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Recovery of carotenoids from brown seaweeds using aqueous solutions of surface-active ionic liquids and anionic surfactants

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ABSTRACT

Carotenoids are lipophilic compounds and their production is one of the most challenging, yet rewarding, activities in algal biotechnology. Some carotenoids (fucoxanthin included) have antioxidant activity and many studies have confirmed their health benefits. Fucoxanthin is considered as one of the most important intracellular active metabolites present in brown macroalgae. In this work, aqueous solutions of different surface-active ionic liquids and anionic surfactants were evaluated for the carotenoids extraction. Aqueous solutions of sodium dodecyl sulfate were selected as the media with the best extractive performance. For the best solvent, the solid-liquid ratio, concentration, and time of extraction were the conditions optimized and the maximum yield of extraction of carotenoids attained found to be between 2.57 ± 0.26 $mg_{carotenoids}/g_{dried\ mass}$ and 3.31 ± 0.02 $mg_{carotenoids}/g_{dried\ mass}$, depending of the algae batch used. This said, this work proposed an efficient water-based process of carotenoids extraction, for both dried (more 37.4% of carotenoids extracted) and wet (more 30.2% of carotenoids extracted) biomass matrices.

1. Introduction

Non-native macroalgae species such as *Sargassum muticum*, are nowadays being identified as renewable resources susceptible of valorization, with their constituent fractions transformed into added-value products [1]. Coupled with the recent increase in the exploitation of natural compounds, the industrial and commercial interest for macroalgae compounds like carotenoids, is also increasing [2–5]. For this reason, their global market demand is growing rapidly, being estimated at USD 1.24 billion in 2016 and expected to be USD 1.53 billion by 2021 [6]. Carotenoids are organic intracellular pigments belonging to the tetraterpenes class consisting of eight isoprene units that occur in chloroplasts and chromoplasts. As the chlorophylls, carotenoids are also part of the photosynthetic process [7]. Fucoxanthin is one of the principal carotenoids present in brown macroalgae. It has a yellowish brown color and belongs to the xanthophyll group. These natural compounds are known by their properties [8] from antioxidant [9], to anti-inflammatory [10], and hepatoprotective [4], cardiovascular [11] and cerebrovascular protective [3] activities. Despite the crescent demand for carotenoids, their industrial application is still limited by the lack of sustainable, yet economically viable and efficient processes of extraction and purification. In this sense, adequate conditions are needed, since carotenoids are molecules highly susceptible to environmental conditions, namely high temperatures and light [12]. Nowadays, the main challenges remain the development of efficient and, if possible, low-cost extraction and purification processes, preferably from fresh biomass, in which the activity and structure of carotenoids are retained. Currently, the most common method to extract carotenoids uses conventional solvents (namely ethanol) [8] to remove the carotenoids from the solid biomass, followed by chromatographic techniques [13,14] applied to recover the fucoxanthin fraction. However, most of these platforms do not guarantee the adequate purity level of the final product [14], which is normally regulated by their final application, are complex (time and energy consuming) [8], and use drastic conditions [14]. Therefore, and considering the invasive nature of *Sargassum muticum*, but the similarity between the carotenoids (in terms of content and type) present in the large variety of brown seaweeds, the development of approaches for their rapid purification, preferentially those based on aqueous media and consequently applicable to fresh biomass, is required.

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In this work, aqueous solutions of surface-active ionic liquids and anionic surfactants were studied aiming at the efficient extraction of carotenoids from the brown macroalgae *Sargassum muticum*. The use of these classes of tensioactive compounds in extraction processes has been previously reported in macro and microalgae [15–18]. In the present work, tensioactive compounds, including the surface-active ionic liquids, are used due to their higher affinity to the most hydrophobic compounds like carotenoids. In one of our previous works [19], non-ionic surfactants were already evaluated for the extraction of carotenoids, but despite the high selectivity of the aqueous solutions of some non-ionic surfactants, the extraction yield was not enhanced when compared with the ethanol-based method. In the same year, we used aqueous solutions of ionic liquids to extract more efficiently phycobiliproteins from a red macroalgae [17], and in this work, we were able to observe that the most tensioactive ILs, namely those with higher alkyl chain length, were the most adequate to extract chlorophylls, the other class of hydrophobic pigments potentially present in seaweeds. In this context, this work aims at to evaluate the potential of aqueous solutions of four surface-active ionic liquids and three anionic surfactants (Table 1) extracting carotenoids from the brown seaweed *Sargassum muticum*. Using the best solvent selected, the complete optimization of the process conditions was then performed, namely regarding the solid-liquid ratio, the concentration of surfactant and the time of extraction. As final output of this work, an efficient process to recover carotenoids from fresh biomass is proposed and proved to be more efficient than those previously described, for both matrices of dry and fresh biomass. Taking into account that the SDS could be considered as toxic and considering the industrial application of the optimized methodology developed in this work, different approaches should be experimentally tested to isolate carotenoids from the surfactant-based aqueous extract.

2. Materials and methods

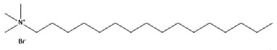
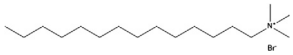
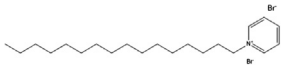
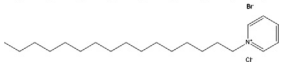
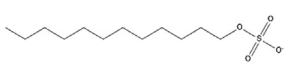
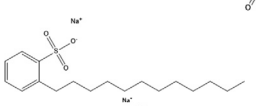
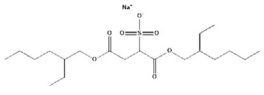
2.1. Materials

The brown macroalgae *Sargassum muticum* used in this work was collected and dried by the ALGAplus, Ltd company. In this work, fresh and dried macroalgae biomass samples were used. The biomass was collected by the ALGAplus staff in the channels of Ria de Aveiro, a coastal lagoon in Aveiro, Portugal. Part of the fresh biomass was cleaned immediately with distilled water and these samples frozen for further use, while the remaining biomass was oven dried by ALGAplus with continuous ventilation, at controlled temperature of 25 °C until constant weight. The biomass samples were then stored in the dark at the University of Aveiro and used for further studies. The solvents used in the conventional extraction method were: ethanol (purity 99%, Fisher Scientific), methanol (purity 100%, CHEM-LAB), ethyl acetate (purity 99%, VWR BDH-Prolabor), chloroform (purity 99%, Carlo Erba), n-hexane (HPLC grade, Carlo Erba e Acros), acetone (purity 100%, VWR Normapur) and acetonitrile of HPLC grade (Fisher Chemical). The chromatography column was prepared with silica gel G-60 (Sigma-Aldrich). Fucoxanthin from Sigma-Aldrich was used as standard for carotenoids, since it is one of the most abundant in this biomass. The surface-active ionic liquids used were tetradecyltrimethylammonium bromide ($[N_{1,1,1,14}]Br$ or TTAB, purity = 99%), acquired at Acros Organics, and the hexadecyltrimethylammonium bromide ($[N_{1,1,1,16}]Br$ or CTAB, purity = 99%), 1-hexadecylpyridinium chloride ($[C_{16}pyr]Cl$ or CPC, purity ≥ 99%), 1-hexadecylpyridinium bromide ($[C_{16}pyr]Br$ of CPB, purity ≥ 99.0%), all purchased from Sigma-Aldrich. The anionic surfactants tested were the sodium dodecyl benzenesulfonate (SDBS), technical grade), and dioctyl sulfosuccinate sodium salt (AOT), purity ≥ 98%), purchased at Sigma-Aldrich and the sodium dodecyl sulfate (SDS) purity ≥ 99%) acquired at Acros Organics. The acronyms, chemical formula, molecular mass, critical micelle concentration (CMC) and chemical structures of these surface-active compounds are provided in Table 1.

2.2. Conventional methodology: Organic solvent extraction (OSE)

The methodology used by Takahashi and collaborators [8] was adapted and applied in this work as the conventional methodology. The dry weight of the algal specimens was established by drying the algae. Then, the algal biomass was subject to mechanical grinding by using a coffee grinder, assisted by successive solvent extractions with ethanol-based solutions. The ethanol-based extracts obtained were then analyzed at 417 nm (maximum peak in the spectrum) for the quantification of the total amount of carotenoids (in $mg_{carotenoids}/g_{dried\ mass}$). Then, these ethanol-based extracts were used in a liquid-liquid separation system prepared by the addition of an aqueous solution of ethyl acetate 1:1 (v/v). The ethyl acetate extract rich in carotenoids was evaporated in a rotary evap-

Table 1
Surface-active ionic liquids and anionic surfactants information: full name, acronyms, chemical formula, molecular mass, CMC and chemical structures.

Type	Name (acronym)	Chemical formula	Molecular weight (g/mol)	CMC in water (mM)	Chemical structure
Surface-active ionic liquids	Hexadecyltrimethylammonium bromide ($[N_{1,1,1,16}]Br$ or CTAB)	$C_{19}H_{42}BrN$	364.45	0.96 [28]	
	Tetradecyltrimethylammonium bromide ($[N_{1,1,1,14}]Br$ or TTAB)	$C_{17}H_{38}BrN$	336.40	1.50 [29]	
	1-hexadecylpyridinium bromide ($[C_{16}pyr]Br$ or CPB)	$C_{21}H_{38}BrN$	384.43	1.5 [30]	
	1-hexadecylpyridinium chloride ($[C_{16}pyr]Cl$ or CPC)	$C_{21}H_{38}ClN$	339.99	0.96 [31]	
Anionic surfactants	Sodium dodecyl sulfate (SDS)	$NaC_{12}H_{25}SO_4$	288.37	8 [32]	
	Sodium dodecyl benzenesulfonate (SDBS)	$C_{18}H_{29}NaSO_3$	348.48	1.6 [33]	
	Dioctyl sulfonsuccinate sodium salt (AOT)	$C_{20}H_{37}NaO_7S$	444.6	2.4 [34]	

orator (Heidolph-Laborota 4000) and the dried extract obtained was re-suspended in methanol (1 mL). The fucoxanthin orange fraction(s) was (were) separated from the ethyl acetate-soluble fraction by percolation of the solution with a preparative silica gel in an open column chromatography (silica G-60) using a chloroform-methanol-water 65:25:4 (v/v/v) solution as the mobile phase. The "orange" fraction was collected, evaporated to dryness and then, the residue was re-suspended again in 1 mL of acetone. This acetone-based extract was percolated in a preparative silica gel in an open column chromatography (silica G-60) with a mobile phase of n-hexane and acetone 6:4 (v/v). The yellowish brown fraction containing fucoxanthin was collected and analyzed by high-performance liquid chromatography (HPLC) to detect the standard peak of absorbance and to quantify the pure fucoxanthin content isolated from the biomass. Pure fucoxanthin was analyzed on HPLC (Shimadzu LC-10A) equipped with a reversed phase C18 column (Vydac 201TP54, 25 cm × 4.6 mm internal diameter), pre-column (Vydac 218GK54 5 uM) and UV–Vis spectrophotometric detector operating at 450 nm. The mobile phase used was a mixture of methanol: acetonitrile 90:10 (v/v), with a flow rate of 1 mL/min. The identification and quantification of fucoxanthin was done by comparison with the standard retention time found (fucoxanthin with high purity level) under the same experimental conditions. These extractions were carried at least in triplicate. The fucoxanthin quantification was done using an external standard calibration curve made with its commercial standard (Sigma-Aldrich). The yield values presented are the average of three sequential sample injections. The average yield of fucoxanthin extracted was expressed in $mg_{fucoxanthin}/g_{dried\ mass}$.

2.3. Alternative methodology: Screening of various surface-active ionic liquids and anionic surfactants on the carotenoids extraction

The grinded biomass of *Sargassum muticum* was mixed with aqueous solutions of four surface-active ionic liquids - CTAB, CPC, CPB, TTAB - and three anionic surfactants, namely SDS, SDBS and AOT. The first experiments corresponded to the screening of the extractive capacity of these seven tensioactive compounds on extracting carotenoids from the algal biomass. This screening was made by evaluating the concentration of each tensioactive compound based on their CMC. Different concentrations of surfactants were tested, considering as starting point the CMC values of each ionic liquid and anionic surfactant under study. Values of one (1*x*CMC), two (*2x*CMC), three (3*x*CMC) and six times the CMC (6*x*CMC*)* were tested for all the surfactants and tests for the best tensioactive compound were also made considering the concentrations from 6*x*CMC to 30*x*CMC. For the extraction time (t), *i.e.* the time of contact between the biomass and the solvent, 90 min were adopted, following the time previously optimized in [19]. The solid-liquid ratio [$R_{(S/L)}$], meaning the mass (mg) of algal biomass *per* volume (mL) of solvent, was fixed at 0.02, again following the condition initially adopted in [19]. The grinded *Sargassum muticum* macroalgae was placed in contact with various aqueous solutions of each tensioactive compound (ionic liquids and anionic surfactants) under constant agitation at 250 rpm, room temperature (25 °C), and in the dark due to the sensitivity of carotenoids to light. Then, these mixtures (biomass and aqueous solutions of tensioactives) were centrifuged (Thermo Scientific, Megafuge 16R) at 5000 rpm, 4 °C, for 40 min. After centrifugation, the supernatant was collected and further analyzed in a UV–vis spectrophotometer (SHIMADZU, UV-1700 Pharma Spec). The whole spectrum from 350 to 700 nm was defined in order to identify and better characterize the typical region of carotenoids, between 400 and 500 nm. The carotenoid content on each supernatant formed after the centrifugation step was quantified at 417 nm (maximum peak of absorbance in each spectrum in the region between 400 and 500 nm) by using adequate calibration curves. The extraction procedure was applied in triplicate. The yield of extraction of carotenoids was determined in triplicate, with the results presented as the average of the three experiments (in $mg_{carotenoids}/g_{dried\ mass}$). The output of this experiment was defined as the starting condition of the optimization step carried using the anionic surfactant media to extract as much as possible the carotenoids content.

2.4. Optimization of the extraction conditions: Response Surface Methodology (RSM)

The processing conditions to extract carotenoids were optimized considering the surface-active compounds with the best extraction performance. The parameters optimized were the concentration of the tensioactive (C_{surf}), the time of extraction (t) and the solid-liquid ratio [$R_{(S/L)}$]. For that purpose, a 2^3 factorial planning was implemented (Table A1 from Supporting information). The focus of the RSM analysis was the identification of the most significant parameters and their main interactions in the extraction process. Therefore, to facilitate the simultaneous analysis of different conditions with relevance in the extraction process, this 2^k factorial planning was carried out, in which there are k factors that can contribute to a different response regarding the final concentration of carotenoids in just one step of extraction. According to a second order polynomial equation (Eq. (1)), the experimental data was treated.

$$\boldsymbol{y} = \boldsymbol{\beta}_0 + \boldsymbol{\beta}_i \boldsymbol{X}_i + \boldsymbol{\beta}_j \boldsymbol{X}_j + \boldsymbol{\beta}_{ii} \boldsymbol{X}_i^2 + \boldsymbol{\beta}_{jj} \boldsymbol{X}_j^2 + \boldsymbol{\beta}_{ij} \boldsymbol{X}_i \boldsymbol{X}_j \quad (1)$$

In this equation, y is the dependent variable concentration of carotenoids (in $mg_{carotenoids}/g_{dried\ mass}$), β_0, β_i, β_j β_{ii}, β_{jj} and β_{ij} are the regression coefficients representing respectively, the intersection, linear, quadratic and interaction of the terms and X_i and X_j are representing the independent variables in the 2^k factorial planning.

The central point (zero level), factorial points (1 and −1, level one) and axial points (level α) were defined, being the 2^3 factorial planning provided in Table A2 from Supporting Information. The axial points are encoded at a distance α (Eq. (2)) from the central point [20], defined as:

$$\boldsymbol{\alpha} = (2^{\boldsymbol{k}})\frac{1}{4} \quad (2)$$

The results obtained were statistically analyzed considering a confidence level of 95%. The model adequacy was determined [21] and three dimensional surface response plots were originated by varying two variables within the experimental range, and maintaining the remaining factors at the central point. The factorial planning used a central point experimentally determined at least 6 times. Moreover, 20 experiments were performed, with the various processing conditions being repeated to guarantee the accuracy of the data, when necessary. The Statsoft Statistica 8.0© software Statsoft© was applied in the statistical analysis, and the response surfaces and contour plots were developed with the same software.

2.5. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) analysis was carried out using a Hitachi SU-70 microscope operating at 15 kV and freeze dried samples were deposited on carbon conducting tape and coated with a carbon layer.

3. Results and discussion

This work aims the development of an efficient process of extraction of carotenoids from *Sargassum muticum*. For that pur-

pose, the conventional use of organic solvents is aimed to be replaced by a water-based solid-liquid extraction for which the use of aqueous solutions of anionic surfactants and surface-active ionic liquids is optimized.

3.1. Conventional methodology: Organic Solvent Extraction (OSE)

In this work, dried algal samples were adopted to perform the optimization studies of carotenoids extraction. However, it should be mentioned that, because this biomass is not produced in aquaculture but wild harvested, its chemical composition, in which pigments are included, is expected to be highly variable [22]. This variability in the amount and even type of carotenoids present was thus taken into consideration during the experiments. In this context, and highlighted the origin and pre-treatment of the biomass, this work can be divided into two main parts considering the process of extraction being developed (organic-based or aqueous-based) or regarding the biomass used to perform the experiments (dried or fresh samples). Despite the different scenarios, our main objective was the development of a simpler and organic solvent free process of (solid-liquid) extraction to recover as much as possible the carotenoids from this low-cost algal biomass and inhibiting the simultaneous extraction of other pigments (*e.g.* chlorophylls). Taking into account the high selectivity recently proved for ionic liquids [19], in this work aqueous solutions of four surface-active ionic liquids were investigated as well as three anionic surfactants. To evaluate the efficiency of the alternative process here developed, the conventional (solid-liquid) extraction using ethanol was tested. The ethanol-based extract rich in carotenoids was subject to several purification steps using chromatography with open columns of silica G-60 to purify the extract and recover the pure fucoxanthin fraction. Before the chromatographic purification, the total content on carotenoids and fucoxanthin from the *Sargassum muticum* (batch S1.0315.D.UA, for details see Table A1 in Supporting information) present in the ethanol-based extracts was quantified by UV–vis spectrophotometry and HPLC obtaining, respectively, 1.87 ± 0.02 $mg_{carotenoids}/g_{dried\ mass}$ and 0.1000 ± 0.004 $mg_{fucoxanthin}/g_{dried\ mass}$, or in other words, 5.35% of fucoxanthin. In this extraction, a solid-liquid ratio of 0.1 was used, *i.e.* an ethanol volume 10 times higher the biomass weight. The process was repeated several times until no carotenoids were detected in the spectrum between 400 and 500 nm (representative of fucoxanthin). The yield of extraction of fucoxanthin obtained is similar to that reported in literature for other *Sargassum* species with experimental results between 0.08 ± 0.05 $mg_{fucoxanthin}/g_{dried\ mass}$ [23] and 1.01 ± 0.39 $mg_{fucoxanthin}/g_{dried\ mass}$ [13]. These differences in the amount of carotenoids extracted are justified by the combination of intrinsic factors like the species (different locations originate different algal compositions [19]), and external conditions such as the environmental stress, seasonality, geographic location and the adaptability of the various *Sargassum* species, principally those due to their invasive nature [19,23–25]. The results obtained by the conventional ethanol-based extraction methodology were used in this work as a control to evaluate the success of the alternative methodology. Meanwhile, we call the attention to the fact that, even when using the same conditions for both methodologies, the experimental results obtained for the yield of extraction of carotenoids vary when different conditions have been analyzed. This variation is justified by the use of distinct batches of biomass, which were used due to the conditioned availability of the *Sargassum* biomass. Due to its invasive nature, this biomass needs to be collected when it is available and then it is stored under controlled conditions by the company ALGAplus. To avoid misunderstandings, the comparisons carried in this work between the two methodologies will be done considering the experimental results obtained for the same batch of algal biomass, which will be indicated during the work.

Aqueous solutions of four surface-active ionic liquids, namely CTAB, TTAB, CPC, and CPB, and three anionic surfactants, the SDS, SDBS and AOT were evaluated to extract carotenoids from *Sargassum muticum*. In a first stage, a screening of the various tensioactive compounds in aqueous solution was performed with increased concentrations in water and according to their CMC values, namely at 1*x*CMC, 2*x*CMC, 3*x*CMC to 6*x*CMC (see CMC values in Table 1), being the experimental data reported in Fig. 1. From these experimental results, it is concluded that the most promising

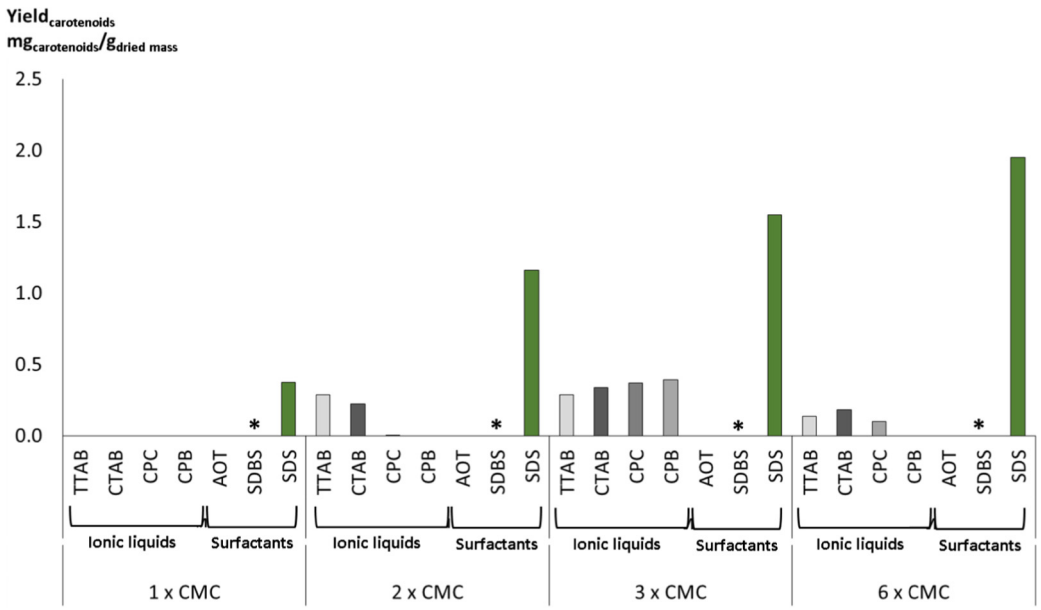


Fig. 1. Effect of increasing the concentration of various surface-active ionic liquids and anionic surfactants in the yield of extraction of carotenoids from dried biomass of *Sargassum muticum*. *SDBS is an exception, because despite the UV–Vis spectra indicates some yield of extraction (Fig. A2 in Supporting information file), the extraction of carotenoids with this surfactant is practically null. Actually, SDBS is extracting some compounds between 300 and 400 nm, *i.e.* outside the fingerprint zone of carotenoids (400–500 nm), which is negatively affecting the spectra in the carotenoids region.

results by far were obtained with SDS, and that the carotenoids yield of extraction increases with the increase of the tensioactive concentration, for both classes of compounds, anionic surfactants and surface-active ionic liquids. The experimental data suggest that there are minimum concentrations of each tensioactive solvent required to start the carotenoids extraction. The minimum concentrations required were not precisely determined but it is clear the difference in the extraction performance when systems with 1*x*CMC, 2*x*CMC, 3*x*CMC and 6*x*CMC are compared, principally for the best (SDS) and worst (CPB) tensioactive compounds. The surfactants can play two major tasks, they can promote/help the cell disruption and/or they can help in the solubilization of the most hydrophobic compounds. In certain cases, and as previously shown [16,17], tensioactive aqueous solutions can easily act as solvents helping in the solubilization of diverse compounds. In this work, when analyzing the systems with TTAB, CTAB, CPC, CPB (surface-active ionic liquids) and AOT (anionic surfactant) at 1*x*CMC, the results do not demonstrate capacity to remove and solubilize the carotenoids from the biomass. In this case, the results seem to suggest that, at these low concentrations, the amount of these tensioactive compounds is not enough to attack the cells. In fact and despite the partial ability of these surface-active ionic liquids to extract carotenoids, the anionic SDS is much better in this purpose. Thus, it seems that SDS presents the most pronounced ability to extract carotenoids, even at low concentrations, with yields of extraction increasing from 0.37 $mg_{carotenoids}/g_{dried\ mass}$ (1*x*CMC) to 1.95 $mg_{carotenoids}/g_{dried\ mass}$ (6*x*CMC).

Aiming at understanding the impact of different solvents and mechanical conditions on the cell disruption, SEM images were taken (Fig. 2), considering three distinct scenarios: (2A) represents the cells dried and powdered, *i.e.* without the presence of any solvent, (2B) corresponds to the cells in the presence of water, and (2C) shows the aspect of the cells in presence of aqueous solutions of SDS at 1xCMC (best solvent). The extractions with water and aqueous solutions of SDS were made according to the standard protocol described in Section 2.3, considering in particular, the extraction time (t) of 90 min and a solid-liquid ratio [$R_{(S/L)}$] of 0.02. The SEM images shows how the use of ionic surfactants improves the extraction of carotenoids. From Fig. 2A we observe that the drying and cutting processes are quite significant promoting the cellular disruption. Since the cells are (at least) partly destroyed, a solvent is necessary to carry the extraction of carotenoids from the biomass. As shown in Figs. 2A and 2B, there is no difference between the cells with and without the presence of water. For the aqueous solutions of SDS (Fig. 2C) a higher

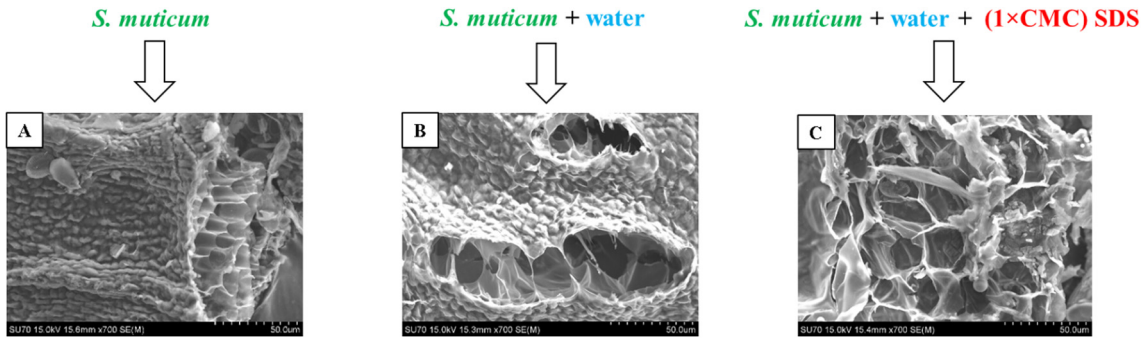


Fig. 2. Scanning Electron Microscopy (SEM) of the *Sargassum muticum* cells. (A) represents the algal cells after the drying process and in powder; (B) represents the residual biomass after extraction of carotenoids with water; (C) represents the algal cells after the extraction performed with an aqueous solution of SDS at 1xCMC. The scale bar for the SEM images is 50 μm and the three images are expanded 700×.

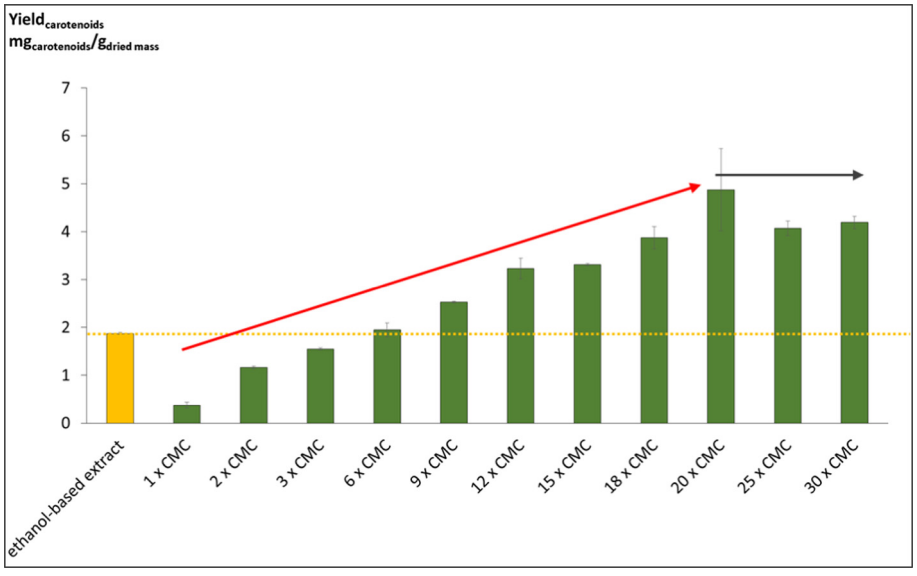


Fig. 3. Effect of various concentrations of SDS in the yield of extraction of carotenoids from dried biomass of *Sargassum muticum*. The yellow dashed line represents the yield of extraction obtained for the ethanol-based methodology. The red and grey arrows illustrate, respectively, the two different profiles obtained for the yield of extraction of carotenoids as function of the surfactant concentration represented in a CMC basis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

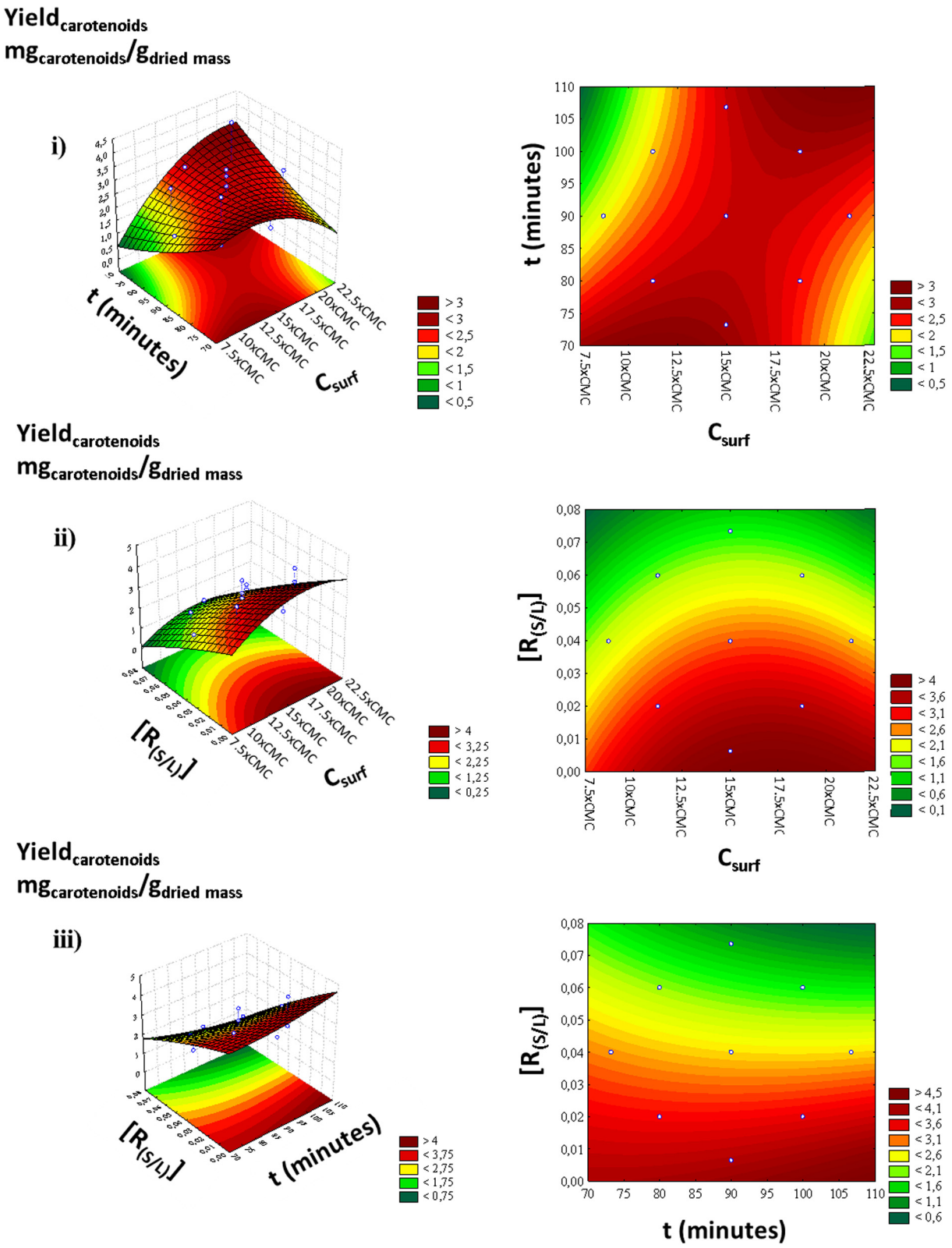


Fig. 4. Factorial planning (2^3): Response surface plots (left) and contour plots (right) on the yield of extraction of carotenoids ($mg_{carotenoids}/g_{dried\ mass}$) with the combined effects of (i) C_{surf} and t in minutes, (ii) C_{surf} and $[R_{(S/L)}]$ and (iii) $[R_{(S/L)}]$ and *t* in minutes, using different aqueous solutions of SDS.

disintegration of the cell wall was observed when this surfactant (1xCMC) is in contact with the macroalgae cells. Actually, it is the probable double action of SDS, namely as a cell disruption and hydrotrope agent, which explains the highest yields of extraction assessed for SDS.

After the selection of SDS as the best solvent, new tests were carried at higher SDS concentrations (from 1xCMC to 30xCMC), aiming at the determination of the maximum value of yield of extraction. The results, depicted in Fig. 3, showed a direct correlation between the SDS concentration increase and the extraction

yield of carotenoids. However, while an increase in the extraction yield is observed for concentrations up to 20xCMC (red arrow) above this concentration the extraction yield is essentially constant up to 30xCMC (grey arrow).

A 2^3 factorial planning study (3 factors and 2 levels) was then carried using the concentration of 15xCMC of SDS, the [$R_{(S/L)}$] of 0.04 and the time of extraction (t) of 90 min as central point (Tables A2 and A3 in Supporting information). All the experimental results obtained and variables optimized are presented in Supporting information. These variables include the model equations, the yield of extraction of carotenoids theoretically determined using the correlation coefficients obtained in the statistical treatment, as well as all the parameters obtained in the statistical analyses (Tables A4, A5 and A6). The accuracy and precision of the model equations used in this factorial planning were validated by comparing the experimental and predicted data achieved for the yield of extraction under the selected conditions (Table A4). Fig. 4 depicts the main results obtained, which indicates the [$R_{(S/L)}$] as the most significant variable. The experimental data suggest that when the [$R_{(S/L)}$] decreases, the yield of extraction of carotenoids increases. Moreover, it is also shown that the use of concentrations of SDS around 15xCMC is adequate for the extraction of carotenoids, because for the concentration range studied, the concentration of surfactant along with the time of extraction are considered as non-statistically significant variables (also established by the Pareto's Diagram; Fig. A3 in Supporting information). As shown in Fig. 3, the yield of extraction of carotenoids at 15*x*CMC (or 0.12 mol/L) obtained was around 2.57 ± 0.26 $mg_{carotenoids}/g_{dried\ mass}$. The maximum yield obtained during the optimization of the process conditions is lower than that previously obtained in the screening of the surfactant concentration shown in Fig. 3 because a different algae batch was used for the optimization. After the optimization of the process conditions, a comparison between the yields of carotenoids extracted using the ethanol-based conventional method (1.87 ± 0.02 $mg_{carotenoids}/g_{dried\ mass}$) and the alternative methodology using SDS (2.57 ± 0.26 $mg_{carotenoids}/g_{dried\ mass}$) was carried. The results obtained show the high efficiency of the water (surfactant)-based methodology, against the use of surface-active ionic liquid aqueous solutions or the ethanol-based methodology. Actually, the use of aqueous solutions of SDS promotes the extraction of more 37.4% of carotenoids.

3.2. Extraction of carotenoids from fresh Sargassum muticum

Because the drying process of the biomass is one of the high-cost steps in the recovery of carotenoids, the study of their extraction from the fresh biomass was performed. In this sense, the same process conditions previously optimized for the dried biomass were adopted. Part of the wild harvested alga was dried (batch S1.4915.D.UA - Table A1 in Supporting information) and the extraction of carotenoids performed following both the conventional and alternative methods. For the alternative method, aqueous solutions of SDS at 15xCMC were used. The main results obtained are reported in Fig. 5, and by comparison between both extraction methods for the dried and fresh biomass using the same biomass batch it is possible to infer about the suitability of the alternative method for the processing of fresh biomass. The data depicted in Fig. 5 shows that the conditions previously optimized for the ethanol-based extraction process and the SDS aqueous solutions are adequate to extract carotenoids from the wet biomass. In fact, these conditions allow even higher extractions of carotenoids from the fresh biomass. These results can be justified by the potential degradation of the carotenoids structure when the dried process was applied. Despite the absence of experimental results proving this theory in this work, some more complete studies are being performed in order to prove the effect of the dry process being largely used by the ALGAplus company. Without any further optimization, the extraction yields obtained were (i) for the ethanol-based methodology 6.48 ± 0.01 $mg_{carotenoids}/g_{dried\ mass}$ for the fresh biomass and 2.38 ± 0.68 $mg_{carotenoids}/g_{dried\ mass}$ for the dried biomass and (ii) using SDS aqueous solutions, 8.44 ± 1.23 $mg_{carotenoids}/g_{dried\ mass}$ for the fresh biomass and 4.09 ± 0.34 $mg_{carotenoids}/g_{dried\ mass}$ for the dried biomass. Envisaging the industrial application of this methodology, the isolation of carotenoids from the surfactant-based aqueous extract is demanded. Actually, and following one of our last works [26] using SDS, the use of a dialysis step is proposed in this work as a simple alternative to promote the isolation of carotenoids from the surfactant, thus allowing its potential recycle and reuse in new extraction cycles.

Summing up, it was possible to prove the higher extraction efficiency of the alternative method developed in this work, by applying aqueous solutions of SDS, when compared with both the conventional methodology (more 30.2% of carotenoids being extracted with the SDS aqueous solution from fresh biomass),

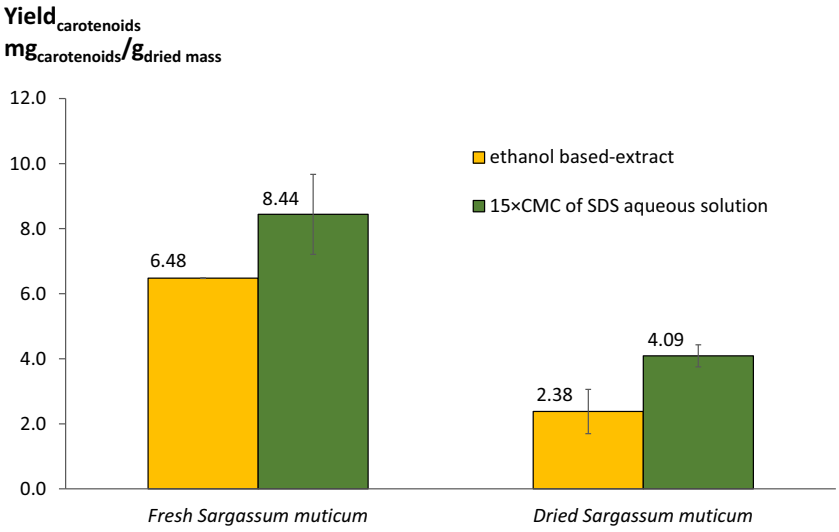


Fig. 5. Yield of extraction of carotenoids ($mg_{carotenoids}/g_{dried\ mass}$) obtained for both the dried and fresh biomass of *Sargassum muticum*, by applying both extraction methods explored in this work. The alternative method using SDS was carried considering the conditions previously optimized for the dried biomass (15xCMC of SDS; time of extraction of 90 min and [$R_{(S/L)}$] of 0.04).

and the alternative method based in non-ionic surfactants recently developed by us [19].

4. Conclusions

Since 2011 that tons of *Sargassum* spp. have been causing severe disruption in several economic activities in the Caribbean coast, which determines its removal and valorization as a critical issue included in the biodiversity conservation [27]. In this work, an alternative method to recover carotenoids from *Sargassum muticum* was developed in a water base, since one of the major limitations regarding the algae processing is their large water content. This alternative methodology, tested using different aqueous solutions of anionic surfactants and surface-active ionic liquids, was optimized. In this work, the anionic surfactant SDS was selected as the best solvent to extract carotenoids, not only compared with the use of pure ethanol, but also evaluated against the application of the surface-active ionic liquids. 15xCMC of SDS in aqueous solution was defined as the most appropriate concentration to be applied, leading to an extraction yield of 2.57 ± 0.26 $mg_{carotenoids}/g_{dried\ mass}$. After the optimization of the process conditions, the alternative water-based extraction developed was proved to be a successful method for both the dried (with more 37.4% of carotenoids extracted in comparison with the ethanol-based methodology) and fresh biomasses (with more 30.2% of carotenoids extracted).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.seppur.2017.05.006.

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