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Effect of storage conditions on lipid oxidation, nutrient loss and colour of dried seaweeds, *Porphyra umbilicalis* and *Ulva fenestrata*, subjected to different pretreatments

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ABSTRACT

Here we evaluated the levels of lipid oxidation products, fatty acids, ascorbic acid and colour of *Porphyra* and *Ulva* after oven-drying at 40 °C, and during subsequent storage for ≥370 days under light, semi-light and dark conditions. Part of the seaweed was pre-soaked in freshwater or pre-coated with a whey protein mixture. Controls consisted of freeze-dried seaweeds. Throughout storage there was a moderate development of the lipid oxidation-derived aldehydes, malondialdehyde, 4-hydroxy-trans-2-hexenal and 4-hydroxy-trans-2-nonenal, while there was a great loss of unsaturated fatty acids and ascorbic acid. Light storage and freeze-drying stimulated the fatty acid loss as well as pigment bleaching, seen as increased a*-values. For *Ulva,* the coating reduced malondialdehyde, 4-hydroxy-trans-2-hexenal and 4-hydroxy-trans-2-nonenal formation during drying and slightly prevented loss of polyunsaturated fatty acids during light storage. Pre-soaking in freshwater had no effect on the seaweed stability, although it reduced the ash content and thereby increased the relative content of ascorbic acid and fatty acids of the biomasses.

1. Introduction

The seaweeds *Porphyra umbilicalis* and *Ulva fenestrata* are promising species for future food use, due to the high content of proteins and the abundance of n-3 polyunsaturated fatty acids (n-3 PUFA), minerals and vitamins. Despite the high content of unsaturated fatty acids, there are only few studies on lipid oxidation in seaweed [1,2]. The oxidation reaction does not only destroy the fatty acids, causing rancidity, but can also co-oxidize pigments and vitamins; altogether reducing both nutritional and sensorial quality of foods. Lipid oxidation has previously been shown to correlate with a decrease in both α-tocopherol [1] and pigments [2] in dried *Porphyra* sp. during 15 days storage at 40 °C. In the second of these studies [2], higher peroxide values (PV) and conjugated dienes were found in light- compared to dark-stored *Porphyra* sp., most likely due to the abundance of pigments acting as photo-sensitizers [3].

Fresh seaweed has been reported to have a relatively short shelf life (~3–14 days) [4–6]. Thereafter, sensorial deterioration and unwanted odour appeared together with growth of microbes, drip loss and colour loss [4–6]. To preserve seaweed for industrial use, sun-drying or conventional air-drying is therefore commonly applied [7–9]. Sun-drying is environmentally friendly and cheap, however, it is highly dependent on the weather conditions [9]. Hot air-drying provides fast heat and mass transfer [9], however, it has been shown to affect the food quality in a negative manner [10]. Freeze-drying has in different studies been found to preserve nutrients, such as polyphenols [11,12], carotenoids [12], ascorbic acid [7], unsaturated fatty acids [7] and amino acids [7,13] in seaweed better than oven-drying (60–70 °C). However, freeze-drying is time consuming and expensive and might therefore not be economically feasible in large-scale production of dried seaweed. Since chemical reactions are not suppressed to the same degree as microbial growth when lowering water activity (a_w); and since lipid oxidation can even increase in rate at $a_w < \sim 0.3$ [14], it is of utmost importance to document how this reaction, as well as its effect on co-oxidation of other compounds in seaweed, proceed during and after drying.

Any pre-treatment of seaweed prior to drying is expected to affect its subsequent quality. As an example, when seaweed has been rinsed in freshwater (FW) prior to further processing to eliminate epiphytes and

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other impurities [4], rapid movement of water across the cell membrane due to the sudden high osmotic pressure was reported to disrupt cells and induce leakage of cellular components, which enable chemical reactions in the wet seaweed [4]. To the best of our knowledge, no studies currently exist where the relation between seaweed pre-treatment and storage stability after drying is studied.

Edible coatings have been used for centuries on fresh fruits as a strategy to prolong shelf life [10]. Since coatings function as barriers, thus, protect the food item against mass transfer and gas exchange, they can reduce oxidation and thereby are promising to apply prior to convective air-drying [10]. Some coatings, e.g. whey-based ones, also protect partly from light exposure due to the opacity [15]. Coatings have shown promising results in retaining the quality of e.g. papaya [10] during drying. However, coatings, to our knowledge, have not been applied before to seaweed. Given the fact that some dried seaweed products such as spice mixes are sold in transparent or partially transparent packages without vacuum applied, new knowledge on the role of coatings for lipid oxidation in seaweed, and its relation to light exposure, is expected to be of significant use for food industry.

In this study, the effect of soaking in FW as well as the effect of applying a whey-based coating to *Porphyra* and *Ulva* prior to oven-drying was evaluated with respect to oxidation of lipids, ascorbic acid loss and colour change. To study the effect of oven-drying per se, also non-soaked and non-coated freeze-dried samples were prepared. All dried samples were stored in light, semi-light or dark conditions for up to 520 days prior to analyses. Lipid oxidation was monitored as formation of malondialdehyde (MDA), 4-hydroxy-trans-2-hexenal (HHE) and 4-hydryoxy-trans-2-nonenal (HNE) along with a decrease in fatty acid content. Colour changes were monitored as L*, a* and b*-values.

2. Materials and methods

2.1. Material

Ascorbic acid, dichloromethane, 2,4-dinitrophenylhydrazine (DNPH), ethylenediaminetetra-acetic acid (EDTA), hexane, metaphosphoric acid (MPA), methanol, phosphoric acid, *tris*(2-carboxyethyl) phosphine (TCEP) and 1,1,3,3-tetraethoxypropane were purchased from Sigma-Aldrich (Stockholm, Sweden). Hydrochloric acid (37%) was purchased from Acros Organics (Fisher Scientific, Gothenburg, Sweden) and HHE and HNE were purchased from Cayman Chemicals (Ann Arbor, MI, USA). The whey-based coating was donated by Smart Future Technologies (Littleton, CO, USA). The coating contained whey, maltodextrin, white corn flour, potassium sorbate, citric acid, sugar, glycerine and water. All ingredients in the coating were GRAS (Generally Regarded as Safe) and thereby considered safe for food use.

2.2. Seaweed raw material

Wild *Porphyra* tufts were collected at Tjärnö (58°52′33.272″N, 11°8′47.202″E) on the 20th of October 2017 and rinsed several times in seawater to remove grazers and loose epiphytes. Tufts were then placed in cultivation tanks. Cultivated gametophytes from *Ulva* were fragmented and temperature shocked to induce the production of reproductive tissue. After two days, released gametes germinated and attached to sterile petri dishes where they grew for two months. Juvenile *Ulva* were placed in cultivation tanks. The seaweeds were cultivated at the Sven Loven Center for Marine infrastructure at Tjärnö, Sweden (58°52′33.2724″N, 11°8′47.202″E), in 100 L cultivation tanks at 12 °C with a neutral light cycle (16 h daylight, 8 h darkness) at a light intensity of 50 μE m^{-2} s^{-1}. Seaweeds received continuous filtered seawater. The lowest filter size used was 1 μm and all seawater was treated with UV prior to entering the cultivation tanks. No additional medium was added.

2.3. Treatments prior to drying

During gentle stirring by hand, freshly harvested *Porphyra* and *Ulva* were soaked for 2 min in i) FW), ii) whey coating dissolved in FW (1% w/v), or iii) whey coating dissolved in saltwater (SW) from the cultivation tank water (1% w/v), at 12 °C (Table 1). The seaweeds were thereafter drained and dried. For 1 kg of wet biomass, 10 L water or coating solution was used, according to instructions from the manufacturer. As controls, seaweeds were harvested, drained and dried without going through any soaking or coating step.

2.4. Drying

Oven-drying was carried out at 40 °C in a drying cupboard (*MK ASZ 25, Maurer, Domaszék, Hungary*). Non-coated samples were dried for 6 h and coated *Porphyra* and *Ulva* samples for 7 h and 40 min as well as 9 h, respectively. The longer drying periods for the coated samples were needed in order to obtain similar a_w as for non-coated samples. As controls, in order to evaluate the effect of oven-drying, non-treated *Porphyra and Ulva* were freeze-dried for 24 h (Heto Drywinner DW 6-55-1, Jouan Nordic A/S, Alleröd, Denmark).

2.5. Storage of dried samples

Directly after drying, the seaweed was transported in Ziploc® plastic bags in darkness at RT to the lab. At arrival 10 g of each sample type was ground using a bead beater (Retsch MM 400, Retsch GmbH, Haan, Germany) at speed 1/30s, vacuum packed in plastic bags (Rubicson, Kjell & Company, Göteborg, Sweden) and stored at −80 °C until further analyses. These samples are referred to as the starting materials (i.e. storage day 0). Remaining non-ground materials were stored in Ziploc® plastic bags (low-density polyethylene plastic) at room temperature (RT) in either darkness or in a room with natural daylight, and occasional additional light from lamps (~7–430 LUX) roughly 4 h/day Monday-Friday, referred to as semi-light. The wide LUX range is due to the Swedish climate with very bright summers and dark winters. Due to a limited sample amount, the coated *Porphyra* samples were only stored under day light conditions. To investigate the effect of full light under controlled conditions, a part of the samples which had been stored in darkness for 90 days were moved to a room with 24-h light (869 ± 9 LUX) where they were stored for an additional 430 days. The three storage conditions thus were: i) storage in darkness (day 0–520), ii) semi-light storage (day 0–520) and iii) light storage (day 90–520). All different treatments, drying conditions and storage conditions are summarized in Table 1.

2.6. Sampling

Samples were taken after 30, 90, 150, 230, 310, 370 and 520 days of storage, with two exceptions which were due to limited sample amounts: (i) at day 310 no *Porphyra* samples were collected, and (ii) at day 520 no *Porphyra* samples stored under full light or in darkness were taken. All taken samples were ground at each specific sampling point, as described above for the starting samples, vacuum packed and stored at −80 °C until further analysis.

2.7. Analysis of water activity, moisture and ash

Water activity (a_w) was analysed at 25 °C using a LabTouch-a_w (Novasina, Hygrometer, Food Diagnostics, Gothenburg, Sweden). All analyses were performed using duplicate samples and data are presented as mean values ± standard deviations (SD). Moisture content was determined gravimetrically for the starting material by drying approximately 100 mg of sample at 105 °C over-night. The ash content was thereafter determined by combusting the residual dried sample at 550 °C for 3 h. To get a measure of the amount of moisture taken up by

Table 1
Pretreatments, drying conditions and storage conditions for Ulva and Porphyra. Plus (+) denotes that the sample was stored under the given condition while minus (−) denotes that no storage was performed during the given condition. FW = freshwater, SW = saltwater.

Species	Sample name	Pretreatment	Drying	Places of storage		
				Dark	Semi-light	Light[a]
Ulva fenestrata	*Ulva* Freeze-dried	None	Freeze-dried	+	+	+
	Ulva Oven-dried	None	Oven-dried	+	+	+
	Ulva Oven-dried-FW	Soaked in freshwater (FW)	Oven-dried	+	+	+
	Ulva Oven-dried-SW coating	Soaked in saltwater (SW) with whey coating	Oven-dried	+	+	+
	Ulva Oven-dried-FW coating	Soaked in freshwater (FW) with whey coating	Oven-dried	+	+	+
Porphyra umbilicalis	*Porphyra* Freeze-dried	None	Freeze-dried	+	+	+
	Porphyra Oven-dried	None	Oven-dried	+	+	+
	Porphyra Oven-dried-FW	Soaked in freshwater (FW)	Oven-dried	+	+	+
	Porphyra Oven-dried-SW coating	Soaked in saltwater (SW) with whey coating	Oven-dried	−	+	−
	Porphyra Oven-dried-FW coating	Soaked in freshwater (FW) with whey coating	Oven-dried	−	+	−

[a] After 90 days in darkness.

the dried samples during subsequent storage at ambient temperature, the moisture content was again analysed at day 370 for a selection of samples. All analyses were performed using triplicate samples and data are presented as mean values ± SD.

2.8. Analysis of lipid oxidation-derived aldehydes

Analysis of free MDA, HHE and HNE was done as described by Tullberg et al. [16], with some modifications. Approximately 50 mg dried and milled seaweed was mixed with 950 μL H_2O, 40 μL BHT (4.5 M in methanol) and 80 μL EDTA (0.02 M in H_2O). The samples were acidified using 1 mL 0.25 M HCl, vortexed and left to precipitate for 5 min, followed by centrifugation at 4000 ×*g* at 4 °C for 2 min. Thereafter 0.4 mL of the supernatant was mixed with 25 μL DNPH (10 mM in methanol), vortexed and incubated at RT for 60 min. Extraction was carried out twice with 0.5 mL dichloromethane and the samples were centrifuged at 16000 ×*g* for 2 min before the lower phase was recovered. Samples were evaporated under N_2-gas at RT and the residues were dissolved in 250 μL methanol and injected into an Agilent 1260 HPLC system, consisting of a binary pump, an auto sampler, a column oven and an UV-detector, coupled to an Agilent 6120 quadrupole mass spectrometer in the APCI negative mode (Agilent Technologies, Waldbron, Germany). The separation of aldehydes was performed on a Phenomenex Luna, 4.6 mm i.d. × 250 mm, 3 μm, C18 column (Phenomenex, Macclesfield, UK). Settings and mobile phases were as described in Tullberg et al. [16]. As standard, a mix of MDA, HHE and HNE in methanol was used. MDA was prepared from 1 mM 1,1,3,3-tetraethoxypropane (TEP) hydrolysed in 1% H_2SO_4 for 120 min at 25 °C. The data were analysed in selected ion monitoring (SIM) mode at the *Agilent ChemStation* software and ions were collected at 234.0, 293.1 and 335.1 *m/z* ratio, corresponding to derivatized MDA, HHE and HNE, respectively. Analyses were performed using duplicate samples and data are presented as mean values ± SD and expressed as μg aldehydes/g dried seaweed.

2.9. Analysis of fatty acids

Fatty acids were analysed on the starting material and on samples stored for 370 days by Chalmers Mass Spectrometry Infrastructure (CMSI). 25 mg of dried and milled seaweed was mixed with 20 μL internal standard (C23:0, 1000 ppm), followed by addition of 1 mL toluene and 1 mL of freshly prepared hydrochloric acid (10% *v*/v in methanol). The samples were incubated at 70 °C for 120 min, then allowed to cool to room temperature, followed by addition of 1 mL water. Thereafter, 1 mL hexane was added, followed by vortexing for at least 60 s and centrifugation at 100 ×*g* for 6 min to allow phase separation. The upper phase was injected on to a Shimadzu TQ-8030 GC–MS/MS system consisting of a Shimadzu GC-2010 Plus gas chromatograph (GC), Shimadzu TQ-8030 triple quadrupole mass spectrometer and a Shimadzu AOC-5000 Plus sample handling system (Shimadzu Europe GmbH, Duisberg, Germany). Data was acquired using Shimadzu GCMSSolutions software version 4.2. One μL of each sample was injected with a split ratio of 1 and separation was done on a 30 m × 0.25 mm × 0.25 μm Zebron ZB-WAXplus column (Phenomenex, Torrance, CA,USA). Injection port temperature was set to 275 °C and the oven temperature program was as follows: initial temperature 100 °C, ramped to 205 °C at a speed of 4 °C/min, followed by ramping to 230 °C at 1 °C/min, where it was held for 5 min. GC was operated in constant linear velocity mode set to 37.2 cm/s. Septum purge flow was set to 3 mL/min. Interface and ion source temperatures were 280 and 230 °C, respectively. The autosampler was kept at 8 °C. Helium was used as the carrier gas. Data was collected between 50 and 550 *m/z* and at single ions at 55, 74 and 87 m/z. Quantification of fatty acids was done based on external calibration curves using response factors from internal standard normalization both for standards and samples. Identification of C22:4n6 was done using the MS-library of the software. Analysis was performed on single samples and the analytical variation for each fatty is given as % CD for the QC (Quality control) samples. The latter samples were prepared by combining a small ́aliquot ́of each sharp sample when weighing them for sample preparation. QC samples were then injected throughout the batch and, thus, reflect reproductivity of the batch during analysis. Results are expressed as mg fatty acids/g dried seaweed.

2.10. Analysis of ascorbic acid

Ascorbic acid was analysed according to the method described by Lykkesfeldt [17]. Approximately 50 mg of dried, milled seaweed was homogenized using a polytron (*Ultra turrax model T18 basic, IKA-Werke GmbH & Co. KG, Staufen, Germany)* in 2 × 2.5 mL MPA (10% *w/v* in water containing 2 mM EDTA) for 30 s at 10000 rpm. The samples were centrifuged at 5000 ×*g* for 5 min and the supernatant was diluted with 1 part McIlvaine buffer (0.46 M Na_2HPO_4, 0.27 M citric acid, pH 4.5) and 9 parts mobile phase (9 mM phosphate buffer containing 2% methanol (v/v) and 0.2% EDTA (w/v), pH 2.8 containing 0.312 mM TCEP) before injected into a HPLC system. The system consisted of two pumps (Jasco PU-2080Plus), a cooled auto sampler at 8 °C (Jasco AS-2057Plus, Easton, MD, USA) and an electrochemical detector (Decade II, Antec Leyden). Separation was performed on a Thermo Aquasil C18 column (150 mm × 4.6 mm, particle size 3 μm, Thermo Fisher Scientific, Gothenburg, Sweden). Standard consisted of L-ascorbic acid in the same mixture of buffers as was used above, and analyses were performed using duplicate samples. Data are presented as mean values ± SD and expressed as μg ascorbic acid/g dry seaweed.

2.11. Analysis of colour

The colour (L*, a* and b*-values) was measured using a colorimeter (Minolta Chroma Meter CR-3, MinoltaCorp, Ramsey, NJ, USA).

Approximately 1 g of ground seaweed was placed in a shallow container (5 mm) with a diameter of 3 cm with the colorimeter probe placed above. Five measurements were done for each sample and data are presented as mean values ± SD.

2.12. Statistics

To determine if there were significant differences between different pretreatment groups, storage conditions and storage times, ANOVA using Tukey's post hoc test (IBM SPSS Statistics 19, IBM Corp., Armonk, NY, USA) was performed. Differences were denoted as significant when $p \leq 0.05$. Aldehydes, fatty acids and ascorbic acid are expressed on basis of the dried sample weight per se, without correcting for its moisture content.

3. Results & discussion

3.1. Water activity, moisture and ash

The water activity (a_w) after drying was significantly lower in freeze-dried than oven-dried samples (Table 2). Moisture content of the seaweed directly after drying varied between 6.0 and 10.1% (Table 2). Based on analyses of a few selected samples also at day 370, it was found that the moisture content at that time point varied between 8.6 and 12.0%, illustrating that there was only a minor amount of water taken up during storage. The wash-out of minerals during soaking in FW resulted in significantly lower ash-levels in soaked samples (14.5–29.7%) compared to the non-soaked ones (33.4–44.1%) (Table 2).

3.2. Lipid oxidation products

For *Ulva,* the freeze-dried samples had significantly higher initial values of MDA and HHE than the oven-dried samples, Figs. 1–2 A-E. For *Porphyra* the same was seen for HHE (Fig. 2 A-E) and HNE (supplementary material). The fact that lipid oxidation has a minimum rate at $a_w \sim 0.3$, below which the rate increases due to more effective metal-catalysis and hydroperoxide breakdown [14], could explain the higher levels of lipid oxidation product in freeze-dried samples ($a_w = 0.15/0.16$) compared to oven dried samples ($a_w = 0.20$–0.29). In addition, an overall increase in the pore surface area of freeze-dried kelp compared to oven-dried kelp has been reported [18] which can be caused by rupture of cell walls due to slow freezing rate, resulting in more void space [19]. It is also very likely that lipid oxidation takes different pathways during the two temperatures applied during oven- vs. freeze-drying, resulting in different profiles of oxidation products [22]. As an example, the higher temperature used during oven-drying compared to freeze-drying may stimulate cross-linking between free aldehydes and e.g. proteins [20–22] rendering the aldehydes undetectable with our method. MDA, HHE and HNE are all known to be very reactive e.g. towards free amino- and sulfhydryl-groups [23].

For *Ulva,* the oven-dried SW-coated samples contained significantly lower initial values of all aldehydes compared to the non-coated samples (Figs. 1–2 B, D and supplementary information), and for oven-dried FW-coated samples, the same was seen for HHE and HNE (Fig. 1 C, E and supplementary information). Thus, it is likely that the whey-based coating worked as a barrier against oxygen migration [15] and protected *Ulva* against lipid oxidation during the drying operation, something which was not seen for *Porphyra*. Also, β-lactoglobulin, which comprises ~50% of the proteins in whey, is thermolabile and in its denatured form the thiol group on cysteine 121 is exposed and available for disulfide formation [15]. This implies that it could also be available e.g. for Michael adduct formation [23], which would "quench" the free aldehydes. Similarly, free amino groups of the whey-coating proteins could lead to Schiff-base formation, also lowering the concentration of free aldehydes [23]. Both reactions are likely to be more pronounced in coated *Ulva* compared to *Porphyra,* due to the longer drying time required. It has earlier been described [24] how aldehydes such as t-2-hexenal can bind covalently to milk proteins; affecting e.g. the aroma intensity of flavoured dairy products.

For MDA, no clear development pattern was seen during storage of the samples, except for the coated *Ulva* samples (Fig. 1D-E), which showed typical oxidation kinetics with an exponential phase between day 150 and 230, resulting in significantly increased MDA values. Thereafter, a more stationary phase appeared. For *Porphyra*, MDA values at some of the initial storage points were higher than the values at day 150 (Fig. 1F-H). This could be due to cross-linking of MDA to proteins in the beginning of the storage period. However, there was a significant increase in MDA between day 230 and day 370 for oven-dried *Porphyra* stored both under light and dark conditions (Fig. 1G); the latter which unexpectedly resulted in the highest MDA levels in the whole storage period.

For most *Ulva* samples there was no difference in HHE levels up to day 150 compared to the initial values, Fig. 2A-E. The exception was the oven-dried SW-coated sample stored light, which had increased significantly in its HHE level at day 150, and also had significantly higher HHE values compared to the corresponding samples stored dark and semi-light. Between day 150 and 230, all *Ulva* samples, except the dark-stored FW-coated sample (Fig. 2E), had increased significantly in their HHE level, and light was found to stimulate the HHE-development. The HHE levels then continued to increase >230 days, except for in light stored samples which in most cases peaked earlier (Fig. 2A-D). Similar pattern was seen for the *Porphyra* samples (Fig. 2F-J). At day 370, both freeze-dried and oven-dried *Porphyra* stored dark had reached significantly higher HHE values compared to the samples stored light (Fig. 2 F-G). Similar to MDA, the highest HHE level (2.4 μg/g) was obtained for oven-dried *Porphyra* (Fig. 2G).

Less HNE compared to HHE formed in the seaweeds, which corresponds to the lower levels of n-6 fatty acids compared to n-3 fatty acids in the used seaweed biomasses (Tables 3–4). HHE and HNE are specific oxidation products from n-3 and n-6 fatty acids, respectively [25]. For dark stored *Ulva*, there was no significant increase in HNE between day 0 and 520, except for in the sample soaked in FW (supplementary information Figure 1). Storage in darkness protected *Ulva* samples from formation of HNE, as was seen also for the HHE-levels up to day 230 (Fig. 2A-E). For *Porphyra*, all samples reached the highest HNE values at day 370 (supplementary information), and as for MDA and HHE, the highest HNE value was obtained in oven-dried *Porphyra* stored dark.

Table 2
Water activity, moisture content and ash content of the starting materials. Data show mean values ± standard deviations. FW = freshwater, SW = saltwater.

Sample	Water activity	Moisture content (%)	Ash content (% of dw)
Ulva Freeze-dried	0.15 ± 0.001^{a}	9.2 ± 0.5^{de}	38.4 ± 0.4^{f}
Ulva Oven-dried	0.21 ± 0.0^{b}	9.0 ± 0.2^{cde}	38.1 ± 0.3^{f}
Ulva Oven-dried-FW	0.20 ± 0.006^{b}	10.1 ± 0.2^{e}	24.9 ± 0.2^{c}
Ulva Oven-dried-SW coating	0.20 ± 0.001^{b}	7.5 ± 2.2^{abcd}	38.4 ± 0.3^{f}
Ulva Oven-dried-FW coating	0.21 ± 0.005^{b}	8.5 ± 0.5^{bcde}	22.2 ± 0.1^{b}
Porphyra Freeze-dried	0.16 ± 0.003^{a}	6.0 ± 0.2^{a}	41.0 ± 0.3^{g}
Porphyra Oven-dried	0.24 ± 0.001^{c}	7.3 ± 0.4^{abcd}	44.1 ± 0.8^{h}
Porphyra Oven-dried-FW	0.26 ± 0.007^{d}	7.0 ± 0.1^{abc}	14.5 ± 0.2^{a}
Porphyra Oven-dried-SW coating	0.29 ± 0.003^{e}	6.7 ± 0.2^{ab}	33.4 ± 0.7^{e}
Porphyra Oven-dried-FW coating	0.27 ± 0.0^{d}	6.1 ± 0.2^{a}	29.7 ± 0.2^{d}

Data in the same column carrying different superscript letters are significantly different ($p < 0.05$).

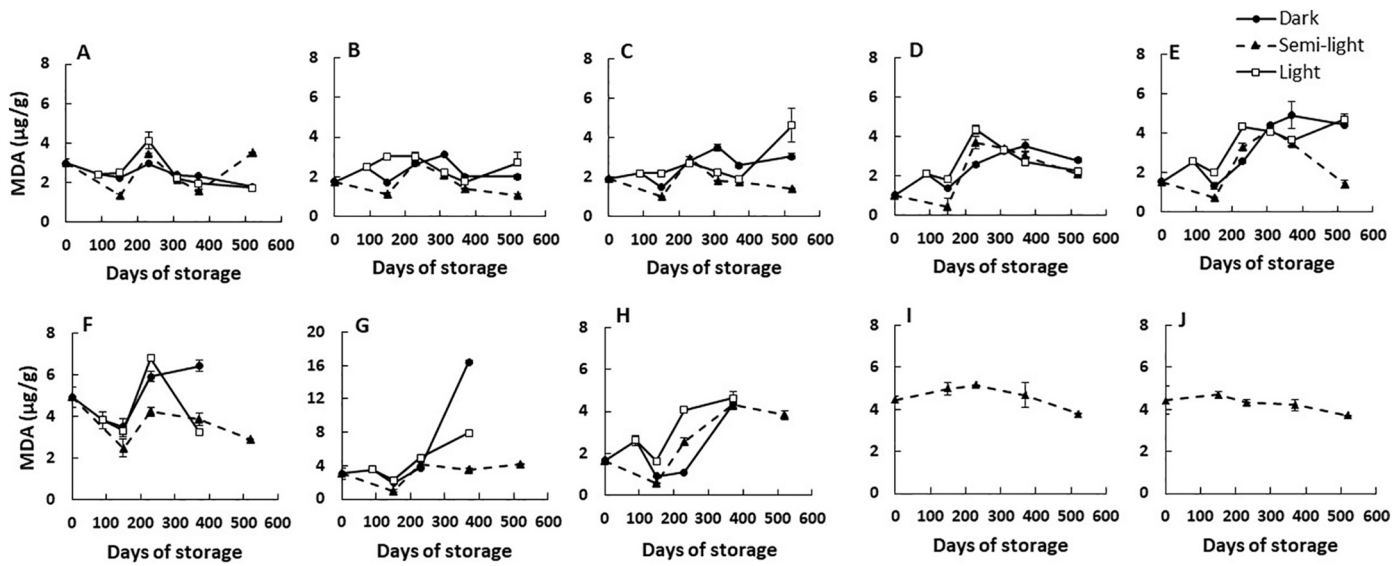

Fig. 1. Malondialdehyde in A) freeze-dried, B) oven-dried, C) oven-dried-FW soaked, D) oven-dried SW-coated and E) oven-dried FW-coated *Ulva* and in F) freeze-dried, G) oven-dried, H) oven-dried-FW soaked, I) oven-dried SW-coated and J) oven-dried FW-coated *Porphyra*. Note the different y-axis in panel G. Error bars show standard deviations ($n = 2$). Note that these were very small for some samples. FW = freshwater, SW = saltwater, MDA = malondialdehyde. Circles, triangles and squares show dark, semi-light and light conditions respectively.

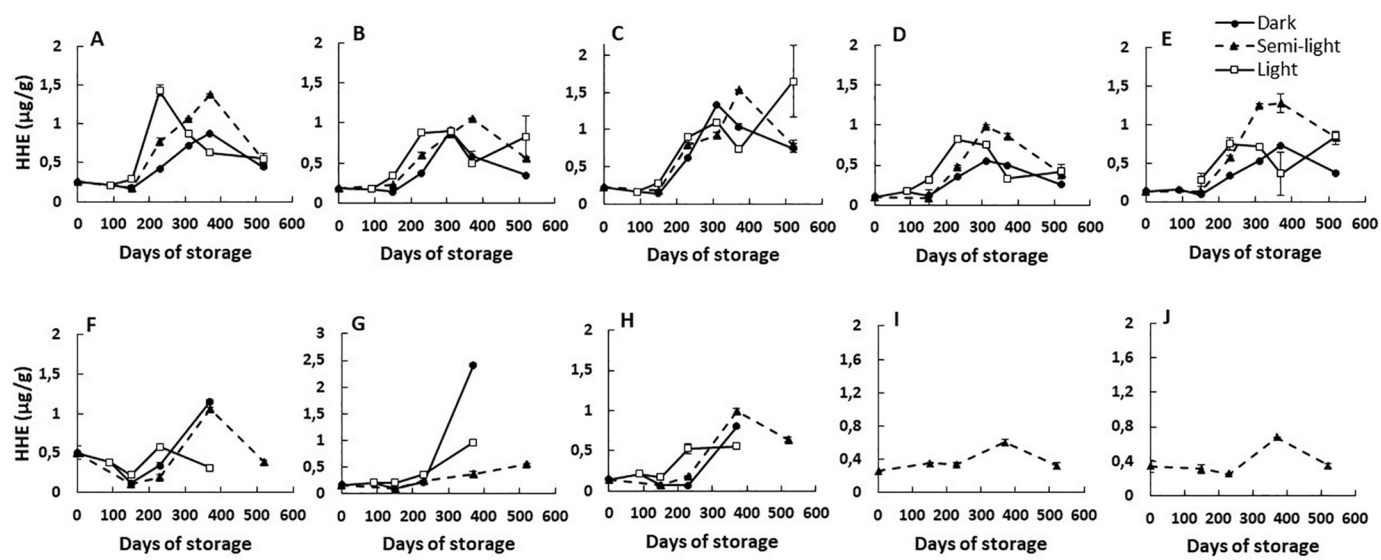

Fig. 2. 4-hydroxy-trans-2-hexenal in A) freeze-dried, B) oven-dried, C) oven-dried-FW soaked, D) oven-dried SW-coated and E) oven-dried FW-coated *Ulva* and in F) freeze-dried, G) oven-dried, H) oven-dried-FW soaked, I) oven-dried SW-coated and J) oven-dried FW-coated *Porphyra*. Note the different y-axis in panel G. Error bars show standard deviations (n = 2). Note that these were very small for some samples. FW = freshwater, SW = saltwater, HHE = 4-hydroxy-trans-2-hexenal. Circles, triangles and squares show dark, semi-light and light conditions respectively.

That light stimulates oxidation in presence of certain pigments is a well-known phenomenon [26], and e.g. chlorophyll and phycoerythrin in seaweed are photo-sensitizers which in the presences of light can become excited and initiate lipid oxidation [26]. Sugawara et al. [3] showed that phycoerythrin induced oxidation of EPA in *Porphyra yezoensis* during exposure to light [3], and Min et al. [27] found that lipid oxidation in soybean oil increased with increasing chlorophyll content under light conditions. Surprisingly, *Porphyra* stored dark reached higher aldehyde levels at many time points than light stored samples. This could be due to the presence of other pro-oxidants, such as lipoxygenase [28], which are not activated by light. Our results for *Porphyra* were contradictory to what Oh et al. [2] found for *Porphyra* sp. They found both higher PV and conjugated dienes in light-stored compared to dark-stored *Porphyra* sp. during 15 days of storage at 40 °C, however, both these measures targets primary lipid oxidation products.

3.3. Fatty acids

The initial content of the quantified total fatty acids was highest in samples soaked in FW, with or without coating, for both species (Tables 3 and 4). This was due to the changed mass balance caused by the washing out of minerals, thereby translating to a lower ash content (Table 2). For all sample types the storage-induced loss of both PUFA and MUFA ranked the samples in the order light storage > semi-light storage > dark storage (Tables 3–4). In agreement with this, Oh et al. [2] found significantly lower EPA content in light-stored compared to dark-stored *Porphyra* sp. already after 6 days when the storage temperature

Table 3
Fatty acid content in freeze-dried, oven-dried, oven-dried-FW soaked, oven-dried SW-coated and oven-dried FW-coated *Ulva* at day 0 and after 370 days of storage under light, semi-light and dark conditions. FW = freshwater, SW = saltwater. CV=Coefficient of variation (%) for each fatty acid caused by the analysis method.

Fatty acids (mg/g)	Freeze-dried				Oven-dried				Oven-dried-FW				Oven-dried SW-coating				Oven-dried FW-coating				
	Start	Dark	Semi-light	Light	Start	Dark	Semi-light	Light	Start	Dark	Semi-light	Light	Start	Dark	Semi-light	Light	Start	Dark	Semi-light	Light	% CV
C14:0	0.09	0.08	0.09	0.09	0.13	0.10	0.10	0.09	0.10	0.12	0.10	0.08	0.10	0.10	0.10	0.11	0.14	0.10	0.12	0.12	5.5
C16:0	2.84	2.85	2.79	2.67	3.01	2.82	2.80	2.79	3.41	3.55	3.30	3.12	2.89	2.67	2.49	2.50	3.27	2.78	3.13	3.11	2.5
C16:1n7	0.12	0.10	0.05	0.04	0.15	0.12	0.06	0.05	0.14	0.15	0.09	0.05	0.13	0.11	0.07	0.05	0.15	0.11	0.09	0.07	5.7
C16:1n9	0.19	0.18	0.15	0.10	0.19	0.18	0.15	0.12	0.28	0.25	0.24	0.19	0.21	0.18	0.14	0.12	0.25	0.22	0.22	0.23	1.5
C16:2n6	0.35	0.32	0.05	0.02	0.35	0.29	0.08	0.07	0.64	0.51	0.25	0.15	0.45	0.30	0.15	0.12	0.58	0.43	0.33	0.31	4.3
C16:4n3	1.84	1.05	0.12	0.06	1.82	1.12	0.25	0.26	2.33	1.49	0.52	0.26	1.92	0.94	0.48	0.38	2.17	1.12	0.83	0.85	2.3
C18:0	0.02	0.02	0.05	0.03	0.02	0.02	0.03	0.03	0.02	0.03	0.04	0.03	0.03	0.03	0.04	0.03	0.07	0.06	0.07	0.07	3.8
C18:1n9	0.09	0.07	0.00	0.00	0.12	0.11	0.04	0.02	0.12	0.11	0.06	0.02	0.15	0.13	0.08	0.05	0.32	0.24	0.16	0.11	1.7
C18:1n7	1.30	1.24	0.48	0.17	1.42	1.26	0.62	0.40	1.58	1.66	0.96	0.42	1.26	1.13	0.82	0.52	1.50	1.17	1.12	0.94	3.9
C18:2n6	1.80	1.56	0.34	0.12	1.83	1.56	0.53	0.40	2.32	2.15	1.07	0.52	2.02	1.51	0.92	0.66	2.35	1.74	1.50	1.33	1.6
C18:3n6	0.28	0.19	0.03	0.00	0.29	0.20	0.05	0.04	0.32	0.27	0.10	0.04	0.30	0.18	0.09	0.07	0.34	0.19	0.15	0.14	1.0
C18:3n3	2.49	1.69	0.24	0.09	2.32	1.72	0.45	0.39	2.87	2.25	0.91	0.40	2.57	1.51	0.84	0.62	2.66	1.63	1.37	1.26	1.2
C18:4n3	0.70	0.38	0.04	0.00	0.64	0.41	0.08	0.09	0.76	0.51	0.16	0.07	0.66	0.32	0.16	0.13	0.75	0.36	0.28	0.28	0.9
C20:1n9	0.00	0.01	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.02	0.00	0.00	0.00	5.7
C20:2n6	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	5.2
C20:3n6	0.04	0.03	0.00	0.00	0.03	0.03	0.00	0.00	0.06	0.04	0.00	0.00	0.05	0.03	0.00	0.00	0.04	0.03	0.00	0.00	1.9
C20:4n6	0.15	0.06	0.00	0.00	0.18	0.11	0.00	0.00	0.23	0.13	0.05	0.00	0.17	0.07	0.04	0.04	0.21	0.08	0.08	0.08	1.9
C20:5n3	0.24	0.08	0.00	0.00	0.28	0.10	0.00	0.00	0.24	0.15	0.00	0.00	0.23	0.07	0.04	0.04	0.27	0.07	0.06	0.07	2.5
C22:0	0.09	0.10	0.11	0.08	0.10	0.09	0.08	0.09	0.14	0.14	0.15	0.12	0.09	0.09	0.06	0.07	0.13	0.10	0.11	0.11	2.5
C22:1n6	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	24.4
C22:4n6	0.06	0.04	0.00	0.00	0.06	0.04	0.00	0.00	0.08	0.06	0.00	0.00	0.06	0.03	0.00	0.00	0.07	0.03	0.00	0.00	
C22:5n3	0.28	0.16	0.00	0.00	0.23	0.13	0.00	0.00	0.32	0.17	0.06	0.00	0.23	0.10	0.00	0.05	0.29	0.12	0.10	0.10	9.2
Total quantified fatty acids (mg/g)	12.97	10.24	4.55	3.47	13.21	10.43	5.32	4.83	15.98	13.80	8.06	5.45	13.54	9.53	6.52	5.57	15.60	10.61	9.71	9.18	
Sum PUFA (mg/g)	8.23	5.56	0.83	0.29	8.04	5.70	1.45	1.24	10.19	7.75	3.13	1.43	8.66	5.07	2.72	2.11	9.75	5.80	4.70	4.42	
Sum MUFA (mg/g)	1.70	1.63	0.68	0.31	1.91	1.69	0.86	0.59	2.12	2.21	1.35	0.68	1.77	1.56	1.11	0.75	2.24	1.77	1.58	1.35	
Sum Saturated (mg/g)	3.03	3.06	3.04	2.87	3.26	3.04	3.01	3.00	3.67	3.84	3.58	3.35	3.12	2.90	2.70	2.71	3.61	3.04	3.42	3.41	
% Lost PUFA		32.5	89.9	96.5		29.1	82.0	84.5		23.9	69.3	86.0		41.5	68.6	75.6		40.5	51.8	54.7	
% Lost MUFA		4.3	60.0	82.0		11.6	54.9	69.2		−4.1	36.2	67.9		11.6	37.4	57.7		21.0	29.4	39.6	
% Lost Saturated		−0.7	−0.2	5.3		6.8	7.6	7.9		−4.6	2.3	8.7		7.1	13.6	13.0		15.8	5.2	5.5	
% Total Loss		17.4	60.2	69.4		18.3	55.5	59.9		9.9	44.8	61.9		26.1	48.0	55.3		29.3	34.0	38.0	

Table 4
Fatty acid content in freeze-dried, oven-dried, oven-dried-FW, oven-dried SW-coated and oven-dried FW-coated *Porphyra* at day 0 and after 370 days of storage under light, semi-light and dark conditions. FW = freshwater, SW = saltwater. CV = Coefficient of variation (%) for each fatty acid caused by the analysis method.

	Freeze-dried				Oven-dried				Oven-dried-FW				Oven-dried SW-coating		Oven-dried FW-coating		
Fatty acids (mg/g)	Start	Dark	Semi-light	Light	Start	Dark	Semi-light	Light	Start	Dark	Semi-light	Light	Start	Semi-light	Start	Semi-Light	% CV
C14:0	0.05	0.04	0.05	0.06	0.04	0.03	0.05	0.07	0.06	0.05	0.07	0.05	0.05	0.05	0.07	0.06	5.5
C16:0	2.91	2.99	2.94	2.97	2.84	3.06	2.89	2.90	4.04	3.79	3.86	3.84	2.84	2.86	3.28	3.23	2.5
C16:1n7	0.06	0.04	0.03	0.00	0.05	0.02	0.03	0.02	0.05	0.04	0.04	0.00	0.05	0.02	0.07	0.02	5.7
C16:1n9	0.07	0.07	0.05	0.04	0.08	0.07	0.07	0.05	0.12	0.11	0.10	0.07	0.07	0.05	0.08	0.07	1.5
C16:2n6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.3
C16:4n3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.3
C18:0	0.09	0.08	0.10	0.12	0.08	0.12	0.10	0.15	0.16	0.16	0.17	0.20	0.13	0.15	0.17	0.16	3.8
C18:1n9	0.59	0.48	0.27	0.07	0.62	0.59	0.39	0.21	0.98	0.84	0.63	0.15	0.72	0.44	0.83	0.50	1.7
C18:1n7	0.04	0.03	0.02	0.00	0.04	0.04	0.03	0.02	0.06	0.06	0.04	0.01	0.05	0.03	0.06	0.03	3.9
C18:2n6	0.21	0.09	0.04	0.01	0.23	0.16	0.09	0.05	0.37	0.31	0.18	0.03	0.38	0.17	0.48	0.21	1.6
C18:3n6	0.11	0.03	0.00	0.00	0.10	0.06	0.02	0.02	0.13	0.10	0.05	0.00	0.10	0.02	0.12	0.03	1.0
C18:3n3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	1.2
C18:4n3	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.9
C20:1n9	0.46	0.40	0.25	0.06	0.46	0.45	0.31	0.20	0.70	0.58	0.46	0.11	0.44	0.31	0.55	0.36	5.7
C20:2n6	0.08	0.03	0.00	0.00	0.08	0.06	0.03	0.02	0.12	0.11	0.06	0.00	0.07	0.00	0.08	0.03	5.2
C20:3n6	1.54	0.37	0.19	0.03	1.26	0.77	0.37	0.24	1.98	1.63	0.77	0.11	1.30	0.29	1.60	0.41	1.9
C20:4n6	1.15	0.20	0.07	0.00	1.05	0.52	0.25	0.18	1.61	1.10	0.57	0.07	1.11	0.20	1.28	0.33	1.9
C20:5n3	4.20	0.73	0.21	0.06	4.20	2.20	0.97	0.79	6.17	4.43	2.11	0.33	3.80	0.70	4.14	1.00	2.5
C22:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.5
C22:1n6	0.07	0.06	0.02	0.00	0.06	0.07	0.03	0.00	0.10	0.10	0.05	0.00	0.06	0.02	0.08	0.03	24.4
C22:4n6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
C22:5n3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.2
Total quantified fatty acids (mg/g)	11.64	5.64	4.23	3.43	11.18	8.23	5.61	4.91	16.66	13.38	9.15	4.97	11.16	5.32	12.93	6.48	
Sum PUFA (mg/g)	7.31	1.45	0.50	0.10	6.91	3.77	1.72	1.30	10.39	7.67	3.74	0.55	6.75	1.38	7.75	2.01	
Sum MUFA (mg/g)	1.28	1.07	0.65	0.17	1.31	1.25	0.85	0.49	2.02	1.72	1.32	0.34	1.38	0.87	1.66	1.02	
Sum Saturated (mg/g)	3.04	3.11	3.08	3.16	2.96	3.21	3.04	3.11	4.25	3.99	4.09	4.09	3.03	3.07	3.52	3.45	
% Lost PUFA		80.1	93.2	98.6		45.4	75.1	81.2		26.2	64.0	94.7		79.5		74.1	
% Lost MUFA		16.3	49.3	86.4		4.7	34.8	62.2		14.7	34.4	83.4		36.8		38.2	
% Lost Saturated		−2.1	−1.2	−3.6		−8.6	−2.7	−5.2		6.3	3.8	3.9		−1.1		1.9	
% Total Loss		51.6	63.6	70.5		26.4	49.8	56.1		19.7	45.1	70.2		52.3		49.9	

was 40 °C.

The loss of PUFA in samples stored light and semi-light was greatest in the freeze-dried samples, which lost almost all their PUFA during storage for 370 days (96.5% and 89.9% as well as 98.6% and 93.2% for *Ulva* and *Porphyra,* respectively). This could be linked to the lower a_w and potentially higher porosity in these samples, which, as discussed in Section 3.2, could stimulate oxidation [14,20,21]. During storage in darkness, the loss of PUFA in freeze-dried *Ulva* was only 32.5% compared to 80.1% in *Porphyra*, which could be due to higher chlorophyll content in green seaweed like *Ulva* compared to red seaweed like *Porphyra* [29,30]. While chlorophyll is a strong pro-oxidant under light conditions, it has also been shown that it can work as an antioxidant in darkness [31,32]. As for the development of aldehydes, storage in light thus seemed to have larger negative impact on *Ulva* than on *Porphyra,* while in darkness, the latter species was more susceptible to lipid oxidation. Schmid et al. [33] also found a major PUFA loss in dried *Laminaria digitata* and *Palmaria palmata* stored in darkness at 20 °C for 22 months, 64% and 63%, respectively. By instead keeping the temperature at 4 °C, the loss was reduced to 0% and 33%, respectively. At −20 °C, no PUFA loss was seen [33]. A lower storage temperate should hence be further investigated also for dried *Ulva* and *Porphyra*.

In *Ulva*, the order of PUFA loss during storage in light was: freeze-dried (96.5%) > FW-soaked oven-dried (86.0%) ~ oven-dried (84.5%) > SW-coated oven-dried (75.6%) > FW-coated oven-dried (54.7%), thus, the coating slightly protected the PUFAs. The same order was seen for MUFA. For *Porphyra*, the coated samples were only stored semi-light and here no protective effect was seen from the coating. In agreement with the theory outlined above, the lower level of photosensitizing pigments in *Porphyra* compared to *Ulva* might imply a lower need for a protecting barrier against light and oxygen.

Viewed against the moderate development of MDA, HHE and HNE during storage of *Ulva* and *Porphyra* (Figs. 1–2 and supporting information), there was a surprisingly large loss of PUFA, especially in samples stored light. It is therefore likely that the oxidation reaction in *Porphyra* and *Ulva* has led to the accumulation of other lipid oxidation products than those monitored here. MDA, HHE and HNE are all very reactive in free form, and the seaweed matrix could offer many possibilities for cross-linking of these aldehydes with e.g. proteins [23] and carbohydrates [34], resulting in an underestimated degree of oxidation. For future studies, saturated aldehydes like propanal and hexanal originating from n-3 and n-6 fatty acids, respectively [35], might therefore be more suitable. These aldehydes have been found for example in oxidized soybean and linseed oil [36,37].

3.4. Ascorbic acid

Samples from both species soaked in FW had higher initial ascorbic acid content (1098 and 559 μg/g for *Porphyra* and *Ulva,* respectively, Fig. 3C and H) compared to the non-soaked samples (799 and 368 μg/g, respectively). As for the fatty acids, this could be explained by the washout of minerals during soaking in FW, and thus, a changed mass balance due to the reduced ash. For *Ulva*, the freeze-dried sample had the highest initial ascorbic acid concentration (602 μg/g), Fig. 3 A, which is in accordance to what was previously seen for *Sargassum hemiphyllum*, where freeze-drying compared to oven-drying retained significantly more ascorbic acid [7]. For *Porphyra,* the drying method had no effect on the initial ascorbic acid level and oven-dried vs. freeze-dried samples had the same initial value (799 μg/g, Fig. 3 F and G). Prior to storage, FW-coated *Ulva* had significantly less ascorbic acid than the corresponding non-coated sample. This was not seen for the SW-coated sample, however, for *Porphyra* both coated samples had significantly lower initial ascorbic acid content than the corresponding non-coated samples (Fig. 3 G-J). This could partly be explained by the longer drying times required for the coated samples. Also, the pH of the coating solution when made in FW was lower (~4.55) than the pH-values previously reported for *Porphyra* and *Ulva* (6.1 and 4.8, respectively) [38]. Metal ions can accelerate ascorbic acid oxidation [39], but this reaction is inhibited by NaCl, especially at lower pH-values [40], which could explain why SW-coated *Ulva* had higher initial value than FW-coated *Ulva*.

For *Ulva*, no decrease in ascorbic acid was seen during the first 90 days of storage in darkness, instead an increase was documented (Fig. 3

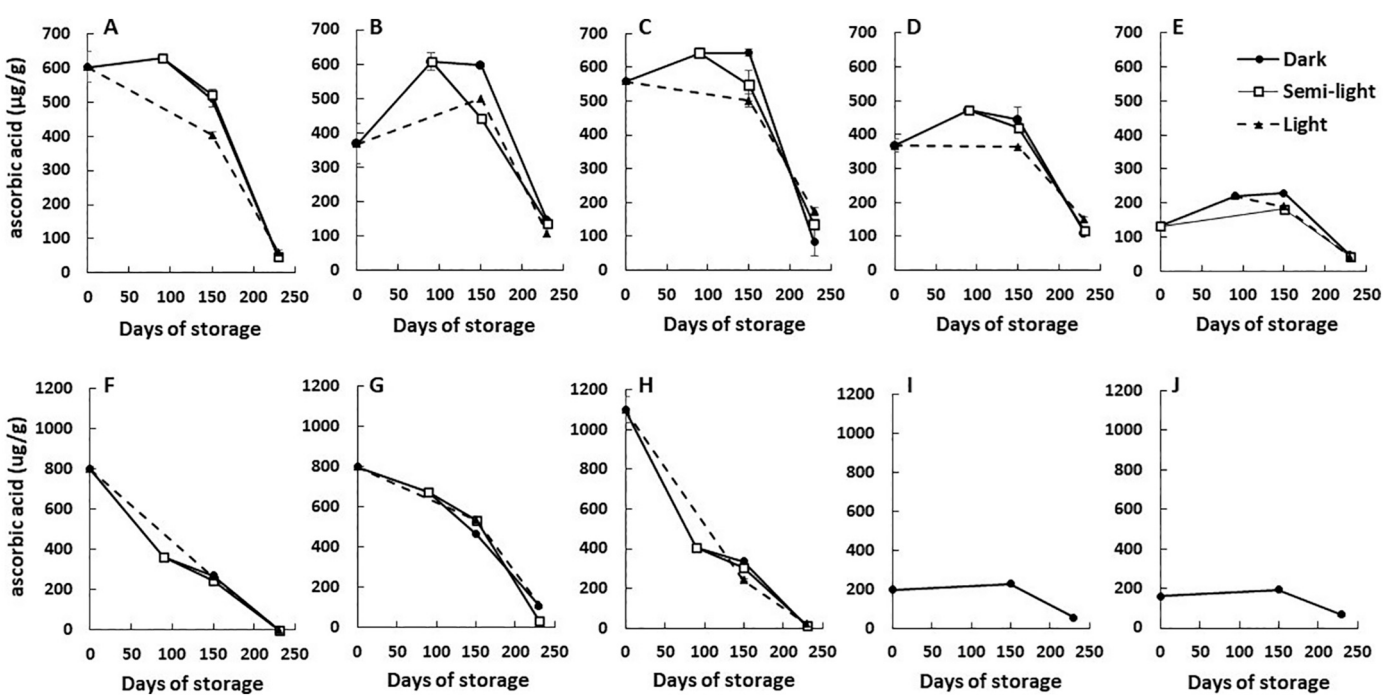

Fig. 3. Ascorbic acid in A) freeze-dried, B) oven-dried, C) oven-dried-FW soaked, D) oven-dried SW-coated and E) oven-dried FW-coated Ulva and in F) freeze-dried, G) oven-dried, H) oven-dried-FW soaked, I) oven-dried SW-coated and J) oven-dried FW-coated Porphyra. Error bars show standard deviations (n = 2). Note that these were very small for some samples. FW = freshwater, SW = saltwater. Circles, triangles and squares show dark, semi-light and light conditions respectively.

A-E). As it is unlikely that ascorbic acid re-form, we believe it is a result of differences in ascorbic acid extraction yield during the sample pre-treatment. It was notified that there were some differences in the milling efficiency of the dried seaweed at different time points. Between day 90 and 150, there was a slight, but not significant, decrease in ascorbic acid in the oven-dried Ulva samples with/without FW-soaking which were moved to light storage conditions (Fig. 3 B–C), something which was not seen in the samples kept in darkness. For *Porphyra*, there was a significant decrease in ascorbic acid already after the first 90 days in darkness. At day 150 the levels were the same regardless of storage conditions. This agreed with the fatty acid and aldehyde results; i.e. light had less effect on *Porphyra* than *Ulva*. Between day 150 and 230 there was a significant ascorbic acid decrease in all samples, regardless of species or light condition. This coincided with the significant increase in aldehydes in the same time span for many of the samples; indicating co-oxidation of ascorbic acid and fatty acids when the former act as a radical scavenger [41]. At day 210, the ascorbic acid levels were so low in all samples that now further analyses were done during the storage.

3.5. Colour

As expected, there was a great difference in colour between *Porphyra* and *Ulva* (Figs. 4–5 and supporting information). Despite this, the colour changes during drying and storage were similar among these species. Freeze-dried samples from both species had significantly higher initial L-values than the oven-dried samples, which could originate in the larger oxygen access and lower a_w discussed earlier. During the storage, there was an increase in a-value, hence, a loss of green colour, in all samples, particularly for the samples stored in light conditions. There was also a storage-induced decrease in b-value (supplementary information Figure 2), i.e. loss of yellow colour, in all samples and together these two changes indicate losses of chlorophyll and carotenoids, respectively. In dried *Porphyra* sp., Oh et al. [2] found a significant decrease in both chlorophyll and carotenoids after 15 days of storage in both darkness and light, however the latter caused largest losses. For freeze-dried *Ulva* there was a significant bleaching, i.e. an increase in L-value, between day 230 and 310 for all storage conditions, but particularly in samples stored light. The same trend was seen for all oven-dried *Ulva* samples, and at day 520, the samples stored light had reached significantly higher L-values compared to the ones stored semi-light and dark. For all *Ulva* samples, except for the FW-coated ones, there was a significant increase in a-value between day 230 and 310, and the light-stored samples reached significantly higher a-values than the other samples. It is likely that there was a co-oxidation of lipids and pigments; a phenomenon well known e.g. during lipoxygenase-mediated oxidation [22,42]. For *Porphyra* there was a tendency towards a larger increase in L- and a-value for samples stored light, however, in accordance with the other parameters analysed, it was not as clear as for *Ulva*.

4. Conclusions

This study illustrated the formation of lipid oxidation products as well as losses of PUFA, ascorbic acid and colour during the storage of dried *Porphyra* and *Ulva*. Soaking the seaweed in FW prior to drying reduced the content of ash, but did not affect any of the measured quality parameters. Applying whey coating induced a loss of ascorbic acid during the actual drying operation, but for *Ulva,* it prevented lipid oxidation during the drying and, to a small extent, PUFA loss during subsequent storage in light. Surprisingly, oven-drying was superior to the more expensive freeze-drying for maintaining the quality of lipids and colour in *Porphyra* and *Ulva*. For both species, the measured chemical and colour changes were accelerated between day 150 and 310 and, especially for *Ulva,* light had a stimulating effect. It is concluded that quality and quantity of lipids, ascorbic acid content as well as colour of dried seaweed is best maintained if storing it for utmost 150 days after drying, and preferable in darkness.

Abbreviations

dw	dry weight
EPA	eicosapentaenoic acid
FAME	fatty acid methyl esters
HHE	4-hydroxy-trans-2-hexenal
HNE	4-hydroxy-trans-2-nonenal
MDA	malondialdehyde
MUFA	monounsaturated fatty acids

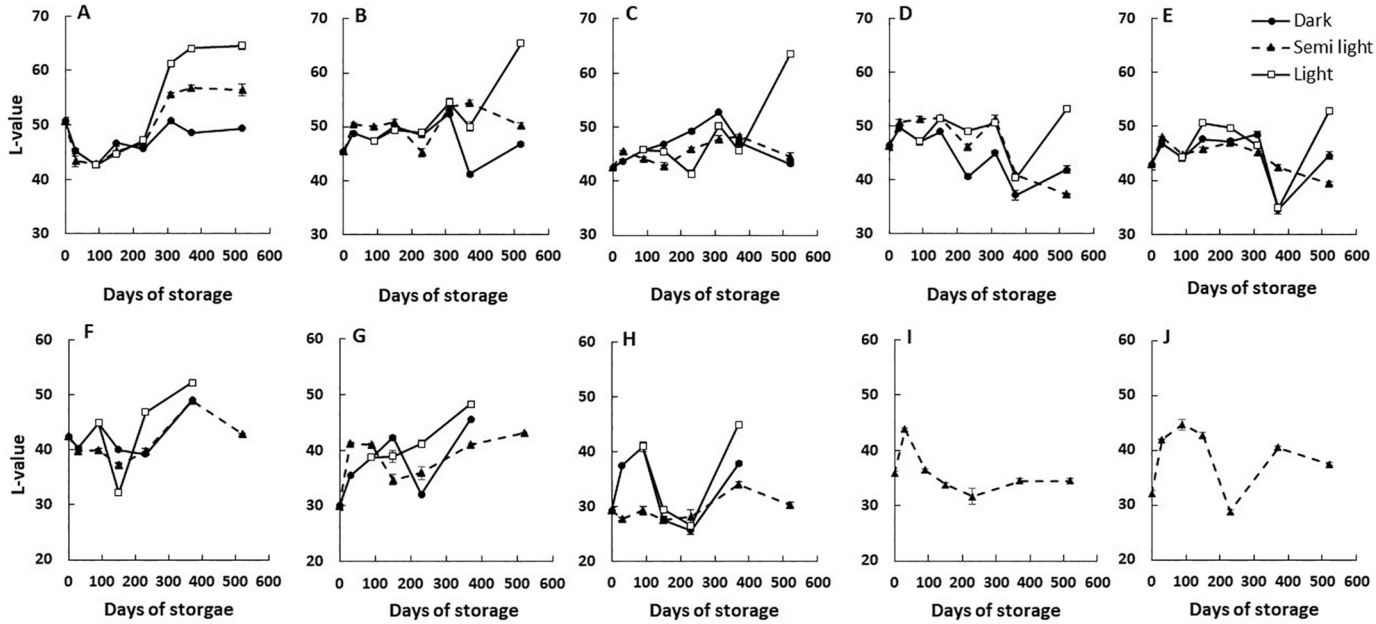

Fig. 4. L*-value A) freeze-dried, B) oven-dried, C) oven-dried-FW soaked, D) oven-dried SW-coated and E) oven-dried FW-coated *Ulva* and in F) freeze-dried, G) oven-dried, H) oven-dried-FW soaked, I) oven-dried SW-coated and J) oven-dried FW-coated *Porphyra*. Error bars show standard deviation ($n = 5$). Note that these were very small for some samples. FW = freshwater, SW = saltwater. Circles, triangles and squares show dark, semi-light and light conditions respectively.

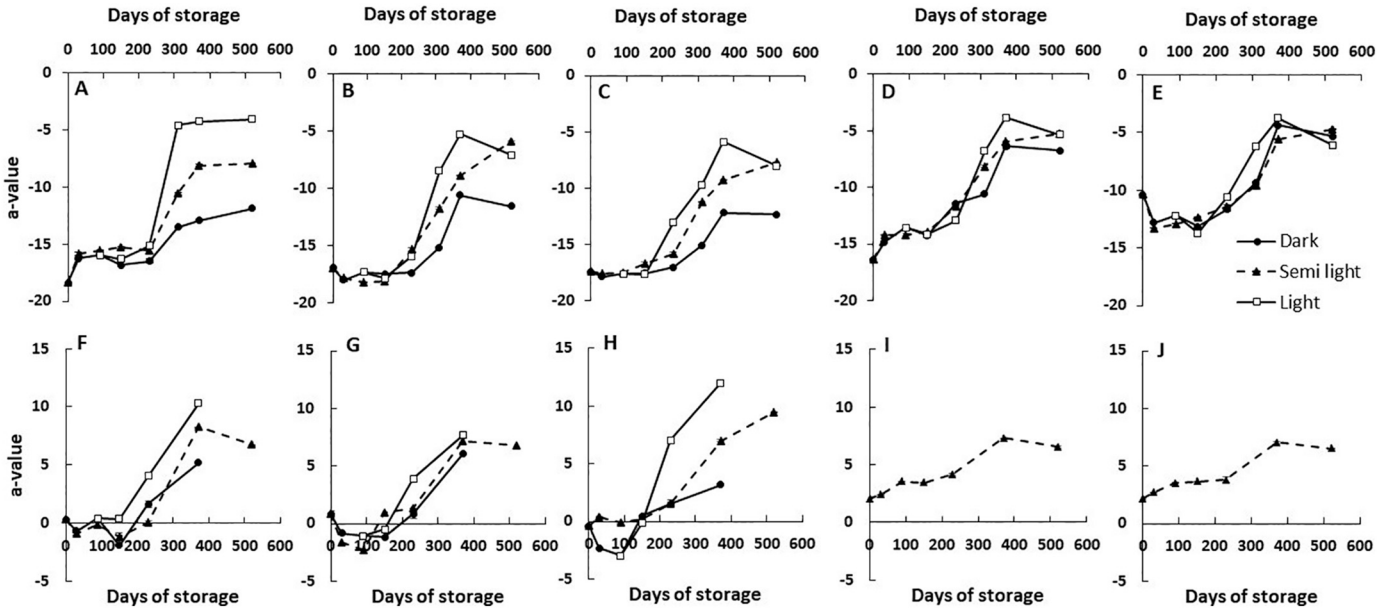

Fig. 5. a*-value of A) freeze-dried, B) oven-dried, C) oven-dried-FW soaked, D) oven-dried SW-coated and E) oven-dried FW-coated *Ulva* and in F) freeze-dried, G) oven-dried, H) oven-dried-FW soaked, I) oven-dried SW-coated and J) oven-dried FW-coated *Porphyra*. Error bars show standard deviation (n = 5). Note that these were very small for some samples. FW = freshwater, SW = saltwater. Circles, triangles and squares show dark, semi-light and light conditions respectively.

n-3	Omega 3
n-6	Omega 6
PUFA	polyunsaturated fatty acids
PV	peroxide value
TCEP	Tris[2-carboxyethyl]phosphine hydrochloride

CRediT authorship contribution statement

Hanna Harrysson: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Visualization. **Johanna Liberg Krook:** Conceptualization, Methodology, Validation, Investigation, Writing – original draft. **Karin Larsson:** Validation, Formal analysis, Investigation, Writing – review & editing. **Cecilia Tullberg:** Writing – review & editing. **Annelous Oerbekke:** Resources. **Gunilla Toth:** Resources, Writing – review & editing. **Henrik Pavia:** Resources, Funding acquisition. **Ingrid Undeland:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2021.102295.

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