




Drying process, storage conditions, and time alter the biochemical composition and bioactivity of the anti-greenhouse seaweed *Asparagopsis taxiformis*

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Abstract

The Azorean red seaweed *Asparagopsis taxiformis* may be used in human and animal diets. This seaweed is deemed to reduce the large production of methane—a major greenhouse gas—by ruminant digestion. Seaweed producers, however, have difficulties in ensuring a reliable and similar product throughout all year and in different years. Changes in biochemical composition and bioactivity are caused not only by natural variability, but also by the particular drying process, storage conditions, and storage time. Regarding the drying process, oven-dried samples had a lower EPA content, $1.9 \pm 0.2\%$ of the total FAs, than freeze-dried samples, $8.6 \pm 1.7\%$. The same occurred with the phenolic contents and particularly with the ethanolic extracts. ABTS antioxidant activity results showed freeze-drying as advantageous. With respect to storage temperature, anti-inflammatory activity was higher in *A. taxiformis* at room temperature after three month storage. Moreover, EPA content in freeze-dried samples decreased to 0.3–1.0% after three month storage. Phenolic content in the ethanolic extracts also declined over storage time. In the case of aqueous extracts, however, variation was in the opposite direction. Antioxidant activity as measured by ABTS showed for almost all samples and types of extracts an increasing trend over time: from 0.26–1.75 to 0.75–4.40 mmol Trolox Eq/100 g dw. Anti-inflammatory activity increased over time from < 30% COX-2 inhibition at the beginning of the trial to > 30% COX-2 inhibition after three month storage. Therefore, there is a relevant bioactive potential in *A. taxiformis* and the drying process and storage conditions and time affect this potential.

Keywords *Asparagopsis taxiformis* · Drying process · Storage conditions and time · Fatty acid profile · Antioxidant activity · Anti-inflammatory activity

Abbreviations

AA Eq	Ascorbic acid equivalent	COX-2	Cyclooxygenase-2
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid	DHA	Docosahexaenoic acid
ARA	Arachidonic acid	DMSO	Dimethyl sulphoxide
		DPPH	2,2-Diphenyl-1-picrylhydrazyl
		ELISA	Enzyme-Linked Immunosorbent Assay
		EPA	Eicosapentaenoic acid
		F	Freezing temperature
		FA	Fatty acid
		FAME	Fatty acid methyl ester
		GA	Gallic acid
		GAE	Gallic acid equivalent
		HSD	Honestly significant difference
		L	Freeze-dried samples
		MUFA	Monounsaturated fatty acid
		O	Oven-dried samples
		PUFA	Polyunsaturated fatty acid
		R	Room temperature
		SFA	Saturated fatty acid
		TFC	Total flavonoid content

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TPC	Total content of phenolic compounds
Trolox Eq	Trolox equivalent
ω 3 PUFA	Omega-3 polyunsaturated fatty acid
ω 6 PUFA	Omega-6 polyunsaturated fatty acid

Introduction

The seaweed *Asparagopsis taxiformis* is a medium-sized red macroalgal species belonging to the order Bonnemaisoniales and family Bonnemaisoniaceae that has a broad global distribution, being found particularly in tropical and sub-tropical coastal marine ecosystems [1, 2]. This species is typically found in the Pacific and Indo-Pacific region, including Mexico, Hawaii, California, China, Japan, and Australia. It is now spreading in the Atlantic Ocean, notably in the Azores Archipelago, and is also found in the Mediterranean Sea [2]. It is an edible seaweed that is consumed in China and India.

Brominated compounds usual in the genus *Asparagopsis* have an effective inhibitory action on archaeobacterial metabolism and growth of methanogenic archaea in the case of *A. taxiformis* [3]. This finding is particularly relevant since, according to Roque et al. (2019) [3], the incorporation of 5% *A. taxiformis* organic matter in dairy cattle feed may lead to reductions of methane production in the rumen surpassing 90%. The incorporation of *A. taxiformis* into feed seems to be a promising strategy in the fight against climate change induced by greenhouse gases, given that methane emissions from cows represent a large share of total global methane emissions and that this gas is long-term a more powerful greenhouse gas than carbon dioxide [4].

Regarding other possible applications and properties, there are few studies on this particular red seaweed species. Studies on other red seaweeds have shown that they synthesize a wide variety of bioactive metabolites, including polysaccharides, proteins, polyunsaturated fatty acids (PUFA), polyphenols and some pigments, which, in turn, underlie biological activities, such as antioxidant, anti-diabetic, anti-inflammatory, anti-tumor activity, and many others [5, 6]. A seaweed species belonging to the same genus, *A. armata*, was shown to have a substantial anti-inflammatory activity as measured by inhibition of phospholipase A2 activity [7]. Concerning *A. taxiformis* itself, there are two important studies on bioactives and properties [8, 9]. Nunes et al. (2017) [8] compared *A. taxiformis* with other red seaweeds as well as green and brown seaweeds and found out that its biomass is relatively rich in fibre and matricial polysaccharides and it contains significant levels of chlorophyll *a* (28.8 ± 3.2 mg/100 g dw), carotenoids (13.1 ± 2.6 mg/100 g dw), and phenolic compounds (57.6 ± 3.9 mg GAE/100 g dw). Within the group of carotenoids, fucoxanthin has been identified in *A. taxiformis* [9]. The FA profile of *A. taxiformis* was

also analysed, but only very low levels of the bioactive eicosapentaenoic acid (EPA, 20:5 ω 3) were determined [9]. Mellouk et al. (2017) [10] also reported low EPA content in the biomass of *A. taxiformis*, but quantified a large share of another bioactive ω 3 PUFA, docosahexaenoic acid (DHA, 22:6 ω 3), exceeding 30% of total FAs. EPA and DHA intakes are associated with decreased morbidity and mortality from cardiovascular and other diseases [11]. In addition, EPA has been claimed to strengthen the anti-inflammatory properties of high-density lipoprotein, among other effects [12].

It is well known that seaweeds have a high percentage of moisture, often exceeding 90% of the wet weight, thus making their biomass highly perishable [13, 14]. Hence, it is critically important that appropriate drying and storage conditions are applied to preserve biomass quality and ensure product safety. Typically, in the industry, algal biomass is dried and stored at a temperature between 0 and 4 °C or approximately -18 °C, depending on whether it is dry or semi-dry, respectively [13, 15]. Applied methods include simple air drying and/or sun-drying or more expensive and/or innovative methods, such as drying in hot air ovens and freeze-drying. All these techniques have advantages and drawbacks [15].

Drying methods are effective techniques for moisture removal, but several studies have shown that the temperatures endured by the algal biomass during these processes may cause significant changes in its properties [16]. Namely, Gupta et al. (2011) [13] analysed the effect of drying on the total content of phenolic compounds (TPC) and flavonoids (TFC) and on the antioxidant activity of the brown seaweed *Himanthalia elongata*. For this purpose, different drying temperatures (25, 30, 35, and 40 °C) were applied for 24 h in a hot air oven. For all temperatures, a reduction in TPC and TFC as well as a decrease in antioxidant activity in comparison to fresh biomass was observed [13]. Other studies [14, 17] have been done on various seaweed species and have indicated significant alterations of composition and bioactivity as a result of different drying and storage conditions. Similar data is not found for *A. taxiformis*, which makes this a relevant knowledge gap, especially taking into account the possible future relevance of this species.

Therefore, the purpose of this work was threefold: to assess the effect of the drying process (oven-drying at 60 °C for 72 h vs freeze-drying at -40 °C and 4×10^{-4} mbar for 48 h); storage temperature (room temperature, 15 ± 5 °C vs frozen storage, -20 °C); and storage time (0–3 months) on relevant parameters of *A. taxiformis* biomass. Accordingly, this study entailed the determination of the FA profile, total phenolic content, and relevant bioactivities of the algal biomass as a function of the three tested variables.

Materials and methods

Human and animal rights

Not relevant in this study.

Seaweed source, collection, and preparation

The studied seaweed species was *Asparagopsis taxiformis*. This is a marketed red seaweed whose taxonomic identification was guaranteed by the Portuguese producer company, seaExpert (Faial, Azores, Portugal). Specific traits that led to this identification were its dark red colour and a creeping base with rigid upright branches, being each branch covered by many fuzzy soft branchlets that get shorter towards the top. Moreover, previous identical seaweed samples of the same area had been identified by sequencing the 5' end of the mitochondrial cytochrome c oxidase gene (COI-5'), the official barcode suggested for red algae [18].

This seaweed originated from the Azores islands (Portugal, located in the mid-North Atlantic), more specifically, Faial Island, being collected from the wild (coordinates: 38°31'24.0"N and 28°38'34.9"W, at 5 m depth and water temperature 16 °C by an apnea diver, which carried out the collection by total removal of the specimens, including the seaweed holdfast) in the morning of 2nd November 2018 (Autumn). The fresh frond seaweed biomass was immediately frozen (−20 °C) after harvest, packaged in plastic bags, and sent to the IPMA laboratory in Lisbon. Afterwards, 5 kg of frozen seaweed was kept stored at −20 °C until further treatment.

Seaweed drying and storage

The frozen biomass of *A. taxiformis* was subjected to two alternative drying techniques: oven-drying and freeze-drying. Accordingly, the frozen seaweed blocks were cut into pieces and approximately 2.7 kg were dried in an oven, while approximately 2 kg were dried in a freeze-drier. Oven-drying was carried out at 60 °C for 72 h in a Memmert equipment without air circulation (Memmert GmbH + Co.KG, Schwabach, Germany). Freeze-drying (after 6 h at −80 °C) was performed at −40 °C and a pressure of 4×10^{-4} mbar for 48 h in a Heto Power Dry LL3000 freeze-dryer (Thermo Fisher Scientific, Waltham, MA, USA). After drying, samples were ground at room temperature in a Grindomix GM 200 mill (Retsch, Haan, Germany) to 100–500 µm particles with 3 cycles of 5 s at 3000 rpm and 2 cycles of 5 s at 5000 rpm and then packaged in sealed bags with some vacuum (200 mbar).

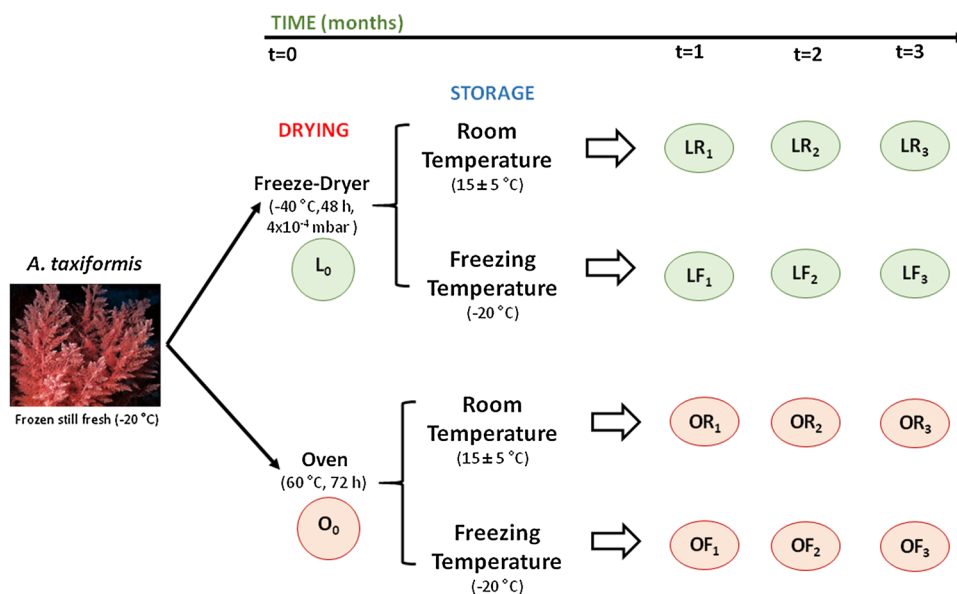
Afterwards, both oven-dried (O) and freeze-dried (L, lyophilized) samples were stored at two alternative temperatures: room temperature, 15 ± 5 °C (OR & LR) and freezing temperature, −20 °C (OF & LF), for three months. The former temperature conditions were chosen to replicate the storage conditions used by industry in Portugal (absence of temperature control and, consequently, typical room temperatures oscillating between 10 and 20 °C). The latter temperature conditions correspond to a situation in which changes during storage time would be expected to be minimal.

At the beginning of the storage study (OR0, OF0, LR0 & LF0) and at every month (OR1, OF1, LR1 & LF1; OR2, OF2, LR2 & LF2) until the end (OR3, OF3, LR3 & LF3), a set of relevant parameters of the *A. taxiformis* biomass were determined. The overall experimental scheme is presented in Fig. 1.

Proximate composition and fibre content

The moisture and ash contents were determined according to Association of Official Analytical Chemists (AOAC) methods [19]. The protein level was quantified according to the Dumas method [20]. Crude lipid content was determined following the Folch extraction method [21]. Fibre content was determined through an enzymatic procedure on the basis of the K-TDFR-100A/K-TDFR-200A Megazyme Kit (Megazyme, Bray, Ireland). First, 1.000 ± 0.005 g of sample was weighed, 40 ml of MES-TRIS blend buffer (pH 8.2) was added, and the resulting mixture was stirred. Afterwards, 50 µl of thermostable α-amylase solution was added and the dispersion-enzyme mixture was incubated with agitation at 98–100 °C for 30 min. Then, mixture was cooled to 60 °C. For second incubation, 100 µl of protease solution was added and attained mixture was left at 60 ± 1 °C for 30 min with agitation. Afterwards, 5 ml of 0.561 N HCl solution was added with constant agitation to the mixture for a final pH in the 4.1–4.8 interval. Third incubation was initiated with the addition of 200 µl of amyloglucosidase solution and required 30 min at 60 ± 1 °C with agitation. The final mixture was filtered through a Gooch fritted crucible (previously weighed with a layer of Celite®) and residue was washed with 70 °C distilled water. The attained filtrate was kept in a previously weighed container for the determination of soluble fibre. Residue was then washed with 10 ml of 95%, v/v, ethanol and 10 ml of acetone. The residue-containing Gooch fritted crucible was dried overnight in an oven at 103 °C. Next day, the crucible was weighed for the calculation of insoluble fibre. Regarding soluble fibre, the container with filtrate was weighed for determining filtrate weight, which was used to determine required volume of 60 °C 95%, v/v, ethanol according to a 1 g:4 ml w:v proportion. After adding 60 °C 95%, v/v, ethanol to the filtrate, the mixture was left at room temperature for 1 h. Afterwards, the mixture was filtered

Fig. 1 Experimental design of the *A. taxiformis* study of quality as a function of drying conditions (oven-drying vs freeze-drying), storage conditions (room temperature, 15 ± 5 °C vs frozen storage, -20 °C), and storage time (t : 0 month-1 month-2 month-3 month). *L* Freeze-dried samples, *O* Oven-dried samples, *R* Room temperature, and *F* Freezing temperature



through a Gooch fritted crucible (previously weighed with a layer of Celite®) and remaining particles in the precipitation container were transferred to the crucible with 78%, v/v, ethanol. Residue was then washed twice with 15 ml of 78%, v/v, ethanol, 15 ml of 95%, v/v, ethanol, and 15 ml of acetone. Following steps were done as described for the insoluble fibre. The contents of insoluble and soluble fibre were corrected through subtraction of protein and ash contents in the residues [19, 20]. Total fibre content was the sum of the insoluble and soluble fibre contents. Analyses were done in duplicate.

Fatty acid profile

Fatty acid methyl esters (FAMES) were prepared from oven-dried and freeze-dried seaweed by acid-catalysed transesterification using the methodology described by Bandarra et al. (1997) [22]. The samples were applied to a DB-WAX (Agilent Technologies, Santa Clara, USA) capillary column (film thickness, 0.25 μm , 30 m \times 0.25 mm i.d.) integrated into a Varian Star 3800 CP gas chromatograph (Walnut Creek, CA, USA), which was equipped with an auto sampler with a split injector (100:1) and a flame ionization detector, both at 250 °C. The separation of the FAMES was performed using helium as the carrier gas and with a temperature programme for the column starting at 180 °C and increasing to 200 °C at 4 °C/min, holding for 10 min at 200 °C, heating to 210 °C at the same rate, and holding at this temperature for 14.5 min. The FAMES were identified by comparing their retention time with those of several Sigma-Aldrich standards (PUFA-3, Menhaden oil, and PUFA-1, marine source from Supelco Analytical). This analysis was done in triplicate.

Total polyphenol content

The phenolic compounds were extracted using a selected solvent. Water and ethanol 96%, w/w, were chosen from among different safe and environmentally acceptable solvents as being the most effective. Accordingly, the selected solvent was added to the oven-dried and freeze-dried seaweed biomass, thus matching the extracts used in the assessment of the antioxidant potential (see “Antioxidant activity as measured by DPPH method” Section), and the total polyphenol content was determined by the Singleton and Rossi method using Folin-Ciocalteu reagent [23]. Gallic acid (GA) was used as a standard and the phenolic content was expressed as gallic acid equivalents (mg GAE/100 g) according to a gallic acid calibration curve (Sigma, Steinheim, Germany). This analysis was done in triplicate.

Antioxidant activity as measured by DPPH method

The antioxidant activity was measured by determining the radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [24]. To prepare the extracts, approximately 1.25 g of oven-dried and freeze-dried seaweed biomass was weighed and homogenized with 25 ml of water or ethanol 96%, w/w using a model Polytron PT 6100 homogenizer (Kinematica, Luzern, Switzerland) at a velocity of 30,000 rpm for 1 min, and agitated for 18 h on an orbital shaker. Following centrifugation (at 5000 \times g at room temperature during 20 min), the supernatant was collected through a filter to a final volume of 25 ml. A 1 ml volume of the extract was prepared in triplicate for each sample and 2 ml of DPPH (Sigma, Steinheim, Germany) in 0.15 mM methanolic solution was added and thoroughly mixed. After

30 min of incubation at room temperature in the dark, the absorbance was measured at 517 nm in a Helios Alpha model (Unicam, Leeds, UK) UV/visible light spectrophotometer. Either water or ethanol 96%, w/w, was used as the blank.

The radical scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition} = (A_0 - A_{\text{sample}})/A_0 \times 100$$

where A_0 – Absorbance of the blank, A_{sample} – Absorbance of the sample.

On the basis of the ascorbic acid calibration curve, results were then expressed in mg of ascorbic acid equivalents (AA Eq) per 100 g of seaweed dry weight. This analysis was done in triplicate.

Antioxidant activity as measured by ABTS method

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging activity was determined using the method described by Re et al. (1999) [25]. The ABTS radical scavenging activity of the samples was expressed as a percentage of inhibition as follows:

$$\% \text{ Inhibition} = (A_0 - A_{\text{sample}})/A_0 \times 100$$

where A_0 – Absorbance of the blank, A_{sample} – Absorbance of the sample.

On the basis of a trolox calibration curve, results were then expressed in mmol of trolox equivalents (Trolox Eq) per 100 g dw of seaweed. This analysis was done in triplicate.

Anti-inflammatory activity

The anti-inflammatory activities of the seaweed *A. taxiformis* were determined in ethanol extracts obtained from approximately 200 mg of sun-dried and freeze-dried seaweed homogenized with 2 ml of ethanol 96%, w/w, using a model Polytron PT6100 homogenizer (Kinematica, Luzern, Switzerland) at a velocity of 30,000 rpm for 1 min. The extracts were subjected to heat treatment (80 °C during 1 h) and then centrifuged (3,000 × g at 4 °C during 10 min). The supernatant was collected and the solvent was evaporated using a vacuum rotary evaporator at a water bath temperature of 65 °C. The residue was directly dissolved in 100% dimethyl sulphoxide (DMSO) to prepare a stock solution with a concentration of 10 mg/ml. The extract was tested at 1 mg/ml using a commercial cyclooxygenase (COX) inhibitory screening assay kit, Cayman test kit-560131 (Cayman Chemical Company, Ann Arbor, MI, USA). The COX-2 inhibitor screening assay directly measures the amount of prostaglandin F₂α generated from arachidonic acid (ARA, 20:4 ω6) in the cyclooxygenase reaction. A volume of 10 μl

each of test extract or DMSO was used. The reaction was initiated by addition of 10 μl 10 mM ARA and each reaction tube was incubated at 37 °C for 2 min. The reaction was terminated by addition of 50 μl 1 N HCl and saturated stannous chloride. Assays were performed using 100 units of human recombinant COX-2. An aliquot was removed and the prostanoid produced was quantified spectrophotometrically (412 nm) via enzyme immunoassay (ELISA) after 18 h incubation, washing, addition of Ellman's reagent, and further 90 min incubation. The results were expressed as the percentage of COX-2 inhibition. For this methodology, four replicates were analysed.

Statistical analysis

To test the normality and the homogeneity of the data variance, the Kolmogorov–Smirnov test and Cochran's C-test were used, respectively. The data were analysed by one-way and factorial ANOVA distributions using Tukey's HSD to determine the difference in the constituent contents and in the biological activities between drying treatments, storage temperature, and storage time. For the anti-inflammatory activity, owing to the different numbers of replicates for each sample, a factorial ANOVA distribution was applied in combination with a Tukey Unequal N HSD. For all the statistical tests, the significance level (α) was 0.05. All the data analyses were performed using STATISTICA 7 (Stat-sof, Inc. USA, 2003). Additionally, the possibility of correlations between EPA and total polyphenol content or bioactivities (antioxidant and anti-inflammatory) as well as between total polyphenol content and bioactivities was tested by the application of linear regression (least quadratic method).

Results

Proximate composition and fibre content

The proximate composition and insoluble, soluble, and total fibre content values are presented in Table 1.

At the beginning of the study, it was observed a higher moisture level in the freeze-dried *A. taxiformis* than in the oven-dried seaweed. This difference was maintained throughout storage time. Storage temperature had only an effect after three month storage, leading to higher moisture levels at room temperature. Regarding ash content, neither time nor temperature produced a variation. Freeze-dried samples presented consistently lower ash contents. Variability in protein content was even more reduced than in ash content. Indeed, there was only a consistent higher protein content in freeze-dried samples at the initial point ($t=0$ month). Concerning lipid content, values were always low, but freeze-dried *A. taxiformis* displayed values slightly

Table 1 Proximate composition and insoluble, soluble, and total fibre contents (in % dry weight) of *Asparagopsis taxiformis* subjected to different drying processes and storage conditions at different times of the storage stability experiment

Sample	Time (month)	Proximate composition and insoluble, soluble, and total fibre contents (% dw)			
		OR	OF	LR	LF
Moisture	0	2.7 ± 0.0 ^{aw}	2.7 ± 0.0 ^{aw}	5.6 ± 0.0 ^{bw}	5.6 ± 0.0 ^{bw}
	1	2.8 ± 0.1 ^{aw}	2.6 ± 0.1 ^{aw}	5.6 ± 0.0 ^{bw}	5.7 ± 0.2 ^{bw}
	2	3.0 ± 0.2 ^{aw}	2.8 ± 0.0 ^{aw}	5.9 ± 0.1 ^{bw}	5.7 ± 0.2 ^{bw}
	3	4.3 ± 0.1 ^{bx}	2.5 ± 0.0 ^{aw}	6.2 ± 0.0 ^{dx}	5.0 ± 0.0 ^{cx}
Ash	0	48.7 ± 0.0 ^{aw}	48.7 ± 0.0 ^{aw}	44.4 ± 0.1 ^{bw}	44.4 ± 0.1 ^{bw}
	1	48.4 ± 0.3 ^{aw}	48.7 ± 0.0 ^{aw}	44.3 ± 0.1 ^{bw}	44.4 ± 0.0 ^{bw}
	2	47.6 ± 0.5 ^{aw}	48.4 ± 0.3 ^{aw}	45.1 ± 0.8 ^{bw}	45.0 ± 0.5 ^{bw}
	3	47.3 ± 0.7 ^{aw}	49.5 ± 0.6 ^{aw}	45.6 ± 0.5 ^{bw}	45.4 ± 0.1 ^{bw}
Protein	0	19.7 ± 0.4 ^{aw}	19.7 ± 0.4 ^{aw}	20.8 ± 0.2 ^{bw}	20.8 ± 0.2 ^{bw}
	1	19.3 ± 0.8 ^{aw}	19.0 ± 0.2 ^{aw}	20.8 ± 0.0 ^{bw}	20.3 ± 0.5 ^{abw}
	2	19.3 ± 0.6 ^{aw}	19.3 ± 0.6 ^{aw}	19.7 ± 0.1 ^{aw}	20.2 ± 0.8 ^{aw}
	3	18.7 ± 0.1 ^{aw}	18.6 ± 0.2 ^{aw}	19.7 ± 0.5 ^{aw}	19.6 ± 0.5 ^{aw}
Lipid	0	1.5 ± 0.2 ^{aw}	1.5 ± 0.2 ^{aw}	3.4 ± 0.3 ^{bw}	3.4 ± 0.3 ^{bw}
	1	1.3 ± 0.4 ^{aw}	1.4 ± 0.6 ^{aw}	3.0 ± 0.8 ^{bw}	3.5 ± 0.4 ^{bw}
	2	1.3 ± 0.1 ^{aw}	1.7 ± 0.0 ^{aw}	2.6 ± 0.5 ^{abw}	2.9 ± 0.9 ^{bw}
	3	1.1 ± 0.2 ^{aw}	2.0 ± 0.7 ^{abw}	2.8 ± 0.3 ^{abw}	3.3 ± 0.4 ^{bw}
Insoluble fibre	0	15.3 ± 0.5 ^{aw}	15.3 ± 0.5 ^{aw}	14.9 ± 0.6 ^{aw}	14.9 ± 0.6 ^{aw}
	1	16.0 ± 0.3 ^{aw}	16.0 ± 0.1 ^{aw}	15.2 ± 0.9 ^{aw}	15.5 ± 0.1 ^{aw}
	2	15.2 ± 0.5 ^{aw}	15.4 ± 0.7 ^{aw}	14.9 ± 0.4 ^{aw}	15.8 ± 1.0 ^{aw}
	3	15.5 ± 0.7 ^{aw}	15.8 ± 0.1 ^{aw}	15.1 ± 0.4 ^{aw}	15.7 ± 0.6 ^{aw}
Soluble fibre	0	4.9 ± 0.1 ^{aw}	4.9 ± 0.1 ^{aw}	5.2 ± 0.4 ^{aw}	5.2 ± 0.4 ^{aw}
	1	4.8 ± 0.3 ^{aw}	5.1 ± 0.2 ^{aw}	4.9 ± 0.0 ^{aw}	4.9 ± 0.6 ^{aw}
	2	4.6 ± 0.5 ^{aw}	5.3 ± 0.9 ^{aw}	5.1 ± 0.6 ^{aw}	4.6 ± 1.1 ^{aw}
	3	4.6 ± 0.1 ^{aw}	4.9 ± 0.2 ^{aw}	4.9 ± 0.7 ^{aw}	5.0 ± 0.1 ^{aw}
Total fibre	0	20.2 ± 0.5 ^{aw}	20.2 ± 0.5 ^{aw}	20.1 ± 0.7 ^{aw}	20.1 ± 0.7 ^{aw}
	1	20.8 ± 0.5 ^{aw}	21.1 ± 0.3 ^{aw}	20.1 ± 1.0 ^{aw}	20.4 ± 0.6 ^{aw}
	2	19.8 ± 0.7 ^{aw}	20.7 ± 1.1 ^{aw}	20.0 ± 0.7 ^{aw}	20.4 ± 1.3 ^{aw}
	3	20.1 ± 0.9 ^{aw}	20.7 ± 0.2 ^{aw}	20.0 ± 0.9 ^{aw}	20.7 ± 0.8 ^{aw}

Values are presented as the average ± standard deviation. Different lowercase letters (a-d) within a row correspond to significant differences ($p < 0.05$) between the drying and storage conditions. Different lowercase letters (w-z) within a column correspond to significant differences ($p < 0.05$) over storage time for each type of extract

L Freeze-dried samples, O Oven-dried samples, R Room temperature, and F Freezing temperature

higher than oven-dried samples with exception of LR at $t=2$ month and $t=3$ month. No differences were found in the case of the insoluble, soluble, and total fibre contents.

Fatty acid profile

The examination of the relative FA composition (as a percentage of the total FAs) shows important differences between oven-dried and freeze-dried *A. taxiformis* as well as variations over storage time—only the initial ($t=0$ month) and final ($t=3$ month) points are displayed (Table 2).

All FA profiles were characterized by a high weight of saturated FAs (SFA)—always exceeding 76% of the total FAs—, low levels of monounsaturated FAs (MUFA)—4–11% of the total FAs—, and very low levels of PUFA

—0–11% of the total FAs. The exception was freeze-dried *A. taxiformis* at $t=0$ month (11% PUFA). The single most important FA was the palmitic acid (16:0), which varied between 57 and 72% of the total FAs. The myristic acid (14:0) was also important, being in the 16–18% range of the total FAs. The most important MUFA was the oleic acid (18:1 ω 9) with 2–5% of the total FAs. The ARA was the main ω 6 PUFA, but only displayed very low contents (< 2% of the total FAs). The same was observed for the total ω 6 PUFA. Furthermore, EPA (20:5 ω 3) was also below 2% of the total FAs with exception of freeze-dried *A. taxiformis* at $t=0$ month, which contained $8.6 \pm 1.7\%$ of the total FAs. The EPA content was lower in oven-dried seaweed at $t=0$ month and for all other samples, which means a reduction in the EPA content of freeze-dried

Table 2 Fatty acid profile (in % of total fatty acids) of *Asparagopsis taxiformis* subjected to different drying processes and storage conditions at the initial ($t=0$ month) and final ($t=3$ month) time of the storage stability experiment

Fatty acid (% total fatty acids)	OR0/OF0	LR0/LF0	OR3	OF3	LR3	LF3
14:0	17.0±0.2 ^a	15.6±1.0 ^a	18.2±2.3 ^a	17.8±2.1 ^a	16.4±1.2 ^a	16.2±1.3 ^a
16:0	59.8±0.6 ^{ab}	57.1±2.3 ^a	66.8±1.3 ^{cd}	63.1±0.8 ^{bc}	71.8±3.6 ^d	69.5±1.5 ^d
18:0	1.3±0.0 ^a	1.3±0.0 ^{ab}	1.4±0.2 ^{abc}	1.3±0.1 ^a	1.7±0.0 ^c	1.6±0.2 ^{bc}
Σ SFA	81.8±1.3 ^{ab}	76.2±3.2 ^a	88.1±1.0 ^{bc}	86.1±3.1 ^{bc}	93.1±3.8 ^c	89.2±1.8 ^c
16:1 ω9	1.9±0.1 ^{bc}	nd ^a	2.1±0.2 ^c	1.6±0.3 ^b	nd ^a	nd ^a
17:1	0.6±0.0 ^{ab}	1.1±0.5 ^{ab}	0.2±0.4 ^a	0.4±0.4 ^a	0.7±0.7 ^{ab}	1.6±0.16 ^b
18:1 ω9	4.5±0.2 ^a	4.0±0.1 ^{ab}	3.6±0.3 ^{bc}	3.1±0.5 ^{cd}	2.8±0.3 ^{cd}	2.1±0.3 ^d
18:1 ω7	1.5±0.1 ^{ab}	2.1±0.4 ^b	1.2±0.1 ^{ab}	1.0±0.2 ^a	0.5±0.5 ^a	0.6±0.5 ^a
Σ MUFA	11.0±0.4 ^a	9.8±1.5 ^{ab}	8.7±0.9 ^{ab}	7.7±0.9 ^{bc}	4.4±1.2 ^d	5.2±1.0 ^{cd}
20:4 ω6	0.5±0.1 ^a	1.7±0.4 ^b	nd ^a	0.2±0.2 ^a	0.1±0.1 ^a	nd ^a
20:5 ω3	1.9±0.2 ^a	8.6±1.7 ^b	0.3±0.6 ^a	0.7±0.7 ^a	0.3±0.3 ^a	1.0±0.9 ^a
Σ PUFA	3.0±0.4 ^a	10.5±2.5 ^b	0.3±0.6 ^a	1.1±1.1 ^a	1.9±1.9 ^a	1.0±0.9 ^a
Σ ω3 PUFA	1.9±0.2 ^a	8.6±1.7 ^b	0.3±0.6 ^a	0.7±0.7 ^a	0.3±0.3 ^a	1.0±0.9 ^a
Σ ω6 PUFA	1.0±0.2 ^{ab}	1.9±0.9 ^b	nd ^a	0.3±0.3 ^{ab}	1.4±1.4 ^{ab}	nd ^a

Values are presented as the average ± standard deviation. *nd* not detected. Different lowercase letters within a row correspond to significant differences ($p < 0.05$) between the relative (%) FA profiles of the seaweed samples

L Freeze-dried samples, *O* Oven-dried samples, *R* Room temperature, and *F* Freezing temperature

samples over storage time regardless of storage temperature. The same pattern was observed for ARA. Oleic acid content declined over storage time regardless of drying process and storage temperature. On the other hand, palmitic acid increased over storage time (by 7 to 15% of the total FAs) with exception of oven-dried *A. taxiformis* stored at -20 °C.

Total polyphenol content

The determined values of polyphenol content in both aqueous and ethanolic extracts are shown in Table 3.

The variations observed in each group of extracts (aqueous vs ethanolic) were clearly distinct. However, for both extract groups, phenolic content was higher in the freeze-dried samples at the beginning of the storage experiment and remained so until the end in the case of the ethanolic extracts. Moreover, in the aqueous extracts, phenolic contents increased for all samples (submitted to different drying and storage conditions) over storage time. This is clear in OF samples, where phenolic content increased from 93 ± 4 mg GAE/100 g dw to more than 140 mg GAE/100 g dw after three month storage. The temporal evolution in the ethanolic extracts was opposite to that of the aqueous extracts, since their phenolic contents declined over storage time, being the reduction observed after the first month. These variations were quite similar. For instance, phenolic content in LR samples decreased from 94 ± 4 mg GAE/100 g dw to 64 ± 2 mg GAE/100 g dw after three month storage.

Table 3 Total polyphenol contents (in mg GAE/100 g dry weight) of *Asparagopsis taxiformis* subjected to different drying processes and storage conditions at different times of the storage stability experiment

Sample	Time (month)	Total polyphenol (mg GAE/100 g dw)			
		OR	OF	LR	LF
Aqueous extract	0	93±4 ^{aw}	93±4 ^{aw}	104±4 ^{bw}	104±4 ^{bw}
	1	101±3 ^{awx}	101±4 ^{aw}	105±4 ^{aw}	108±3 ^{aw}
	2	131±3 ^{ay}	138±7 ^{ax}	133±5 ^{ax}	154±4 ^{by}
	3	119±8 ^{axy}	144±0 ^{bxc}	152±8 ^{cy}	125±5 ^{abx}
Ethanolic extract	0	75±1 ^{aw}	75±1 ^{aw}	94±4 ^{bw}	94±4 ^{bw}
	1	50±4 ^{ax}	49±1 ^{ax}	63±1 ^{bx}	64±2 ^{bx}
	2	47±1 ^{ax}	47±1 ^{ax}	69±3 ^{bx}	67±2 ^{bx}
	3	49±1 ^{ax}	47±1 ^{ax}	64±2 ^{bx}	62±1 ^{bx}

Values are presented as the average ± standard deviation. Different lowercase letters (a-d) within a row correspond to significant differences ($p < 0.05$) between the drying and storage conditions. Different lowercase letters (w-z) within a column correspond to significant differences ($p < 0.05$) over storage time for each type of extract

L Freeze-dried samples, *O* Oven-dried samples, *R* Room temperature, and *F* Freezing temperature

Antioxidant activity

The antioxidant activity determined by the DPPH and ABTS methods for both groups of extracts (aqueous and ethanolic) are presented in Tables 4 and 5, respectively.

Regarding DPPH results, as observed in the phenolic contents (“Total polyphenol content” Section), there were differences in the values and variations for each extract

Table 4 DPPH antioxidant activity (in mg AA Eq/100 g dry weight) of *Asparagopsis taxiformis* subjected to different drying processes and storage conditions at different times of the storage stability experiment

Sample	Time (month)	DPPH antioxidant activity (mg AA Eq/100 g dw)			
		OR	OF	LR	LF
Aqueous extract	0	14 ± 3 ^{aw}	14 ± 3 ^{aw}	17 ± 1 ^{aw}	17 ± 1 ^{ax}
	1	24 ± 0 ^{ax}	21 ± 1 ^{bx}	22 ± 1 ^{abx}	17 ± 1 ^{cx}
	2	32 ± 0 ^{ax}	38 ± 1 ^{by}	41 ± 3 ^{by}	43 ± 2 ^{by}
	3	29 ± 4 ^{bx}	46 ± 5 ^{cy}	22 ± 3 ^{abwx}	10 ± 1 ^{aw}
Ethanol extract	0	52 ± 1 ^{aw}	52 ± 1 ^{aw}	34 ± 1 ^{bw}	34 ± 1 ^{bw}
	1	47 ± 1 ^{ax}	45 ± 0 ^{by}	33 ± 1 ^{cw}	24 ± 0 ^{dy}
	2	45 ± 0 ^{ay}	46 ± 0 ^{axy}	42 ± 0 ^{bx}	32 ± 0 ^{cx}
	3	45 ± 0 ^{ay}	46 ± 0 ^{ax}	42 ± 0 ^{bx}	35 ± 1 ^{cw}

Values are presented as the average ± standard deviation. Different lowercase letters (a–d) within a row correspond to significant differences ($p < 0.05$) between the drying and storage conditions. Different lowercase letters (w–z) within a column correspond to significant differences ($p < 0.05$) over storage time for each type of extract

L Freeze-dried samples, *O* Oven-dried samples, *R* Room temperature, and *F* Freezing temperature

group. There were some strong oscillations, particularly in the aqueous extracts. For the latter, there was a clear growing trend in the case of the oven-dried samples, but stronger in the OF samples, which increased from 14 ± 3 mg AA Eq/100 g dw to 46 ± 5 mg AA Eq/100 g dw over the whole storage time. In the aqueous extracts of freeze-dried samples no such increase was observed and a reduction was registered for LF. With respect to the ethanolic extracts, variations were more modest, being detected a slight decreasing trend in the oven-dried seaweed regardless of storage temperature.

Table 5 ABTS antioxidant activity (in mmol Trolox Eq/100 g dry weight) of *Asparagopsis taxiformis* subjected to different drying processes and storage conditions at different times of the storage stability experiment

Sample	Time (month)	ABTS antioxidant activity (mmol Trolox Eq/100 g dw)			
		OR	OF	LR	LF
Aqueous extract	0	0.84 ± 0.14 ^{aw}	0.84 ± 0.14 ^{aw}	1.17 ± 0.13 ^{aw}	1.17 ± 0.13 ^{aw}
	1	2.37 ± 0.24 ^{ax}	1.81 ± 0.25 ^{bx}	2.11 ± 0.06 ^{abx}	2.42 ± 0.22 ^{ax}
	2	2.17 ± 0.15 ^{ax}	3.52 ± 0.25 ^{by}	2.67 ± 0.40 ^{ax}	3.51 ± 0.35 ^{by}
	3	2.13 ± 0.05 ^{ax}	4.40 ± 0.23 ^{cz}	2.50 ± 0.10 ^{abx}	2.89 ± 0.51 ^{bx}
Ethanolic extract	0	0.26 ± 0.04 ^{aw}	0.26 ± 0.04 ^{aw}	1.75 ± 0.22 ^{bw}	1.75 ± 0.22 ^{bw}
	1	0.93 ± 0.08 ^{ay}	1.18 ± 0.06 ^{ay}	2.33 ± 0.18 ^{bx}	2.46 ± 0.09 ^{bx}
	2	0.62 ± 0.06 ^{ax}	0.80 ± 0.05 ^{bx}	1.65 ± 0.35 ^{cw}	2.44 ± 0.02 ^{dx}
	3	0.75 ± 0.06 ^{ax}	0.90 ± 0.08 ^{ax}	1.67 ± 0.03 ^{bw}	2.42 ± 0.05 ^{cx}

Values are presented as the average ± standard deviation. Different lowercase letters (a–d) within a row correspond to significant differences ($p < 0.05$) between the drying and storage conditions. Different lowercase letters (w–z) within a column correspond to significant differences ($p < 0.05$) over storage time for each type of extract

L Freeze-dried samples, *O* Oven-dried samples, *R* Room temperature, and *F* Freezing temperature

Concerning ABTS, aqueous and ethanolic extracts trended in the same direction, all showing strong increases over storage time with the sole exception of the ethanolic extracts of LR samples. More specifically, in the case of ethanolic extracts, freeze-dried *A. taxiformis* had always higher antioxidant activity than oven-dried seaweed. Indeed, whereas OR and OF samples presented ABTS values in the 0.26–1.18 mmol Trolox Eq/100 g dw range, LR and LF samples exhibited ABTS results in a clearly higher interval, 1.65–2.46 mmol Trolox Eq/100 g dw. An equivalent contrast in the case of aqueous extracts was absent due to the very steep increase of ABTS antioxidant activity of OF seaweed from 0.84 ± 0.14 mmol Trolox Eq/100 g dw to 4.40 ± 0.23 mmol Trolox Eq/100 g dw, which was more than the double of OR at $t = 3$ month, 2.13 ± 0.05 mmol Trolox Eq/100 g dw. However, taking into account all storage temperature contrasts, this independent variable did not seem as influential as drying process and storage time.

Anti-inflammatory activity

The anti-inflammatory activity expressed as percentage of COX-2 inhibition and reflecting its response to the three independent variables of the experimental design (drying process, storage temperature, and storage time) is presented in Table 6. Moreover, after averaging OR, OF, LR, and LF samples, a more clear-cut dependence of anti-inflammatory activity on storage time is achieved and illustrated by Fig. 2. Tested extract concentration was 1 mg/ml.

At the beginning of the storage study, oven-dried samples displayed higher anti-inflammatory activity than freeze-dried samples, but differences between drying processes were progressively lost and were absent after three month storage. With respect to storage temperature, it was not possible to identify any unequivocal effect for the two

Table 6 Anti-inflammatory activity (% inhibition of COX-2) in ethanolic extracts of *Asparagopsis taxiformis* subjected to different drying processes and storage conditions at different times of the storage stability experiment

Sample	Time (month)	Anti-inflammatory activity (% inhibition of COX-2)			
		OR	OF	LR	LF
Ethanolic extract	0	29 ± 7 ^{aw}	29 ± 7 ^{aw}	6 ± 3 ^{bw}	6 ± 3 ^{bw}
	1	20 ± 5 ^{aw}	3 ± 3 ^{aw}	20 ± 8 ^{awx}	44 ± 4 ^{by}
	2	15 ± 3 ^{aw}	26 ± 6 ^{abw}	30 ± 5 ^{bxy}	32 ± 7 ^{bxy}
	3	40 ± 11 ^{ax}	30 ± 12 ^{aw}	40 ± 7 ^{ay}	26 ± 4 ^{ax}

Values are presented as the average ± standard deviation. Different lowercase letters (a–d) within a row correspond to significant differences ($p < 0.05$) between the drying and storage conditions. Different lowercase letters (w–z) within a column correspond to significant differences ($p < 0.05$) over storage time

L Freeze-dried samples, O Oven-dried samples, R Room temperature, and F Freezing temperature

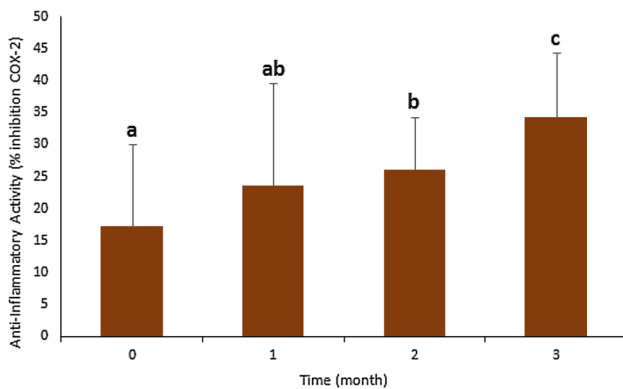


Fig. 2 Variation of the anti-inflammatory activity (% inhibition of COX-2) in ethanolic extracts of *Asparagopsis taxiformis* over storage time. Each month value is the average of seaweed samples submitted to different drying and storage conditions. Different lowercase letters correspond to significant differences ($p < 0.05$) between different time points. Standard deviation is indicated by the vertical bars

pairs, OR vs OF and LR vs LF. However, after three month storage and averaging OR with LR as well as OF with LF, a significant ($p < 0.05$) difference was detected with a 12% additional activity in *A. taxiformis* at room temperature. A complex evolution of the anti-inflammatory capacity over time was observed with the exception of LR, which showed an almost continuous increase of the COX-2 inhibition from $t = 0$ month ($6 \pm 3\%$) to $t = 3$ month ($40 \pm 7\%$). However, if all OR, OF, LR, and LF values are combined for each sampled storage time (Fig. 2), an overall increasing trend is observed from the beginning to the second month and, finally, to the third month, thus doubling the initial value (17–34% range).

Discussion

Proximate composition and fibre content

The proximate composition and fibre content values were stable over storage time and were not particularly sensitive to storage temperature. The drying method generated some differences, particularly in the case of the moisture and ash contents. Oven-drying ($60\text{ }^{\circ}\text{C}$ for 72 h) was more effective than freeze-drying ($-40\text{ }^{\circ}\text{C}$ and 4×10^{-4} mbar for 48 h) in removing moisture from *A. taxiformis*. As a probable consequence, ash contents were higher in the oven-dried seaweed. Such effect was missing in the case of the protein and lipid contents. A hypothetical explanation for the observed lower protein and lipid contents in oven-dried *A. taxiformis* could be the occurrence of dripping during the 72 h in the oven (during freeze-drying water remains frozen and no such dripping is possible). However, this explanation is weakened by the absence of a similar effect on the ash content. Finally, fibre content did not show any variation, not even a relative concentration—as occurred in the case of ash content—or lower values by dripping loss—as hypothesized for protein and lipid contents—in the oven-dried samples. Regarding this latter possibility, fibre (at least, the insoluble part) would be less prone to be lost, given its structural role in *A. taxiformis* biomass. Studies on other seaweed species showed some similarities. Namely, Chan et al. (1997) [26] reported a higher moisture level in freeze-dried *Sargassum hemiphyllum* when compared with the oven-dried seaweed. However, ash content in *S. hemiphyllum* was higher in freeze-dried samples, thus differing from the variations observed in *A. taxiformis*. More studies on this subject are necessary, given their current scarcity.

Fatty acid profile

There are not many studies on *A. taxiformis*. More specifically, concerning lipid fraction components, studies are scarcer. The current study is the first one to encompass drying and storage conditions and storage time for this seaweed species. There is a study on the FA profile of *A. armata* [27], which displays a composition similar to those of the current study. Indeed, palmitic acid content exceeded 50% of the total FAs and total SFA level surpassed 80% of the total FAs. Moreover, total MUFA was the second most important group and total PUFA content was very low, $4.7 \pm 0.4\%$ of total FAs. ARA and EPA were the main $\omega 6$ and $\omega 3$ PUFA, respectively, just as in the current study. Specifically, EPA content was $2.9 \pm 0.2\%$ of total FAs. All these FA levels agree with *A. taxiformis*

results, which is probably a result of the taxonomic proximity and its influence on the FA composition. The biomass of *A. armata* was freeze-dried, thus a higher EPA content in freeze-dried *A. taxiformis* ($t = 0$ month) may probably be an interspecific difference. Nonetheless, even higher EPA contents are found for other red seaweeds [28–30]. An extreme example is the red seaweed *Palmaria palmata* (order Palmariales) with almost 70% EPA [28]. Moreover, the EPA content of red seaweeds has been found to depend on seasonality [30] and location [31].

The drying process is considered influential on EPA and PUFA contents, which are prone to oxidation and degradation [32, 33]. This may explain the lower ARA and EPA contents in oven-dried samples (OR and OF). A study on *S. hemiphyllum* [26] concluded that the nutritional composition of seaweed may be greatly affected by different drying methods, being total PUFA content higher in freeze-dried seaweed than in oven-dried seaweed.

Schmid et al. (2016) [34] investigated the effect of storage temperature and time on the stability of PUFA in ground and freeze-dried seaweed biomass. Samples were stored for a total duration of 22 months (with intermediate samplings after one and three months) at three different temperatures (-20 °C, 4 °C, and 20 °C). For the red seaweed *P. palmata*, levels of ARA, EPA, and PUFA were only stable after storage at -20 °C. This observation differed from current study, where this same temperature did not prevent loss of these FAs after three month storage. However, species are different and initial levels also differed between *P. palmata* and *A. taxiformis*. At 20 °C, there was some loss of EPA and PUFA after one month storage, which became massive after 22 month storage [34]. As in current study, palmitic acid content increased over storage time at 20 °C as well as at 4 °C.

In general, it is considered that lipid degradation by oxidation is a major concern during storage of dried biological materials. Lipid oxidation may take place by different mechanisms, such as autoxidation, photosensitized or enzymatic oxidation [35]. Autoxidation is a free radical chain reaction encompassing initiation, propagation, and termination steps. In the initiation step a spontaneous abstraction of hydrogen of the FA by oxygen occurs. When the hydrogen atom is attached to carbon atoms between two double bonds, its dissociation energy is low, leading to FA radicals. This entails that PUFAs are more susceptible to oxidation [35]. Though the initiation step is an endothermic reaction that requires substantial activation energy [35], even at -20 °C this did not prevent PUFA loss in *A. taxiformis* biomass. The increase of palmitic acid content may be at least partially ascribed to the mathematical consequences of a relative decline in the proportion of other FAs.

Total polyphenol content

The variation of the phenolic contents in aqueous and ethanolic extracts in opposite directions over storage time suggest a possible conversion of less hydrophilic (soluble in ethanol) phenolic compounds into more hydrophilic ones. Since the phenolic contents reported in the relevant literature for a biomass rich in these compounds range from 100 to 500 mg GAE/100 g dw [36], the aqueous extracts of *A. taxiformis* may be considered a satisfactory source of phenolic substances. It is important to remark that a number of intrinsic factors can affect the concentration of phenolic compounds, namely the geographical location, climate, and phase of algal ripening at harvest, being claimed that higher phenolic contents in seaweed are associated with higher UV exposure [37].

Seaweed drying is considered a process that may decrease phenolic content either by binding polyphenols with other compounds (e.g. proteins) or by altering the chemical structure of polyphenols [38]. These biochemical alterations are accelerated by a higher drying temperature. This seems to corroborate results at $t = 0$ month. Moreover, Le Lann et al. (2008) [14] analysed the effects of sun-drying (72 h), oven-drying (50 – 60 °C, 40 h), and freeze-drying (72 h) on the biomass quality of *Sargassum muticum* and *Bifurcaria bifurcata*. These authors reported that phenolic content was higher in freeze-dried samples than in oven-dried samples. Similar results were also found by Wong and Cheung (2001) [39] for three brown *Sargassum* species. A decline of phenolic content by 28% in red seaweed *Porphyra* sp. after oven-drying has also been described [17]. In contrast, Ling et al. (2015) [40] observed for methanolic extracts of the red seaweed *Kappaphycus alvarezii* a higher phenolic content in oven-dried samples (80 °C, 24 h) compared to freeze-dried seaweed.

It may be conjectured a relationship between phenolic content in ethanolic extracts—more lipophilic phenolic substances— and PUFA contents (in % of total FAs). Indeed, it is known that PUFAs are more prone to oxidation than SFAs and MUFAs [41]—see also “Fatty acid profile” Section. Phenolic compounds constitute a possible response due to their antioxidant properties. Lipophilic phenolics are even more important since they can easily mingle with lipid molecules and position themselves near the FAs of phospholipids. Accordingly, a decline in phenolic content of the ethanolic extracts due to oven-drying or prolonged storage may entail a lower protection of PUFA against oxidation. In fact, it was found a consistent trend when total phenolic content in the ethanolic extracts was related to EPA (a very susceptible PUFA with five double bonds) content ($R^2 = 0.8$).

Antioxidant activity

With respect to the effect of the drying process, Le Lann et al. (2008) [14] found significantly higher activity as measured by DPPH in the freeze-dried samples compared to oven-dried samples. These authors claimed that the heat generated by the oven probably induced some denaturation of phenolic compounds that hampered their bioactivity. On the other hand, Ling et al. (2015) [40], on the basis of DPPH and ABTS results, stated that oven-dried (80 °C) samples of *K. alvarezii* displayed stronger scavenging activity and reducing ability as compared to tested freeze-dried samples. Such findings seem to contradict the thermal degradative effect on phenolic substances and other antioxidants. However, as mentioned in “Total polyphenol content” Section, this study [40] had measured a higher phenolic content in oven-dried samples. Moreover, studies on brown seaweeds [42, 43] have observed an overall strengthening of the antioxidant capacity and a substantial increase in the phenolic content with exposure to higher temperatures. Of course, this is not the reason for the higher DPPH activity of the ethanolic extracts attained from oven-dried *A. taxiformis*, since phenolic content in these extracts was lower than in those from freeze-dried samples. Hence, this subject warrants further study.

Concerning the storage study, variation of DPPH in some samples and of ABTS in almost all samples over time was opposite to what would be expected in a scenario of progressive loss of quality of the *A. taxiformis* biomass. Though such evolution is uncommon, it is not unparalleled. Indeed, a stability study on plant material using hydroalcoholic extracts by Montoro et al. (2006) [44] reported that after three month storage antioxidant activity increased relative to that of the initial time, while the major antioxidants' content declined. Conversion of some of these compounds into others that are more bioactive was a possible explanation. The same may have happened in the current study on *A. taxiformis* and the increase of the concentration of phenolic substances in the aqueous extracts over storage time while concentration in the ethanolic extracts declined may be an indication of such conversion phenomena.

There was divergence in the antioxidant activity as determined by different methodologies. Such divergence reflects the relative strengthening of the antioxidant activity as measured by ABTS with respect to the DPPH methodology. Considering that applied techniques are not equivalent and reflect different antioxidant properties, such lack of consistency could be expected. In fact, ABTS has been considered more sensitive than DPPH, usually yielding different results [45].

Neither DPPH nor ABTS values show any degree of correlation with the total polyphenol content. This suggests that each phenolic compound may have a different impact on

antioxidant activity as measured by DPPH or ABTS. This warrants further study directed to the analysis of the phenolic profile of *A. taxiformis*. Moreover, other compounds in this seaweed species, such as carotenoids [9], may exert a role in generating antioxidant activity.

Anti-inflammatory activity

The anti-inflammatory activity of seaweed is still poorly studied and the assay techniques vary, comprising in vitro tests and in vivo models [46–48]. This causes difficulties whenever comparisons across studies are made. Nevertheless, there are studies pointing to the existence of anti-inflammatory bioactives in seaweed [49, 50] and substantial bioactivity in red seaweed [47]. This latter study reported that an ethanol extract from *Gracilaria verrucosa* showed anti-inflammatory activity as measured in astrocyte cell cultures. Kim et al. (2013) [51] observed in an in vitro study that floridoside, a natural glycerol galactoside naturally isolated from the red seaweed *Laurencia undulata*, was able to suppress pro-inflammatory responses in microglia by markedly inhibiting the production of nitric oxide and reactive oxygen species. The methods used in these studies were different from that used in the current study, in which the activity of the COX-2 enzyme was reduced by inhibition. Furthermore, sometimes different solvents were used in the preparation of the extracts of other studies. It should be remarked that a preliminary study with the initial samples ($t=0$ month) of *A. taxiformis* showed that anti-inflammatory activity was more significant in the ethanolic extracts of *A. taxiformis*. Hence, for all samples, ethanolic extracts were prepared and anti-inflammatory activity measured in them. One of the few studies using the COX-2 activity inhibition methodology was carried out for green seaweed and used aqueous extracts [52]. These authors found anti-inflammatory activity in 10%, w/v, extracts, the residue of which was dissolved in DMSO (1 mg/ml): COX-2 inhibition varied between 31 and 45%, which is relatively similar to the results attained with the *A. taxiformis* ethanolic extracts. Campos et al. (2019) [53] also used the COX-2 inhibition method and observed anti-inflammatory activity in aqueous extracts of the brown seaweed *Petalonia binghamiae* ($40 \pm 7\%$ inhibition). For the red seaweed *Osmundea pinnatifida*, according to the same study, neither aqueous nor ethanolic extracts generated anti-inflammatory activity.

Whereas the drying process and storage temperature did not drive clear changes in the levels of anti-inflammatory activity of *A. taxiformis* ethanolic extracts, a global effect of storage time on anti-inflammatory activity was observed and indicated an enhancement of this property (Fig. 2). As with the antioxidant activity, this differs from the most usual outcome of biomass quality loss. An explanation may lie in the nature of the compounds generating the anti-inflammatory

activity. Indeed, different classes of compounds may have anti-inflammatory effects, phenolic compounds, carotenoids, phytosterols, alkaloids or polysaccharides. Though ω 3 PUFA and, particularly, EPA display an anti-inflammatory action [12] these compounds do not inhibit COX-2. Moreover, there was no strong correlation between the total phenolic content and anti-inflammatory activity. Thus, anti-inflammatory activity in *A. taxiformis* extracts may be the result of different compounds, necessarily soluble in ethanol, which excludes polysaccharides given their low solubility in ethanol. Therefore, other compounds, such as carotenoids and phytosterols, may underlie the observed anti-inflammatory activity and conversion phenomena during storage time may have generated more bioactive molecules.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest involving any of the authors.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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