Journal of Applied Phycology

Nutritional value of the kelps Alaria esculenta and Saccharina latissima and effects of short-term storage on biomass quality

--Manuscript Draft--

Manuscript Number:	JAPH-D-16-00632R1
Full Title:	Nutritional value of the kelps Alaria esculenta and Saccharina latissima and effects of short-term storage on biomass quality
Article Type:	SI: ISS-2016 Copenhagen
Section/Category:	Original Research
Keywords:	alginate, bioactive compounds, carbohydrates, chemical composition analysis, edible seaweeds, fucose, fucoxanthin, laminaran, macroalgae, mannitol, minerals, polyphenols, potassium, preservation, processing, protein, sodium
Corresponding Author:	Pierrick Stévant, MSc Møreforsking Ålesund Ålesund, NORWAY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Møreforsking Ålesund
Corresponding Author's Secondary Institution:	
First Author:	Pierrick Stévant, MSc
First Author Secondary Information:	
Order of Authors:	Pierrick Stévant, MSc
	Hélène Marfaing, MSc
	Turid Rustad, Ph.D
	Ingrid Sandbakken, MSc
	Joël Fleurence, Ph.D
	Annelise Chapman, Ph.D
Order of Authors Secondary Information:	
Funding Information:	Norges Forskningsråd (244244) — Dr Annelise Chapman
Abstract:	Storage of macroalgae in seawater, prior to further processing, is a standard initial pre-treatment step after harvest to avoid rapid degradation of the biomass. In the context of using seaweeds in human food and animal feed products, such practice may affect the nutritional value and the overall quality of the biomass. The effects of seawater storage on the chemical composition (i.e. mineral fraction, carbohydrates, proteins, polyphenols and fucoxanthin) and surface color of two cultivated kelps, Alaria esculenta and Saccharina latissima, was investigated over a 22-hour period. Storage treatments resulted in a rapid decrease in dry weight during the first two hours (-21.4% and -20.4% in A. esculenta and S. latissima, respectively) with subsequent stabilization. Although it is not clear whether the reduction of dry weight was caused by the release of nutritional compounds from seaweed biomass or water uptake during storage treatment, the results from chemical analyses suggest the combined effect of both mechanisms. Seawater storage increased the ash and sodium contents and reduced carbohydrates and polyphenol levels in both species. Among carbohydrates, the levels of mannitol and glucose (laminaran) were particularly reduced in S. latissima samples while the fucose level, reflecting fucoidans, was reduced in A. esculenta. The protein content remained relatively stable in both species. These results provide evidence of the effect of seawater storage on the quality of the edible kelps A. esculenta and S. latissima.

	The results will contribute to selecting post-harvest strategies adequate for maintaining biomass quality, minimizing losses of valuable compounds and increasing profitability for industrial stakeholders.
Response to Reviewers:	First the authors would like to thank the reviewers for their valuable comments which lead to a more nuanced discussion regarding the effects of seawater storage on the quality parameters of Alaria esculenta and Saccharina latissima. Overall we think it improved the quality of the article. Please see the attachment file for the answers to specific comments to the reviewers.

Nutritional value of the kelps *Alaria esculenta* and *Saccharina latissima* and effects of short-term storage on biomass quality

Pierrick Stévant [a,c1], Hélène Marfaing [b], Turid Rustad [c], Ingrid Sandbakken [d], Joël Fleurence [e] and Annelise Chapman [a]

[a] Møreforsking Ålesund AS, PO Box 5075, N-6021 Ålesund, Norway

[b] CEVA (Centre d'Etude et de Valorisation des Algues), B.P. 3, F-22610 Pleubian, France

[c] The Norwegian University of Science and Technology NTNU, N-7491 Trondheim, Norway

[d] SINTEF Materials and Chemistry, N-7465 Trondheim, Norway

[e] MMS (Mer Molécule Santé), Université de Nantes, B.P. 92208, F-44322 Nantes cedex 3, France

[1] Corresponding author: email: pierrick.stevant@moreforsk.no

Abstract

Storage of macroalgae in seawater, prior to further processing, is a standard initial pre-treatment step after harvest to avoid rapid degradation of the biomass. In the context of using seaweeds in human food and animal feed products, such practice may affect the nutritional value and the overall quality of the biomass. The effects of seawater storage on the chemical composition (i.e. mineral fraction, carbohydrates, proteins, polyphenols and fucoxanthin) and surface color of two cultivated kelps, *Alaria esculenta* and *Saccharina latissima,* was investigated over a 22-hour period. Storage treatments resulted in a rapid decrease in dry weight during the first two hours (-21.4% and -20.4% in *A. esculenta* and *S. latissima,* respectively) with subsequent stabilization. Although it is not clear whether the reduction of dry weight was caused by the release of nutritional compounds from seaweed biomass or water uptake during storage treatment, the results from chemical analyses suggest the combined effect of both mechanisms. Seawater storage increased the ash and sodium contents and reduced carbohydrates and polyphenol levels in both species. Among carbohydrates, the levels of mannitol and glucose (laminaran) were particularly reduced in *S. latissima* samples while the fucose level, reflecting fucoidans, was reduced in *A. esculenta*. The protein content remained relatively stable in both species. These results provide evidence of the effect of seawater storage on the quality of the edible kelps *A. esculenta* and *S. latissima*.

The results will contribute to selecting post-harvest strategies adequate for maintaining biomass quality, minimizing losses of valuable compounds and increasing profitability for industrial stakeholders.

Key words: alginate, bioactive compounds, carbohydrates, chemical composition analysis, edible seaweeds, fucose, fucoxanthin, laminaran, macroalgae, mannitol, minerals, polyphenols, potassium, preservation, processing, protein, sodium

Introduction

The use of marine macroalgae as a food item and a functional ingredient has gained increasing interest in the Western world over the past decades. Seaweeds can be cultivated on a large-scale in coastal areas, and may therefore offer superior alternatives to production of terrestrial biomass and related challenges, such as high demand on fresh water and area conflicts. Moreover, macroalgal production rates exceed those of agricultural production (Brinkhuis et al., 1987) hence the interest for sustainable cultivation of seaweed biomass for food and feed applications. In Europe, efforts for large-scale cultivation of seaweeds have largely focused on the kelp species *Saccharina latissima* and *Alaria esculenta* because of their ability to reach high biomass yields (Kraan et al., 2000; Handå et al., 2013), and for their valuable nutritional content.

The nutritional value and health benefits of including seaweeds in the diet are well documented (Déléris et al., 2016). High content of minerals, vitamins and trace elements (Mabeau and Fleurence, 1993; Rupérez, 2002; MacArtain et al., 2007; Holdt and Kraan, 2011) has been an argument for developing food supplements based on macroalgae. Previous studies highlighted the potential for using seaweeds as a functional ingredient in manufactured food products e.g. as a salt-replacing ingredient, by lowering the sodium to potassium (Na/K) ratio, hence resulting in a healthier mineral profile (López-López et al., 2009a; López-López et al., 2009b), in contrast to traditional products with high Na/K ratios. Besides, seaweeds are a rich source of various natural antioxidants such as polyphenols which reach particularly high levels in brown algae (up to 25 % dry weight, Magnusson et al., 2017) and play a role in preventing lipid oxidation (Wang et al., 2010). In addition, seaweed polyphenols are also described in the literature for their anti-allergic properties (Fleurence and Ar Gall, 2016) which leads to the multiple applications of seaweed phenolic compounds e.g. in pharmaceutical, food and cosmetic industries. Likewise, fucoxanthin, a xanthophyll pigment abundant in kelp species, is a potent antioxidant (Fung et al., 2013) with anti-obesity and anti-diabetic effects (Maeda et al., 2005; Maeda et al., 2008). Other compounds such as polysaccharides (dietary fibers, Dawczynski et al., 2007), proteins, amino-acids (Fleurence, 2004; Mæhre et al., 2014) and lipids (Sánchez-Machado et al., 2004) are highly relevant towards food and feed applications for both terrestrial and marine organisms (Soler-Vila et al., 2009; Evans and Critchley, 2014). Although seaweeds have been underutilized as food in Western countries, they have enjoyed increasing popularity over recent decades mainly introduced by Asian dishes. Previous studies have revealed the potential of native seaweed species along the Atlantic coast of Europe, e.g. the red algae *Palmaria palmata* and the

kelps *Saccharina latissima* and *Alaria esculenta*, to be used as food ingredients in a wide range of foodstuffs both as vegetables and flavor enhancers (Mouritsen, 2012; Chapman et al., 2015).

Industrial applications of seaweeds are multiple, and the use of macroalgal biomass as raw material for the provision of valuable compounds is growing rapidly (Kumar et al., 2008). Integrated processes maximizing biomass utilization by recovering a stream of products from seaweed biomass have been studied with promising results (Hou et al., 2015; Baghel et al., 2016). However, the high water content of seaweeds (70 to 90 %, Jensen, 1993) represents a challenge for conserving and transporting large amounts of biomass (as in industrial production) from harvesting to processing sites. Seaweeds are characterized by a rapid microbial decomposition once harvested (Enríquez et al., 1993), and thus require appropriate preservation methods to maintain biomass quality and ensure product safety. Moreover, the year-round cultivation of kelps is often hindered by the onset of biofouling during the summer, causing extensive destruction of the crop (Forbord et al., 2012; Bruhn et al., 2016) and forcing producers to harvest in May-June. Primary processes of harvested seaweeds such as drying, can effectively stabilize the biomass but require technology and may be difficult to implement close to harvesting sites. Moreover, drying large biomass volumes is extremely energy demanding, lowering the environmental and economical sustainability of the process chain. Drying also affects the chemical content (Chan et al., 1997; Gupta et al., 2011) with consequences for the product's nutritional value as well as extraction yields of bioactive compounds during further processing. Alternative postharvest treatments with the purpose of maintaining biomass quality include seawater storage (Paull and Chen, 2008), cold storage (Liot et al., 1993) and silage (Herrmann et al., 2015), but generally the effects of early treatments of macroalgal biomass is highly understudied. Finding sustainable processes that can stabilize rapidly large quantities of seaweeds is therefore a crucial step for the development of a new bio-economy based on cultivated macroalgal biomass.

The present work reports on the impact of short-term seawater storage on the biomass quality of two cultivated kelps, namely *A. esculenta* and *S. latissima*. Quality was defined as the seaweeds' content of bioactive compounds including mineral fraction, carbohydrates, proteins, polyphenols, fucoxanthin, as well as surface color. The aim of this study was to assess whether seawater storage can assist in temporarily maintaining the quality of seaweed biomass to be used as food ingredient, and in other industrial applications.

Materials and methods

Biomass harvesting Samples of *A. esculenta* and *S. latissima* were harvested from CEVA's cultivation site (latitude: 48.836362 N, longitude: -3.044157 W) at Pleubian, off the Northern coast of Brittany in France on May 27th and 28th 2016, respectively. If epiphytic brown seaweeds (Ectocarpaceae) were observed on the distal part of some blades, these sections were cut off and discarded. The biomass was stored in mesh bags during boat transport to the laboratory. Care was taken not to overload the bags with seaweeds to avoid spontaneous fermentation processes. All biomass samples were received and treated within 2 h post-harvest.

Storage and sample treatment Batches of 5 kg of harvested seaweeds were transferred to tanks supplied with air bubbling to ensure water mixing, and filled with 100 L seawater, as this stocking density previously had shown good results in maintaining the organoleptic qualities and microbiological characteristics of *Ulva* sp. and *P. palmata* (CEVA, unpublished results). The seawater was pumped from the same bay as the cultivation site, filtrated (10 µm) and maintained at a temperature of 18 ˚C ± 1 ˚C throughout the experiment. Samples of 500 g of seaweed biomass were analyzed for their chemical content both, prior to (t_0) and after 22 h storage. The initial sampling at t_0 corresponds to biomass arrival at the laboratory after harvest and transport. In addition, samples were taken after 1, 2 and 6 h treatment for determination of the dry weight (DW), ash content as well as color analysis. All treatments were performed in triplicate. Sampled blades were gently blotted to remove excess water, vacuum-packed and frozen until freeze-drying (C38L Cryorivoire), then ground to 250 µm (using a knife-mill) prior to chemical analyses. The DW was determined gravimetrically as the residue remaining after freeze-drying and subsequent oven-drying to remove residual moisture.

Ash content The samples' ash content was determined using a standard procedure (AFNOR, 1977) in which samples were combusted at 550 °C for 12 h in a laboratory muffle furnace. Ashes were quantified gravimetrically after combustion.

Sodium (Na), Potassium (K) analysis Na and K contents were analyzed following an official reference method (AOAC 984.27, AOAC, 2000) in which samples were combusted overnight in a muffle furnace. The ashes were solubilized in nitric acid (HNO_3, 65 %) under high heat and pressure using a laboratory microwave oven. The Na and K of the solutions were quantified by inductively coupled plasma atomic emission spectroscopy (ICP-OES, Perkin Elmer Optima 7300DV).

Carbohydrate analysis Neutral sugars (D-glucose, D-galactose, D-mannose, D-xylose, L-fucose, L-rhamnose), D-mannitol and uronic acid (D-glucuronique, D-mannuronic, poly-D-guluronic and poly-D-mannuronic) composition were determined by high-performance liquid chromatography (HPLC) analysis after depolymerization under

methanol- acid hydrolysis reaction (methanolysis) as described by Quemener et al. (2000). Ground freeze-dried seaweed samples of 15 mg were transferred into 2 mL MeOH–HCl solution (prepared by adding acetyl chloride in methanol, 17/3 v/v). Methanolysis was conducted at 100 °C for 4 h, after which neutralization was achieved by adding silver carbonate (successively 100 mg then 50 mg) until pH reached 4-5. The solutions were evaporated at 47 °C for 16 h, then dissolved in distilled water and filtered prior to HPLC analysis (Grace smart RP18, 5 µm, 4.6×250 mm). Chromatographic peaks were identified by comparison with high purity reference sugars purchased from Sigma-Aldrich (Steinheim, Germany) except for the poly-D-guluronic and poly-D-mannuronic standards prepared at CEVA's laboratory.

Protein content Total nitrogen (N) was determined in ground freeze-dried samples using a CHNS-O elemental combustion system (Costech Instruments ECS 4010) at a temperature of approximately 1000 °C, where the samples' N is converted to N gas/oxides. Results were expressed in gram N per 100 g of dried sample. A N-to-protein conversion factor of 5, recommended as suitable to predict the protein content of brown seaweeds (Angell et al., 2016), was used.

Polyphenolic content The polyphenolic content of algal extracts was determined colorimetrically using the Folin–Ciocalteu reagent according to the method of Ragan and Glombitza (1986). The extraction was performed using 250 mg of ground freeze-dried seaweed samples in 10 mL solvent (acetone/water, 80/20 v/v). The mixture was incubated for 1 h in the dark at room temperature. After decantation, the supernatant was recovered and re-extracted under the same conditions. Both supernatants were pooled prior to filtration (0.45 µm). The filtrate represented the seaweed sample extract. Then, 200 µL of seaweed extract was mixed with 1300 µL distilled water and 100 µL Folin-Ciocalteu reagent followed by the addition of Na_2CO_3 (29%). After incubation at 45 °C for 30 min in the dark, the absorbance was recorded at 760 nm using a UVIKON-XL spectrophotometer (Bio-Tek Instruments, USA), with phloroglucinol used as the standard reference (Sigma-Aldrich, Steinheim, Germany). A standard curve with serial phloroglucinol solutions (ranging from 0 to 100 µg ml^{-1}) was used for calibration. The polyphenol contents were expressed as gram phloroglucinol equivalent per 100 g of dried sample. Analyses were performed in duplicate with 10 % relative uncertainty of measure.

Fucoxanthin content The extraction of fucoxanthin from seaweed samples was carried out in ethanol/water solvent 60/40 for 2 h in ice bath protected from light (1 % seaweed powder in solvent). After decantation, the seaweed sample residue was subjected to a second extraction following the same conditions. The supernatants were pooled prior to analysis. The fucoxanthin content in the extracts was determined by reversed phase HPLC in a YMC

Carotenoid column (250x4.6 mm i.d. 5.5 µm particle size, INTERCHIM, France) with UV detection at 448 nm. Acetonitrile, methanol and water was used as mobile phase. A commercial fucoxanthin standard (C5753, Caroténature,) was used for quantification.

Surface color The surface color of seaweed samples was analyzed by a computerized image technique known as computer vision system (CVS) as described by Girolami et al. (2013), using a digital camera (Canon EOS 60D) and a 35 mm lens (Canon EF 35mm f/2) mounted in a black box isolated from any external light. Lighting was achieved with two fluorescent light bulbs with a color temperature of 6500K (D_{65}, standard light source commonly used in food research) positioned at an angle of 45° from the sample to obtain uniform lighting. The color was analyzed quantitatively using Photoshop (Photoshop CC 2015, Adobe Systems Inc.) and expressed in CIE L^* (whiteness or brightness), a^* (redness/greenness) and b^* (yellowness/blueness) coordinates, as described by Yam and Papadakis (2004). A minimum of three blades from each sample were photographed and the results averaged prior to calculating the total color difference (ΔE) using Eq. (1), where L^*_0, a^*_0 and b^*_0 are color coordinates of the samples before treatment.

$$\Delta E = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2} \quad (1)$$

Statistical analysis Raw data were pre-processed for descriptive statistics and results expressed as mean ± standard error (n = 3). All data sets from chemical and color analysis were tested for normality using the Shapiro-Wilk's test and homogeneity of variances using the Levene's test. A repeated measures analysis of variance (RM ANOVA) at $p < 0.05$ was used to detect significant differences among storage duration treatments on the quality parameters analyzed at all sampling times. The paired sample *t*-test was used to detect significant differences on quality parameters analyzed only at t_0 and at the end of the storage treatment. All statistical analyses were performed on R (R Development Core Team, 2008).

Results

The effects of short-term storage in seawater on the kelp species *A. esculenta* and *S. latissima* were assessed by compositional analysis. The chemical composition of the harvested biomass prior to and after 22 h storage treatments is shown in table 1.

Both species were characterized by a high ash content, reflecting macro-minerals and trace elements, reaching approximately 25 % of the DW of the biomass. Analysis of individual minerals revealed high levels of Na and K in both species, with low Na/K ratios, especially in *S. latissima*, which contained nearly twice as much K as Na. The total carbohydrate content, which was quantified as the sum of each individual sugar, was slightly higher in *S. latissima* than in *A. esculenta*. The sum of guluronic and mannuronic acids (known as G- and M-units) measured in the samples, which are the monomeric units composing alginate, was used to quantify the alginate content. It represented close to 50 % of the total carbohydrates in both species. Mannitol was abundant in *S. latissima*, reaching 17 % DW, while *A. esculenta* contained more glucose and fucose than *S. latissima*. Galactose, mannose and glucuronic acid were also found in small amounts, close to or under the detection limit of 0.5 mg $100g^{-1}$ DW. Similar protein levels were estimated for both kelp species, reaching over 10 % DW, using 5 as N-to-protein conversion factor. The samples of *A. esculenta* contained almost 5 times more polyphenols than samples of *S. latissima* and twice as much fucoxanthin. A decrease in DW was observed in both *A. esculenta* and *S. latissima* throughout seawater storage although this trend was only significant in *A. esculenta* (RM ANOVA, $p < 0.05$) probably due to the relatively high variation in the results of DW analysis in *S. latissima* at t_0. The decrease occurred mainly during the first two hours of the treatment resulting in 21.4 % and 20.4 % DW losses for both species respectively after 2 h (fig. 1). A marginal increase in DW was observed in both kelps after 6 h storage. Expressing the results from chemical analyses as part of the DW of the biomass reflects on the relative proportions of each compound analyzed and does not highlight their absolute variation throughout the storage period when significant losses of dry matter occur. A decrease in DW can be the result of a release of compounds and/or seawater uptake from the biomass.

The samples of *A. esculenta* and *S. latissima* taken after 22 h storage in seawater were both characterized by higher relative ash contents (expressed in g 100 g^{-1} DW, 12 % ± 7 % and 14 % ± 10 % respectively, table 1) and significantly higher Na levels (33 % ± 5 % and 19 % ± 5 % respectively) compared to initial levels prior to treatment, while the K levels remained relatively stable. The Na/K ratio was unaffected in *S. latissima* and slightly increased in *A. esculenta*. The relative content in total carbohydrates was reduced by 7 % ± 4 % and 13 % ± 2 % in *A. esculenta* and *S. latissima* respectively. An important reduction of mannitol (-29 % ± 12 %) and glucose levels (-44 % ± 13 %) was observed in *S. latissima* although these trends were not validated by the paired sample *t*-test due to high variations within triplicates. The levels of fucose were significantly reduced in *A. esculenta* (-22 % ± 3 %) as well as polyphenol levels

(-26 % ± 3 %) while the protein and fucoxanthin contents were stable throughout storage of this species. A reduction in polyphenol (-29 % ± 6 %) and fucoxanthin levels (-17% ± 6%) was registered in *S. latissima* samples after 22 h storage in seawater while the relative proportion of protein as part of the DW slightly increased (9 % ± 2 %).

Variations in the biomass' surface color, defined by the color coordinates *L**, *a** and *b**, were recorded during storage using a CVS. The RM ANOVA detected significant variations in *L** and *b** in both species during seawater storage ($p < 0.05$). Both *L** and *b** followed an irregular pattern throughout treatment of *A. esculenta*, while values for the same color coordinates increased (i.e. gained lightness and yellowness) in *S. latissima* blades until 6 h storage, then decreased towards initial values (fig. 2). Variations in the *a** parameter did not significantly contribute to the overall color variation in both species. Despite different color profiles prior to storage, i.e. lighter and more yellow blades of *A. esculenta* compared to *S. latissima*, both kelps displayed relatively similar values among the three coordinates after 22 h of storage. The total color variation (ΔE) reflected the variation in each of the three chromatic coordinates (*L**, *a** and *b**) during storage as compared to initial values measured at t_0. Despite a relatively high variation among the blades photographed at each sampling time, the observed differences in color were greater after 6 h storage in both species (fig. 3). In the case of *A. esculenta*, ΔE did not reflect the absolute variations of both *L** and *b** coordinates, which oscillated around their initial values during the first 6h of storage. The comparatively smaller ΔE value of *S. latissima* at 22 h compared to 6 h treatment was explained by a decrease in *L** and *b** at 22 h to values closer to the initial color profile of the biomass at t_0.

Discussion

The results from chemical analyses of *Alaria esculenta* and *Saccharina latissima* revealed concentrations of a range of nutritional compounds, including carbohydrates, minerals, proteins and fucoxanthin, as previously reported in the literature (Holdt and Kraan, 2011; Schiener et al., 2015).

Carbohydrates amounted for over 40 % of the DW in both species and were mainly composed of alginate. Alginate, which is the main skeletal component of the intercellular matrix in brown algae, represented 49 % and 47 % of the total carbohydrates in *A. esculenta* and *S. latissima* respectively, although the alginate levels reported in this study were lower than those found in other kelps species, e.g. *Laminaria hyperborea* (Draget et al., 2002). Being

indigestible, alginates are regarded as a source of dietary fiber with beneficial health effects both on colonic and cardiovascular health (Brownlee et al., 2005).

Fucose is mainly found in sulphated form, constituting the backbone of a group of water-soluble polysaccharides originally called fucoidans or fucans found in the cell wall and intercellular space in brown seaweeds (Skriptsova, 2015). Fucoidans represents a diverse group of sugars with large structure variations among sources and within seaweed species, and with a wide range of documented biological activity, including anti-tumoral and immune-stimulating effects (Hayashi et al., 2008; Pádua et al., 2015). Although the fucoidan content of the two kelp species was not investigated in this study, the level of fucose can be indicative of the fucoidan content and compared to values reported in the literature. Ale and al. (2011) measured higher levels of fucose in *Fucus vesiculosus* (13.9 g $100g^{-1}$ DW), suggesting that *A. esculenta* and *S. latissima* may not be a major source of fucoidans.

Glucose in Laminariales species is mainly found within cell walls in form of cellulose (insoluble dietary fibers, Rupérez and Saura-Calixto, 2001) and laminaran, a soluble storage glucan found to accumulate in kelps during summer and autumn (Black, 1950; Adams et al., 2011; Schiener et al., 2015). The glucose content obtained from the methanol- acid hydrolysis reaction (methanolysis) directly reflects the laminaran content of the samples. The levels measured in this study in *A. esculenta* (8.5 g $100g^{-1}$ DW) and *S. latissima* (5.0 g $100g^{-1}$ DW) were respectively lower and comparable to the laminaran levels measured from the same species harvested in Scotland at this time of the year (Schiener et al., 2015). Mannitol, another major storage carbohydrate, was also found at high levels, particularly in *S. latissima*, reaching 17.6 % of the DW. This water-soluble monosaccharide which is abundant within the intracellular matrix of kelp species, follows the same seasonal pattern as laminaran and can reach up to ca. 25 % DW in *L. hyperborea* and *S. latissima* (Black, 1950; Schiener et al., 2015). Mannitol is widely used as a sweetener in the food industry and is likely contributing to the flavor profile of edible kelps, although this has not yet been thouroughly investigated. The main interest for seaweed mannitol and laminaran is related to the production of bio-energy since both compounds can be hydrolyzed and converted to fructose and glucose respectively, which can be further used in a fermentation process to produce ethanol (Hou et al., 2015).

The ash content was high in both kelps (approximately 25 % DW) and close to levels reported in previous studies for the same species (Mæhre et al., 2014; Schiener et al., 2015). Therefore, these kelps may be a good source of minerals, as generally described in the literature (Rupérez, 2002). Both were rich in Na and K with Na/K ratios below 1.0, which is interesting in a nutritional perspective given that diets with high Na/K ratios (i.e. rich in Na salts) are associated

with health risks, such as high blood pressure and cardiovascular diseases (Perez and Chang, 2014). This highlights the potential of using edible seaweeds as a functional ingredient for salt replacement in the food industry, resulting in healthier mineral profiles in food products (López-López et al., 2009b).

In order to estimate the total protein content from the analysis of total N, a conversion factor of 6.25 has been widely used for seaweed food items. However, Lourenço et al. (2002) highlighted the inaccuracy of this factor in seaweeds due to relatively high amounts of non-protein N. In a recent study, Angell et al. (2016) established a universal N-to-protein ratio of 5 for seaweeds, based on the results of a meta-analysis of a large body of literature reporting the protein and amino-acid content of seaweeds, among taxonomic groups and geographical regions. This conversion factor was also used in this study to estimate the protein content of the samples. Similar protein levels were found in *A. esculenta* and *S. latissima*, which were comparable to those reported earlier for the same species harvested in spring (Mæhre et al., 2014; Schiener et al., 2015). Although the protein content of brown seaweeds is generally lower than levels found in red algae (Fleurence, 2004), all essential amino acids are present (also methionine), and may cover human and animal requirements (Mæhre et al., 2014), making cultivated kelp species a good source of essential amino acids for food and feed.

The total phenolic content was higher in *A. esculenta* than in *S. latissima* but lower than levels reported in the literature for other brown seaweed species such as *Fucus* spp., *Ascophyllum nodosum* and *Sargassum muticum* (Connan et al., 2006; Wang et al., 2009; Magnusson et al., 2017). *A. esculenta* also displayed relatively high levels of fucoxanthin, in the range of those found in another Alariaceae, i.e. *Undaria pinnatifida*, suggested as raw material for commercial extraction (Quitain et al., 2013; Kanda et al., 2014). This carotenoid pigment has a high value in both nutraceutical and pharmaceutical fields due to bioactivities e.g. anti-oxidant (Fung et al., 2013), and anti-obesity activities (Maeda et al., 2005). Cultivated *A. esculenta* may be a potential candidate for future commercial extraction of this pigment.

In this study, the chemical composition of *A. esculenta* and *S. latissima* was altered during storage in seawater, illustrated primarily by a substantial diminution in DW in both species, occurring mainly during the first two hours of treatment. However, the results from this study could not confirm whether the DW reduction was caused by (i) the release of nutritional compounds from seaweed biomass or (ii) water uptake during storage treatments as a consequence of osmotic activity. Additional analyses are necessary in order to estimate the effect of each process at play. Expressing the results as part of the DW of the biomass directly reflects the relative proportions of the compounds

analyzed and their variations during treatment, as well as the general quality of the biomass prior to and after storage. A general hypothesis about the influence of seawater storage on the quality of *A. esculenta* and *S. latissima* can be drawn from these results.

The variation in phytochemical content in both kelps, i.e. an increase in mineral content and a general decrease in carbohydrates, polyphenols and fucoxanthin pigment, suggest an impact of seawater storage on the nutritional value of these species rather than the sole effect of seawater uptake to explain the significant decrease in dry matter content. Higher ash contents observed in both species after 22 h storage reflect the relative increase in the total mineral content along with increased Na contents, while the K contents remained relatively stable. Uptake of seawater, including salts can result from the osmotic reaction to equalize the concentration difference of charged molecules between the seaweed biomass and the storage seawater. However, the increase in Na alone do not explain the accumulation in total mineral (ashes) within the biomass. Other macro-minerals such as calcium (Ca) and magnesium (Mg) were not analyzed in this study and may have contributed to higher ash contents after treatment. The relative increase in total minerals may be derived from the combined effects of an uptake of salts, and the relative decrease in other compounds constituting the rest of the dry matter, released by the seaweed biomass during storage.

The decrease in total carbohydrates was more pronounced in *S. latissima* than in *A. esculenta* due to high losses of mannitol and glucose during storage while the relative proportions of alginate and fucose increased relatively. Exudation is clearly visible and characteristic when harvesting *S. latissima* which releases a brown "sap" shortly after harvest. This process is an active physiological defense mechanism following the stress induced by harvesting the biomass. The exudate in kelps typically contains low molecular weight and water soluble compounds such as mannitol, laminaran and polyphenols (Newell et al., 1980; Abdullah and Fredriksen, 2004). The exudation is likely responsible for the brown color observed in the storage water of *S. latissima*. Coloration of the water was not observed to the same extent in the case of *A. esculenta*, and may explain some of the differences between the two species concerning variations of the phytochemical constituents. The results clearly indicate other mechanisms than solely water solubility to explain the release or the retention of a compound, since the mannitol, laminaran (glucose) and fucose contents were not affected in the same way in both kelps. Following the results of a study showing no differences in mannitol content between samples of *L. digitata* unwashed and washed with tap water, Adams et al. (2014) suggested the ability of cell pores to close and limit the leakage of intracellular mannitol. A similar mechanism may have occurred in *A. esculenta* during the storage in seawater. It should be noted that various types of fucoidans are localized differently in

cell walls and in the intercellular matrix (Mabeau et al., 1990). A larger proportion of fucoidans localized intercellular in *A. esculenta* may explain the significant decrease in the level of fucose, since these compounds are expected to be more readily released than cell wall bound fucoidans. Conversely, a larger fraction of the fucoidans and proteins bound to cell walls would explain their higher relative content in *S. latissima* after 22 h storage in seawater.

Color analysis is a common practice in the food industry and is used extensively for agricultural food products. It quickly provides information that can be correlated to other quality attributes such as sensory, nutritional and visual or non-visual defects (Francis, 1995) and can also monitor product changes from processing (Guiné and Barroca, 2012). The results of color analysis using the CVS method did not display patterns corresponding to the variation in DW i.e. major changes during the first two-hour treatment. However, some of the valuable compounds such as fucoxanthin and polyphenols are present at low concentration in the biomass and may be affected at different rates than other compounds contributing to the DW variation. These two specific compounds, one being a pigment, the other an antioxidant would be expected to have an influence on the surface color of the biomass. Thus, the CVS may be a good indicator of the variation of these two compounds although more occurrence data is needed to establish the relationship between the surface color of kelps and their chemical composition.

The extent to which the bioactive compounds analyzed were released during storage could not be quantified precisely in this study since the variation in DW may also be the result of water uptake. However, the sole effect of water uptake would not result in variations in the relative proportions of phytochemical constituents e.g. minerals and carbohydrates, observed in this study, suggesting the impact of seawater storage on the nutritional value of *A. esculenta* and *S. latissima*. In the future, applying a methodology including the analysis of the storage water which should contain the leaked compounds will allow a more precise estimation of the mass balance between the seaweed biomass and storage water. Fresh weight measurements of individual kelp blades prior to and after storage will directly reflect water uptake during the process.

Care was taken to minimize the stress endured by the biomass during the process of harvesting and transport, and the conditions of this study were comparable to the routines in use during harvesting seaweed biomass for commercial or research purposes. Direct air exposure probably causes drought stress inducing physiological disorders such as an excess in reactive oxygen species (ROS), changes in cellular osmolarity and membrane damage (Burritt et al., 2002; Flores-Molina et al., 2014) which can ultimately lead to leakage of cellular compounds (Burritt et al., 2002). Seaweeds

have developed complex enzymatic and non-enzymatic antioxidant mechanisms to mitigate these effects and acclimatize to adverse environments (Bischof and Rautenberger, 2012) but species having higher intertidal distributions display higher antioxidant capacity and greater tolerance response to desiccation (Flores-Molina et al., 2014). Both *A. esculenta* and *S. latissima* are naturally bound to the lower intertidal and subtidal zones, and may only be exposed to air at rare occasions. Moreover, cultivated individuals are grown on a submerged substrate and do not experience air exposure, which may increase their sensitivity to desiccation stress. However, knowledge is still missing regarding intraspecific differences in tolerance to abiotic stress, between cultivated seaweeds and their wild counterparts. The marginal increase in DW after 6 h storage observed in this study may be related to the ability of seaweeds to recover from stress by taking up nutrients from their environment.

Although significant alterations in the chemical composition of both species were observed in this study, it is not entirely clear whether seawater storage or possibly the stress induced by harvesting procedures were responsible for the leakage of compounds. However, the kelps, especially *S. latissima*, clearly exuded valuable compounds during storage. In large-scale industrial processes, this will lead to reduced extraction yields in the recovery of a single or multiple products from seaweed biomass, with economic consequences. Alternatively, the lost compounds can be recovered from the storage water by membrane filtration, although processing large water volumes will entail extra costs. Other short-term preservation methods such as cold storage and silage, where the exudate is not diluted and can easily be recovered, may be a preferable alternative. Both species, and especially *S. latissima*, appear to be prime candidates to be used as a salt replacing ingredient in the food industry due to their low Na/K ratios. In this regard, the value of *S. latissima* was not compromised by storage treatments in seawater for 22 h whereas a higher Na content increased the Na/K ratio in *A. esculenta*. The effect of seawater storage on other compounds of nutritional interest such as Ca, Mg, iron (Fe), as well as lipids and polyunsaturated fatty acids are envisaged in future studies. Seawater storage may decrease the value of the biomass to be used for the recovery of high value products such as fucoidan, polyphenols and fucoxanthin, although this effects will vary among seaweed species. The protein content of both species, which is of interest in food and feed applications remained relatively unaffected. Seawater storage can be an acceptable and convenient short-term storage method; however, industry players must be aware of the possible consequences highlighted in this study. Logistic models to optimize harvesting and primary processing should focus on minimizing biomass stress and emphasizing rapid stabilization of the biomass to avoid losses of highly nutritious compounds.

Acknowledgements

This work was conducted as part of the PROMAC project (244244), funded by the Research Council of Norway, and part of the Sustainable Innovation in Food- and Bio-based Industries Programme. Pierrick Stévant was supported by a doctoral fellowship from Sparebanken Møre. The authors are also grateful to two anonymous reviewers for their valuable comments and contribution in improving the manuscript.

References

Abdullah MI and Fredriksen S (2004). Production, respiration and exudation of dissolved organic matter by the kelp *Laminaria hyperborea* along the west coast of Norway. J Mar Biol Assoc U.K. 84(05): 887-894.

Adams JMM, Ross AB, Anastasakis K, Hodgson EM, Gallagher JA, Jones JM, Donnison IS (2011). Seasonal variation in the chemical composition of the bioenergy feedstock *Laminaria digitata* for thermochemical conversion. Bioresour Technol 102(1): 226-234.

Adams JMM, Schmidt A and Gallagher, J. A. (2014). The impact of sample preparation of the macroalgae *Laminaria digitata* on the production of the biofuels bioethanol and biomethane. J Appl Phycol 27(2): 985-991.

AFNOR (1977). Norme NF V18-101: Aliments des animaux - Dosage des cendres brutes.

Ale MT, Meyer AS (2013). Fucoidans from brown seaweeds: an update on structures, extraction techniques and use of enzymes as tools for structural elucidation. RSC Adv 3(22): 8131-8141.

Angell AR, Mata L, de Nys R and Paul NA (2016). The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. J Appl Phycol 28(1): 511-524.

AOAC Iternational (2000). Official methods of analysis of AOAC international (17th ed.). Maryland, USA: Association of Official Analytical Chemistry.

Baghel RS, Trivedi N, Reddy CRK (2016). A simple process for recovery of a stream of products from marine macroalgal biomass. Bioresour Technol 203: 160-165.

Bischof K, Rautenberger R (2012). Seaweed Responses to Environmental Stress: Reactive Oxygen and Antioxidative Strategies. In: Wiencke C and Bischof K (ed), Seaweed Biology: Novel Insights into Ecophysiology, Ecology and Utilization. Springer Berlin Heidelberg**,** pp 109-132.

Black W (1950). The seasonal variation in weight and chemical composition of the common British Laminariaceae. J Mar Biol Assoc U.K. 29(01): 45-72.

Brinkhuis BH, Levine HG, Schlenk CG, Tobin S (1987). *Laminaria* cultivation in the far east and North America. In Bird and Benson (ed), Seaweed cultivation for renewable resources. Development in aquaculture and fisheries science. Elsevier, New York**,** pp 107-146.

Brownlee IA, Allen A, Pearson JP, Dettmar PW, Havler ME, Atherton MR, Onsøyen E (2005). Alginate as a source of dietary fiber. Crit Rev Food Sci 45(6): 497-510.

Bruhn A, Tørring DB, Thomsen M, Canal-Vergés P, Nielsen MM, Rasmussen MB, Eybye KL, Larsen MM, Balsby TJS. and Petersen JK (2016). Impact of environmental conditions on biomass yield, quality, and bio-mitigation capacity of *Saccharina latissima*. Aquac Environ Interac 8: 619-636.

Burritt DJ, Larkindale J, Hurd CL (2002). Antioxidant metabolism in the intertidal red seaweed *Stictosiphonia arbuscula* following desiccation. Planta 215(5): 829-838.

Chan JCC, Cheung PCK, Ang PO (1997). Comparative Studies on the Effect of Three Drying Methods on the Nutritional Composition of Seaweed *Sargassum hemiphyllum* (Turn.) C. Ag.†. J Agr Food Chem 45(8): 3056-3059.

Chapman AS, Stévant P, Emblem Larssen W (2015). Food or fad? Challenges and opportunities for including seaweeds in a Nordic diet. Bot Mar 58(6).

Connan S, Delisle F, Deslandes E, Ar Gall E (2006). Intra-thallus phlorotannin content and antioxidant activity in Phaeophyceae of temperate waters. Bot Mar 49(1).

Dawczynski C, Schubert R, Jahreis G (2007). Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chem 103(3): 891-899.

Déléris P, Nazih H, Bard JM (2016). Seaweeds in Human Health. In: Fleurence J and Levine I (ed), Seaweed in Health and Disease Prevention. Academic Press, Elsevier, Amsterdam**,** pp 319-367.

Draget KI, Smidsrød O, Skjåk Bræk G (2002). Alginates from algae. In: De Baets S, Vandamme EJ, Steinbuchel A (ed), Biopolymers. Wiley, Weinheim, pp 215-244.

Enríquez S, Duarte CM, Sand-Jensen K (1993). Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94(4): 457-471.

Evans FD, Critchley AT (2014). Seaweeds for animal production use. J Appl Phycol 26: 891-899.

Fleurence J (2004). Seaweed proteins. In: Yada RY (ed), Proteins in food processing. Woodhead publishing, Cambridge, pp 197-213.

Fleurence J, Ar Gall E (2016). Antiallergic Properties. In: Fleurance J and Levine I (ed), Seaweed in Health and Disease Prevention. Academic Press, Elsevier, Amsterdam, pp 389-406.

Flores-Molina MR, Thomas D, Lovazzano C, Núñez A, Zapata J, Kumar M, Correa JA, Contreras-Porcia L (2014). Desiccation stress in intertidal seaweeds: Effects on morphology, antioxidant responses and photosynthetic performance. Aquat Bot 113: 90-99.

Forbord S, Skjermo J, Arff J, Handå A, Reitan KI, Bjerregaard R, Lüning K (2012). Development of *Saccharina latissima* (Phaeophyceae) kelp hatcheries with year-round production of zoospores and juvenile sporophytes on culture ropes for kelp aquaculture. J Appl Phycol 24(3): 393-399.

Francis FJ (1995). Quality as influenced by color. Food Qual Prefer 6(3): 149-155.

Fung A, Hamid N, Lu J (2013). Fucoxanthin content and antioxidant properties of *Undaria pinnatifida*. Food Chem 136(2): 1055-1062.

Girolami A, Napolitano F, Faraone D, Braghieri A (2013). Measurement of meat color using a computer vision system. Meat Sci 93(1): 111-118.

Guiné RPF, Barroca MJ (2012). Effect of drying treatments on texture and color of vegetables (pumpkin and green pepper). Food Bioprod Process 90(1): 58-63.

Gupta S, Cox S, Abu-Ghannam N (2011). Effect of different drying temperatures on the moisture and phytochemical constituents of edible Irish brown seaweed. LWT - Food Sci Technol 44(5): 1266-1272.

Handå A, Forbord S, Wang X, Broch OJ, Dahle SW, Størseth TR, Reitan KI, Olsen Y, Skjermo J (2013). Seasonal- and depth-dependent growth of cultivated kelp (*Saccharina latissima*) in close proximity to salmon (*Salmo salar*) aquaculture in Norway. Aquaculture 414-415: 191-201.

Hayashi K, Nakano T, Hashimoto M, Kanekiyo K, Hayashi T (2008). Defensive effects of a fucoidan from brown alga *Undaria pinnatifida* against herpes simplex virus infection. Int Immunopharmacol 8(1): 109-116.

Herrmann C, FitzGerald J, O'Shea R, Xia A, O'Kiely P, Murphy JD (2015). Ensiling of seaweed for a seaweed biofuel industry. Bioresour Technol 196: 301-313.

Holdt SL, Kraan S (2011). Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol 23(3): 543-597.

Hou X, Hansen JH, Bjerre AB (2015). Integrated bioethanol and protein production from brown seaweed *Laminaria digitata*. Bioresour Technol 197: 310-317.

Jensen A (1993). Present and future needs for algae and algal products. In: Chapman ARO, Brown MT, Lahaye M (ed), Fourteenth International Seaweed Symposium. Springer Netherlands. 85**:** 15-23.

Kanda H, Kamo Y, Machmudah S, Wahyudiono EY, Goto M (2014). Extraction of fucoxanthin from raw macroalgae excluding drying and cell wall disruption by liquefied dimethyl ether. Mar Drugs 12(5): 2383-2396.

Kraan S, Verges Tramullas A, Guiry M (2000). The edible brown seaweed *Alaria esculenta* (Pheophyceae, Laminariales) hybridization growth and genetic comparisons of six Irish populations. J Appl Phycol 12: 577-583.

Kumar CS, Ganesan P, Suresh PV, Bhaskar N (2008). Seaweeds as a source of nutritionally beneficial compounds. J Food Sci Tech Mys 45(1): 1-13.

Liot F, Colin A, Mabeau S (1993). Microbiology and storage life of fresh edible seaweeds. J Appl Phycol 5(2): 243-247.

López-López I, Bastida S, Ruiz-Capillas C, Bravo L, Larrea MT, Sánchez-Muniz F, Cofrades S, Jiménez-Colmenero F (2009a). Composition and antioxidant capacity of low-salt meat emulsion model systems containing edible seaweeds. Meat Sci 83(3): 492-498.

López-López I, Cofrades S, Ruiz-Capillas C, Jiménez-Colmenero F (2009b). Design and nutritional properties of potential functional frankfurters based on lipid formulation, added seaweed and low salt content. Meat Sci 83(2): 255-262.

Lourenço SO, Barbarino E, De-Paula JC, da S. Pereira LO, Lanfer Marquez UM (2002). Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. Phycol Res 50: 233-241.

Mabeau S, Fleurence J (1993). Seaweed in food products: biochemical and nutritional aspects. Trends Food Sci Tech 4: 103-107.

Mabeau S, Kloareg B and Joseleau J-P (1990). Fractionation and analysis of fucans from brown algae. Phytochemistry 29(8): 2441-2445.

MacArtain P, Gill CIR, Brooks M, Campbell R, Rowland IR (2007). Nutritional Value of Edible Seaweeds. Nutr Rev 65(12): 535-543.

Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K (2005). Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. Biochem Biophys Res Commun 332(2): 392-397.

Maeda H, Tsukui T, Sashima T, Hosokawa M, Miyashita K (2008). Seaweed carotenoid, fucoxanthin, as a multi-functional nutrient. Asia Pac J Clin Nutr 17(S1): 196-199.

Magnusson M, Yuen AKL, Zhang R, Wright JT, Taylor RB, Maschmeyer T and de Nys R (2017). A comparative assessment of microwave assisted (MAE) and conventional solid-liquid (SLE) techniques for the extraction of phloroglucinol from brown seaweed. Algal Res 23: 28-36.

Mouritsen OG, Williams L, Bjerregaard R, Duelund L (2012). Seaweeds for umami flavour in the New Nordic Cuisine. Flavour 1(1): 4.

Mæhre HK, Malde MK, Eilertsen KE, Elvevoll EO (2014). Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed. J Sci Food Agric 94(15): 3281-3290.

Newell R, Lucas M, Velimirov B and Seiderer L (1980). Quantitative significance of dissolved organic losses following fragmentation of kelp *Ecklonia maxima* and *Laminaria pallida*. Mar Ecol Prog Ser 2: 45-59.

Pádua D, Rocha E, Gargiulo D, Ramos AA (2015). Bioactive compounds from brown seaweeds: Phloroglucinol, fucoxanthin and fucoidan as promising therapeutic agents against breast cancer. Phytochem Lett 14: 91-98.

Paull RE, Chen NJ (2008). Postharvest handling and storage of the edible red seaweed *Gracilaria*. Postharvest Biol Tec 48(2): 302-308.

Perez V, Chang ET (2014). Sodium-to-Potassium Ratio and Blood Pressure, Hypertension, and Related Factors. Adv Nutr 5(6): 712-741.

Quemener B, Marot C, Mouillet L, Da Riz V, Diris J (2000). Quantitative analysis of hydrocolloids in food systems by methanolysis coupled to reverse HPLC. Part 1. Gelling carrageenans. Food Hydrocolloid 14(1): 9-17.

Quitain AT, Kai T, Sasaki M, Goto M (2013). Supercritical Carbon Dioxide Extraction of Fucoxanthin from *Undaria pinnatifida*. J Agr Food Chem 61(24): 5792-5797.

R Development Core Team (2008). R: A Language and Environment for Statistical Computing. Vienna, Austria. R Foundation for Statistical Computing. http://www.R-project.org.

Ragan MA, Glombitza KW (1986). Phlorotannins, brown algal polyphenols. In: Round FE, Chapman DJ (ed), Progress in phycologcal research. Biopress ltd, Bristol. 4, pp 130-230.

Rupérez P (2002). Mineral content of edible marine seaweeds. Food Chem 79: 23-26.

Rupérez P, Saura-Calixto F (2001). Dietary fibre and physicochemical properties of edible Spanish seaweeds. Eur Food Res Technol 212(3): 349-354.

527 Sánchez-Machado DI, López-Cervantes J, López-Hernández J, Paseiro-Losada P (2004). Fatty acids, total lipid,
528 protein and ash contents of processed edible seaweeds. Food Chem 85(3): 439-444.

529 Schiener P, Black KD, Stanley MS, Green DH. (2015). The seasonal variation in the chemical composition of the kelp
530 species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*. J Appl Phycol 27(1):
531 363-373.

532 Skriptsova AV (2015). Fucoidans of brown algae: Biosynthesis, localization, and physiological role in thallus. Russ J
533 Mar Biol 41(3): 145-156.

534 Soler-Vila A, Coughlan S, Guiry M, Kraan S (2009). The red alga *Porphyra dioica* as a fish-feed ingredient for
535 rainbow trout effects on growth, feed efficiency and carcass composition. J Appl Phycol 21: 617-624.

536 Wang T, Jónsdóttir R, Kristinsson HG, Thorkelsson G, Jacobsen C, Hamaguchi PY, Ólafsdóttir G (2010). Inhibition
537 of haemoglobin-mediated lipid oxidation in washed cod muscle and cod protein isolates by *Fucus vesiculosus* extract
538 and fractions. Food Chem 123(2): 321-330.

539 Wang T, Jónsdóttir R, Ólafsdóttir G (2009). Total phenolic compounds, radical scavenging and metal chelation of
540 extracts from Icelandic seaweeds. Food Chem 116(1): 240-248.

541 Yam KL, Papadakis SE (2004). A simple digital imaging method for measuring and analyzing color of food surfaces.
542 J Food Eng 61(1): 137-142.

543

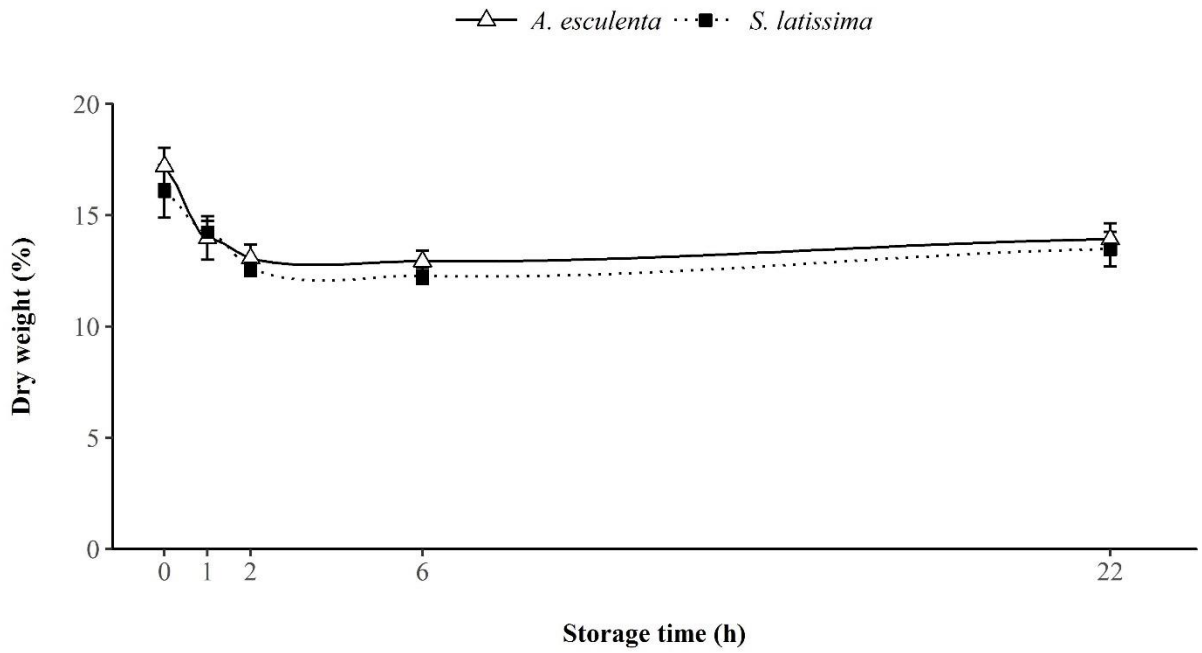
544 **Table 1:** Chemical composition of the seaweed biomass prior to (t_0), and after 22 h storage in seawater tanks.
545 Concentrations are expressed in g 100 g^{-1} DW, except for the fucoxanthin content expressed in mg kg^{-1} DW and the
546 dimensionless Na/K ratio. Values are given as mean ± standard error (n = 3). The symbol * indicates a significant
547 different level of a compound measured after 22 h storage as compared to the initial value measured at t_0 (paired
548 sample *t*-test, $p < 0.05$).

	A. esculenta		*S. latissima*	
	t_0	**t=22h**	**t_0**	**t=22h**
Dry weight (%)	17.2 ± 0.8	13.9 ± 0.7	16.1 ± 1.2	13.5 ± 0.8
Minerals				
Ash	24.2 ± 1.4	27.0 ± 1.6	26.2 ± 2.6	30.0 ± 2.1
Na	3.9 ± 0.2	5.2 ± 0.2 *	3.6 ± 0.2	4.3 ± 0.2
K	4.2 ± 0.3	4.4 ± 0.5	6.5 ± 1.1	7.2 ± 0.8
Na/K	0.94 ± 0.03	1.22 ± 0.13	0.56 ± 0.06	0.60 ± 0.04
Carbohydrates				
Total carbohydrates	40.7 ± 1.5	37.7 ± 1.5	46.1 ± 2.46	40.0 ± 1.0
Alginate	19.9 ± 0.5	18.6 ± 0.4	21.5 ± 0.5	23.1 ± 1.5
Mannitol	10.5 ± 0.4	10.4 ± 0.3	17.6 ± 1.2	12.3 ± 2.2
Glucose	8.5 ± 1.9	7.5 ± 1.4	5.0 ± 2.0	2.7 ± 0.6
Fucose	1.25 ± 0.03	0.98 ± 0.04 *	0.76 ± 0.03	0.89 ± 0.07

Proteins	10.5 ± 0.2	9.9 ± 0.1	10.6 ± 0.3	11.6 ± 0.2
Polyphenols	3.43 ± 0.08	2.55 ± 0.09 *	0.69 ± 0.04	0.49 ± 0.04 *
Fucoxanthin	871 ± 53	829 ± 45	431 ± 19	360 ± 27

549

550

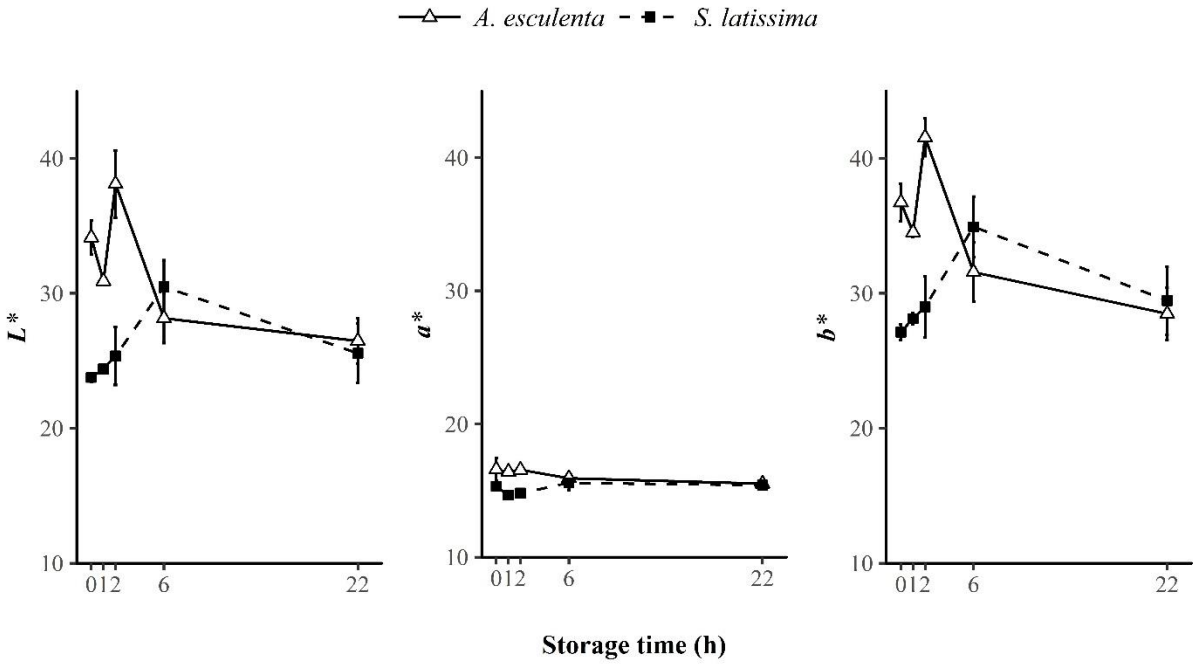


551

552 **Fig 1:** Changes in dry matter content of *A. esculenta* and *S. latissima* stored in seawater tanks. Values are given as

553 mean ± standard error (n = 3).

554



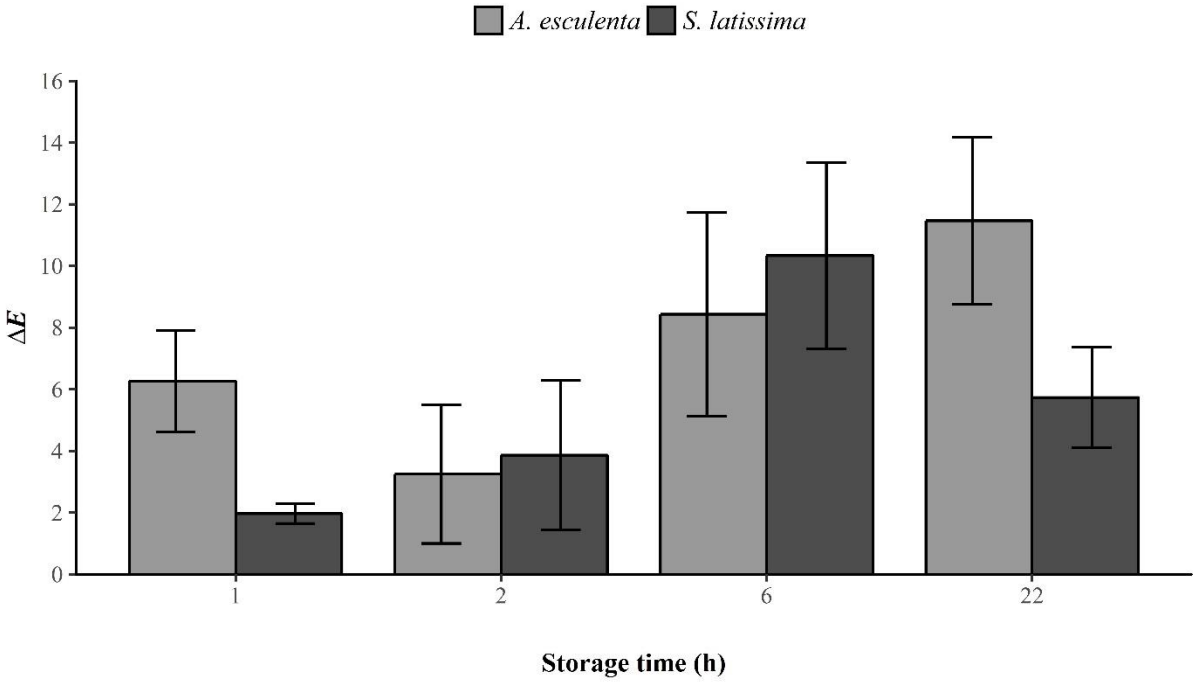
555

556 **Fig. 2**: Variations of the color coordinates *L** (lightness), *a** (redness/greenness) and *b** (yellowness/blueness) of *A.*

557 *esculenta* and *S. latissima* during seawater storage measured by computer vision system (CVS). Values are given as

558 mean ± standard error (n = 3).

559



560

561 **Fig. 3:** Total color variation (Δ*E*) of *A. esculenta* and *S. latissima* during seawater storage. Values are given as mean

562 ± standard error (n = 3).

563

Figure 1

Click here to download line figure Figure_1.eps

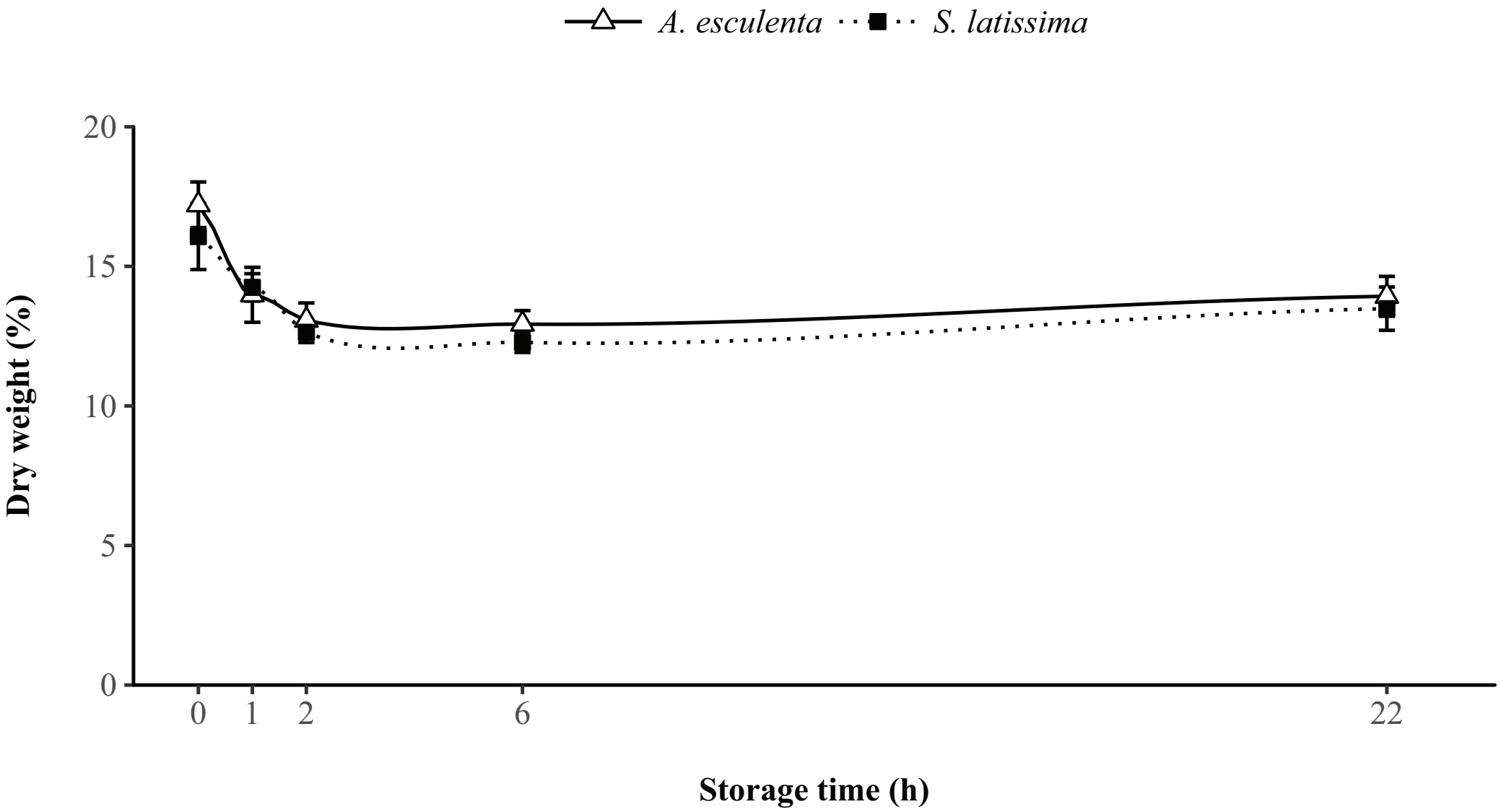


Figure 2

Click here to download line figure Figure_2.eps

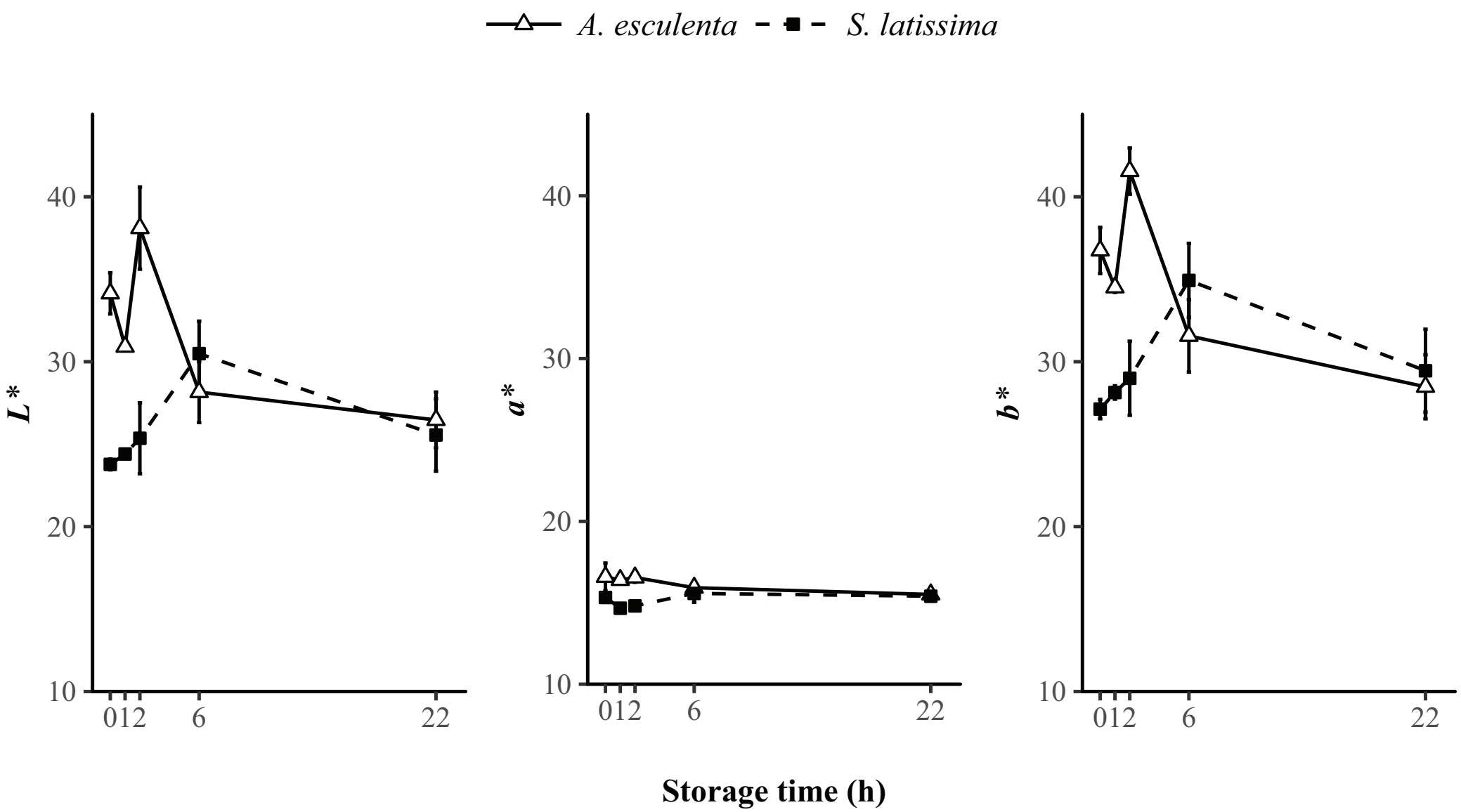
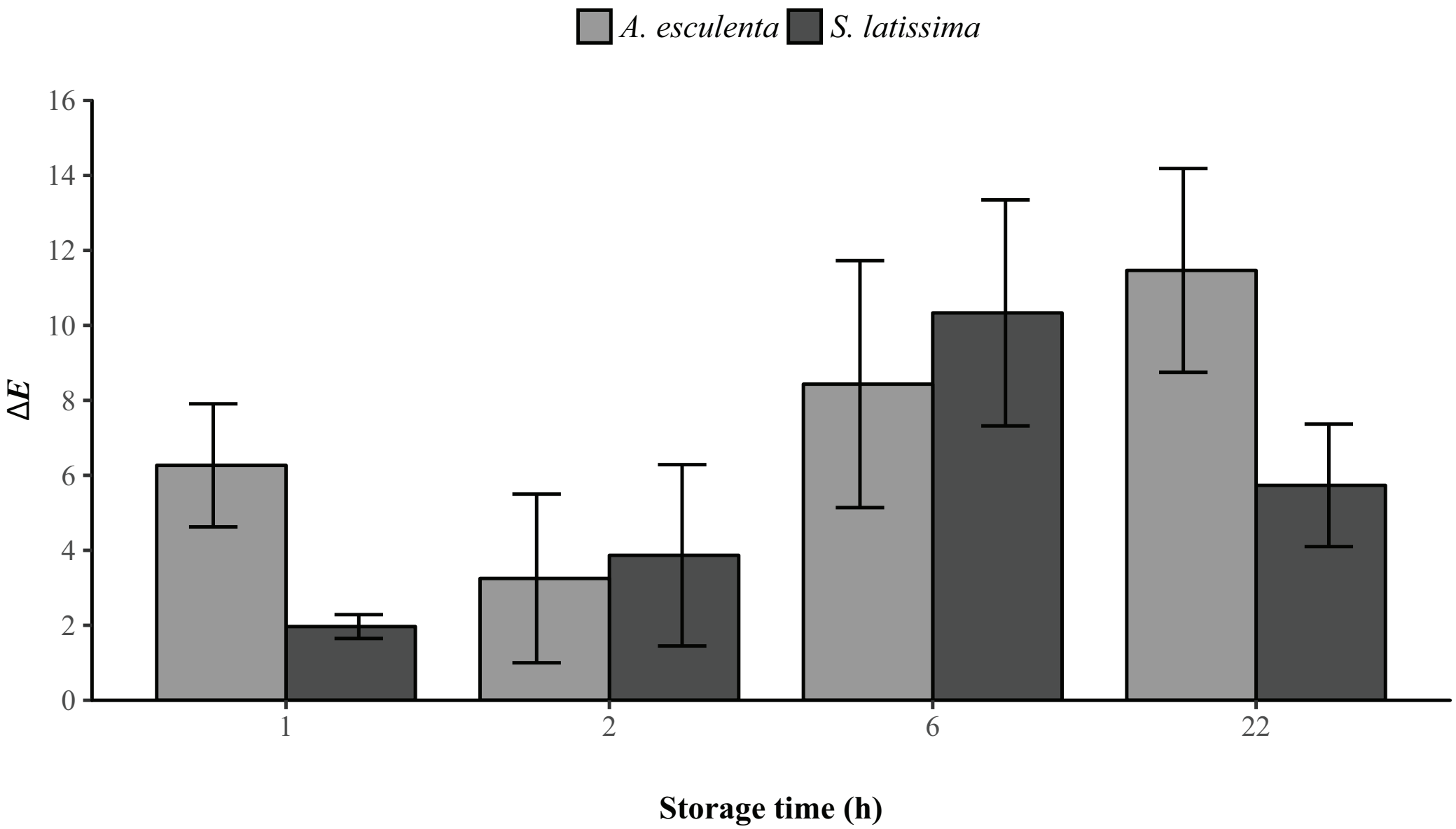


Figure 3

Click here to download line figure Figure_3.eps



Answers to reviewers

Reviewer #1, line 110: What was the temperature during transport?

The temperature was not recorded during boat transport of the biomass at the time of harvest, however, data log from the French meteorological institute reported maximal temperature of approximately 16˚C in the municipality for both days of harvest.

Reviewer #1, line 117: What was the water and storage temperature, was it controlled?

The storage water was maintained at a temperature of 18˚C ± 1˚C throughout the experiment. The water temperature on cultivation site was not recorded during harvest, however, data log reports sea surface temperature comprised between 15.5 and 16˚C off the coast of Northern Brittany. The cultivation site is located in a relatively shallow bay which may have slightly higher temperatures following a relatively sunny spring that year.

Reviewer #1, line 124: With respect to the sensitivity of the fresh weight to variations (e.g. drip water) I suggest not to use calculations based on fresh weight like formula 1.

Sampled blades were gently blotted to remove excess water (this detail did not appear in the first version of the manuscript) to limit the variability due to drip water. However, based on the relevant reviewers' comments regarding the potential effect of water uptake, the results expressed as part of the FW were not used in the new version of the manuscript.

Reviewer #1: Did the fresh weight increase during storage (perhaps salinity was lower, osmotic potential of the blades results in uptake water)

Variations in FW due to possible water uptake were not measured in this study. Additional methodology in order to estimate this effect on the variation in %DW are proposed in the discussion (line 346-353). Although the salinity in the tank was not recorded, major differences in salinity between the cultivation site and storage water are unlikely due to stable salinity conditions (35,893 in average) of the seawater pumped from the same bay as the cultivation site.

Reviewer #1: What is the color of the water after storage? Does it contain the leaked compounds?

A characteristic brown color was observed in the storage water of *S. latissima* which was not observed to the same extent in the case of *A. esculenta*. The storage water was not analyzed due to the dilution of the compounds in 100L hence, the difficulties in the detection from analyses.

Reviewer #1: What is the integrity of the blades before and after storage? Any visible degradation?

No visible degradation was observed after storage in both species. Exudation usually occurs shortly after harvest of *S. latissima* and was observed here, although it does not seem to affect the integrity of the blade. The analysis of blade texture (tensile strength) is envisaged in future studies.

Reviewer # 1: Some compounds (like mannitol) occur intercellular and could leak out easier than f.i. proteins or cell wall bound alginate. Why those respond the same? I suggest a more general hypothesis about the mechanism of action instead of the very specific discussion per compound, which is interesting to know but not convincing as an argument for this total effect. Furthermore, I would suggest a mass balance with the water which should contain the lost compounds.

As a result of this comment, the result and discussion parts were modified. The results expressed in %DW and variations observed after storage were used to draw hypothesis of the combined effects of the release of compounds as water uptake on the reduction of dry matter.

Reviewer #2: The analyses included in the study would allow for including two more important quality parameters: the Na/K ratio as well as the M/G ratio of the alginate. I find that addition and discussion of this data would further improve the pertinence of the manuscript.

Details regarding the variations in the Na/K ratio were added to the new version of the manuscript. Regarding the M/G ratio, the results from HPLC are given as the total M+G. Further analysis using quantitative magnetic resonance spectroscopy (NMR) are necessary to give a reliable result of the M/G ratio which were not conducted in this study.

Reviewer #2: I find the definition of t_0 unclear. Is t_0 defined at harvest or a 2 hours when kelps biomass was delivered at the lab? Please specify, and the latter, please include a discussion of what happens in the 2 hours in transport from harvest to lab.

t_0 sampling was done at biomass receival at the lab. This details was added to the new version of the manuscript (line 120). A general hypothesis about possible stress during transport was developed in the discussion part (line 354).

Reviewer #2: You mention that the molecules lost to the seawater in storage cannot be recovered. Wouldn't that be possible by some filtration or separation technology? Perhaps even the storage could act like a pre-treatment technology in a cascade biorefinery?

Yes, filtration procedures are possible to recover lost compounds in the storage water although processing large volumes will entail extra costs and reduce profitability. Perhaps fresh water treatment to recover larger amount of soluble compounds are preferable in the case of a cascade biorefinery?

Reviewer #2: The biomass is aerated during storage. Is that a normal procedure in storage and transportation of kelp biomass?

Aeration was provided to ensure proper mixing within storage tanks as previous experiments have shown that spontaneous fermentation process can happen relatively quickly when the biomass is packed and especially in the case of *S. latissima*.

Reviewer #2: Line 313-315, you mention that the color change at 6 hours does not reflect the loss of biomolecules. I do not find sufficient support in the results for this argument. Yes, the DM content stabilizes after 2 h, however some of the valuable biomolecules - polyphenols and fucoxanthin are in very low concentrations and may be lost at a different rate than minerals/mannitol. These two specific compounds - one being a pigment, the other an antioxidant would be expected to have influence on the color of the seaweed. Thus, the CVS method may be a good indicator of when these two compounds are lost from the biomass - however your results cannot document this, since you have only analyses at 0 and 22 h. I strongly recommend a more nuanced and critical discussion of this.

The discussion of the results from color analyses were modified following this relevant suggestion (line 335).

Reviewer #2: A conversion factor of 5.38 is used for converting tissue nitrogen into protein based on Lourenco 2002. None of the 4 tropical species of brown algae used in Lorencos study are kelps. A newer paper, Angel et al, 2016, reviews N-protein conversion factors and recommend on that basis to use a factor of 5, albeit the means/medians for brown algae are 4.56/4.81. As this study compares the effect of a treatment this is not crucial, however the choice of factor should be discussed.

This universal factor of 5 was used in the new version of the manuscript.

Reviewer #2: Polyphenol content of seaweeds are reviewed in a new paper by Magnusson et al (Algal Research), this reference should be studied and included, as it is very informative and relevant.

It is indeed an interesting and relevant reference. It was added to the text (line 71).

Reviewer #2: The abstract could benefit from further specific results included on the actual loss of biomolecules.

The abstract was modified according to the more general hypothesis regarding the effect of seawater storage on quality of kelp biomass, including the compounds that were mainly affected.

Reviewer #2: The keywords could include the specific biomolecules, i.e. alginate, fucoxanthin, polyphenols, fucose.

The keyword list was modified accordingly.

Reviewer #2: Minor specific comments:

- **Insert space between numbers and units, i.e. 2 h and 5 kg**
- **Line 116, please specify if the seawater was filtered or UV treated, and if so specify details**
- **Line 140-141, please specify reference sugars and supplier**
- **Line 154, please specify supplier of standard phloroglucinol**
- **Lines 155-156, please specify instrument used for analysis, and if blanks were analyzed**
- **Line 171-174, please specify how triplicates were "pooled". Were results averaged before or after calculation of delta E?**

  Modifications were made according to comments and details were added to the text.

- **Is ANOVA the correct analysis to use, when samples are not independent (the same sample, sampled over time)?**

  The use of ANOVA was reconsidered and yes, when considering each seaweed batch as the functional unit, samples are not independent, thus the repeated measures ANOVA is the adapted statistical test in our case.

- **Line 252, I do not think you have the results to make this conclusion. The dry matter leaks within the first 2 hours, however different sugars are bound differently in the algae cells, some free in the cytosol, some in the cell walls. These may very well leak out at different pace**

  The more general hypothesis about the effect of storage on quality parameters of seaweeds in the new version of the manuscript also includes the effects of the compounds' localization within the biomass in order to explain their release or retention.

- **When words are abbreviated and abbreviations are defined in (), the abbreviations only should be used in the following text (i.e. nitrogen, potassium)**
- **See suggestions and comments made in the pdf version of the manuscript**

  Modifications were made in the text according to the above suggestion and comments in the pdf version of the first submitted manuscript. Here are some more details:
  - Temperature and pressure used for Na and K analysis: this information was not disclosed by the external laboratory which conducted the analysis.
  - Polyphenol analysis: the blanks were controlled but the absorbance was negligible hence there were no need for subtraction from the measurement of the seaweed extracts.