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PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF EXTRACTS FROM *Bifurcaria bifurcata* ALGA, OBTAINED BY DIVERSE EXTRACTION CONDITIONS USING THREE DIFFERENT TECHNIQUES (HYDROTHERMAL, ULTRASOUNDS AND SUPERCRITICAL CO_2)

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Abstract

Extracts of *Bifurcaria bifurcata* seaweed were obtained by diverse conditions. Different extraction techniques, such as hydrothermal and ultrasounds, and three different solvents (water, ethanol and water/ethanol (50:50)) depending on technique were used. Moreover, supercritical CO_2 (SC-CO_2) with 10% of ethanol as co-solvent using different extraction times (30, 45 and 60 min) was also used as extraction technique. Extraction yield, phenolic content and antioxidant activity were measured for each extract. Hydrothermal extraction obtained better extraction yields than ultrasound extraction. Regarding the effect of solvent composition, water/ethanol (50:50) in hydrothermal treatment (HW50E50) and water/ethanol (50:50) in ultrasound treatment (UW50E50) showed the highest extraction yields. The worst extraction yields were shown by the extraction with SC-CO_2. Water/ethanol (50:50) showed to be more efficient extracting phenolic compounds than water, although the highest extraction was achieved by ethanol. On the other hand, ultrasound-assisted extraction seemed to be more efficient extracting phenolic compounds than hydrothermal extraction. From the results obtained, it can be concluded that the use of ultrasound extraction technique and the use of water/ethanol as extracting solvent seemed to be the best extraction condition.

Keywords: Bifurcaria bifurcata, hydrothermal, supercritical CO_2, ultrasound

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1. Introduction

The exploitation of antioxidant bioactive compounds is of great interest for consumers, industries and researchers due to the beneficial effects associated with their regular consumption and their potential use as food additives, in pharmaceutic and cosmetic industries to avoid oxidation processes (Patil et al., 2009). Marine sources of bioactive compounds have been gaining attention among the dietary and natural sources, particularly for its polyphenolic composition (Barba, 2017; Poojary et al., 2017). Phlorotannins is the main group of phenolic compounds in brown and other alga species. This

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group has been related to potential health benefits such as antidiabetic, antihypertensive and anti-inflammatory. Phlorotannins also exhibit antioxidant activity with promising application in pharmaceutical, food and chemical industry (Ibañez and Cifuentes, 2013; Stengel et al., 2011; Thomas and Kim, 2011). Brown seaweeds have high concentrations of nutrients (Lorenzo et al., 2017). In addition, they also possess high amounts of phenolic compounds (from 1 to 14% dry weight (DW)), being Ascophyllum and Fucus the two genera with the highest contents (Holdt and Kraan, 2011).

Bioactive compounds are isolated from algal biomass by different methods. Conventional extraction methods have been used to extract bioactive compounds from plant materials for a long time (Barba et al., 2014; Blaga et al., 2018; Liza and Abdul, 2010; Tanase et al., 2018). These conventional extraction methods (extraction in Soxhlet apparatus, solid-liquid extraction, and liquid-liquid extraction) have some disadvantages: demand of high volumes of solvent, difficult solvent separation after extraction, degradation of thermolabile compounds when extraction is done at high temperatures, time-consuming or energy intensive protocols (Dai and Mumper, 2010; Poojary et al., 2017).

In contrast to the classic extraction methods, extraction assisted by innovative processing technologies is developing fast due to the ongoing consumer demands for clean and green extraction technics with minimal use of organic solvents and value addition to food processing by-products (Barbosa, 2005; Galanakis, 2013). The main advantage of these novel technologies is the preservation, or at least the reduction of biological activity, after extraction (Michalak and Chojnacka, 2015).

Ultrasound-assisted extraction is a non-conventional extraction technology widely used in the extraction of bioactive compounds from natural sources (Corbin et al., 2015; Roselló-Soto et al., 2015; Zhu et al., 2017). This technology increases the extraction efficiency and reduces energy requirements and solvent consumption compared to traditional methods (Macías et al., 2009). Ultrasound technology is based on the production of ultrasonic jet toward the solid surfaces, which induces cell disruption and particle size reduction. Thereby, ultrasound increases the contact area between solid and solvent and also facilitates the penetration of solvent and its contact with soluble compounds (Kentish and Feng, 2014).

Other alternative extraction method to conventional procedures, and usually proposed, is the use of supercritical fluid extraction (SFE) (Barba et al., 2014; Michalak et al., 2015). Supercritical fluids have been gaining increasing attention because of its environmentally friend and improved reaction media character. Supercritical fluids are also cheap, non-toxic, non-flammable, non-explosive, and offer essential advantages compared to other substances (Lang and Wai, 2001). In the SFE, the solvent is conditioned at temperature and pressure above its critical point which improves transport properties: decreases viscosity and increases diffusivity. The use of CO_2 as a supercritical fluid offers numerous advantages: it is non-toxic, noncorrosive, easily separated from the extract (Pan et al., 2012), cheap, available, inert to the product, non-flammable, and shows improved affinity to volatile compounds (Crampon et al., 2011).

Although the use of total phenolic compounds and total antioxidant capacity have been reviled over the last years due to their non-specificity and the lack of scientific evidence regarding the beneficial effects of polyphenol-rich foods can be attributed to the antioxidant properties of these food, these parameters are still useful. For example, they can constitute a screening tool to predict the yield of antioxidant compounds, especially after extraction processes, which are for example used for the preparation of food additives, at the pharmaceutical and/or cosmetic level in the formulation of preparations to prevent their oxidation.

In this study, different extraction conditions were tested on *Bifurcaria bifurcata* brown macro-alga. Two different extraction techniques, hydrothermal and ultrasounds, with three different extraction solvents, water, ethanol and water/ethanol (50:50) depending on the technique were used. An extraction technique using supercritical CO_2 with 10% of ethanol at different times, 30, 45 and 60 min, were also used. The aim was to assess the different extraction conditions (technique-solvent or technique-time in case of supercritical CO_2) according to the phenolic extraction and antioxidant activity of the extracts. In addition, extraction yield was also of interest.

2. Material and methods

2.1. Algal material

The brown seaweed *Bifurcaria bifurcata* used in the present study was kindly supplied by Portomuiños company (A Coruña, Spain). It was collected in the Atlantic Ocean, in the area of Camariñas (A Coruña, Spain). The seaweed was dried (40°C) and grounded to obtain a powder with a particle lower than 0.8 mm, using a conventional mincer. Then, the seaweeds were passed through a 0.8 mm mesh sieve and stored under vacuum (75%) in plastics bags at -20°C until further analysis.

2.2. Obtaining of Bifurcaria bifurcata extracts using hydrothermal and ultrasonic techniques

The ground alga (5 g) was mixed with 50 mL of distilled water (HW100) and with 50 mL of water/ethanol (50:50) (HW50E50) in glass bottles (hydrothermal technique), and with 50 mL of distilled water (UW100), 50 mL of water/ethanol (50:50) (UW50E50) and ethanol (UE100) in Erlenmeyer flasks (ultrasound technique). In the hydrothermal technique, the bottles were introduced in an autoclave (Raypa Stericlav-S 150 L, Terrassa, Spain) and the

extraction conducted at 121ºC for 30 min. In the ultrasound technique the Erlenmeyer flasks were introduced in an ultrasonic bath (Branson ultrasonic M3800-E, Dietzenbach, Germany) and the extraction conducted at room temperature for 30 min. Then, the sets for both techniques were centrifuged at 4000 rpm for 10 min at 4ºC, and filtered through a lab filter paper to remove the residue. The extracts obtained were stored at -20ºC until needed for analysis.

2.3. Obtaining of Bifurcaria bifurcata extracts by extraction with supercritical CO_2

A one-liter cylinder extractor with two 500 mL separators (Thar Designs SFE-1000 F-2-C10, Pittsburgh, USA) was used for the extraction experiments. The CO_2 was precooled using a circulating bath (PolyScience, USA, model 9506) prior being pumped with a P-200A piston pump (Thar Design Inc.) (flow rate: 25 g CO_2 min^{-1}). The co-solvent, ethanol, was pumped by a HPLC pump (Scientific Systems, Inc., USA, model Series III). The flow rate was adjusted to achieve concentrations of a 10% of ethanol. The extraction by SC-CO_2 was conducted at temperature of 40 ºC and pressure of 35 MPa. The extracts were collected at 30, 45 and 60 min (SC–CO_2–30; SC–CO_2–45; and SC–CO_2–60, respectively), and stored at -20ºC until needed for analysis analysis.

2.4. Measurement of the extraction yield

An aliquot of 5 mL from each extract was taken and evaporated in a drying oven at 100ºC overnight. The weight of the final residue was used to calculate the extraction yield by gravimetry. Result was expressed as g extract 100 g^{-1} DW. The dry weight of *Bifurcaria bifurcata* seaweed was calculated subtracting its moisture content, which was measured by the International Organization for Standardization (ISO) recommendation for moisture content (ISO 1442, 1997).

2.5. Determination of the phenolic content

Phenolic content was determined for all extracts obtained by all extraction conditions and solvents. This determination was based on a procedure described by Medina et al. (2009) as follows: 15 µL of samples were mixed with 170 µL of Milli-Q water, adding 12 µL of Folin-Ciocalteu reagent and 30 µL of sodium carbonate. The mixtures were incubated for 1 h at room temperature in the dark. After the reaction period, 73 µL of Milli-Q water were added with a multichannel pipette. Absorbance was measured at 765 nm. Phenolic content was expressed as g phloroglucinol equivalents (PGE) 100 g^{-1} DW.

2.6. Measurement of the antioxidant activity

Antioxidant activity was measured using the oxygen radical absorbance capacity (ORAC) assay described by Ou et al. (2001) and modified by Dávalos et al. (2003). In order to carry out the reaction, 75 mM phosphate buffer (pH 7.4) was used, being the final reaction volume of 200 mL. Twenty microliters of antioxidant were mixed with 120 mL of fluorescein (70 nM) and incubated for 15 min at 37ºC. After that, 60 mL of 12 mM 2,2-azobis (2-methylpropionamidine) dihydrochloride (AAPH) were added. Then, the plate was placed into the reader and the fluorescence was recorded each minute for 120 min (excitation and emission wavelengths of 485 and 520 nm, respectively). The plate was automatically stirred before each measurement. A blank sample consisting of phosphate buffer instead of the antioxidant extract, and eight calibration solutions (using Trolox as antioxidant) were also determined. Results were calculated on the basis of the differences in areas under the fluorescein decay curve between the blank and the sample, and were expressed as µmol of Trolox equivalents (TE) g^{-1} DW.

2.7. Statistical analysis

The differences in extraction yield, phenolic content and antioxidant activity among the extraction conditions used in *Bifurcaria bifurcata* seaweed were examined using an ANOVA test. Least-squares means were compared among extraction conditions using the Duncan's post hoc test (significance level $P < 0.05$). The values were given in terms of mean values ± standard deviations (n=2). All statistical analysis were performed using IBM SPSS Statistics® 21 software.

3. Results and discussion

3.1. Extraction yield

Yields of extracts from *Bifurcaria bifurcata* are presented in Table 1. The highest extraction yield was obtained by HW50E50 followed by UW50E50 and HW100 extraction conditions (41.82; 39.11 and 37.48 g extract 100 g^{-1} DW, respectively). The combination of ultrasound with pure solvents (UW100 and UE100 for water and ethanol, respectively) gave extracts of reduced yield (22.27 and 14.67 g extract 100 g^{-1} DW, respectively), while the lowest extraction yields were obtained for SC-CO_2 extraction technique (average yields lower than 4 g extract 100 g^{-1} DW). In a similar way, Hwang and Do Thi (2014) found that solid-liquid extraction by hydrothermal aqueous extraction at 100 ºC enhanced extraction yield compared to 37ºC in dried (41 *vs* 25%, respectively), roasted (41 *vs* 32%, respectively), and seasoned (26 *vs* 21%, respectively) *Porphyra tenera* red alga. The extraction yield values reached by them in dried and roasted laver at 100ºC was very similar to ours with a close temperature (37.48±0.77 vs 41.3-40.6 g 100g^{-1} DW, respectively). On the other hand, the use of ultrasounds with the same solvent by us achieved a lower value (22.27±0.77 g 100g^{-1} DW). However, when Hwang and Do Thi (2014) extracted with 100% water at 37 ºC, they reached extraction yield values similar to ours

in all algae processed methods (dried, roasted and seasoned) (22.27±0.77 vs 25.5-32.1-21 g $100g^{-1}$ DW, respectively). Another interesting result observed by these authors is the effect of solvent composition on extraction yield. *Porphyra tenera* red alga extracts obtained from aqueous extraction at 37°C had higher extraction yield than extracts with 70% ethanol as solvent for dried (25 *vs* 18%, respectively), roasted (32 *vs* 20%, respectively), and seasoned (21 *vs* 16%, respectively) alga. Tierney et al. (2013) reported the same finding on *Ascophyllum nodosum*, *Pelvetia canaliculata*, *Fucus spiralis* brown algae and *Ulva intestinalis* green alga collected in Irish coast (from 19 up to 30% for water extracts and between 3 and 24% for water/ethanol extracts).

On contrary, our results showed that using 50% ethanol improved the yields than using 100% water, both at 121ºC and at room temperature by ultrasounds (41.82±2.30 vs 37.48±0.77 g $100g^{-1}$ DW and 39.11±1.54 vs 22.27±0.77 g $100g^{-1}$ DW). López et al. (2011) also reported that the mixture of an organic solvent such as methanol and water in a solid-liquid extraction improved the extraction yield in the *Stypocaulon scoparium* brown alga in comparison to 100% water solvent. The solid-liquid extraction in several alga species from Danish coast using 100% water (14-51%) showed higher extraction yields than using 100% ethanol (3-28%) at room temperature (Farvin and Jacobsen, 2013). This finding is in agreement with ours using the ultrasound technique (22.27±0.77 vs 14.67±0.77 g 100^{-1} DW).

The lower extraction yield observed for SC-CO_2 treatments may be explained by the balance between the interactions of alga solutes with CO_2 and alga matrix components. Our previous research about the proximate composition of *Bifurcaria bifurcata* and other alga species (*Ascophyllum nodosum* and *Fucus vesiculosus*) extracts revealed that most of the components in these algae were suggested to be of high polarity (Agregán et al., 2017). On the other hand, the characteristics of CO_2 under supercritical conditions (7.2 MPa at 31°C) are favorable for extraction of thermally labile and non-polar compounds (Abbas et al., 2008). Although the SC-CO_2 treatments were performed in pressure (> 25 MPa) and temperature (< 50°C) to facilitate the extraction of polar and high molecular weight compounds (Díaz-Reinoso et al., 2006), the results observed in the present study suggest that additional energy and mixture of solvents were necessary to facilitate the release of polar and high molecular weight compounds from alga matrix under the studied conditions for SC-CO_2 (35 MPa at 40°C).

3.2. Phenolic content

Phenolic contents from different extracts of *Bifurcaria bifurcata* are also presented in Table 1. The highest values for total phenolic content were obtained for HW50E50 and UW50E50 (5.65 and 5.46 g PGE 100 g^{-1} DW, respectively), followed by HW100 (2.92 g PGE 100 g^{-1} DW). The SC-CO_2 procedures were inefficient for all extraction times due to the lowest total phenolic content recovery in comparison to other extraction techniques, wherein average phenolic content in such extracts were in the range of 0-0.06 g PGE 100 g^{-1} DW.

Interestingly, the highest total phenolic content was found for the samples where the highest extraction yield was obtained. It is possible to suggest that increased extraction yield also contributed to release of phenolic compounds from algal structure. It is also suggested that other non-phenolic compounds such as water-soluble polysaccharides, proteins and organic acids were extracted (Chirinos et al., 2007). The extraction of phenolic compounds was favored by hydrothermal and ultrasound processing, particularly for the water/ethanol mixture.

Table 1. Extraction yield, phenolic content and antioxidant activity of *Bifurcaria bifurcata* extracts obtained by hydrothermal, ultrasound, and SC-CO_2 extraction techniques

Extraction conditions		***Extraction yield***	***Phenolic content***	***Antioxidant activity***
Technique	***Solvent/time***	***(g 100 g^{-1} DW)***	***(g PGE 100 g^{-1} DW)***	***(µmol TE g^{-1} DW)***
Hydrothermal	100% water	37.48±0.77[a]	2.92±0.05[a]	372.87±58.25[a]
	W/E (50:50)	41.82±2.30[a]	5.65±0.61[a]	426.88±80.13[a]
	SEM	1.44	0.81	32.57
	Sig.	ns	*	ns
Ultrasound	100% water	22.27±0.77[b]	2.28±0.16[a]	253.06±0.08[a]
	W/E (50:50)	39.11±1.54[c]	5.46±0.34[b]	552.24±72.81[b]
	100% ethanol	14.67±0.77[a]	2.57±0.24[a]	227.06±29.91[a]
	SEM	4.58	0.65	67.53
	Sig.	***	**	**
Supercritical CO_2	30 min	0.49±0.11[a]	0.00±0.00[a]	nd
	45 min	1.05±0.05[a]	0.01±0.00[a]	nd
	60 min	3.10±0.54[b]	0.06±0.01[b]	nd
	SEM	0.51	0.01	
	Sig.	**	**	

*W/E: water/ethanol; DW: dry weight; PGE: phloroglucinol equivalent; nd: not determined; SEM: standard error of mean. [a-c]Means in the same column not followed by a common superscript letter are significantly different (P < 0.05; Duncan test). Sig.: significance: *(P < 0.05); **(P < 0.01); ***(P < 0.001); ns (not significant)*

The UW100 and UE100 treatments (2.28±0.16 and 2.57±0.24, respectively) displayed reduced efficiency in extraction of phenolic compounds in comparison to hydrothermal and ultrasound treatments (50:50). Hwang and Do Thi (2014) observed that extraction of phenolic compounds from *Porphyra tenera* were increased due to the change of water to 70 % ethanol solution as solvent. Auezoba et al. (2013) also found one of the highest phenolic contents in a solid-liquid extraction among all extractant solvents used, on *Saccharina bongardiana* brown alga, with aqueous mixtures of the organic polar solvents ethanol and methanol, in the ratio of 70% (0.619 g PGE 100 g^{-1} DW for water/ethanol and 0.708 g PGE 100 g^{-1} DW for water/methanol). These values were very lower than ours using water/ethanol (50:50) both in the hydrothermal and the ultrasound extraction technique (5.65±0.61 and 5.46±0.34 g PGE 100 g^{-1} DW, respectively). However, some parameters, such as seaweed-solvent ratio, extraction time or agitation, all of them different among the studies, must be also taken into account. In a similar way, Tierney et al. (2013) observed that extraction of phenolic compounds from almost all Irish macroalgae species was increased by acetone/water solution (80:20) as solvent in comparison to water and even to ethanol/water solution (80:20). López et al. (2011) reported a higher total phenolic content using water/methanol (50:50) as extraction solvent than using 100% ethanol and methanol, on *Stypocaulon scoparium* alga during a solid-liquid extraction. Nevertheless, contrary to our study, they reported that 100% water extract reached the highest phenolic content, although parameters, such as solid-liquid ratio, agitation or temperature did not coincide in both studies. Kuda et al. (2005) used water at 121ºC and ethanol at room temperature on seaweeds from Noto Peninsula (Japan), displaying very high phenolic contents when they used the water. However, temperature could have affected to the phenolic compounds extraction.

The improved extraction of phenolic compounds in HW50E50 and UW50E50 can be explained by their higher solubility in polar organic solvents such acetone, ethanol and methanol (Farvin and Jacobsen, 2013; Wang et al., 2012). In addition, increased temperature has been associated to enhanced extraction of polyphenols in other algae species. Belda et al. (2016) reported that extraction of polyphenols from *Himanthalia elongata* brown alga was facilitated by increasing temperature (60°C) in comparison to lower temperatures (25 and 40°C) for 2 h. Moreover, cell wall softening and degradation facilitate the release of trapped compounds, such as polyphenols due to increasing temperature and ultrasound treatments (Balboa et al., 2013; Parniakov et al., 2015).

The reduced extraction of phenolic compounds by SC-CO_2 treatments may be associated with reduced capacity of CO_2 to extract hydrophilic compounds as observed for extraction yield results (Table 1) since CO_2 has non-polar character under supercritical conditions (Abbas et al., 2008). Although the penetration and diffusion (crucial events for successful extraction of target compounds) of SC-CO_2 in algal matrix is suggested to occur during treatments applied in the present study, the level of interaction of SC-CO_2 with phenolic compounds may be lower than the interaction of phenolic compounds with algal components and, therefore, impair the extraction of phenolic compounds by SC-CO_2 extraction.

In addition, the phenolic content of algal samples is mainly composed by phlorotannins. This group of phenolic compounds comprises more than 700 structures, is highly hydrophilic and has phloroglucinol as monomeric unit. Classification of phlorotannins can be done according to degree of polymerization: monomers, dimers, trimmers, tetramers and phlorotannins have a degree of polymerization of 1, 2, 3, 4 and ≥ 5, respectively. Another classification of phlorotannins is based in inter-molecular linkage: ether linkage (fuhalols/phlorethols); phenyl linkage (fucols); ether and phenyl linkage (fucophloroethols); and dibenzodioxin linkage (eckols). Such compounds can contribute to defences against stress and herbivores (Li et al., 2011; Singh and Bharate, 2006).

3.3. Antioxidant activity

Antioxidant activity measured by ORAC method from different extracts from *Bifurcaria bifurcata* is presented in Table 1. In view of the low polyphenol content obtained in the extraction with SC-CO_2, the decision of not measuring its antioxidant activity was taken. The UW50E50 treatment produced the extract with the highest value of antioxidant activity followed by HW50E50 and HW100 (552.24, 426.88, and 372.87 μmol TE g^{-1} DW, respectively). From these results, it is also shown that the combination of solvents (water/ethanol) and the application of additional energy (hydrothermal and ultrasound) led to production of natural extracts with increased antioxidant activity. Hwang and Do Thi (2014) found that the use of 70% ethanol instead of 100% water improved the antioxidant activity of extracts from dried, roasted and seasoned *Porphyra tenera* red alga using DPPH radical ($DPPH^{\bullet}$) scavenging assay. Nevertheless, using ABTS radical cation ($ABTS^{\bullet+}$) decolorization assay only the 70% ethanol extract from the seasoned alga showed higher antioxidant activity. In the same way, Tierney et al. (2013) reported higher DPPH scavenging activities in ethanol/water (80:20) and acetone/water (80:20) extracts than in water extracts. However, different results are given by the authors when they use ferric reducing antioxidant power (FRAP) and ferrous ion chelating (FIC) assays. Our results agree with those reported by Rajauria et al. (2013), who found that mixtures of methanol with water reached higher antioxidant activities than using water and methanol as pure solvents. The highest activities were achieved

with mixtures around of the 50% of the solvents. On the other hand, López et al. (2011) noted higher antioxidant activity values in the water/methanol (50:50) extract than in the 100% methanol and ethanol extracts. Nonetheless, 100% water extract showed the highest antioxidant activity.

Lee et al. (2013) stated that ultrasound increased the antioxidant activity of *Ecklonia cava* extracts in comparison to conventional extraction (agitation in a shaking incubator), particularly for alkyl radical and H_2O_2 scavenging assays. Time of extraction was reduced in extracts submitted to ultrasound treatment from 24 h (shaking incubator) to 12 h (ultrasound) which was also considered as an important improvement by these authors. Differently, Hwang and Do Thi (2014) observed that increasing temperature during extraction stage was associated with reduced antioxidant activity. The authors obtained reduced antioxidant activity for extracts submitted to thermal processing at 100°C with water as solvent in comparison to extraction at 37°C with 70 % ethanol solution as solvent.

Additionally, extraction temperature also influenced the type of compounds extracted from algal matrix and may lead to differences in antioxidant activity, as it was observed for total flavonoid content in that study.

Although the data are not significantly conclusive, a correlation among phenolic content and antioxidant activity seemed to be observed. Previous studies have reported a strong correlation between polyphenols and antioxidant activities in macroalgae, suggesting that polyphenols are some of the main contributors to antioxidant activity (Vinayak et al., 2011; Wang et al., 2009; Zhang et al., 2010).

4. Conclusions

The results obtained in the present study indicated that the mixture water/ethanol (50:50) used as solvent has the ability to increase extraction yield, and particularly the amount of recovered polyphenols with antioxidant activity from *Bifurcaria bifurcata* extracts.

Ultrasound is an interesting alternative to increase the extraction of antioxidant bioactive compounds from this algal matrix, while SC-CO_2 is suggested to be avoided for antioxidant extraction purposes, even under optimized operation conditions. Testing the optimal extraction conditions obtained in this study in other brown seaweeds, ultrasound intensities, temperature and extraction period would be of interest for further research.

In this way, extracts with high antioxidant activity could be obtained, minimizing their cost, and thus favoring the commercial exploitation of *Bifurcaria bifurcata* with significant benefits from an economical and environmental point of view.

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