Targeted DES extraction can enrich compounds of interest while reducing compounds responsible for off flavors and odors. In this context, DES are particularly promising. They enable pretreatment and selective extraction that preserves sensitive molecules, can reduce or mask negative sensory notes, and may improve nutritional and functional properties as well as the bioavailability of bioactives. Unlike conventional solvents, some DES can remain in finished formulations, if provided safety and biodegradability are demonstrated, avoiding costly solvent removal.

The synergy between DES and algal or cyanobacterial bioprocessing can improve organoleptic properties, increase bioavailability of bioactives, and align with zero-waste biorefineries.

Conclusions and future perspectives

This review examined the use of DESs and NADESs for extracting high-value bioactive compounds from algae and cyanobacteria. The evidence indicates that these solvents can offer clear advantages for algal and cyanobacterial bioprocessing, but performance depends on solvent design and operating conditions and optimization is required for specific feedstocks and target fractions. Their ability to operate under mild conditions and to solubilize a wide range of biomolecules further supports their relevance for sustainable processing.

Within these boundaries, algae and cyanobacteria remain highly attractive resources owing to their environmental sustainability, nutritional value, and diverse bioactive properties. By carefully selecting DES for pretreatment, selective extraction, and stabilization, these biological advantages can be maximized while mitigating undesirable taste and odor. Moreover, where safety and regulatory frameworks permit, certain NADES may even be retained in the final product, thereby eliminating the need for an additional solvent-removal step.

Most studies to date have focused on European biomass, with *Arthrospira*, *Chlorella*, *Nannochloropsis*, and *Scenedesmus* most frequently investigated, underscoring both their biotechnological potential and availability. To enable comparability and scale-up, studies should consistently report processing conditions (pretreatment and extraction), adopt harmonized protocols for quantifying component concentrations and biological activities, and provide numerical data that enable cross-study calculation of recovery (*e.g.*, extract yield in mg per g dry biomass and compound content in mg per g extract).

Key challenges, detailed in the preceding section, still limit broader deployment: high viscosity and hygroscopicity, analytical incompatibilities, composition-dependent cytotoxicity and uneven regulatory acceptance, and the need for rigorous sustainability assessment. Safety evaluation and regulatory alignment should begin early, while claims of "greenness" must be supported by full life-cycle assessment and techno-economic analysis that account for energy use, emissions, and potential toxicity. Emerging LCA evidence shows that DES sustainability is formulation-dependent, with some systems exhibiting higher environmental burdens than conventional solvents, underscoring the need for a case-by-case assessment.

Future research should focus on optimizing DES systems for specific algal and cyanobacterial strains and target fractions, scaling to industrial throughputs, and integrating them with other technologies for assisted extraction. Widespread adoption will require scalable processes, clear regulatory standards, and robust ecological and economic evidence. Despite current hurdles, DES integrated with algal biotechnology are well positioned for impact in high-value markets such as nutraceuticals, pharmaceuticals, and cosmetics. With targeted research, cross-disciplinary collaboration, and realistic sustainability assessments, DES and microalgae can move from promise to practice.

Author contributions

Conceptualization: K. P., J. V., A. R. D., data curation: K. P., formal analysis: K. P., J. V., funding acquisition: J. V., investigation: J. V., K. P., methodology: K. P., J. V., project administration: J. V., resources: J. V., software: K. P., supervision: J. V., A. R. D., validation: J. V., visualization: J. V., writing – original draft: K. P., J. V., writing – review & editing: J. V., A. R. D.

Conflicts of interest

There are no conflicts to declare.

Abbreviations

AcA	Acetic acid
ATPS	Aqueous two-phase systems
Bet	Betaine
BDO	1,4-Butanediol
ChCl	Choline chloride
CA	Citric acid
DcA	Decanoic acid
DES	Deep eutectic systems

DoA	Dodecanoic acid
EG	Ethylene glycol
Fen	Fenchyl alcohol
F	Fructose
Ger	Geraniol
Glu	Glucose
Gly	Glycerol
HxA	Hexanoic acid
Im	Imidazole
LcA	Lactic acid
LuA	Lauric acid
LevA	Levulinic acid
MA	Malic acid
MAE	Microwave-assissted extraction
Men	Menthol
NADES	Natural deep eutectic solvents
NoA	Nonanoic acid
OcA	Octanoic acid
OD	1,2-Octanediol
OleA	Oleic acid
PEG	Polyethylene glycol
PLE	Pressurized liquid extraction
Pro	Proline
PD	1,2-Propanediol
PDO	1,3-Propanediol
PEF	Pulsed electric field
AcNa	Sodium acetate
TMAC	Tetramethylammonium chloride
Thy	Thymol
U	Urea
UAE	Ultrasound-assisted extraction
W	Water
Xyl	Xylitol

Data availability

The data supporting this article are included within the manuscript.

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References

1 P. L. Show, Global market and economic analysis of microalgae technology: Status and perspectives, *Bioresour. Technol.*, 2022, **357**, 127329.
2 F. K. Sarsekeyeva, A. K. Sadvakasova, S. K. Sandybayeva, B. D. Kossalbayev, Z. Huang, B. K. Zayadan, N. R. Akmukhanova, Y. K. Leong, J. S. Chang and S. I. Allakhverdiev, Microalgae- and cyanobacteria-derived phytostimulants for mitigation of salt stress and improved agriculture, *Algal Res.*, 2024, **82**, 103686.
3 T. Suganya, M. Varman, H. H. Masjuki and S. Renganathan, Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: a biorefinery approach, *Renewable Sustainable Energy Rev.*, 2016, **55**, 909–941.
4 J. Hernández-Urcera, A. Romero, P. Cruz, V. Vasconcelos, A. Figueras, B. Novoa and F. Rodríguez, Screening of microalgae for bioactivity with antiviral, antibacterial, anti-inflammatory and anti-cancer assays, *Biology*, 2024, **13**, 255.
5 A. Ferreira, J. Vladic, D. de Oliveira Corrêa, V. L. L. Butzke, P. L. Martins, B. Ribeiro, C. Marques-dos-Santos, F. G. Acién and L. Gouveia, Innovative approach in sustainable agriculture: Harnessing microalgae potential via subcritical water extraction, *Environ. Technol. Innovation*, 2024, **36**, 103797.
6 L. Novoveská, S. L. Nielsen, O. T. Eroldoğan, B. Z. Haznedaroglu, B. Rinkevich, S. Fazi, J. Robbens, M. Vasquez and H. Einarsson, Overview and challenges of large-scale cultivation of photosynthetic microalgae and cyanobacteria, *Mar. Drugs*, 2023, **21**, 445.
7 F. Sultana, M. A. Wahab, M. Nahiduzzaman, M. Mohiuddin, M. Z. Iqbal, A. Shakil, A. Mamun, S. R. Khan, L. Wong and M. Asaduzzaman, Seaweed farming for food and nutritional security, climate change mitigation and adaptation, and women empowerment: A review, *Aquacult. Fish.*, 2023, **8**, 463–480.
8 G. A. Colusse, J. Carneiro, M. E. R. Duarte, J. C. D. Carvalho and M. D. Noseda, Advances in microalgal cell wall polysaccharides: a review focused on structure, production, and biological application, *Crit. Rev. Biotechnol.*, 2022, **42**, 562–577.
9 T. M. Bernaerts, L. Gheysen, I. Foubert, M. E. Hendrickx and A. M. Van Loey, The potential of microalgae and their biopolymers as structuring ingredients in food: A review, *Biotechnol. Adv.*, 2019, **37**, 107419.
10 A. T. Hoang, R. Sirohi, A. Pandey, S. Nižetić, S. S. Lam, W.-H. Chen, R. Luque, S. Thomas, M. Arici and V. V. Pham, Biofuel production from microalgae: challenges and chances, *Phytochem. Rev.*, 2023, **22**, 1089–1126.
11 S. Adarshan, V. S. S. Sree, P. Muthuramalingam, K. S. Nambiar, M. Sevanan, L. Satish, B. Venkidasamy, P. G. Jeelani and H. Shin, Understanding macroalgae: A comprehensive exploration of nutraceutical, pharmaceutical, and omics dimensions, *Plants*, 2023, **13**, 113.

12 M. A. Sinetova, E. V. Kupriyanova and D. A. Los, Spirulina/Arthrospira/Limnospira—Three names of the single organism, *Foods*, 2024, **13**, 2762.
13 J. Vladic, S. Radman, Ž Žižak, I. Besu, I. Jerković, L. G. Speranza, A. Furqan Hala, S. Kovačević, H. Perreira and L. Gouveia, Clean production of microalgae high-value lipid fraction: Influence of different pretreatments on chemical and cytotoxic profiles of Chlorella vulgaris supercritical extracts and life cycle assessment, *J. Cleaner Prod.*, 2025, **491**, 144823.
14 A. N. Shikov, E. D. Obluchinskaya, E. V. Flisyuk, I. I. Terninko, Y. E. Generalova and O. N. Pozharitskaya, The impact of natural deep eutectic solvents and extraction method on the co-extraction of trace metals from Fucus vesiculosus, *Mar. Drugs*, 2022, **20**, 324.
15 E. L. Smith, A. P. Abbott and K. S. Ryder, Deep eutectic solvents (DESs) and their applications, *Chem. Rev.*, 2014, **114**, 11060–11082.
16 L. Benvenutti, A. A. F. Zielinski and S. R. S. Ferreira, Which is the best food emerging solvent: IL, DES or NADES?, *Trends Food Sci. Technol.*, 2019, **90**, 133–146.
17 J. B. González-Campos, A. Pérez-Nava, M. Valle-Sánchez and L. H. Delgado-Rangel, Deep eutectic solvents applications aligned to 2030 United Nations Agenda for Sustainable Development, *Chem. Eng. Process.*, 2024, **199**, 109751.
18 United Nations, Transforming our world: The 2030 agenda for sustainable development, https://sdgs.un.org/2030agenda, (accessed August 2025).
19 P. Moreno Martínez, V. M. Ortiz-Martínez, S. Sánchez Segado, M. J. Salar-García, A. P. de los Ríos, F. J. Hernández Fernández, L. J. Lozano-Blanco and C. Godínez, Deep eutectic solvents for the extraction of fatty acids from microalgae biomass: Recovery of omega-3 eicosapentaenoic acid, *Sep. Purif. Technol.*, 2022, **300**, 121842.
20 D. Moldes, P. F. Requejo, M. Vega, S. Bolado, R. H. Wijffels and A. Kazbar, Protein extraction from seaweed Saccharina latissima with deep eutectic solvents, *Microchem. J.*, 2024, **205**, 111275.
21 W. M. A. Wan Mahmood, A. Lorwirachsutee, C. Theodoropoulos and M. Gonzalez-Miquel, Polyol-based deep eutectic solvents for extraction of natural polyphenolic antioxidants from Chlorella vulgaris, *ACS Sustainable Chem. Eng.*, 2019, **7**, 5018–5026.
22 Z. M. Jiang, L. J. Wang, Z. Gao, B. Zhuang, Q. Yin and E. H. Liu, Green and efficient extraction of different types of bioactive alkaloids using deep eutectic solvents, *Microchem. J.*, 2019, **145**, 345–353.
23 J. Vladic, S. Kovačević, S. Rebocho, A. Paiva, S. Jokić, A. R. Duarte and I. Jerković, A new green approach for Lavandula stoechas aroma recovery and stabilization coupling supercritical CO_2 and natural deep eutectic solvents, *Sci. Rep.*, 2023, **13**, 12443.
24 S. Khodaverdian, B. Dabirmanesh, A. Heydari, E. Dashtban-moghadam, K. Khajeh and F. Ghazi, Activity, stability and structure of laccase in betaine based natural deep eutectic solvents, *Int. J. Biol. Macromol.*, 2018, **107**, 2574–2579.
25 O. Zannou and I. Koca, Optimization and stabilization of the antioxidant properties from alkanet (Alkanna tinctoria) with natural deep eutectic solvents, *Arabian J. Chem.*, 2020, **13**, 6437–6450.
26 J. Vladic, I. Jerković, V. Pavić, D. Cvetković, S. Jokić, K. Aladić, K. Pastor and A. R. C. Duarte, Exploring the potential of deep eutectic systems for the preservation of the chemical profile and antibacterial potential of dill (Anethum graveolens L.) supercritical CO_2 extracts, *J. Supercrit. Fluids*, 2025, **218**, 106499.
27 J. B. Barbieri, C. Goltz, F. B. Batistão Cavalheiro, A. T. Toci, L. Igarashi-Mafra and M. R. Mafra, Deep eutectic solvents applied in the extraction and stabilization of rosemary (Rosmarinus officinalis L.) phenolic compounds, *Ind. Crops Prod.*, 2020, **144**, 112049.
28 Food and Agriculture Organization of the United Nations (FAO) https://www.fao.org/asiapacific/news/news-detail/fao-report–global-fisheries-and-aquaculture-production-reaches-a-new-record-high/en, (accessed August 2025).
29 E. A. Asevedo, B. M. E. das Chagas, S. D. de Oliveira Júnior and E. S. dos Santos, Recovery of lipids and carotenoids from Dunaliella salina microalgae using deep eutectic solvents, *Algal Res.*, 2023, **69**, 102940.
30 B. R. Danilović, N. G. Đorđević, I. T. Karabegović, D. Z. Troter, D. S. Savić and V. B. Veljković, Enhancing lipid extraction from green microalgae Chlorella sp. using a deep eutectic solvent pretreatment, *Chem. Ind. Chem. Eng. Q.*, 2021, **27**, 313–317.
31 N. Nemani, S. M. Dehnavi and G. Pazuki, Extraction and separation of astaxanthin with the help of pre-treatment of Haematococcus pluvialis microalgae biomass using aqueous two-phase systems based on deep eutectic solvents, *Sci. Rep.*, 2024, **14**, 5420.
32 M. Kent, H. M. Welladsen, A. Mangott and Y. Li, Nutritional evaluation of Australian microalgae as potential human health supplements, *PLoS One*, 2015, **10**, e0118985.
33 A. Kuech, M. Breuer and I. Popescu, *Research for PECH Committee - The future of the EU algae sector*, European Parliament, Policy Department for Structural and Cohesion Policies, Brussels, 2023.
34 European Marine Observation and Data Network (EMODnet), Map of the Week - Algae production facilities, 3 July 2020, https://emodnet.ec.europa.eu/en/map-week-algae-production-facilities, (accessed August 2025).
35 European Commission, Towards a Strong and Sustainable EU Algae Sector 2022, https://oceans-and-fisheries.ec.europa.eu/publications/communication-commission-towards-strong-and-sustainable-eu-algae-sector_en, (accessed August 2025).
36 European Maritime, Aquaculture and Fisheries Fund (EMFAF), Study to support a Sustainable EU Algae Industry Executive Summary, CINEA/2023/OP/0006/SI2.906327, June 2025, DOI: DOI: 10.2926/2125698, https://cinea.ec.europa.eu/document/download/7485d1e8-e685-4d8c-b068-

c635cae8d73a_en?filename=Ex.summary_study%20to%20support%20a%20sustainable%20eu%20algae%20industry-HZ0125111ENN.pdf, (accessed August 2025).
37 I. Gallego, N. Medić, J. S. Pedersen, P. K. Ramasamy, J. Robbens, E. Vereecke and J. Romeis, The microalgal sector in Europe: Towards a sustainable bioeconomy, *New Biotechnol.*, 2025, **86**, 1–13.
38 N. Ozel, A. Inam and M. Elibol, Exploring deep eutectic solvents for enhanced extraction of bioactive compounds from microalgae biomass, *J. Mol. Liq.*, 2024, **407**, 125237.
39 C. Fan, Y. Liu, Y. Shan and X. Cao, A priori design of new natural deep eutectic solvent for lutein recovery from microalgae, *Food Chem.*, 2022, **376**, 131930.
40 Algal Pigments Market Research Report: By type (chlorophylls, carotenoids, phycoerythrin, phycocyanin), by application (food and beverage, cosmetics and personal care, pharmaceuticals, nutraceuticals, industrial), By Source (Spirulina, Chlorella, Dunaliella, Haematococcus pluvialis, other sources), by form (powder, liquid, extract, capsules, tablets) and by regional (North America, Europe, South America, Asia Pacific, Middle East and Africa) - Forecast to 2034, https://www.marketresearchfuture.com/reports/algal-pigments-market-25730, (accessed August 25, 2025).
41 Research and Markets, Algae Proteins - Global Strategic Business Report, https://www.researchandmarkets.com/reports/5029927/algae-proteins-global-strategic-business-report#src-pos-1, (accessed August 2025).
42 I. A. Severo, G. S. de Lira, R. R. Ambati, R. A. Gokare, J. V. C. Vargas, J. Ordonez and A. B. Mariano, Disruptive potential of microalgae proteins: Shaping the future of the food industry, *Future Foods*, 2024, **9**, 100318.
43 R. Craveiro, F. Dusschooten, A. R. Nabais, I. Boboescu, C. Lo, L. A. Neves and M. Sá, Deep eutectic systems for carbonic anhydrase extraction from microalgae biomass to improve carbon dioxide solubilization, *J. CO_2 Util.*, 2022, **65**, 102225.
44 M. Cokdinleyen, G. Domínguez-Rodríguez, H. Kara, E. Ibáñez and A. Cifuentes, New green biorefinery strategies to valorize bioactive fractions from Palmaria palmata, *Mar. Drugs*, 2024, **22**, 467, DOI: 10.3390/md22100467.
45 K. Li, C. Jiang, S.-I. Han, S. Kang, J. Chen, D. Won, Y. Kang, B. Bae, Y.-E. Choi, H. S. Kim and J. Lee, Green and efficient method to acquire high-value phycobiliprotein from microalgal biomass involving deep eutectic solvent-based ultrasound-assisted extraction, *Food Chem.*, 2024, **449**, 139196.
46 C. Lo, R. H. Wijffels and M. H. M. Eppink, Lipid extraction from fresh Nannochloropsis oceanica using semi-hydrophobic eutectic solvents, *Algal Res.*, 2023, **72**, 103117.
47 S. Dubey, C. W. Chen, A. K. Patel, S. K. Bhatia, R. R. Singhania and C. D. Dong, Development in health-promoting essential polyunsaturated fatty acids production by microalgae: a review, *J. Food Sci. Technol.*, 2024, **61**(5), 847–860.
48 A. Jabłońska-Trypuć, E. Wołejko, M. D. Ernazarovna, A. Głowacka, G. Sokołowska and U. Wydro, Using algae for biofuel production: a review, *Energies*, 2023, **16**(4), 1758.
49 P. A. García-Soto, M. I. S. de Santiago, M. J. Salar-García, S. Sánchez-Segado and V. M. Ortiz-Martínez, Study of the effect of water content in deep eutectic phases on the extraction of fatty acids from microalgae biomass, *Appl. Sci.*, 2023, **13**, 12680.
50 L. Wils, M. Yagmur, M. Phelippe, B. Montigny, B. Clément-Larosière, J. Jacquemin and L. Boudesocque-Delaye, Alternative solvents for the biorefinery of Spirulina: Impact of pretreatment on free fatty acids with high added value, *Mar. Drugs*, 2022, **20**, 600.
51 L. Wils, M. Yagmur, N. Bellin, M. Phelippe, A. Chevalley, C. Bodet and L. Boudesocque-Delaye, Innovative alkanediol-based eutectic solvents for extracting/pre-formulating dermatologically valuable free fatty acids from Spirulina and Porphyridium cakes, *Mar. Drugs*, 2024, **22**, 281.
52 J. Resende, F. H. B. Sosa, J. A. P. Coutinho, J. Rocha, A. J. D. Silvestre and S. A. O. Santos, Sustainable phytosterol extraction from Codium tomentosum using eutectic solvents, *ACS Sustainable Chem. Eng.*, 2024, **12**, 9037–9044.
53 D. Xu, J. Chow, C. C. Weber, M. A. Packer, S. Baroutian and K. Shahbaz, Evaluation of deep eutectic solvents for the extraction of fucoxanthin from the alga Tisochrysis lutea - COSMO-RS screening and experimental validation, *J. Environ. Chem. Eng.*, 2022, **10**, 108370.
54 E. D. Obluchinskaya, O. N. Pozharitskaya, L. V. Zakharova, A. V. Daurtseva, E. V. Flisyuk and A. N. Shikov, Efficacy of natural deep eutectic solvents for extraction of hydrophilic and lipophilic compounds from Fucus vesiculosus, *Molecules*, 2021, **26**, 4198.
55 Á. Morón-Ortiz, P. Mapelli-Brahm and A. J. Meléndez-Martínez, Sustainable green extraction of carotenoid pigments: Innovative technologies and bio-based solvents, *Antioxidants*, 2024, **13**, 239.
56 W. Pitacco, C. Samorì, L. Pezzolesi, V. Gori, A. Grillo, M. Tiecco, M. Vagnoni and P. Galletti, Extraction of astaxanthin from Haematococcus pluvialis with hydrophobic deep eutectic solvents based on oleic acid, *Food Chem.*, 2022, **379**, 132156.
57 D. Yang, W. Qiu, Y. Xu, Z. Hu and L. Wang, Optimisation and modelling of ultrasonic-assisted extraction of canthaxanthin from Chromochloris zofingiensis using eutectic solvents, *Ind. Crops Prod.*, 2023, **202**, 117002.
58 R. Martins, C. Mouro, R. Pontes, J. Nunes and I. Gouveia, Ultrasound-assisted extraction of bioactive pigments from Spirulina platensis in natural deep eutectic solvents, *Bioresour. Bioprocess.*, 2023, **10**, 88.
59 S. Hilali, L. Van Gheluwe, M. Yagmur, L. Wils, M. Phelippe, B. Clément-Larosière, B. Montigny, J. Jacquemin, E. Thiery and L. Boudesocque-Delaye, NADES-based biorefinery of Spirulina (Arthrospira platensis): A new path for sustainable high value-added metabolites, *Sep. Purif. Technol.*, 2024, **329**, 125123.
60 L. Wils, C. Leman-Loubière, N. Bellin, B. Clément-Larosière, M. Pinault, S. Chevalier, C. Enguehard-Gueiffier, C. Bodet and L. Boudesocque-Delaye, Natural deep eutectic

solvent formulations for spirulina: Preparation, intensification, and skin impact, *Algal Res.*, 2021, **56**, 102317.

61 A. S. Fernandes, P. A. Caetano, E. Jacob-Lopes, L. Queiroz Zepka and V. V. de Rosso, Alternative green solvents associated with ultrasound-assisted extraction: A green chemistry approach for the extraction of carotenoids and chlorophylls from microalgae, *Food Chem.*, 2024, **455**, 139939.

62 M. D. Gkioni, V. Andriopoulos, E. Koutra, S. Hatziantoniou, M. Kornaros and F. N. Lamari, Ultrasound-assisted extraction of Nannochloropsis oculata with ethanol and betaine: 1,2-propanediol eutectic solvent for antioxidant pigment-rich extracts retaining nutritious the residual biomass, *Antioxidants*, 2022, **11**, 1103.

63 Y. Xu, Q. Wang and Y. Hou, Efficient purification of R-phycoerythrin from marine algae (Porphyra yezoensis) based on a deep eutectic solvents aqueous two-phase system, *Mar. Drugs*, 2020, **18**, 618.

64 L. Van Gheluwe, S. Odou, M. Yagmur, I. Théry-Koné, M. Phelippe, A. Chevalley and L. Boudesocque-Delaye, Single-step extraction/pre-formulation process for B-phycoerythrin using glycerol-based eutectic solvents: A step toward more sustainable production of phycobiliproteins, *Sustainable Chem. Pharm.*, 2024, **40**, 101654.

65 B. A. Pereira, C. T. Matos, L. Costa, L. M. Ferreira, J. G. Crespo and C. Brazinha, Sustainable processing of microalgae protein: Design of biphasic partitioning systems based on natural deep eutectic solvents for C-phycocyanin recovery from model aqueous solutions, *Sep. Purif. Technol.*, 2025, **353**, 128510.

66 S. B. Hashemi, A. Rahimi and M. Arjmand, Extraction of nutraceutical bioactive compounds from native algae using solvents with a deep natural eutectic point and ultrasonic-assisted extraction, *CRPASE*, 2023, **9**, 2848.

67 R. Fassi Fihri, A. Ez-Zoubi, L. Mbarkiou, A. Amar, A. Farah and E. O. Bouchamma, Antibacterial and antioxidant activities of Chlorella vulgaris and Scenedesmus incrassatulus using natural deep eutectic solvent under microwave assisted by ultrasound, *Heliyon*, 2024, **10**, e35071.

68 P. A. Shah, V. Chavda, D. Hirpara, V. S. Sharma, P. S. Shrivastav and S. Kumar, Exploring the potential of deep eutectic solvents in pharmaceuticals: Challenges and opportunities, *J. Mol. Liq.*, 2023, **390**, 123171.

69 R. Gera, C. J. Moll, A. Bhattacherjee and H. J. Bakker, Water-induced restructuring of the surface of a deep eutectic solvent, *J. Phys. Chem. Lett.*, 2022, **13**, 634–641.

70 E. Benítez-Correa, J. M. Bastías-Montes, S. Acuña-Nelson and O. Muñoz-Fariña, Effect of choline chloride-based deep eutectic solvents on polyphenols extraction from cocoa bean shells and antioxidant activity of extracts, *Curr. Res. Food Sci.*, 2023, **7**, 100614.

71 Y. Dai, G.-J. Witkamp, R. Verpoorte and Y. H. Choi, Tailoring properties of natural deep eutectic solvents with water to facilitate their applications, *Food Chem.*, 2015, **187**, 14–19.

72 H. Monteiro, A. Paiva, A. R. C. Duarte and N. Galamba, Structure and dynamic properties of a glycerol–betaine deep eutectic solvent: When does a DES become an aqueous solution?, *ACS Sustainable Chem. Eng.*, 2022, **10**, 3501–3512.

73 S. Bhattacharjee, R. Dikki, B. Gurkan and R. B. Getman, Effect of water on eutectic solvents: structural properties and physical interactions with CO_2, *J. Mol. Liq.*, 2024, **410**, 125569.

74 L. K. Savi, M. C. G. C. Dias, D. Carpine, N. Waszczynskyj, R. H. Ribani and C. W. I. Haminiuk, Natural deep eutectic solvents based on citric acid and sucrose: effect of water inclusion on thermal, physical and rheological properties, *Int. J. Food Sci. Technol.*, 2019, **54**, 898–907.

75 A. Mero, S. Koutsoumpos, P. Giannios, I. Stavrakas, K. Moutzouris, A. Mezzetta and L. Guazzelli, Comparison of physicochemical and thermal properties of choline chloride- and betaine-based deep eutectic solvents: influence of component nature and molar ratios, *J. Mol. Liq.*, 2023, **377**, 121563.

76 L. V. Tavares, D. de Alencar, S. B. Rodríguez-Reartes, F. W. Tavares and F. Llovell, Assessing viscosity in sustainable deep eutectic solvents and cosolvent mixtures: an artificial neural network-based molecular approach, *ACS Sustainable Chem. Eng.*, 2024, **12**, 7987–8000.

77 V. Jančíková, M. Jablonský, K. Voleková and I. Šurina, Summarizing the effect of acidity and water content in deep eutectic solvent-like mixtures: a review, *Energies*, 2022, **15**, 9333.

78 A. Roosta, R. Haghbakhsh, A. R. C. Duarte and S. Raeissi, Deep eutectic solvent viscosity prediction by hybrid machine learning and group contribution, *J. Mol. Liq.*, 2023, **388**, 122747.

79 J. F. Fabre, N. U. F. Niangoran, C. Gaignard, D. Buso, Z. Mouloungui and R. Valentin, Extraction of C-PC from Arthrospira platensis: Use of ultrasounds, organic solvents and deep eutectic solvents, *Eur. Food Res. Technol.*, 2024, **250**, 1149–1161.

80 K. J. Lanjekar and V. K. Rathod, Application of ultrasound and natural deep eutectic solvent for the extraction of glycyrrhizic acid from Glycyrrhiza glabra: Optimization and kinetic evaluation, *Ind. Eng. Chem. Res.*, 2021, **60**, 9532–9538.

81 J. Zhou, M. Wang, J. A. Saraiva, A. P. Martins, C. A. Pinto, M. Á. Prieto, J. Simal-Gandara, H. Cao, J. Xiao and F. J. Barba, Extraction of lipids from microalgae using classical and innovative approaches, *Food Chem.*, 2022, **384**, 132236.

82 R. V. Kapoore, T. O. Butler, J. Pandhal and S. Vaidyanathan, Microwave-assisted extraction for microalgae: from biofuels to biorefinery, *Biology*, 2018, **7**, 18.

83 E. Quitério, C. Grosso, R. Ferraz, C. Delerue-Matos and C. Soares, A critical comparison of the advanced extraction techniques applied to obtain health-promoting compounds from seaweeds, *Mar. Drugs*, 2022, **20**, 677.

84 J. Bintanel-Cenis, M. A. Fernández, B. Gómara and L. Ramos, Critical overview on the use of hydrophobic (deep) eutectic solvents for the extraction of organic pollutants in complex matrices, *Talanta*, 2024, **270**, 125599.

85 G. Bechis, G. Mastellone, A. Marengo, B. Sgorbini, C. Cagliero and P. Rubiolo, Hydrophobic natural eutectic solvents for the gas chromatographic determination of suspected allergens in fragrances by dispersive liquid-liquid microextraction, *Separations*, 2022, **9**, 318.
86 M. Hayyan, Y. P. Mbous, C. Y. Looi, W. F. Wong, A. Hayyan, Z. Salleh and O. Mohd-Ali, Natural deep eutectic solvents: cytotoxic profile, *SpringerPlus*, 2016, **5**(1), 913.
87 Q. Zaib, M. J. Eckelman, Y. Yang and D. Kyung, Are deep eutectic solvents really green?: A life-cycle perspective, *Green Chem.*, 2022, **24**, 7924–7930.
88 S. Nejrotti, A. Antenucci, C. Pontremoli, L. Gontrani, N. Barbero, M. Carbone and M. Bonomo, Critical assessment of the sustainability of deep eutectic solvents: a case study on six choline chloride-based mixtures, *ACS Omega*, 2022, **7**, 47449–47461.