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Research Article

Sustainable Phytosterol Extraction from *Codium tomentosum* Using Eutectic Solvents

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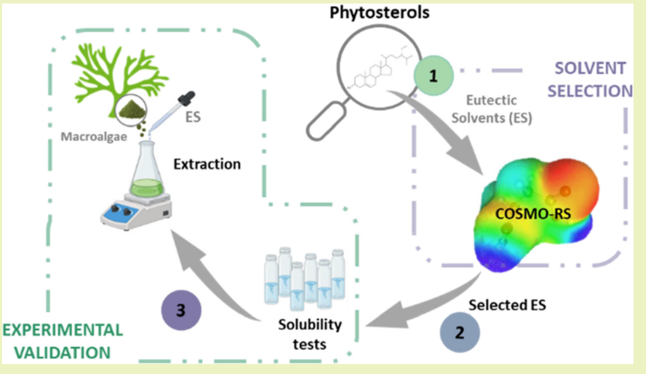
ABSTRACT: Macroalgae are a promising source of natural compounds like phytosterols, known for their cholesterol-lowering effects and other biological properties. However, they have often been challenging to extract efficiently due to a lack of sustainable extraction techniques. This study aimed to exploit eutectic solvents (ES) in the sustainable extraction of phytosterols. The conductor-like screening model for real solvents (COSMO-RS) was used as an initial screening tool to select the most suitable combination from a pool of 35 HBA and 64 HBD (2240 mixtures in total), thus reducing the number of experiments required. ES composed of terpenes, fatty acids, and some alcohols were found to be the most suitable solvents for phytosterols extraction. After validation using a standard mixture of sterols, the selected ES were successfully used to extract phytosterols from the macroalga *Codium tomentosum*. A maximum extraction yield of 3.16 ± 0.01 mg phytosterols/g dry macroalgae weight was obtained with ES menthol:octanoic acid (Men:AcC8), which was further selected to perform a sequential extraction procedure, allowing to obtain an enriched extract with a 4.4-fold increase in the phytosterol content after four extraction cycles. Overall, this study provides a promising, sustainable, and environmentally friendly approach to obtain bioactive phytosterols enriched extracts from macroalgae.



KEYWORDS: *phytosterols extraction, eutectic solvents, COSMO-RS, bioactive compounds, green extraction, Codium tomentosum*

INTRODUCTION

There is an increasing trend for revisiting natural resources, including marine organisms toward the discovery of bioactive components[1] within the emerging Blue and Circular Economy and biorefinery concepts. Macroalgae, also called seaweed, are a promising and almost unlimited source of bioactive and exclusive bioactive compounds, promising for multiple applications.[2] Although the global use of macroalgae is a billion-dollar industry in terms of biotechnology development,[3] this market is largely focused on polysaccharides, pigments or minerals, neglecting less abundant, yet highly promising bioactive molecules, namely, lipophilic compounds, polar lipids, proteins, carbohydrates, vitamins, fibers, and phenolic compounds.[4]

Phytosterols, a group of sterols derived from plants, which are one of the high-value class of compounds that can be extracted from macroalgae, have gained increased attention due to their blood cholesterol-lowering properties[5,6] along with other health-promoting properties, such as anti-inflammatory, antibacterial, or antiproliferative activities,[5] which make them very promising for high-value applications, such as nutraceuticals or pharmaceuticals.

Macroalgae represent a rich source of phytosterols, exhibiting a diverse composition that varies significantly depending on the species, which can also be useful in taxonomic classification.[7,8] While the red (Rhodophyta) and brown (Phaeophyta) macroalgae generally possess a single primary phytosterol (cholesterol and fucosterol, respectively), the green macroalgae (Chlorophyta) are significantly more diverse and exhibit a complex mixture of phytosterols in their composition compared with the other macroalgae groups.

However, despite ongoing advances, there is still a lack of ecofriendly and efficient extraction methods to obtain phytosterols from these sources. Commonly used methods are still based on conventional methods using mostly organic and often toxic solvents, which might compromise their use as nutraceuticals and pharmaceuticals.

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Table 1. Compositions, Solvent Formation Time, and Abbreviations for the Prepared ES

HBA: HBD	molar ratio	water addition (%)	solvent formation time (min)	acronym
menthol:octanoic acid	1:1		5	Men:AcC8
menthol:decanoic acid	1:1		8	Men:AcC10
menthol:thymol	1:1		7	Men:Thy
octanoic acid:decanoic acid	1:1		4	AcC8:AcC10
thymol:octane-1,8-diol	1:1	5	15	Thy:AlC8
choline chloride:glycerol	1:1	5	12	ChCl:Gly

Innovative extraction solutions, fulfilling the principles of Green Chemistry[9] to reduce or eliminate hazardous substances and minimize energy consumption, such as those using green and alternative solvents, are seen nowadays to be promising in the extraction of bioactive components. Eutectic solvents (ES), most often known as deep eutectic solvents (DES), were defined for the first time in 2003 by Abbott et al.,[10] as liquid mixtures of two or more compounds that are capable of associating with each other, as a hydrogen bond acceptor (HBA) or a hydrogen bond donor (HBD), and form a eutectic mixture. These mixtures have unusual solvent properties and, as they can be formed from readily available compounds (in many cases from natural origin), can be considered as inexpensive (this is especially true when compared to the high price of common ionic liquids), versatile and green alternative solvents; in addition, ES are easy to prepare and their sustainability, biodegradability, and biocompatibility can vary depending on the specific HBA and HBD properties.[11] They have been successfully applied in the extraction of anthocyanins, flavonoids, phenolic acids, sugars, proteins, triterpenic acids, pigments, and fatty acids from natural resources.[12−14] More recently, some authors reported the application of ES and their corresponding aqueous solutions in the extraction of bioactive compounds from algae (mostly microalgae),[15−17] but to the best of our knowledge these solvents have never been applied in the extraction of phytosterols from macroalgae. The existing literature only reports the extraction of phytosterols with ES from cow's milk and from the mushroom *Agaricus bisporus* (*A. bisporus*) and none of these studies employed predictive models, such as conductor-like screening model for real solvents (COSMO-RS), to enhance the optimization of ES selection for the extraction process.[18,19]

The present work aims to develop a sustainable extraction methodology to obtain phytosterols enriched extracts from macroalgae using ES. Macroalga *Codium tomentosum* (*C. tomentosum*), where phytosterols are one of the most abundant class of lipophilic compounds,[20] was selected as biomass. In order to decrease the number of experiments necessary to select the best ES for that purpose, COSMO-RS was used as an initial screening tool to predict the relative solubility of β-sitosterol, used as a model compound, in the ES. After the validation of the predicted solubility data with a mixture of standards (including β-sitosterol), the selected ES were used to extract phytosterols from *C. tomentosum*, and the results were compared with those obtained by conventional Soxhlet extraction with dichloromethane (DCM). Finally, for the most suitable ES, a sequential extraction using renewed biomass was studied aiming at maximizing the phytosterols content on the solvent.

EXPERIMENTAL SECTION

Materials. *Codium tomentosum* samples collected in October 2022 (C2.111022F) were provided by ALGAplus Lda, a company dedicated to the production of edible seaweeds in an integrated multitrophic aquaculture system from a production site located at Ria de Aveiro coastal lagoon, Northern Portugal, 40°36′43″ N, 8°40′43″ W. The biomass was hand-washed with distilled water to remove salts, epiphytes, and/or microorganisms, frozen at −80 °C and lyophilized. The dried biomass was ground in a laboratory mill and stored in hermetically sealed bags in a desiccator until further use.

DCM (p.a., ≥99% purity) was supplied by Fischer Scientific (Pittsburgh, PA, USA). Pyridine (p.a., ≥99.5% purity), N,O-bis(trimethylsilyl)trifluoroacetamide (99% purity), trimethylchlorosilane (99% purity), tetracosane (99% purity), stigmasterol (95% purity), β-sitosterol (≥70% purity), octanoic acid (≥98% purity), and choline chloride (≥98% purity) were obtained from Merck (Madrid, Spain). Menthol (99% purity) and decanoic acid (99% purity) were supplied by Thermo Scientific (USA). Thymol (99.5% purity) was supplied by TCI (USA). Octane-1,8-diol (98% purity) was supplied by Alfa Aesar (USA). Glycerol was supplied by VWR. All reagents were of analytical grade or of the highest available purity.

COSMO-RS Model: Screening of ES. The COSMO-RS calculations involved a two-step process. Initially, the COSMO-BP-TZVP template of the TmoleX software package41 (interface of TURBOMOLE), which includes a def-TZVP basis set, DFT with the B−P83 functional level of theory, and the COSMO solvation model (infinite permittivity) was employed to optimize the geometry of each molecule, including the HBAs, HBDs, and the sterol model.[21] Following this, COSMO-RS calculations were performed using the software COSMOtherm (Version 21.0) package31 with the BP_TZVP_21.ctd parametrization.[22] In COSMO-RS calculation, ES were treated as binary mixtures of HBD and HBA at a fixed stoichiometric ratio.[23] COSMO-RS was utilized to predict the activity coefficient at infinite dilution of the sterol model (β-sitosterol) in 2240 combinations of HBAs and HBDs at 25 °C. The activity coefficient at infinite dilution ($\gamma\infty$) served as the criterion for selecting the most potential HBA/HBD combinations to be experimentally tested. A database of HBAs and HBDs commonly employed in the extraction of bioactive compounds was compiled based on literature reports. This database comprised 35 HBA and 64 HBD, as detailed in the Supporting Information, Table S1.

ES Preparation. Based on the COSMO-RS results, we combined four HBAs (Menthol (Men), thymol (Thy), octanoic acid (AcC8), and choline chloride (ChCl)), along with five HBDs (octanoic acid (AcC8), decanoic acid (AcC10), thymol (Thy), octane-1,8-diol (AlC8), and glycerol (Gly)), to form ES. These ES were then assessed as potential solvents for extracting phytosterols from *C. tomentosum*.

The six ES tested (Table 1) were prepared by mixing the respective precursors (HBA and HBD), at a molar ratio of 1:1, followed by heating at 70 °C with constant stirring until a homogeneous transparent and uniform liquid was formed (solvent formation time). After that, the mixtures were kept in these conditions for 1 h before returning to room temperature. In the case of Thy:AlC8 and ChCl:Gly, 5% water was added in order to maintain the mixture liquid at room temperature.

Validation of the COSMO-RS Prediction with Standards. To validate the solubility trends predicted from the COSMO-RS, the six ES selected were tested in the solubilization of a commercial mixture of phytosterols (≥70% β-Sitosterol and minor amounts of

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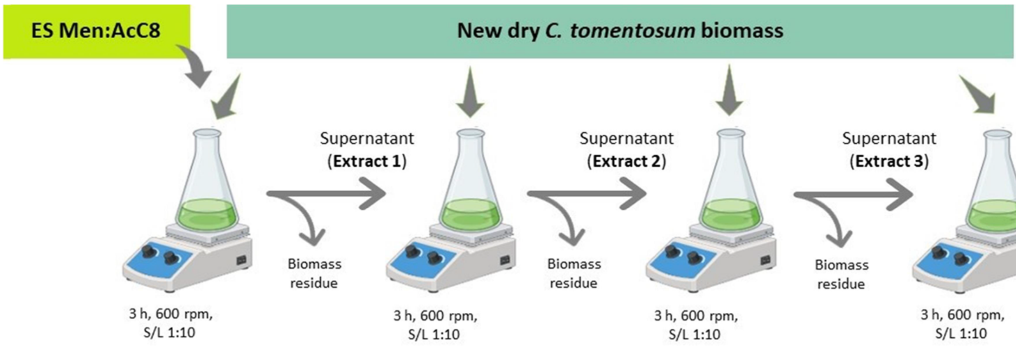

Figure 1. Scheme of the sequential extraction (four cycles) using ES Men:AcC8 as extraction solvent, with a ratio of 1:10 in all the extractions.

stigmastanol, campesterol, cholestane-3-ol and stigmasterol) by adding this mixture to the ES until saturation. After filtration, the soluble fraction was quantified by gas chromatography−mass spectroscopy (GC−MS) and the results were expressed in milligrams of phytosterol mixture/mL. Samples were prepared in triplicate (n = 3).

Extractions of Phytosterols. Extraction of 1 g of lyophilized macroalgae samples using different ES as extraction solvent (solid/liquid ratio of 1:10), at room temperature (25 °C), for 3 h under constant stirring (600 rpm) were performed in triplicate (n = 3). Phytosterols contents in the extracts were quantified by GC−MS and expressed as stigmasterol equivalents (mg/g dw).

Conventional Soxhlet extraction of lyophilized macroalgae was also performed for comparative purposes, according to the procedure proposed by Santos et al.[20] by extracting 5 g of lyophilized macroalgae samples with DCM in a Soxhlet apparatus for 8 h. Solvent was then evaporated to dryness, and lipophilic extracts were weighted. All experiments were performed in triplicate. Extracted phytosterols were expressed in milligrams of phytosterols/g of dry macroalgae weight (dw).

Sequential Extraction of Phytosterols. For the most suitable ES, a sequential extraction was performed, as represented in Figure 1. The sequential extraction was carried out by extracting 2 g of lyophilized *C. tomentosum* biomass, using the ES Men:AcC8 (S/L, 1:10), at room temperature, for 3 h under constant stirring (600 rpm). After the first extraction cycle, the mixture was centrifugated (6000 rpm, 15 min) and the supernatant was collected. An aliquot was withdrawn for GC−MS analysis, and the remaining Men:AcC8 solution was reused for the next extraction step, keeping extraction conditions constant. Four extraction cycles were performed.

GC−MS Analysis. Prior to GC−MS analysis, three aliquots of 150 μL of each ES extract, or approximately 15 mg of each dried extract, in the case of Soxhlet extracts with DCM, were derivatized to convert hydroxyl groups into trimethylsilyl (TMS) derivatives using a previously optimized methodology.[20] The extracts were dissolved in 250 μL of pyridine containing 0.6 mg tetracosane (internal standard), and then 250 μL of *N*,*O*-bis(trimethylsilyl)tri-fluoroacetamide and 50 μL of trimethylchlorosilane were added. The mixture was kept at 70 °C for 30 min. The GC−MS analysis was carried out using a GC−MS-QP2010 Ultra (Shimadzu, Kyoto, Japan) equipped with a DB-1 J&W capillary column (30 m × 0.32 mm inner diameter, 0.25 μm film thickness). The chromatographic conditions included an initial temperature of 80 °C for 5 min, followed by a temperature gradient of 4 °C/min to a final temperature of 260 °C, and then a temperature gradient of 2 °C/min to a final temperature of 285 °C for 13 min. The injector temperature was set at 250 °C, and the transfer-line temperature was set at 290 °C with a split ratio of 1:50. Helium was used as the carrier gas at a linear velocity of 40.0 cm/s. Compounds were identified as TMS derivatives by comparing their mass spectra with the GC−MS spectral library (Wiley 275 and U.S. National Institute of Science and Technology (NIST14)). Their mass spectrometry fragmentation profiles and characteristic retention times obtained under the same GC conditions were also compared with published data.[30] For semiquantitative analysis, the GC−MS was calibrated with pure reference stigmasterol and the response factor against tetracosane was calculated as an average of three GC−MS runs.

RESULTS AND DISCUSSION

COSMO-RS: Screening of ES. Owing to the numerous possible combinations of HBA and HBD to form ES, COSMO-RS was used in this work to predict the best solvents for the extraction of phytosterols. β-Sitosterol, one of the most abundant phytosterols in macroalgae[24] and also present in *C. tomentosum*,[20] was used as a model compound. The infinite dilution activity coefficient ($\gamma\infty$) was used as the criterion to evaluate the dissolution capability of a solute in a solvent, and its prediction can serve as a molecular descriptor to preselect a solvent.[25] As the solubility of a compound in a solvent is inversely proportional to the $\gamma\infty$, the smaller the value of ln $\gamma\infty$, the higher the solubility.[26] The ln $\gamma\infty$ values were estimated for a total of 2240 possible mixtures, considering equimolar combinations of 35 HBAs and 64 HBDs, at 25 °C. The results are shown in Figure 2, where the ln $\gamma\infty$ values were represented using a color scale. A lower ln $\gamma\infty$ value indicated a higher solvent capacity, with the mixtures exhibiting the highest solvating capacity located in the dark blue regions.

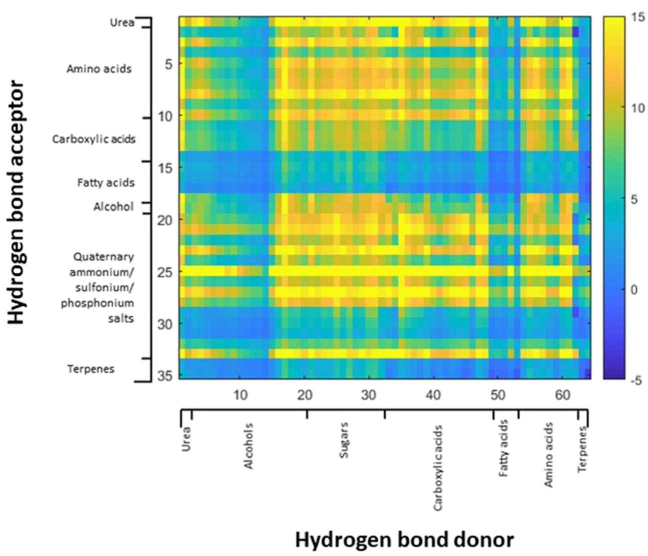

Figure 2. Predicted activity coefficients at infinite dilution (ln $\gamma\infty$) of β-sitosterol in eutectic solvents (1:1), at 25 °C using COSMO-RS. The labels of each corresponding hydrogen bond acceptor and hydrogen bond donor are in Table S1.

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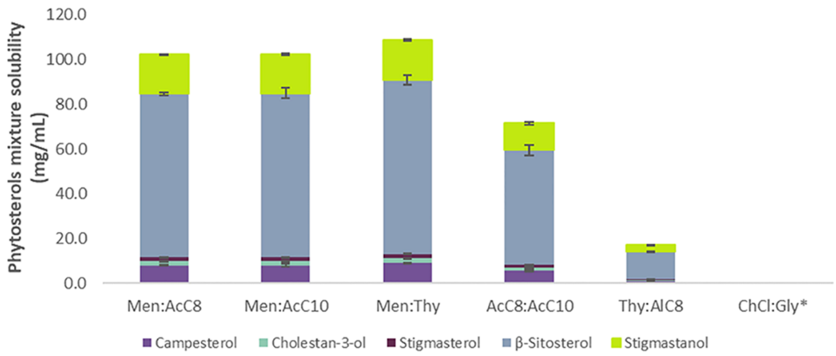

Figure 3. Validation of the predicted COSMO-RS results through the solubilization of a commercial mixture of phytosterols in the six ES selected. The results are expressed as expressed in mg phytosterols/mL. * Phytosterols were not detected.

The results reveal that the choice of HBA and HBD impacted the solubility of β-sitosterol. ES formed by fatty acids (15−18 compound number in Table S1), quaternary ammonium salts (29−31), or terpenes (34,35) as HBAs when combined with alcohols (11−14), fatty acids (15−18), or terpenes (34,35) as HBDs showed to lead to low ln $\gamma\infty$ values for β-sitosterol, suggesting that the solubility was dominated by the hydrophobicity of the ES. Considering the physicochemical properties of phytosterols,[27] particularly its nonpolar and fat-soluble nature, it was expected that the best ES to dissolve β-sitosterol would be those prepared from hydrophobic HBAs and HBDs, and the addition of water to the ES composition will have a negative impact on their solubility. Therefore, octanoic acid (15—Table S1), menthol (34—Table S1), and thymol (35—Table S1) combined with octane-1,8-diol (11—Table S1), octanoic acid (50—Table S1), decanoic acid (52—Table S1), and thymol (64—Table S1) were selected for the subsequent experimental screening. The ES choline chloride:glycerol (HBA 20, HBD 3—Table S1) was selected to represent the yellowish region (lower solubility).

Validation of the COSMO Prediction with Standards. Based on the COSMO-RS results, six ES were selected as potential solvents in the solubilization of a commercial mixture of phytosterols (≥70% β-sitosterol and minor amounts of stigmastanol, campesterol, cholestane-3-ol, and stigmasterol). The solvents were used at a molar ratio of 1:1 and the content of each compound in the ES was evaluated by GC−MS.

The solubility results, shown in Figure 3, demonstrate that, as predicted by COSMO-RS, all the selected ES, with the exception of ChCl:Gly were able to solubilize the sterols mixture, with solubilities ranging from 17.0 ± 0.5 to 109.0 ± 2.8 mg/mL. These ranges of solubility are higher than those observed for β-sitosterol in organic solvents.[28] The highest solubilities of the commercial mixture of phytosterols (102.4 ± 3.9 to 109.0 ± 2.8 mg/mL) were observed when menthol-based ES were used. Furthermore, it was verified that the components of the phytosterol mixture dissolve in identical ratios across all the ES. The order of solubilities of the mixture of phytosterols in the studied ES was: Men:Thy > Men:AcC10 ≈ Men:AcC8 > AcC8:AcC10 > Thy:AlC8 > ChCl:Gly. According to COSMO-RS predictions, the ES ACC8:ACC10 was expected to be a better solvent (yet for a single compound) than Men:AcC10; however, in the validation essays, Men:AcC10 solubilized 102.4 ± 3.9 mg/mL of the sterol mixture, whereas AcC8:AcC10 solubilized only 71.6 ± 3.7 mg/mL (Table 2). Although the results have demonstrated that the experimental data agree in general with the COSMO-RS prediction, the six ES previously selected were evaluated in the extraction of phytosterols from macroalgae in order to understand the extraction efficiency of each ES, as well as the interference of other compounds present in the macroalgae matrix.

Table 2. COSMO-RS Predicted ln $\gamma\infty$ Values for the Selected ES (1:1) at 25°C, and Their Validation through Experimental Solubility Measurements of a Commercial Mixture of Phytosterols as Well as Through the Amount of Phytosterols Extracted from the Macroalga *C. tomentosum*

ES	ln $\gamma\infty$ values (COSMO-RS)	solubility of phytosterols (mg/mL)	*C. tomentosum* phytosterols extraction (mg/g dw)
Men:Thy	−0.768	109.0 ± 2.8	2.72 ± 0.02
Men:AcC8	−0.115	102.4 ± 0.8	3.16 ± 0.01
AcC8:AcC10	−0.086	71.6 ± 3.7	1.43 ± 0.03
Men:AcC10	0.080	102.4 ± 3.9	2.43 ± 0.11
Thy:AlC8	0.759	17.0 ± 0.5	1.87 ± 0.06
ChCl:Gly	9.880		

Extractions of Phytosterols. The six previously selected ES were tested in the extraction of phytosterols from *C. tomentosum*, and their performance was compared with the conventional Soxhlet extraction with DCM. Figure 4 represents the phytosterols content obtained with each ES and with Soxhlet (as analyzed by GC−MS). The extraction yield obtained with DCM Soxhlet extraction was 6.73 ± 0.09% (w/w), which is very similar to that previously reported for *C. tomentosum*.[20] Two phytosterols, namely, 24-methylenocholesterol and stigmasterol, were detected and quantified in the DCM extract, with contents of 0.25 ± 0.02 and 2.86 ± 0.07 mg/g dw, respectively. Concerning the extraction with ES, only stigmasterol was detected and quantified, possibly due to the dilution of the compounds in the extracts. The contents ranged from 1.43 ± 0.03 with AcC8:AcC10 to 3.16 ± 0.01 mg/g dw with the Men:AcC8.

Concerning only the stigmasterol content, Men:AcC8 allowed to extract a considerable higher amount (3.16 ± 0.01 mg/g dw) than that obtained with DCM (2.86 ± 0.07 mg/g dw). In fact, the stigmasterol content extracted with this ES is in the same range of total phytosterols able to extract using DCM, highlighting the potential of ES to extract this family of compounds. Moreover, in the extraction with Men:AcC8 there is a significant decrease in the extraction time (from 8 h in Soxhlet extraction to 3 h with the ES). Concerning the comparison of the efficiency of extraction

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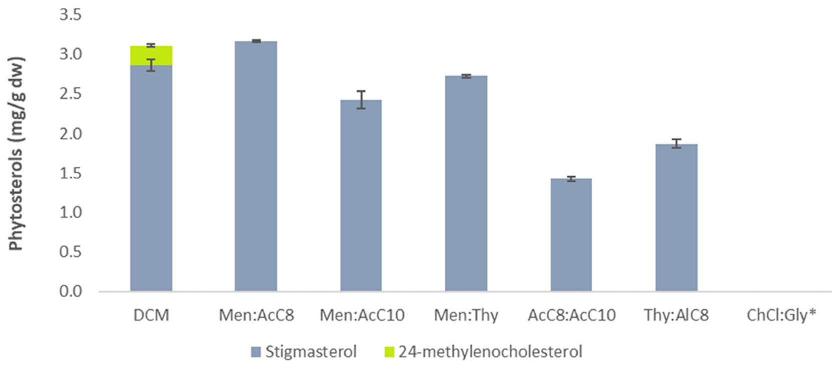

Figure 4. Results in terms of the extraction yields of phytosterols using the six ES selected. The results were expressed in mg/g dw. * Phytosterols were not detected.

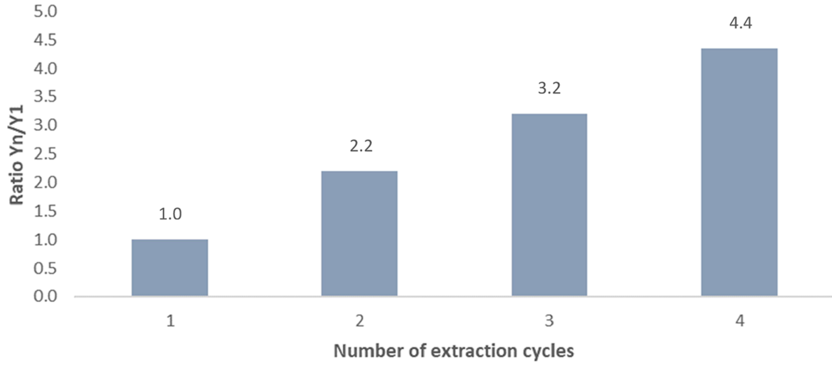

Figure 5. Results in terms of the total amount of phytosterols present in the ES extract in each cycle, where Y_n $(1 \leq n \geq 4)$ and Y_1 are the amounts obtained in the first extraction cycle.

between the tested ES, the following order was observed: Men:AcC8 > Men:Thy > Men:AcC10 > Thy:AlC8 > AcC8:AcC10 > ChCl:Gly.

The phytosterol extraction results showed a more significant deviation from the COSMO-RS predictions than those observed with the commercial mixture of phytosterols, most likely because of the more complex nature of the natural plant matrix. In the extraction essays, the ES containing menthol as HBA, with different HBDs, showed the best extraction yields (Table 2), with Men:AcC8 showing the best results (3.16 ± 0.01 mg/g dw). Moreover, all of the tested ES using menthol as HBA are hydrophobic eutectic solvents (HES), highlighting the higher efficiency of these solvents to extract phytosterols.

The potential of HES was reported for the first time in 2015 by Kroon's et al.[29] in which they combined quaternary ammonium salts and decanoic acid to prepare the HES. Marrucho et al.[30] also contributed to the study of HES by reporting the first HES composed of two neutral compounds, namely, menthol and naturally occurring organic acids, for the removal of different biomolecules from water. After that, other studies have reported the use of HES in the extraction and isolation of heavy metals,[31] bisphenol A,[32] pharmaceuticals,[33] biomolecules,[34−36] and volatile fatty acids[29] from aqueous solutions. These HES are regarded as suitable solvents to extract compounds with low water solubility. Menthol-based HES have also been widely studied and are acknowledged to be efficient in the extraction of bioactive compounds, such as β-carotene[12] and triterpenic acids.[13] With regard to the extraction of phytosterols, these HES were successfully used in the extraction of ergosterol from *A. bisporus* mushrooms byproducts.[19] In this case, the use of HES allowed to extract higher amounts of ergosterol than those reported by microwave-assisted extraction and ultrasound-assisted extraction, both with ethanol.[37,38] The authors also claimed that the high phytosterol extraction efficiency of HES containing menthol could be related to some degree of stereochemical affinity. Notwithstanding, the higher levels of phytosterols extracted from *A. bisporus* mushrooms using HES, compared to that obtained from *C. tomentosum* macroalgae, in the present work, may be related with the different phytosterols contents present in these two biomasses.[20,39] However, for both biomasses, the use of HES proved to be more efficient than the solvents or methods used previously.

Finally, the findings suggest that the utilization of the COSMO-RS predictions in this study is appropriate for evaluating the capacity of ES to extract phytosterols from macroalgae, serving as a preliminary step prior to conducting experimental work in the laboratory.

Sequential Extraction of Phytosterols. After selection of the Men:AcC8 as the most efficient ES in the extraction of phytosterols from *C. tomentosum* biomass, the possibility of maximizing the phytosterols content on the ES extract (or the possible saturation), as expected from the results described above for a commercial mixture of phytosterols, was tested.

Thus, first Men:AcC8 was used to extract *C. tomentosum* biomass for a longer period of time (12 h) and the phytosterol recovery increment showed to be negligible, being within the error of that obtained with the extraction of 3 h, which also corroborates the efficiency of the extraction process. Thus, since the ES is far from being saturated and the low density of biomass did not allow to increase the S/L ratio, we decided to perform a sequential extraction using renewed biomass and the recovered ES.

The sequential extraction was performed up to four times, with renewed biomass, and the ES extract from the previous step was reused. As a result of this process, it was possible to observe that the phytosterol content increases roughly linearly in each extraction step, and after 4 steps corresponds to a 4.4-fold increase in the initial concentration of phytosterols in the extract (Figure 5). After the second extraction step, it was possible to detect and quantify 24-methylenecholesterol in the ES extract which might explain the fact that the overall increase in the phytosterol content at the end of the 4 cycles was higher than 4. With these results, it is expected that increasing the number of extraction cycles, Men:AcC8 can be further concentrated in phytosterols to address specific concentration requirements for further processing. These results would be very helpful not only for future studies considering the exploitation of sterols from *C. tomentosum* but also for studies involving the use of ES to recover phytosterols from other natural resources, even those with considerably increased amounts of these components. Finally, and still considering *C. tomentosum*, future work must be done to design and optimize this extraction process, considering the final application desired for the extract (Figure 5).

CONCLUSIONS

In the present work, ES were studied in the solubilization and extraction of phytosterols from macroalgae.

COSMO-RS was used to predict the solubility of β-sitosterol in a total of 2240 possible mixtures. From these, six ES were selected to experimentally validate the computational prediction trends. Due to the nonpolarity of phytosterols, menthol-based HES were successfully applied for the extraction of these compounds and showed similar extraction efficiencies compared to conventional organic solvents, with a significant reduction in extraction time. It is important to note that the current extraction process with ES has not been optimized, and there is still room for further improvements in extraction yields. The best extraction yield (3.16 ± 0.01 mg/g dw) was obtained with ES Men:AcC8 and owing to its biodegradability and pharmaceutically acceptable toxicity, it represents a safer, more sustainable, and environmentally friendly alternative to the conventional organic solvents. Furthermore, this ES can be used to extract phytosterols at least four times without regeneration or saturation of the ES, with a fourfold increase in the yield of phytosterols extraction. The present study underscores the potential of ES, particularly those that are hydrophobic, in the extraction of bioactive compounds, such as phytosterols from marine sources. It also reinforces the utility of predictive tools such as COSMO-RS in expediting and enhancing the research process.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.4c00145.

List of the initial 35 HBA and 64 HBD used in the COSMO-RS predictions, with the respective number-compound correspondence (PDF)

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J.R.: investigation, formal analysis, writing—original draft; F.H.B.S.: validation, writing—review & editing; J.A.P.C.: validation, Writing—review & editing; J.R.: supervision, writing—review & editing, funding acquisition; A.J.D.S.: supervision, writing—review & editing, funding acquisition; S.A.O.S.: supervision, writing—review & editing, funding acquisition, conceptualization. The authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

ES, eutectic solvents; DES, deep eutectic solvents; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; COSMO-RS, conductor-like screening model for real solvents; $\gamma\infty$, activity coefficient at infinite dilution; Men, menthol; Thy, thymol; AcC8, octanoic acid; AcC10, decanoic acid; ChCl, choline chloride; AlC8, octane-1.8-diol; Gly, glycerol; DCM, dichloromethane; TMS, trimethylsilyl; GC−MS, gas chromatography−mass spectroscopy; dw, dry weight; S/L, solid/liquid ratio

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