



Supercritical CO₂ extraction of lipids and bioactives from *Gigartina pistillata*

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Received: 4 March 2025 / Revised: 20 April 2025 / Accepted: 23 April 2025 / Published online: 20 May 2025
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Abstract

A sequence of pressurized stages has been proposed for the extraction of bioactives and biopolymers from *Gigartina pistillata* commercially dried biomass. The optimal supercritical CO₂ extraction operation conditions were selected at 15 MPa, 50 °C and 10% ethanol as polar modifier to maximize total yield and phenolic content in the extract, and the maximum total carotenoid yielded at 35 MPa, 50 °C and 10% ethanol, whereas the polyunsaturated fatty acids content was enhanced under operation at 25 MPa, 50 °C and 5% or at 60 °C and 10% ethanol. Operating at optimal extraction time, up to 2.7% total yield was attained, which was 5.66% of the value with conventional solvent extraction. The carrageenan yields obtained from the residual solids by non-isothermal hydrothermal treatment during heating up to 160 °C were lower than those obtained from the initial seaweed. However, the carrageenan extracted from the waste solids exhibited stronger gel properties, with higher viscoelastic features (2-folder) than those commercially available carrageenan.

Keywords Rhodophyta · Supercritical extraction · Lipids · Phenolics · Carrageenan

Introduction

Gigartina pistillata (S.G. Gmelin) Stackhouse is an underutilized carragenophyte with commercial potential for the production of hybrid λ - and κ -carrageenans (Amimi et al. 2007; Pereira et al. 2009; Pereira 2013). With a high carrageenan content (around 60%) it is among red algae receiving attention as alternative sources for the extraction of new carrageenan types with different functionalities (Pereira et al. 2009; Pereira 2013). In addition to the gelling, emulsifying, thickening, and stabilizing properties, *G. pistillata* carrageenan fractions have been reported to possess other properties, such as plant biostimulants (Mzibra et al. 2021) or antitumor potential against colorectal cancer cells (Cotas

et al. 2020). From studies in the Atlantic coast of Portugal and Morocco it has been observed that the thallus composition of this seaweed is quite stable along most part of the year, with the growth period and maximal dry matter and carrageenan content found in spring–summer (Amimi et al. 2007; Pereira 2013).

Red seaweed biomass can also be a sustainable source of valuable components such as proteins, phenolics, lipids, carotenes and minerals, which should be utilized to attain an integral valorization of the whole seaweed biomass (Torres et al. 2019a; Rudke et al. 2020). Despite the low lipid content in seaweeds (Gómez-Ordóñez et al., 2010; Susanto et al. 2019), the balance of ω -6/ ω -3 ratio is favourable for the preparation of concentrated polyunsaturated fatty acids (PUFA) products, which are gaining interest to enrich Western diets in ω -3 fatty acids (Gosch et al., 2014; Řezanka et al. 2021). However, PUFA are highly susceptible to oxidation, a process induced by free radicals, which could be delayed or avoided by the addition of natural antioxidants, such as those found in seaweeds (Sabeena Farvin and Jacobsen 2012).

Simultaneous extraction of lipidic and phenolic fractions from seaweed can be attained with a green technology such as supercritical fluid extraction (SFE), which offers advantages over conventional solvent extraction, by shortening extraction time and facilitating the separation of a solvent

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free product. Since carbon dioxide is safe and nontoxic, it is the solvent preferred when thermolabile components must be recovered. Also, oxygen-free environment protects them from oxidation. These compounds are more effectively extracted under relatively mild critical operation (Tc: 31.1 °C and Pc: 7.29 MPa) (Crampon et al. 2011; Sivagnanam et al. 2015). Both solvent power and selectivity can be tuned by adequate selection of the operation pressure, temperature and the concentration of polar modifiers, which allow the extraction of polar compounds, such as polar lipids, pigments and phenolic compounds (Crampon et al. 2011; Ospina et al. 2017; Rozo et al. 2019; Siahaan et al. 2020). This technology can influence the lipid composition and has been used to favour the selective extraction of unsaturated fatty acids over saturated ones (Cheung 1999) and could be as efficient as conventional solvent extraction (Cheung et al. 1998). Terme et al. (2017) observed that pure CO₂ extracted mainly glycolipids and the addition of ethanol as polar modifier increased the fraction of neutral lipids from *Solieria chordalis* and these extracts also showed higher antiradical properties (Terme et al. 2018). Supercritical carbon dioxide (sc-CO₂) extraction has been used for obtaining antioxidant-rich extracts with potential bioactivity from seaweeds (Rozo et al. 2019; Kumar et al. 2020). The fatty acids in the sc-CO₂ extracts from brown and red seaweeds were responsible for the inhibition of plant pathogens growth on in vitro cultures and in vivo on wounded fruit (De Corato et al. 2017). The phenolic compounds found in the supercritical extracts can be responsible for the antioxidant activity (ABTS, TBARS, protection against LDLs oxidative damage) (Rozo et al. 2019) such as *p*-hydroxybenzaldehyde, 2,2'-methylenebis(6-*tert*-butyl-4-methylphenol) or vanillylacetone identified in *Gloiopeltis tenax* extracts (Zheng et al. 2012). The supercritical extracts also contained sesquiterpenes, ketones, fatty acids and their esters, phenols, and sterols, which contribute to the antioxidant properties (Pal and Gosh 2012). The simultaneous extraction of oil and compounds with potential to protect from oxidation was also feasible (Ospina et al. 2017).

The supercritical stage can be integrated in a more complex biorefinery process, a concept already proposed for other brown (Balboa et al. 2015) and red (Rudke et al. 2020) seaweeds. The lipidic extraction could be considered one of the first stages in a biorefinery process, a scheme already validated in a previous study where a separated extraction of the lipid and phenolic fractions from *Mastocarpus stellatus* was tried, using pure CO₂ in the first stage and ethanol-modified in the second; the exhausted solids were processed by microwave assisted subcritical water extraction to recover the carrageenan and protein soluble fractions (Cid et al. 2021). The simultaneous extraction of antioxidant compounds with the lipids could confer additional oxidative stability to the oily extract. This would be an advantage when using them as nutraceuticals, for

the protection of the polyunsaturated fatty acids and the additional biological properties. Other similar processing schemes have been reported, including the supercritical CO₂ extraction of β -carotene and linoleic acid followed by the subcritical water extraction of carrageenan and polyphenols from *Eucheuma cottonii* and *Gracilaria* sp (Setyorini et al. 2018), and the supercritical CO₂ extraction of the oily or non-polar fraction from *Kappaphycus alvarezii* before pressurized ethanolic extraction of the phenolic fraction and the further aqueous extraction of carrageenan fraction, with ultrasound or with pressurized hot water extraction (Rudke et al. 2022; Hans et al. 2023). The aim of this study is to optimize the supercritical carbon dioxide extraction of the lipidic, phenolic and carotenoid fractions from *Gigartina pistillata* and the further extraction of carrageenan by a previously optimized hydrothermal extraction stage.

Materials and methods

Material

Wild *Gigartina pistillata* (S.G. Gmelin) Stackhouse (Gp) from the north west of the Iberian Peninsula was kindly provided by Portomuiños (Cerceda, A Coruña, Spain). The air dried and lightly ground seaweeds (> 1 mm size) were stored in closed plastic bags in a dark and dry place before use.

Extraction

Conventional extraction

The dried and ground *G. pistillata* samples were extracted with 80% ethanol in a Soxhlet apparatus for 8 h. After extraction the solvent was eliminated using a vacuum rotary evaporator (Buchi R-114, Switzerland).

Supercritical CO₂ extraction

In each experiment 100 g of Gp were packed into a 1 L extraction vessel (Thar Designs SFE-1000 F-2-C10, USA). The CO₂ was pumped by a P-200 A piston pump (Thar Design Inc.) at a flow rate of 25 g CO₂ min⁻¹. In the extraction experiments carried out in the presence of absolute ethanol as modifier, this was pumped by a HPLC pump (Scientific Systems, USA, model Series III) and mixed with the CO₂ before being introduced into the extraction cell. Once the desired extraction conditions were reached in the extractor, dynamic extraction of the algae was carried out for 1 h. After this, the extract was collected in the first separator at ambient pressure and 20 °C. The separator was further washed with ethanol to recover the extract and this solution was combined with the extract collected. Ethanol

was vacuum evaporated using a rotary evaporator and the extracts were kept at -20°C in the dark until analysis.

A Box-Behnken design with three factors and three levels was employed to evaluate the influence of the operating conditions on the supercritical extraction of *G. pistillata*. The selected variables for their influence on sc- CO_2 processing of seaweeds were pressure, temperature and solvent polarity (Ospina et al. 2017; Rozo et al. 2019). The independent variables in coded and actual levels are listed in Table 1.

The operational conditions assayed for each individual experiment are listed in Table 2.

A second-order polynomial function was adopted to fit the experimental data according to Eq. 1:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j>i}^2 \beta_{ij} X_i X_j \quad (1)$$

where Y is the studied response, X_i and X_j are the coded value of the independent variable, β_0 , β_i and β_{ij} are the regression coefficients for intercept, linear, quadratic, and interaction terms, respectively.

Table 1 Independent variables and their coded and actual values in the SFE design experiment

Factor	Variable	Coded level		
		−1	0	1
Pressure (MPa)	X_1	15	25	35
Temperature ($^{\circ}\text{C}$)	X_2	40	50	60
Ethanol (%)	X_3	0	5	10

Table 2 Experimental design and observed responses for extraction yield (EY), total phenolic extraction yield (TPY), total carotenoids extraction yield (TCY), as well as the ABTS radical scavenging capacity (TEAC) and PUFA content of the extracts

Run	P (MPa) (X_1)	T ($^{\circ}\text{C}$) (X_2)	EtOH (%) (X_3)	EY (%)	TPY (mg GAE (100 g) $^{-1}$ Gp)	TCY ($\mu\text{g C}$ (100 g) $^{-1}$ Gp)	TEAC (mg Trolox g $^{-1}$ extract)	PUFA (%)
1	15 (−1)	40 (−1)	5 (0)	0.057	0.782	3.884	28.529	4.37
2	35 (1)	40 (−1)	5 (0)	0.043	0.704	6.364	30.167	5.37
3	15 (−1)	60 (1)	5 (0)	0.080	1.264	1.248	29.531	5.04
4	35 (1)	60 (1)	5 (0)	0.054	0.997	5.266	30.621	4.99
5	15 (−1)	50 (0)	0 (−1)	0.059	0.760	0.635	13.868	3.31
6	35 (1)	50 (0)	0 (−1)	0.037	0.441	2.137	12.625	4.75
7	15 (−1)	50 (0)	10 (1)	0.082	1.524	6.893	48.371	7.56
8	35 (1)	50 (0)	10 (1)	0.057	0.727	11.290	33.231	5.20
9	25 (0)	40 (−1)	0 (−1)	0.025	0.273	1.751	21.651	2.77
10	25 (0)	60 (1)	0 (−1)	0.041	0.533	1.601	21.254	2.35
11	25 (0)	40 (−1)	10 (1)	0.062	0.944	9.173	26.297	9.66
12	25 (0)	60 (1)	10 (1)	0.072	1.048	7.719	30.052	10.04
13	25 (0)	50 (0)	5 (0)	0.045	0.879	7.080	41.223	7.36
14	25 (0)	50 (0)	5 (0)	0.042	0.718	7.707	37.948	13.68
15	25 (0)	50 (0)	5 (0)	0.051	0.853	6.878	23.885	8.38

The studied responses were the extraction yield (EY, %), the total phenolic yield (TPY, mg GAE (100 g) $^{-1}$ Gp), the total carotenoid yield (TCY, mg C (100 g) $^{-1}$ Gp), the polyunsaturated fatty acid content (PUFA, %) and the ABTS radical scavenging capacity (ABTS, mg Trolox g $^{-1}$ extract). An analysis of variance (ANOVA) was employed to evaluate the statistical significance of the results. Design Expert 11 (Stat-Ease, Inc) software was employed in calculations.

Pressurized hot water extraction of carrageenan

The sc- CO_2 extracted solids were processed at previously selected aqueous extraction under pressurized conditions. The solids were mixed with distilled water at a liquid to solid ratio 1:30 and heated up to 160°C in the rapid heating profile of a pressurized reactor (Parr Instruments, USA). Once attained the target temperature, the reactor vessel was cooled down and the suspension was vacuum filtered. The solids were discarded, and the solubilized carrageenan was further precipitated with 96% ethanol.

Analytical methods

Total extraction yield was gravimetrically determined. Total phenolic yield was measured according to the Folin-Ciocalteu method using gallic acid (Sigma Aldrich, USA) as standard and results were expressed as mg GAE (100 g) $^{-1}$ Gp.

ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging was determined according to the method described by Re et al. (1999) by adding 1 mL of diluted ABTS to 10 μL of extracts or Trolox

(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich, USA). The mixture was incubated at 30 °C. After 6 min, absorbance was read at 734 nm, and results were expressed as Trolox Equivalent Activity Antioxidant (TEAC), mg Trolox g⁻¹ extract.

Total carotenoid yield was determined spectrophotometrically. The extract was dissolved in 10 mL of absolute ethanol and the absorbance at 450 nm was determined. β -carotene was used for the calibration curve in the concentration range 0.0005–0.0035 mg mL⁻¹. The results were expressed as μ g carotenes (100 g)⁻¹ of Gp.

Fatty acid methyl esters (FAME) were prepared according to UNE-EN ISO 12966–3:2016, and then analyzed by a gas chromatograph-mass spectrometer (GC–MS Trace GC Ultra, Thermo Fisher) equipped with a Zebron ZB-WAX Phenomenex column (60 m $\times$ 0.25 mm i.d.; 0.25 μ m) coupled to a race DSQ (Thermo Fisher) quadrupole mass spectrometer. The following temperature profile was applied 50 °C (2 min), gradient increase at 10 °C min⁻¹ up to 240 °C, which was maintained for 40 min. Injection temperature, mode, and volume were 250 °C, split ratio 1:10, and 1 μ L, respectively. FAMEs were identified by comparing their mass spectra with those of authentic standards, as well as these provided by the NIST MS Search 2.0 library. The average values of two independent determinations are expressed as a percentage of total FAME.

Rheology

Carragenan-based gelled matrices were formulated at 0.5–2% of biopolymer content in the presence of potassium chloride (0.1 M) following the conditions and procedure detailed previously (Ponthier et al. 2020). The developed matrices were cold stored at 4 °C for 24 h before any further analysis to ensure the full maturation of the systems (Moreira et al. 2011). Rheological testing of the prepared systems was conducted at 25 °C on a controlled-stress rheometer (MCR302, Anton Paar Physica, Austria). A sandblasted parallel plate (25 mm diameter, 1 mm gap) was selected to monitor the viscoelastic characteristics, in terms of elastic (G') and viscous (G'') moduli within the linear viscoelastic region. In order to define this region (< 51 Pa), the corresponding stress sweeps were performed at fixed frequencies (0.1 and 10 Hz). Samples were placed on the plate, sealed with light paraffine oil and rested for 5 min prior to the measurements. Small amplitude oscillatory shear measurements were made at 15 Pa from 0.1 to 10 Hz. All tests were made at least in duplicate.

Results

Supercritical CO₂ extraction

Table 2 shows the experimental results under the 15 conditions evaluated, according to a Box-Behnken experimental

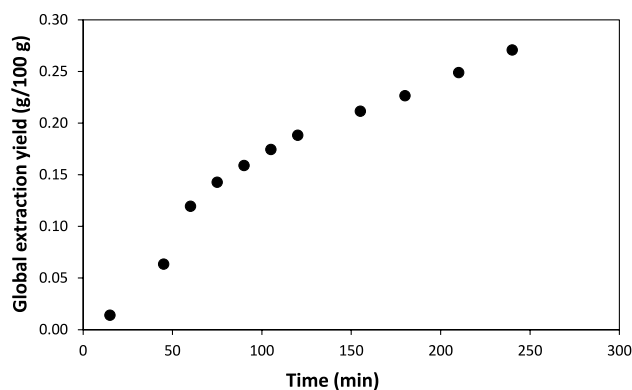


Fig. 1 Supercritical CO₂ extraction kinetics during operation with *Gigartina pistillata* at 25 MPa, 50 °C with 5% of ethanol as cosolvent

design (Table 1). Based on a preliminary experiment, the extraction time was fixed at 1 h, a period corresponding to the initial extraction stage or constant extraction rate (CER) period, where more marked differences among the tested conditions are expected (Fig. 1).

Global extraction yield

The extraction yield with conventional extraction with ethanol 80% (v/v) was 5.66. Maximum overall yields have been obtained under conditions of experiment 7 (15 MPa, 50 °C, 10% ethanol) and experiment 3 (15 MPa, 60 °C, 5% ethanol), almost double than those attained at lower temperature. A slight decrease with pressure was observed on the total extraction yields. The calculated regression coefficients obtained for each response using coded variables are summarized in Table 3.

Composition of the extracts

To contrast the effects of the studied factors and their interactions on the responses, the Pareto diagrams of the linear, quadratic, and cross effects are illustrated in Fig. 2. The black and white colours indicate the type of influence (numerically positive or negative, respectively) of each factor/interaction. All effects whose bars are longer than the statistical significance line (dotted red line) can be considered significant at a confidence level of 95% (or 90% for PUFA content).

Ethanol concentration is highly significant in all response functions (Eqs. 2–6) as also observed when plotted in Fig. 2. The non-significant coefficients were removed from the general quadratic equation (except those necessary to maintain the hierarchy of the model) and the final reduced models were refitted to the data. The final expressions for each response converted to the uncoded variables are described by Eqs. 2–6.

$$\text{EY}(\%) = 0.089 - 0.0062 \cdot P + 0.00074 \cdot T + 0.0027 \cdot \text{EtOH} + 0.00010 \cdot P^2 \quad (2)$$

$$\text{TPY} (\text{mg GAE } (100 \text{ g})^{-1} \text{ Gp}) = 0.295 - 0.0182 \cdot P + 0.0142 \cdot T + 0.0559 \cdot \text{EtOH} \quad (3)$$

$$\text{TPY} (\mu\text{g C } (100 \text{ g})^{-1} \text{ Gp}) = -44.968 + 0.846 \cdot P + 1.495 \cdot T + 0.724 \cdot \text{EtOH} - 0.0138 \cdot P^2 - 0.0156 \cdot T^2 \quad (4)$$

$$\text{TEAC} (\text{mg Trolox g}^{-1} \text{ extract}) = 20.178 + 1.714 \cdot \text{EtOH} \quad (5)$$

$$\text{PUFA} (\%) = -11.399 + 1.339 \cdot P + 0.482 \cdot \text{EtOH} - 0.0268 \cdot P^2 \quad (6)$$

The response surface for the predicted extraction yield in the range studied is shown in Fig. 3a as a function of temperature and ethanol concentration. A continuous and linear effect of increasing ethanol concentration and a less marked effect of temperature is observed, reaching almost 0.1% at 60 °C with 10% ethanol.

The experimental values for the total phenolic yield is shown in Table 2. The maximum phenolic yield was observed in samples from experiment at 15 MPa and 50 °C with 10% ethanol, with 1.5 mg GAE (100 g)^{−1} Gp. The phenolic content for this extract was almost 20 mg GAE g^{−1}. In the case of the total carotenoids yields (Table 2), the maximum values corresponded to 11.29 μg C (100 g)^{−1} Gp at similar conditions, just increasing the the preasure at 35 MPa. The maximum values of antioxidant capacity were achieved in experiment 7 (15 MPa, 50 °C and 10%

ethanol) and in the central point (25 MPa, 50 °C and 5% ethanol).

Once selected the optimal conditions for sc-CO₂ extraction, the extraction was performed at prolonged times. The final extraction yield was 2.7%. The fatty acid percentage was influenced by the pressure (Eq. 6). Oleic and palmitic acids were the most abundant, followed by palmitoleic and stearic acids (Table 4). The SFA ranged from 35.19% (at 15 MPa) to 54.18% (at 25 MPa). An opposite trend was observed for MUFA which varied between 36.11% (at 25 MPa) and 61.50% (at 15 MPa), and the maximum content was achieved without ethanol as modifier. Eicosapentaeonic acid (EPA,C20:5-ω3) was preferentially extracted at 25 MPa.

Data of total PUFA content shown in Table 2 show maximum values in the central point, at 25 MPa, 50 °C and 5% ethanol, and at 25 MPa, 60 °C and 10% ethanol. The regression coefficient corresponding to the linear effect of ethanol and the double effect of pressure were significant at 95%, but temperature was not significant in the range studied (Table 4 and Fig. 3e). The response surface defined by Eq. 6 plotted in Fig. 3d, shows a continuous increase with the ethanol concentration a maximum at 25 MPa.

Extraction of carrageenan from the waste solids

After hydrothermal treatment, the carrageenan extraction yield from the waste solids subjected to ethanolic precipitation was 24.3 ± 0.3%. Despite the low percentage

Table 3 Coded regression coefficients of predicted quadratic polynomial models for each response. Values in bold represent significant coefficient at a 95% confidence interval (or 90% for PUFA content)

Coef	Y1: EY		Y2:TPY		Y3: TCY		Y4: TEAC		Y5: PUFA	
	Estimate	<i>p</i> -value	Estimate	<i>p</i> -value	Estimate	<i>p</i> -value	Estimate	<i>p</i> -value	Estimate	<i>p</i> -value
β ₀	0.0462	0.000	0.8166	0.000	7.222	0.000	34.350	0.001	9.81	0.000
β _P	−0.0108	0.0024	−0.1824	0.019	1.550	0.000	−1.710	0.6260	0.0031	0.9975
β _T	0.0074	0.0117	0.1422	0.044	−0.667	0.017	0.602	0.8620	0.0296	0.9762
β _E	0.0137	0.0008	0.2795	0.003	3.619	0.000	8.570	0.0480	2.41	0.0512
β _{PT}	−0.0030	0.3157	−0.0472	0.557	0.384	0.209	−0.137	0.9776	−0.2599	0.8534
β _{PE}	−0.0009	0.7582	−0.1194	0.173	0.723	0.0420	−3.470	0.4888	−0.9495	0.5091
β _{TE}	−0.0015	0.6013	−0.0388	0.628	−0.326	0.276	1.040	0.8323	0.2006	0.8865
β _{PP}	0.0106	0.0128	0.1420	0.129	−1.427	0.0038	−1.220	0.8118	−2.93	0.0886
β _{TT}	0.0015	0.6200	−0.0218	0.792	−1.604	0.0022	−3.430	0.5109	−1.93	0.2237
β _{EE}	0.0024	0.4390	−0.0954	0.277	−0.556	0.102	−6.110	0.2624	−1.67	0.2833
Models										
F-value	37.19		6.12		57.29		11.18		4.32	
<i>p</i> -value	< 0.0001		0.0030		< 0.0001		0.0053		0.030	

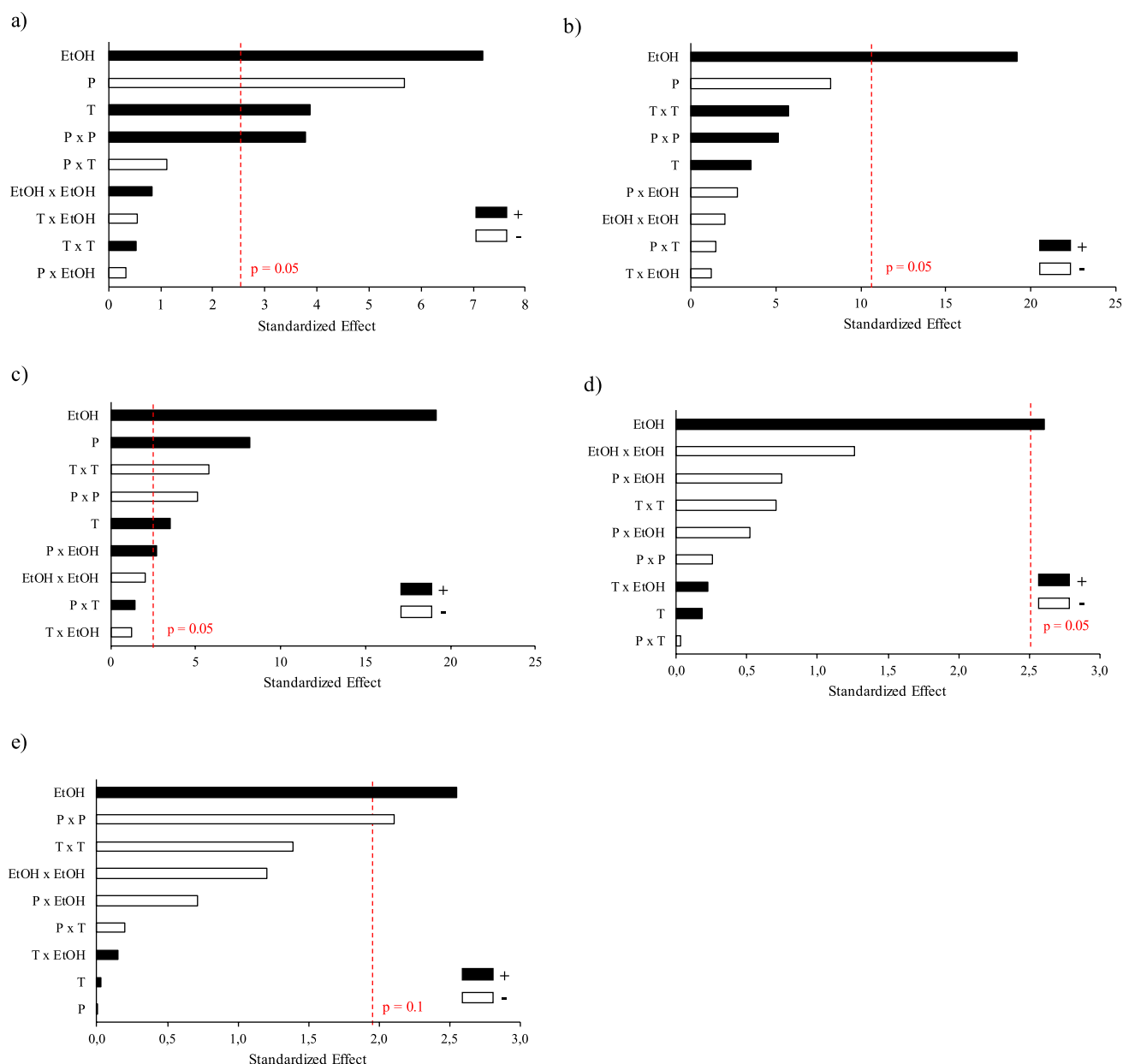


Fig. 2 Pareto charts for SFE of *Gigartina pistillata* showing the influence of the variables and their interactions on (a) total extraction yield, (b) total phenolic extraction yield, (c) total carotenoid extraction yield, (d) ABTS radical scavenging capacity and (e) PUFA content

of biopolymer present in the solids when compared with the high content reported on the untreated seaweed (up to 60%) (Pereira 2013), it exhibited strong mechanical features (Fig. 4).

The rheological testing showed characteristic gel profiles, with the viscous modulus lower than the elastic one over the tested frequency range. Both viscoelastic moduli were almost frequency invariant. At fixed frequency, the G' and

G'' moduli decreased with increasing carrageenan content. In all cases, the viscoelastic values were indicative of strong and stable gels. Similar tendencies were identified for the complex viscosity variation.

Figure 5 shows a scheme of the optimal conditions found in this study to the extraction of crude carrageenan, including other high valuable compounds, after sc- CO_2 extraction followed by hydrothermal treatment.

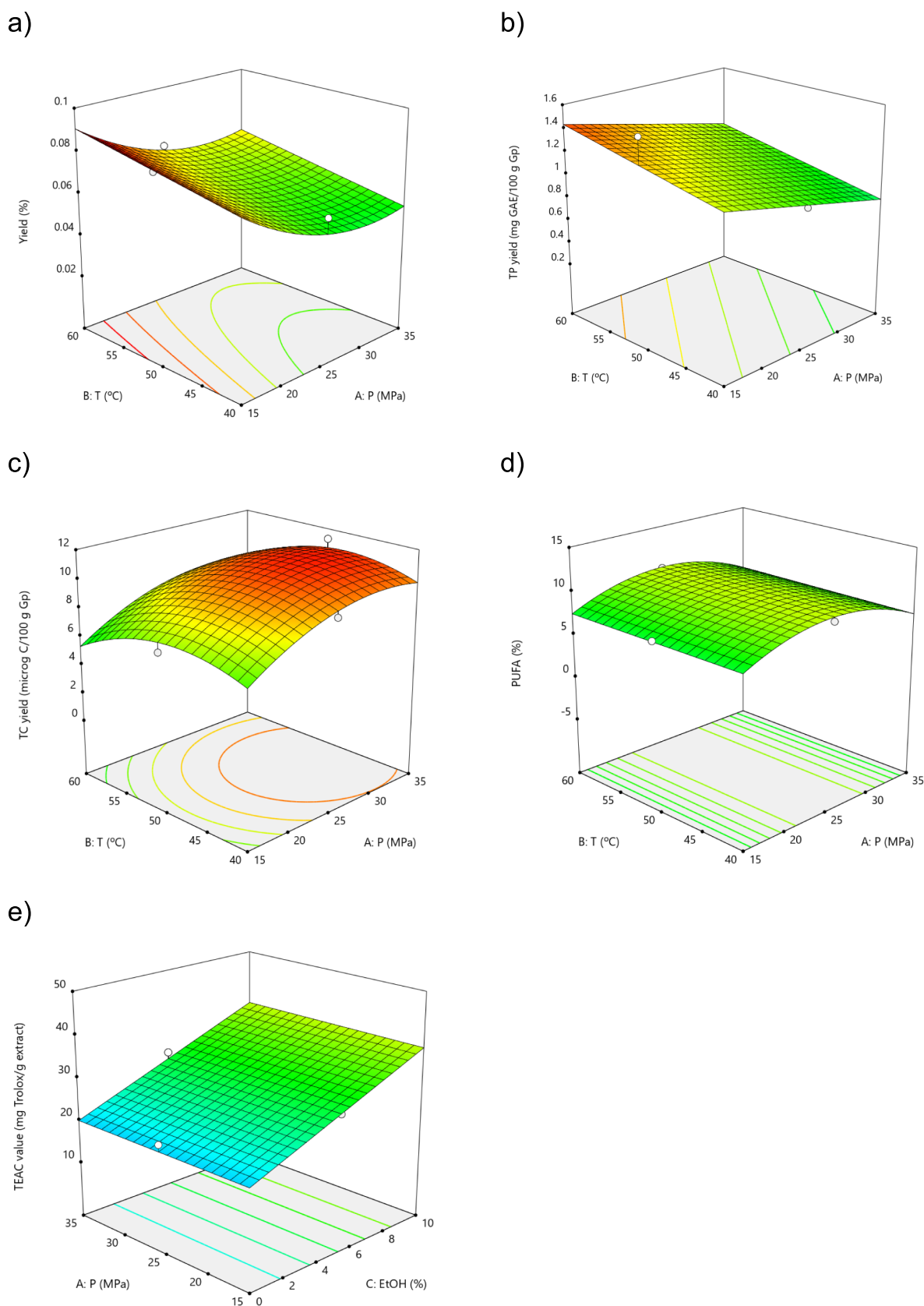


Fig. 3 Predicted dependence of **a)** extraction yield, **b)** total phenolic extraction yield, **c)** total carotenoid extraction yield, **d)** PUFA content on temperature and pressure at an ethanol concentration of 10%, and **e)** TEAC value depending on pressure and ethanol concentration at 60 °C

Table 4 Lipid profile of the extracts obtained with sc-CO₂ under the operational conditions defined in the experimental design

	Experiment														
Fatty acid (%)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Myristic acid	4.76	5.01	4.89	5.13	4.34	4.64	6.00	6.04	4.56	5.38	6.49	6.22	5.85	5.39	5.75
Palmitic acid	36.90	38.99	37.01	41.47	30.85	36.35	46.14	47.23	35.70	37.14	47.69	47.63	43.59	41.24	45.31
Stearic acid	6.53	6.50	8.81	8.68	8.52	9.19	7.12	6.91	8.48	8.17	8.31	7.27	7.46	5.88	7.65
Palmitoleic acid	15.98	11.76	6.39	7.42	6.26	6.43	8.28	7.45	7.66	7.23	6.20	6.59	7.64	11.47	8.16
Oleic acid	31.45	32.37	37.86	32.31	46.72	38.64	24.89	27.18	40.83	39.74	21.65	22.24	28.11	22.34	24.75
Linoleic acid	2.47	2.84	3.33	2.23	3.31	2.82	1.49	1.30	2.77	2.35	1.11	1.74	1.14	5.93	1.51
Arachidonic acid	1.91	2.52	1.70	2.76	-	1.93	3.93	3.90	-	-	4.90	4.57	3.46	4.50	4.30
EPA	-	-	-	-	-	-	2.14	-	-	-	3.65	3.73	2.76	3.25	2.58
SFA (%)	41.66	44.00	41.90	46.60	35.19	40.99	52.14	53.27	40.27	42.52	54.18	53.85	49.44	46.63	51.06
MUFA (%)	53.97	50.64	53.06	48.41	61.50	54.26	40.30	41.54	56.96	55.13	36.16	36.11	43.20	39.69	40.56
PUFA (%)	4.38	5.36	5.03	4.99	3.31	4.75	7.56	5.20	2.77	2.35	9.66	10.04	7.36	13.68	8.38
ω-6 (%)	4.38	5.36	5.03	4.99	3.31	4.75	5.42	5.20	2.77	2.35	6.01	6.31	4.60	10.43	5.81
ω-3 (%)	-	-	-	-	-	-	2.14	-	-	-	3.65	3.73	2.76	3.25	2.58
ω-6/ω-3	-	-	-	-	-	-	2.53	-	-	-	1.65	1.69	1.67	3.21	2.25

SFA Saturated fatty acid; MUFA Monounsaturated fatty acid; PUFA Polyunsaturated fatty acid; EPA Eicosapentanoic acid

Discussion

The most relevant differences between the tested conditions on the analysed variables were identified on the constant extraction rate (CER) period (around 1 h), which is consistent with the achievements found by Rozo et al. (2019). These authors also found the CER period occurring up to 80 min for *H. musciformis*, with a global yield of 70%. During this period extraction occurs mainly as the dissolving and convective mechanisms to remove the easily accessible solutes from the surface of the solid matrix. Other authors have selected more prolonged periods, i.e. 240 min (Cheung 1999; Rozo et al. 2019), considering also the diffusion stages and Cheung (1999) did not find high weight variations for the last 3 h.

The global extraction yields obtained in this work are in the range of those reported for *Gracilaria mammillaris* (5.12%) with ethanol (Ospina et al. 2017). The oil extracted with petroleum ether was 0.57% for this species collected in the same geographical area (Gómez-Ordóñez et al., 2010) and a total lipid content of 2.96% has been obtained in chloroform/methanol from *Solieria chordalis* (Terme et al. 2017) and 0.24% with *n*-hexane (Ospina et al. 2017). Using supercritical CO₂ the oil extraction yields reported for *Porphyra umbilicalis*, *Eucheuma denticulatum* and *Gelidium pusillum* were 1.58, 1.37 and 1.70%, respectively (De Corato et al. 2017). Up to 92% of the value attained with chloroform:methanol (2:1, v/v) has been reported for *Sargassum hemiphyllum* (Cheung et al. 1998). Usually sc-CO₂ provides lower oil yields than Soxhlet extraction (Cheung 1999; Terme et al. 2017; Kim et al. 2024). The sc-CO₂ extraction efficiency of oil from *Undaria pinnatifida* yielded

6.31% oil at 30 MPa and 50 °C, 20% lower than with hexane (Kim et al. 2024). However, values comparable to those attained with conventional solvent extraction have been reported. Supercritical CO₂ extracted around 90% of the oil extracted with conventional solvents from *S. japonica* and the same or higher yields from *Sargassum horneri* (Sivagnanam et al. 2015). Cheung (1999) obtained good yields (67.1 mg g⁻¹ dry weight) from *Hypnea charoides* at 323.15 K, 37.9 MPa, with 1 mL CO₂ min⁻¹ for 1 h.

Concerning the obtained extraction yields, despite the increase in pressure causes an increase in solvent density, and consequently on the solubility of the target compounds, the observed effects were masked by other different competing effects occur regarding the pressure effect. At a constant temperature, the sc-CO₂ density increases with increasing pressure, enhancing the solute solubilization, but at high pressure the sc-CO₂ diffusivity may decrease due to the difficult mobility of the molecules to diffuse into the pores of the solid matrix and also to the compacting effect and the lowered void fraction and the penetration ability of sc-CO₂ into the matrix (Kazazi and Rezaei 2010; Yang et al. 2013). The small solute diffusion rates at higher pressures explains the low extraction efficiency when compared with the yields than would be expected by the solubility enhancements at higher pressures (Kazazi and Rezaei 2010). This was attributed to an increase in the collisions between the solutes and carbon dioxide-modifier due to an increase in density at high pressures. The diffusion coefficients increased with increasing temperature due to the increased mobility of the solute in the carbon dioxide-modifier, but at a fixed concentration of modifier, the diffusion coefficients could decrease with increasing pressure (Medina 2012). The solid bed

Fig. 4 Small amplitude oscillatory shear measurements for the formulated carrageenan-based matrices at different biopolymer content (%): 0.5 (circles), 1 (triangles), 1.5 (diamonds) and 2 (squares). G' and G'' : closed, G' : open symbols. Lines correspond to the commercial carrageenan at the 2% of biopolymer content

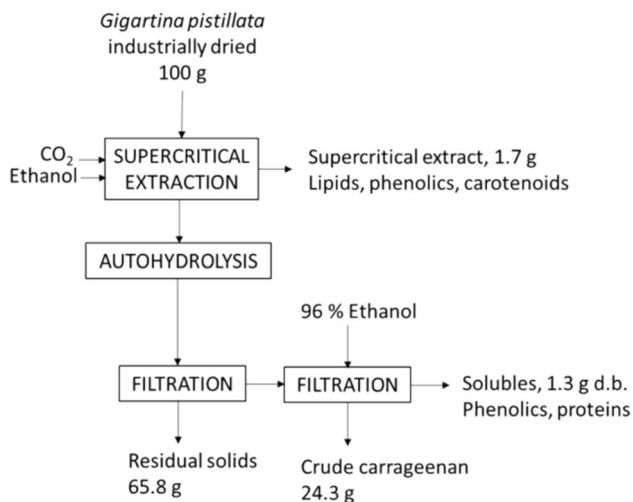
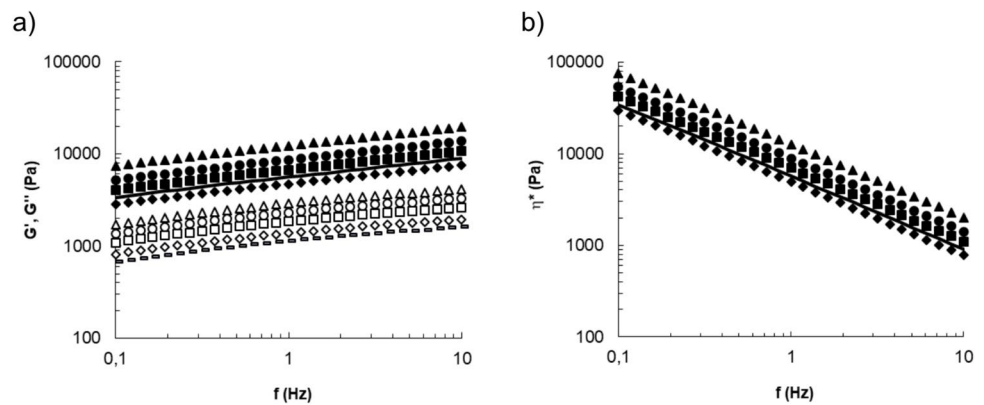


Fig. 5 Flow diagram of the cascade process to extract different fractions from *Gigartina pistillata* with sc-CO₂ and hot water pressurized extraction

compaction can interfere in SFE due to an increase in mass transfer resistance and solvent channelling effects. The negative effect at high pressures could be due to the increased repulsive solute–solvent interactions and to the decreased fluid diffusion coefficient. Whereas the increased pressure favours the solubilisation of solutes due to the enhanced density of the solvent, when a modifier at 10–30% is used, the increased pressure does not increase the fluid density so markedly and the effective diffusivity can be lowered thus lowering the solute mass transfer. This negative influence of increasing pressure values has been found with 20% ethanol for heartwood flavonoids (Atwi-Ghaddar et al. 2023). In the present case, the solid algal material exhibited a small flake, tubular like geometry and compaction could have a negative effect on the extraction performance. These effects could be responsible for the slight but significant negative influence of increased pressure on the overall and total phenolics extraction yield (Table S1).

Temperature has a significant effect, due to different competing effects, the promoted thermal motion of solutes in the matrix facilitating desorption at higher temperature and the saturated vapour pressure increases enabling the solutes dissolution. However, the decreased solvent density with increased temperature lowers the solvent power (Atwi-Ghaddar et al. 2023). Increased temperature could have a detrimental effect on the stability of bioactives and also the double effect of temperature because at higher temperature increases the vapor pressure of the solute and improved the extraction yield but could also reduce the density of carbon dioxide. The results are depending on the predominant effect (Wang et al. 2011).

Cheung et al. (1998) have reported an increase in total yield from *S. hemiphyllum* using pure CO₂ at 40 °C from 31 to 37 MPa and at 50 °C from 24 to 31 MPa. Also, at 30 and 50 °C, Conde et al. (2015) observed an increase with pressure in the total yield from *Sargassum muticum* using ethanol modified CO₂ (10% ethanol), but not with pure CO₂. In studies with red seaweeds, Cheung (1999) reported a significant increase of total extraction yields from 24 to 31 MPa at 40 and at 50 °C from *H. charoides*. Roza et al. (2019) confirmed the effect of pressure on the content of antioxidant compounds from *H. charoides*, being more efficient those obtained at 30 MPa than those extracted at 10–20 MPa, when 8% ethanol was used as modifier. Cid et al. (2021) found an increase in the range 10 to 20 MPa from *M. stellatus*. The use of high pressure and temperature conditions are usually proposed. At industrial scale, operation at 35 MPa or higher may be economically advantageous, since both the extraction time and the solvent consumption could be lowered (Crampon et al. 2011).

The influence of ethanol on the extraction yield in the present study could be mainly ascribed to the influence on the solvent polarity, but during operation at more prolonged times other effects could be also important, such as the effects on the interaction with the matrix to enhance desorption of solutes. For safety, toxicity and environmental reasons, ethanol is the preferred polar modifier to enhance

the extraction performance (Zheng et al. 2012; Terme et al. 2017). The role of ethanol has been also observed in other studies with seaweeds, although the influence of increasing the concentration has not been determinant in all studied systems (Terme et al. 2017). Whereas 2% ethanol extracted 41% of the chloroform:methanol extractables, considered as 100% lipid recovery, the use of 8% yielded only 32% from *S. chordalis*. However, the use of pure sc-CO₂ yielded only 4% of the total extractable lipids (Terme et al. 2017). Operating at 29 MPa and 45 °C, these authors found that pure CO₂ provided 4% of the chloroform:methanol yield from *S. chordalis* at 2 MPa, whereas 2 and 8% ethanol modified CO₂ yielded 41 and 32% of the chloroform:methanol yield. These data suggest the need of an adequate selection of the modifier concentration. Supercritical fluid extractions at 20 MPa and 50 °C for 2 h from *G. mammillaris* yielded 0.43%, and Soxhlet with *n*-hexane in 6 h yielded 0.75% (Ospina et al. 2017).

Rozo et al. (2019) confirmed the interest of using the highest tested ethanol concentration (8%) to maximize the extraction yield from *H. musciformis* potential of sc-CO₂ to extract the phenolic fraction from red seaweeds and the antioxidant activity (ABTS, TBARS, protection against LDLs oxidative damage). Kumar et al. (2020) have remarked the promising potential of supercritical carbon dioxide modified with ethanol to obtain antioxidant-rich extracts from *T. conoides* and *S. wightii* in 1 h compared to conventional methods requiring 72 h using 40% ethanol and water, respectively. Ethanol, used as polar modifier, can increase the polarity favouring the extraction of polar lipids and neutral compounds, and also increases the neutral lipid extraction because it can easily penetrate in the cell wall (Terme et al. 2017), whereas chloroform/methanol mixture extracted mainly neutral lipids and glycolipids from *S. chordalis* and glycolipids from *S. muticum* and pure supercritical CO₂ preferentially extracted glycolipids (Terme et al. 2017).

The optimal performance for the phenolic content of the extracts were slightly lower than those found in the SFE extracts of brown seaweeds (2.8–32.5 mg g⁻¹ extract) (Kumar et al. 2020). These authors found that supercritical extracts compared favourably to conventional solvents for the extraction of total phenolics, flavonoids and total antioxidant activity from *S. wightii* and *T. conoides*, and the antioxidant properties were also higher for sc-CO₂ extract and also for those in 40% ethanol. The phenolic content and antioxidant potency were higher in the oil extracted by sc-CO₂ with ethanol as a co-solvent from brown seaweeds compared with the conventional solvent extraction with hexane, ethanol, or acetone: methanol (1:1, v/v) (Sivagnanam et al. 2015). They produced extracts with 0.60 and 0.64 mg GAE g⁻¹ extract from *Saccharina japonica* and *S. horneri* extracts, respectively, obtained by using sc-CO₂ modified

with ethanol, but from *Ascophyllum nodosum* at 25 MPa and 50 °C lower values were attained (Sivagnanam et al. 2024). However, ethanol modified supercritical CO₂ extraction at 25 MPa and 60 °C provided higher β-carotene content from *Eucheuma cottonii* and from *Gracilaria* sp (Setyorini et al. 2018).

Other studies have reported the interest of this technology for the extraction of bioactives. Zheng et al. (2012) reported 30 MPa and 45 °C for the sc-CO₂ extraction with ethanol as modifier for the extraction of antioxidant and antimicrobial compounds from *Gloiopeltis tenax*. Sivagnanam et al. (2015) reported operation at 25 MPa, 45 °C, 27 g CO₂ min⁻¹, 1 mL ethanol min⁻¹ for 2 h to extract the phenolic and antioxidant fraction from *S. japonica* and *S. horneri*. De Corato et al. (2017) used a semipilot equipment (equipped with two vessels of 9 L each) and processed 2 kg dry biomass from brown and red seaweeds operating at 37.9 MPa, 50 °C, 34 kg CO₂ h⁻¹. Ospina et al. (2017) applied a central composite design to optimize the operational conditions to maximize the extraction yield, total content of phenols and carotenes and antioxidant activity from the red seaweed *G. mammillaris* and selected 30 MPa, 60 °C and 8% ethanol. Terme et al. (2017) worked at pilot scale, with around 500 g of ground seaweed at 29 MPa, 45 °C, 10 kg CO₂ h⁻¹ and 2% ethanol during 2 h. Kumar et al. (2020) processed *S. wightii* and *T. conoides* at 25 MPa, 50 °C, 30 g CO₂ min⁻¹, 6% ethanol, 1 h to extract the phenolic fraction. Rozo et al. (2019) selected 30 MPa, 8% ethanol for the extraction of the phenolic fraction with antioxidant activity from *H. musciformis*.

Regarding antioxidant features, Rozo et al. (2019) suggests that the pressure, temperature and ethanol concentration and their interactions are significant on the ABTS radical scavenging properties. The most influencing variable was ethanol content, the antioxidant activity (ABTS and TBARS assays) especially when the ethanol concentration increased from 5 to 8%. They also reported the influence of combination of the extraction parameters to modify the properties of the extract due to variations in composition of the extracts. These authors found a correlation between the antioxidant activity and the number of polyphenols in the samples. However, the presence of other components, such as carotenoids and other pigments could be responsible for the observed activity.

Terme et al. (2018) associated the in vitro antioxidant to the lipid fractions (neutral lipids, glycolipids and phospholipids) from *S. chordalis* and *S. muticum*. All the lipid classes showed free radical scavenging activity at the concentration of 1 mg mL⁻¹, the most potent being the neutral lipid fraction was extracted from *S. chordalis* with a chloroform:methanol mixture. The neutral lipid fraction extracted with sc-CO₂ showed a lower activity than those obtained with solvents, and the addition of ethanol to sc-CO₂ did not enhance the antioxidant activity. The activity of the pure sc-CO₂ was

twice lower than with chloroform:methanol; the addition of ethanol to sc-CO₂ produced a less active fraction and was not influenced by increasing the amount of ethanol despite an increase of the glycolipid content from *S. chordalis* and the content of phospholipids decreased when ethanol was added to sc-CO₂, but 8% ethanol in sc-CO₂ might extract phospholipids with a greater free radical scavenging activity. The non-polar lipids are the most active fraction when they are extracted with solvent mixture and polar lipids either glycolipids or phospholipids are the most active lipid class when the extraction was conducted with supercritical carbon dioxide.

Ethanol used as modifier for the extraction of lipids from *S. japonica* and *S. horneri* provided higher phenolic content and antioxidant capacity (antiradical and reducing properties) than in the oil obtained by conventional extraction using *n*-hexane, ethanol, and acetone:methanol (1:1, v/v), although this latter provided higher oil yields (Sivagnanam et al. 2015). Supercritical CO₂ was effective for the extraction of compounds with antiradical properties, and protecting from oxidation, due to the presence of (-)-epicatechin and phloretin (Rozo et al. 2019). Some fatty acids have also been reported to contribute to the antioxidant activity, among them tetradecanoic acid, *n*-hexadecanoic acid, linoleic acid and oleic acid, present in *G. tenax* (Pal and Gosh 2012). Supercritical carbon dioxide extraction of *G. tenax* showed remarkable antioxidant activity (radical scavenging activity of hydroxyl radical and 2,2-diphenyl-1-picrylhydrazyl, DPPH), and protection of lipid oxidation as the inhibition capacity in the β -carotene/linoleic acid system and antimicrobial activity (Zheng et al. 2012).

Lipid and fatty acid contents are low in red algae; however, they are high content in ω 3 fatty acids, mainly linolenic, eicosapentaenoic and docosahexaenoic acids, and show a low ω 6/ ω 3 ratio (Torres et al. 2019b). This higher ratio of ω 3 to ω 6 fatty acids has been particularly indicated as beneficial at a nutritional level, as well as for reducing blood cholesterol, and for protection against cardiovascular and coronary heart diseases, osteoarthritis, diabetes, as well as having anti-inflammatory and anti-thrombotic properties (Schmid et al. 2018). The ratio of arachidonic + linoleic (ω -6) and eicosapentaenoic acids (ω -3) ranged from 1.65 to 3.21, which, being lower than 4, will help prevent the development of the above pathologies.

Supercritical CO₂ extraction provided higher PUFA content in the extracts than conventional solvents for *S. horneri*, but acetone:methanol provided higher content from *S. japonica* (Sivagnanam et al. 2015). Operation at 40 °C provided higher PUFA yields from *H. charoides* than 50 °C and the increase of pressure show favourable effects (Cheung 1999). The same behaviour was found for *S. hemiphyllum*, except for C18:3 and C18:4, which showed maximum values at 50 °C (Cheung et al. 1998). Whereas 13 fatty acids were

identified in the supercritical extracts, only hexadecanoic acid, octadecanoic acid and palmitelaidic acid were detected in hexane extracts (Ospina et al. 2017).

Furthermore, selectivity towards neutral and polar lipids could be tuned with the use of a polar modifier. Terme et al. (2017, 2018) observed that the chloroform/methanol used in the Bligh and Dyer method yielded 37% neutral lipids and 38% glycolipids from *S. chordalis* whereas pure supercritical CO₂ extracted mainly glycolipids (60%) and with 2% ethanol enhanced the neutral lipid fraction up to 56%. The chloroform:methanol extracts from *S. chordalis* were more potent DPPH scavengers than the supercritical extracts, and among lipid, the most active were neutral, followed by glycolipids and by phospholipids. However, for supercritical extracts, the most active were phospholipids, followed by glycolipids and only the addition of 8% improved the activity of phospholipids (Terme et al. 2018).

Overall, similar conditions to those found in this work have been reported for the extraction of both lipids and bioactives from other red and brown seaweeds. Cheung (1999) obtained maximum lipid yield (between 58 and 67.1 mg g⁻¹ dry weight) from *H. charoides* at 37.9 MPa, 40 °C with 1 mL CO₂ min⁻¹ for 4 h.

The SFE the oily or non-polar fraction from *K. alvarezii*, performed at 20 MPa and 50 °C for 2 h, yielded 0.43%, half of the value extractable with hexane in Soxhlet, which is performed at higher temperatures and more prolonged time (Rudke et al. 2022). These authors also found supercritical was more selective extraction than hexane, regarding the number of compounds identified, but the number of fatty acids in supercritical extract was four times higher. In another study the total amount of lipids recovered with SFE method (40 °C and 25 MPa for 1.5 h) was 0.21%, one third of the value with *n*-hexane, but the lipids extracted with SFE contained more saturated and more PUFAs than those in conventional solvents (Hans et al. 2023). In this work linoleic acid, eicosapentaenoic acid, arachidonic acid, γ -linolenic acid, and docosahexaenoic acid were present in higher concentrations than in *n*-hexane extracts.

Extraction of phytochemical compounds (such as β -carotene, linoleic acids, carrageenan, and polyphenols) from *E. cottonii* and *Gracilaria* sp (Setyorini et al. 2018) and from *M. stellatus* (Cid et al. 2021) with supercritical CO₂ followed by subcritical water have been previously proposed. This sequential processing showed an interesting behaviour in the extracted carrageenans from the mechanical point of view. The gels strength of the extracted carrageenans from the waste solids was comparable to those previously formulated with carrageenans from *G. skottbergii* and their commercial counterparts (Torres et al. 2018; Fontes-Candia et al. 2020). It should be remarked that neither water release nor variation of the rheological properties was observed after one month

cold storage. *Gigartina* is also regarded as a source of protein and peptides with nutritional and functional properties which could enhance the mechanical features of the extracted carrageenan (Echers et al. 2022).

Conclusions

The supercritical fluid extraction of lipids, phenolics and carotenoids from *Gigartina pistillata* can be addressed using ethanol as polar modifier. The influence of temperature was also observed on the yield and content of the target components, however the influence of pressure was more marked on the polyunsaturated fatty acids. The overall yields attained were lower than those reported for other red seaweeds and the solids remaining after the supercritical fluid extraction yielded lower carrageenan but this product showed very strong rheological properties.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10811-025-03534-9>.

Author Contribution CRediT authorship contribution statement Ismael Rodríguez-González: Data curation, Formal analysis, Investigation, Writing – original draft. Beatriz Díaz Reinoso: Formal analysis, Methodology, Supervision, Validation, Writing – review & editing. María Dolores Torres: Formal Analysis, Methodology, Investigation, Validation, Writing – review & editing. Elena Falqué: Formal Analysis, Methodology, Supervision, Validation, Writing – review & editing. Herminia Domínguez: Funding acquisition, Methodology, Supervision, Validation, Writing – review & editing.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature.

Data availability Data can be provided under request.

Declarations

Competing Interests The authors declare no competing interests

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