Journal of Applied Phycology
https://doi.org/10.1007/s10811-024-03210-4

RESEARCH

# Nutritional value, bioactive composition, physico-chemical and sensory properties of *Ulva* sp. and *Fucus vesiculosus* depending on post-harvest processing: a drying comparison study

**Cecilie Bay Wirenfeldt[1,2] · Ditte B. Hermund[1] · Aberham Hailu Feyissa[1] · Grethe Hyldig[1] · Susan Løvstad Holdt[1]**

Received: 17 May 2023 / Revised: 6 February 2024 / Accepted: 9 February 2024


**Abstract**
Drying is an important post-harvest process to preserve seaweed as they are highly susceptible to spoilage due to their high moisture content. Drying can be performed in multiple ways by changing the temperature, pressure, air flow, and humidity. Therefore, the choice of drying method can affect the quality of the product in terms of sensory, chemical, and physicochemical properties. Seaweeds contain nutrients (protein, lipids, carbohydrates, vitamins, and minerals) and bioactive compounds. The compounds impact properties such as texture, taste, odor, and appearance. However, there is currently limited knowledge about how different drying methods affect the quality of seaweed products. In this paper we demonstrate, how different drying methods: i) convective drying (52 °C), ii) microwave-vacuum drying (-40 to 40 °C at 10 Pa), and iii) freeze-drying (-20 to 20 °C at 20 Pa) influence the food quality of *Fucus vesiculosus* and *Ulva* sp. by investigating physico-chemical properties such as water holding capacity, water absorption, and color, the changes in some of the chemical compounds such as macronutrients, fatty acids, amino acids, antioxidants, and pigments, as well as the taste, odor, appearance, and texture within sensory attributes. This study found that different drying methods have a species-dependent influence on the quality of seaweed, with *Ulva* sp. showing more similarities of using microwave-vacuum and freeze-drying methods, while the drying method for *F. vesiculosus* should be selected based on the desired food quality due to significant variations between the drying methods.

**Keywords** Amino acids · Antioxidant · Color · Convective air drying: Freeze drying · Microwave-vacuum drying · Pigments · Proximate · Texture

## Introduction

Seaweeds are used as food for several purposes such as extracted ingredients (gelling agents), supporting the flavor (umami), nutritional composition (protein, minerals) or as a crispy snack as it is. Holdt and Kraan (2011) summarizes the nutritional composition and other interesting bioactive compounds of brown, red, and green seaweeds, including protein and amino acids, lipid and fatty acids, polysaccharides and dietary fibers, vitamins, and minerals. Some of these compounds are also important regarding the physicochemical properties, e.g., water holding capacity and texture, which are related to the sensory properties of the seaweed.

The protein content varies between seaweed species. In brown seaweeds, the protein content is generally low (1-24 % protein), compared to red and green seaweeds (10-47 % protein, d.w.) (Mohamed et al. 2012). The protein content of seaweeds varies greatly with season and harvest location. Hence, for *Fucus* sp. the protein content ranges between

✉ Susan Løvstad Holdt
suho@food.dtu.dk

Cecilie Bay Wirenfeldt
cewini@food.dtu.dk

Ditte B. Hermund
dbala@food.dtu.dk

Aberham Hailu Feyissa
abhfe@food.dtu.dk

Grethe Hyldig
grhy@food.dtu.dk

[1] Technical University of Denmark, National Food Institute, 2800 Kongens Lyngby, Kemitorvet, Denmark

[2] Department of Biotechnology and Food Science, NTNU Norwegian University of Science and Technology, Sem Sælands vei 6/8, 7491 Trondheim, Norway

Published online: 22 May 2024

1.4-17.0 % d.w., while for *Ulva* sp., it has a range of 4-44 % d.w. (Holdt and Kraan 2011). The proteins found in seaweeds contain all the essential amino acids as well as aspartic acid and glutamic acid, which are associated with umami flavor (Yamaguchi 1991). Brown seaweed (*Durvillaea antarctica*) has been shown to have three times more umami flavor compared to green seaweed (*Ulva* sp.), related to a high amount of aspartic acid and glutamic acid (Figueroa et al. 2022). Processing of seaweeds can reduce the free amino acids and thereby change the flavor (Wirenfeldt et al. 2022).

The lipid content of seaweeds is generally low (0.5-3.1 % d.w. for *Fucus* sp. and 0.3-1.6 % d.w. for *Ulva* sp. (Holdt and Kraan 2011). However, seaweeds are rich in some of the same long-chain polyunsaturated omega-3 fatty acids associated with fish and seafood, especially EPA (C20:5, n-3). Seaweeds can therefore be a great source of supply of EPA for the maintenance of health (Murata and Nakazoe 2001). However, EPA containing foods are compromising a long shelf-life due to EPA's high susceptibility to oxidative degradation during processing and storage (Arab-Tehrany et al. 2012).

Phlorotannins are the major group of phenolic compounds of brown seaweeds. Phlorotannins constitute an extremely heterogeneous group of molecules (structure and polymerization degree heterogeneity) providing a wide range of potential biological activity, e.g., antioxidant, anti-coagulant and anti-enzymatic (Karthik et al. 2016). *Fucus* sp. are especially rich in phlorotannins (approximately 14 % d.w.) (Holdt and Kraan 2011), which have evidently antioxidant activity (Hermund et al. 2018), and potential candidates for the development of unique natural antioxidants for further industrial applications as functional foods (Li et al. 2009). Moreover, other bioactive compounds from seaweeds are the carotenoids, which possess functional properties and have been associated with antioxidant activity (Stahl and Sies 2003). Green seaweeds contain carotenoids like β-carotene, lutein, and some xanthophylls, whilst brown seaweed species contain β-carotene and fucoxanthin (Haugan and Liaaen-Jensen 1994). Fucoxanthin is the dominant carotenoid in brown seaweeds ranging from 172 to 720 mg $kg^{-1}$ d.w., with a maximal concentration in *Fucus serratus* (Holdt and Kraan 2011).

Drying is the oldest method of preserving food, and even today it remains a critically important and widely used process operation for long-term storage by removing water to extend the shelf-life of food products. The process results in a food product with low moisture content and low water activity, reducing the possibility of chemical reactions that lead to off-flavors and discoloration, hindering enzymatic activity and the growth of microorganisms, and may even eliminate bacteria (Claussen et al. 2007). Seaweeds are highly susceptible to spoilage (Wirenfeldt et al. 2022) due to their high water content ranging from 73 to 94% (Holdt and Kraan 2011). Therefore, drying is an essential process to achieve shelf-stable seaweed products. Although several drying methods are available, some compromise quality for a fast drying rate. The choice of drying method will have varying effects on the quality of the product, particularly in terms of chemical, sensory, and physicochemical properties.

Convective air drying is a process that utilizes either natural or forced convection of air to remove surface moisture from food. The efficiency of the drying process is mainly affected by air temperature, airflow rate, and humidity levels. Although higher temperature and airflow can increase heat and mass transfer, they can also damage the quality of food by causing case-hardening, nutrient loss, and flavor deterioration. Thus, there are other alternative methods available to achieve better quality. Freeze-drying is a superior method that maintains the quality of the food. This method relies on sublimation and operates at low-temperature, reduced-pressure conditions. The product is first frozen to -20 to -60 °C and then placed in a low-pressure chamber. Through freeze-drying, the structure of the food is persevered leading to a porous structure, and minimal loss of flavor and nutrients. Due to its ability to retain food quality, freeze-drying is widely regarded as the preferred method for drying of high-quality food (Mujumdar 2014). However, it is an energy-intensive and time-consuming method. As an alternative, microwave-vacuum drying uses microwave radiation and low pressure to dry products faster than freeze-drying. Unlike freeze-drying, which dries from the external part of the food to the internal, microwave-vacuum drying generates energy within the food matrix (volumetric or in-out), the food is particularly heated where the water is present, which causes the water to diffuse out to the surface as vapor. As a result of its fast-drying rate, microwave-vacuum drying presents an attractive alternative to freeze-drying (Scaman et al. 2014).

*Fucus vesiculosus* and *Ulva* sp. are interesting from a food perspective due to their unique composition of essential nutrients and bioactive compounds (Burtin 2003). These two seaweed species grow as wild populations in the Danish inner waters and are of industrial interest. Consequently, it is critical to characterize how post-harvest processing affects the food quality such as the nutrients and bioactive compounds.

The aim of this study was to investigate the effect of microwave-vacuum drying, freeze-drying and convection drying of the two species *Ulva* sp. and *F. vesiculosus*. The quality of the dried seaweeds was compared with regards to color, sensory (flavor, texture, appearance, and taste), moisture, minerals, protein, amino acid and fatty acid profiling, pigments, antioxidant and water holding and binding capacity. The assessment resulted in scientific insight into the drying methods, and their effects on the seaweed food

quality which is crucial for also industrial drying and use of seaweed.

## Materials and methods

### Seaweed material and experimental design

Two seaweed species were harvested from wild populations in October 2020 in Danish inner waters. The brown seaweed *Fucus vesiculosus* was harvested by Hansen & Lindstrøm ApS in Juelsminde, Denmark (N55°70′ E10°03′) and the green seaweed *Ulva* sp. was harvested by Dansk Tang ApS in Isefjord, Denmark (N55°56′ E11°46′). The seaweeds were run through a vegetable chopper (Kronen, KG 253, 0.65 KW, volume: 3.2 L) with the seaweed going through a disc cutter size 2 cm x 2 cm. All seaweed material was stored at -20 ºC until further processing (two weeks for convective air drying (CVD) and freeze drying (FD), and 2.5 months for microwave vacuum drying (MVD)). Each batch of drying was 1.5 kg wet weight.

The convection drying was carried out at an industrial setup at Hansen & Lindstrøm ApS (designed by Hansen & Lindstrøm ApS, Denmark). The seaweed or product was thawed and the excess water was removed. The product was dried with convective airflow (52 °C, 11 % relative humidity). The thickness of the biomass was 3 cm at beginning, and rotated by hand every 20 min. *Ulva* sp. was dried for 70 min and *F. vesiculosus* for 68 min. For freeze-drying the product was frozen and the initial drying temperature of the product was -20 °C (the pressure in the chamber was 20 Pa), and at the end of drying, the product reached a final temperature of 20 °C. Microwave-vacuum drying was performed in a rotary drum with a pressure of 10 Pa (Püschner Microwave Vacuum Freeze Drier, model "µWaveVac0250fd", Germany) at Sintef, Trondheim, Norway. The initial temperature of the product was -40 ºC and reached a final temperature of 40 ºC. All drying methods were performed in triplicates (i.e., done three times).

### Physicochemical properties of *Ulva* sp. and *F. vesiculosus*

The physicochemical properties such as water activity ($a_w$), color, water absorption, and water holding capacity were investigated for the two seaweed species after drying.

**Water activity** Water activity ($a_w$) was measured by using a water activity meter (Aqua Lab model 4TE, Decagon devices Inc., USA).

**Color measurement** Color was measured by a Chroma meter (CR-200, Konica Minolta, Japan) recording the CIE L* a* b* color scale. Approximately 5 g of samples were added to a Petri dish with a white surface (L*=89.0, a*=-4.01, b*=4.27) underneath. The samples were measured at five random locations for each sample.

**Water absorption and water holding capacity** Water absorption was quantified by mixing ground dry samples (0.2-0.3 g) with 10 mL distilled water by vortex mixing in a 15 mL centrifuge tube. The analysis was performed in duplicates. The tubes were incubated at room temperature overnight (22 h). Water absorption was calculated by decanting excess water and using Eq. 1:

$$Water\ absorption\ =\ \frac{(m_{SW}+m_p-m_0)}{m_0} \quad (1)$$

where $m_{sw}$ is the mass of the seaweed and the water that has been absorbed by the seaweed, $m_p$ is the mass of the particles lost when decanting excess water and $m_0$ is the initial mass of the ground seaweed samples.

Water holding capacity was determined by centrifugation by applying the tubes containing the swelled seaweed at 3,000 x*g* for 20 min and then decanting excess water, and using eq. 2:

$$Water\ holding\ capacity\ =\ \frac{(m_c-m_0)}{m_0} \quad (2)$$

where, $m_c$ is the mass of seaweed after centrifugation and decanting.

**Qualitative image analysis** Imaging of dried seaweed (flakes placed in 90 mm Petri dishes) was recorded with a VideometerLab2 device (Videometer A/S, Denmark). The camera was calibrated by three plates: a white for reflectance correction, a dark for background correction, and a dotted plate for pixel position calibration.

### Chemical composition of *Ulva* sp. and *F. vesiculosus*

All chemical analyses were carried out in duplicate (n = 2) unless otherwise stated, and results reported as means ± standard deviation (SD). The moisture content and ash concentration were determined gravimetrically, according to (AOAC 938.08, 1990).

**Pigment composition** Methanolic extracts were obtained as described in Safafar et al. (2015) using 50 mg dry sample to 5 mL pure methanol. The extracts were analyzed for pigments using high performance liquid chromatography (HPLC) (Agilent 1,100 Liquid Chromatograph) with diode array detector (DAD) (Agilent Technologies, USA). Separation was carried out on a Zorbax Eclipse C8 column 150 mm × 46 mm × 3.5 µm (Phenomenex Inc., USA) at

60 °C. The mobile phase was a mixture of 75 % methanol + 25 % of 0.028 M tertiary butyl ammonium acetate in water and methanol at a flow rate of 0.9 mL $min^{-1}$ with a total acquisition time of 40 min. DHI pigment standard mix (DHI LAB Products, Denmark) was used for the identification of peaks. Detection of chlorophylls and carotenoids was carried out at 660 nm and 440 nm, respectively, and for internal standard (BHT) at 280 nm. Pigments are reported as mg $g^{-1}$ of the extract.

**Amino acids and protein content** To quantify total amino acids, 30 mg of the dried sample was hydrolyzed with 6 M HCl at 110 °C for 18 h. To measure the free amino acids, dried samples weighing around 50 mg were mixed vigorously (votex for 10 s) with 1 mL of 5 % trichloroacetic acid and left overnight at a temperature of 5 °C. The next day the samples were centrifuged at 5,000 ×g for 5 min at room temperature (approximately 21 °C). The process of derivatization and chromatography for both total and free amino acids was carried out in accordance with the methodology outlined by Bak et al. (2019).

The calculation of the total protein content followed the approach recommended by Angell et al. (2016), which involved adding up the total moles of amino acids and subtracting the mass of water (18 g $H_2O$ $mol^{-1}$ amino acid) that was released during the acid hydrolysis (Diniz et al. 2011). To access the quality of the amino acids the EAA ratio was determined as the sum of EAA divided by the total AA found in the sample.

**Fat content and fatty acid composition** Lipid phase extraction and fat quantification were performed on the dry seaweed powder according to the method by Bligh and Dyer (1959) with minor changes. Briefly, 2 g of dried homogenized samples were added to 10 mL distilled water, 30 mL methanol, and 15 mL chloroform and homogenized for 30 s, followed by 30 s homogenization with addition of 15 mL chloroform and then a 30 s homogenization with 15 mL distilled water. The mixture was centrifuged at 2,800 rpm for 10 min. Afterwards, the water and methanol phases were discarded. A known amount of the chloroform phase was added to a glass container and left to evaporate overnight in a fume hood. The following day the container was weighed, with the remaining content representing the fat content of the sample.

The extraction and quantification of the fatty acid methyl esters (FAME) were performed as described by Jacobsen et al. (2022).

**Calculation of carbohydrates** The total carbohydrate content was determined using the "carbohydrate by difference" method. Specifically, the calculation was performed as follows:

$$Total\ carbohydrates(\%) = 100 - (protein + lipid + ash + water)\%$$

## Antioxidant capacity of *F. vesiculosus*

In brief, methanolic extracts were obtained by weighing approximately 10 mg of the dry *F. vesiculosus* powder in a centrifugation tube and adding 10 mL of methanol.

The content of potentially antioxidant phenolic compounds was estimated by determining the total phenolic content (TPC) on the methanolic extracts. The methodology was modified from Farvin and Jacobsen (2013) and carried out as follows: the methanolic extract was diluted x10 prior to analysis. To 100 µL of diluted extract 0.75 mL Folin Ciolcalteu phenol reagent (10 % *v/v* in water) was added and mixed. After 5 min, 0.75 mL sodium-carbonate solution (7.5 % $Na_2CO_3$ *w/v* in water) was added and mixed. The reaction was incubated for 90 min at room temperature (dark). The absorbance was measured at 725 nm by a UV-vis spectrophotometer. Gallic-acid (2,3,4-trihydrobenzoic acid) was used for quantification (calibration curve: 0-250 µg $mL^{-1}$). The results are expressed in gallic acid equivalents (GAE) (µg GAE $g^{-1}$ dw). Analysis was carried out in triplicates (n = 3).

The radical scavenging capacity of the methanolic extracts was quantified using 2,2-diphenyl-1-picrylhydrazyl (DPPH), by applying the method described by Yang et al. (2008) modified for use in a 96-well microplate. 100 µL extract solution (8 different dilutions of the extract) and 100 µL 0.1 mM DPPH (in 96 % ethanol) were mixed in the microtiter plate. Reaction mixtures were incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm using a microplate reader (BioTek Eon, BioTek Instruments Inc., USA) and Gen5 2.09 data analysis software. BHT was included as a positive control (63 % inhibition in a concentration of 0.2 mg $mL^{-1}$). $EC_{50}$ values were calculated (efficient concentration to obtain 50 % inhibition) by linear regression (Y=50) and expressed as mg dw $mL^{-1}$. The analysis was carried out in triplicates (n = 3).

## Sensory analysis of *Ulva* sp. and *F. vesiculosus*

The sensory characterization of the different sensory attributes was performed with an objective sensory panel at DTU Food in a sensory lab that fulfills the international standards and guidelines for the design and construction of sensory assessment rooms (ISO 8589, 2007; NMKL Procedure No. 6, 2023). The assessors in the sensory panel were tested and trained according to ISO 8586 (2012) and ISO 13299 (2016).

The first sessions were used to develop a vocabulary to describe the sensory characteristics describing the attributes of appearance, smell, taste, and texture of the samples.

Furthermore, the panel was trained to measure the intensity of each attribute on an unstructured 15 cm line scale with anchor points at 1.5 cm and 13.5 cm. The dried seaweed samples were served in petri dishes. All samples were served in random order and the assessors were served peeled cucumber and water to clean their mouths between samples.

The final vocabulary was: Appearance: Thickness, Crumpled, Transparency, Uniform color. Odor: Sea, Seaweed, Green/hay, Fresh fish. Flavor: Seaweed, Sweet, Umami, Salty, Metal, Bitter, Green. Texture: Crispy, Firm, Clotted, Astringency, Adhesiveness.

### Statistics and data treatment

Data analyses and statistics were performed using the R software (R-Core-Team 2022). Analysis of variance (ANOVA) was conducted to assess differences between the three drying methods. The homogeneity of variance was tested using Levene's test. In the event of significant differences, a Tukey's post hoc test was carried out to identify significant differences between samples at a 5 % level of significance ($p \leq 0.05$). Principal component analysis (PCA) was performed on standardized data for all variables (free and total amino acids, pigments, sensory, and fatty acids) and resulted in a score plot. Moreover, $EC_{50}$ values were determined and EAA ratios were calculated.

## Results and discussion

### Macronutrients

The compositions of macronutrients were quantified for the two types of seaweed after convection drying (CD), freeze drying (FD) and microwave vacuum drying (MVD) (Table 1). Drying of *F. vesiculosus* led to a final water content between 8.4-11.0 % wet weight (w.w.) and with no significant differences between the drying methods (ANOVA: $F(2,6) = 5.0$, $p = 0.053$). However, for *Ulva* sp. freeze drying led to a significantly lower water content compared to the other drying methods (ANOVA: $F(2,6) = 31$, $p < 0.001$). Moreover, the texture of *Ulva* sp. was obviously affected, as the sensory panel rated the firmness and crispiness higher for the convective dried *Ulva* sp. compared to the freeze dried (Fig. 2).

During drying, the phenomena of case-hardening can occur when the outer surface of a material dries out before the inner part, reaching the glassy state much faster than the core leaving more water trapped inside (Mujumdar 2014). Boateng and Yang (2021) showed that oven drying of *Gingo biloba* seeds lead to increased hardening of the surface and decreases moisture transfer, thus reducing the drying of the samples, compared to freeze drying. Case-hardening also affects the texture with a harder crust of the product. However, for the brown seaweed *Saccharina latissima* Sappati et al. (2017) did not find case-hardening during drying, but that study was performed as drying kinetics at laboratory scale based on thin layer drying. However, the higher dry matter for CD and MVD of *Ulva* sp., could be explained by case-hardening. Since, the pieces of *Ulva* stuck together at e.g., CD, to form lumps instead of thin pieces of *Ulva* the possible explanation of case-hardening was introduced. However, additional experiments of glass transition are needed to confirm or reject this.

The fat content of *Ulva* sp. was significantly higher when using MVD compared to the other drying methods (ANOVA: $F(2,6) = 32$, $p < 0.001$). Moreover, the fat content of *F. vesiculosus* was significantly higher when using CD and MVD (ANOVA: $F(2,6) = 6.2$, $p = 0.035$). Microwaves are a well-known assisted extraction technology for increasing the extraction yield of lipids (Zhou et al. 2021). Microwaves break the cells and make the lipids more accessible for extraction resulting in a higher yield, as indicated by the higher fat content when using MVD of *Ulva* sp. compared to FD, which is a gentler drying technique and might leave some fat embedded in the cell structure after lipid extraction.

**Table 1** Composition of water (% ww) and the other macronutrients (ash, protein (based on amino acids), fat, and carbohydrates) per dry weight (% dw) for *Fucus vesiculosus* and *Ulva* sp. dried by convection (CD), freeze-drying (FD) or microwave-vacuum drying (MVD)

| Species | Drying method | Water (% ww) | Ash (% dw) | Protein (% dw) | Fat (% dw) | Carbohydrates (% dw) |
|---|---|---|---|---|---|---|
| *F. vesiculosus* | CD | $11\pm0.8^{a}$ | $15\pm0.7^{b}$ | $1.1\pm0.1^{a}$ | $4.1\pm0.2^{a}$ | 68.8 |
| | FD | $8.9\pm1.4^{a}$ | $19\pm2.5^{a}$ | $1.2\pm0.1^{a}$ | $3.5\pm0.3^{b}$ | 67.4 |
| | MVD | $8.4\pm0.8^{a}$ | $17\pm0.4^{a,b}$ | $1.6\pm0.1^{a}$ | $4.1\pm0.1^{a}$ | 68.9 |
| *Ulva* sp. | CD | $11\pm1.0^{y}$ | $15\pm0.9^{z}$ | $6.1\pm0.1^{x}$ | $1.5\pm0.2^{x}$ | 66.4 |
| | FD | $5.1\pm1.4^{x}$ | $26\pm0.8^{x}$ | $5.8\pm0.2^{x}$ | $1.7\pm0.1^{x}$ | 61.4 |
| | MVD | $11\pm0.6^{y}$ | $20\pm1.6^{y}$ | $5.4\pm0.1^{y}$ | $2.2\pm0.1^{y}$ | 61.4 |

All data is represented by the average ± standard deviation (n=3). The superscript letters represent significant differences between the drying methods.

The higher fat content of *F. vesiculosus* when using both CD and MVD indicates that CD has the same cell-breaking effect as MVD however only on *F. vesiculosus* and not on *Ulva* sp.

Protein content of *F. vesiculosus* was not significantly affected by the different drying methods ($p = 0.17$). The protein content of *Ulva* sp. was significantly lower ($p = 0.0017$) when using MVD compared to CD and FD, indicating that microwaves might affect the protein content and thereby also the amino acids (protein based on total amino acid). In a previous study by Xiang et al. (2020) the effect of microwaves on protein structure and browning reactions was discussed. The heat from microwaves could cause amino acids to react with reducing sugars forming Maillard products. If this is the case, the amino acid content will decrease and thereby the protein content of *Ulva* sp. dried by MVD will also decrease. Maillard reactions in the seaweed during drying would also result in browning of the seaweed. However, this reaction is dependent on the drying conditions, such as time and temperature. The maximum temperature during MVD was 40 °C, which was lower than CVD (52 °C).

The protein contents in both *F. vesiculosus* and *Ulva* sp. are very low compared to other studies. *Ulva* sp. usually has a protein content between 10 and 25 % d.w. (Fleurence 1999), but the protein content in seaweed varies with season (Bak et al. 2019). Juul et al. (2022) reported a total amino acid (TAA) content of freeze-dried *Ulva* sp. of 9.3 % of the dry matter. A review found that the protein content of *F. vesiculosus* would vary from 1-11 % d.w. and the fat content would be 1.2-4.0 % d.w. (Catarino et al. 2018). Carbohydrate content was calculated based on the content of ash, water, protein, and fat. The reported content of carbohydrates varies between 15 and 65 % (d.w.) for *Ulva* sp. and 62-66 % for *Fucus* sp. (Rioux and Turgeon 2015). This is less than what was found in the present study. The calculations of total carbohydrates by difference is highly dependent on the content of other food components such as protein, and does also include fibers and other compounds, which are not strictly carbohydrates. Hence, this method to determine carbohydrates have some uncertainties and that is possibly, why this variety was observed.

## Physico-chemical properties

Food materials with water activity ($a_w \leq 0.25$) are considered dry, powdery, and chemically stable, except for lipid oxidation. They have a lack of molecular mobility, which hinders biological processes, making them highly stable with respect food safety (Sikorski et al. 2007). According to Table 2, all the samples were on the border of this threshold except freeze-dried *Ulva* sp. The water activity of the final dried products followed the water content also in terms of the statistics. Also, here the freeze-dried *Ulva* sp. reached significantly lower water activity compared to the others (ANOVA: $F(2,6) = 32$, $p < 0.001$). This is well below the threshold of 0.25 and suggests that freeze-drying of *Ulva* sp. is very efficient and might remove some of the bound water in this seaweed.

For *F. vesiculosus,* the water holding capacity (ANOVA: $F(2,15) = 1.4$, $p = 0.27$) and water absorption (ANOVA: $F(2,15) = 1.9$, $p = 0.18$) did not differ between the drying methods. Interestingly, *Ulva* sp. could reabsorb water 10-12 times its weight and hold 6.0-8.0 times its weight; this probably due to the way that *Ulva* fronds trap the water when it is packed flat and potentially also the higher protein content of *Ulva* compared to *F. vesiculosus*. These numbers are supported by Jannat-Alipour et al. (2019), who found the water holding capacity to be 9.5 for 60 °C convection dried *Ulva intestinalis* and utilized this property for surimi products.

The visual appearance of seaweed products after three different drying methods was qualitatively evaluated by examining all replicates ($n$=3) of each drying method. The products were photographed (Fig. 1). Differences in color between the products were observed, with CD resulting in a darker product for both species. This is backed up by color measurements in Table 3, which showed that for *F. vesiculosus*, the lightness (L*) was significantly different, with CD resulting in the darkest color, followed by MVD (ANOVA: $F(2,42) = 34$, $p < 0.001$). For *Ulva* sp., CD resulted in a significantly darker product (ANOVA: $F(2,42) = 35$, $p < 0.001$), whereas the other measured did not differ. The observed color differences were likely due to temperature,

**Table 2** Physio-chemical properties (water activity, water holding capacity and water absorption) of *Fucus vesiculosus* and *Ulva* sp. dried by convection (CD), freeze drying (FD) or microwave-vacuum drying (MVD)

| Species | Drying method | Water activity | Water holding capacity (g water/g sample) | Water absorption (g water/g sample) |
|---|---|---|---|---|
| *F. vesiculosus* | CD | $0.31\pm0.05^{a}$ | $5.4\pm1.5^{a}$ | $6.9\pm0.3^{a}$ |
| | FD | $0.18\pm0.08^{a}$ | $4.6\pm0.6^{a}$ | $6.3\pm0.7^{a}$ |
| | MVD | $0.23\pm0.02^{a}$ | $4.5\pm0.7^{a}$ | $6.6\pm0.7^{a}$ |
| *Ulva* sp. | CD | $0.24\pm0.04^{y}$ | $8.0\pm0.7^{y}$ | $12\pm1^{x}$ |
| | FD | $0.069\pm0.031^{x}$ | $6.0\pm1.9^{x}$ | $10\pm3^{x}$ |
| | MVD | $0.27\pm0.03^{y}$ | $6.9\pm0.5^{xy}$ | $11\pm1^{x}$ |

All data is represented by the average ± standard deviation ($n$=3). The superscript letters represent significant differences between the drying methods

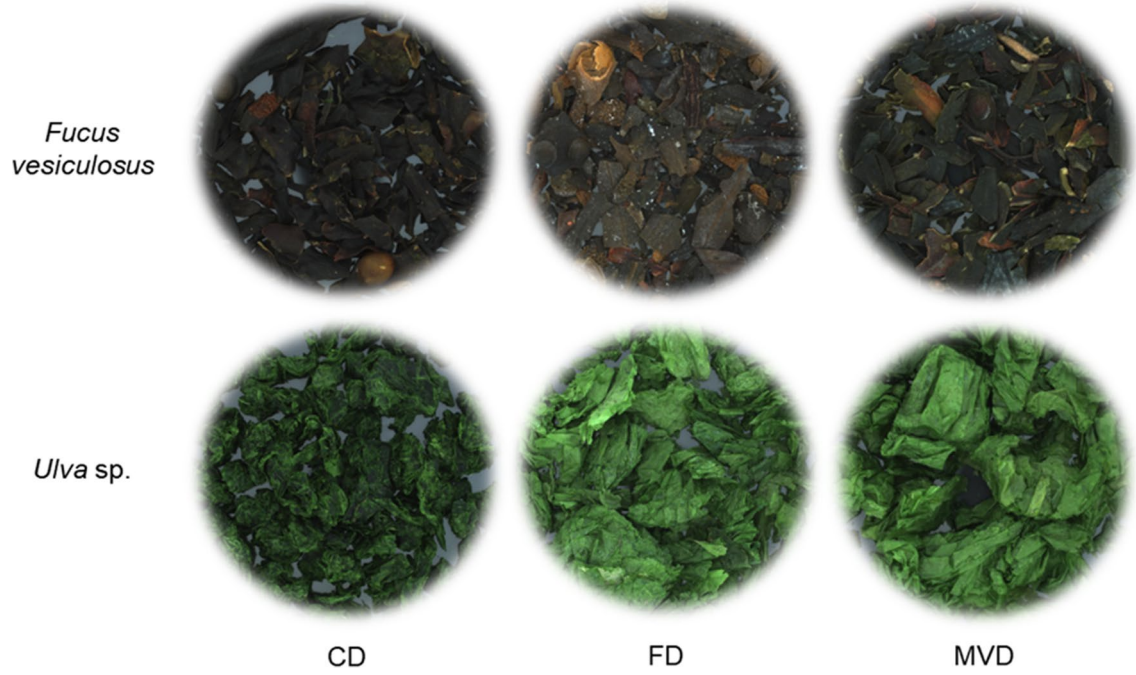


**Fig. 1** Qualitative visual inspection (using Videometer) of *F. vesiculosus* and *Ulva* sp. after drying by convection (CD), freeze drying (FD) or microwave-vacuum drying (MVD)

**Table 3** Color (L*, a* and b*) of *Fucus vesiculosus* and *Ulva* sp. after drying by convection (CD), freeze drying (FD) or microwave-vacuum drying (MVD)

| | | L* | a* | b* |
|---|---|---|---|---|
| *F. vesiculosus* | CD | $30.8\pm2.4^{c}$ | $-1.74\pm0.27^{a}$ | $-0.36\pm1.23^{c}$ |
| | FD | $40.5\pm4.8^{a}$ | $-1.83\pm1.03^{a}$ | $5.77\pm3.23^{a}$ |
| | MVD | $34.7\pm1.89^{b}$ | $-2.84\pm0.69^{b}$ | $3.17\pm1.86^{b}$ |
| *Ulva* sp. | CD | $37.8\pm5.7^{y}$ | $-7.35\pm2.25^{y}$ | $4.34\pm3.63^{y}$ |
| | FD | $49.5\pm3.5^{x}$ | $-13.5\pm1.9^{x}$ | $14.3\pm3.2^{x}$ |
| | MVD | $47.87\pm2.8^{x}$ | $-14.0\pm1.5^{x}$ | $16.5\pm2.5^{x}$ |

L*= dark to light (0-100), a*=green (-) to red (+), b*=blue (-) to yellow (+). All data is represented by the average ± standard deviation ($n$=3). The superscript letters represent significant differences between the drying methods

with CD at 60 °C causing color changes and product shrinkage. Hence, the observation of the lost protein (Table 1) by MVD of *Ulva* sp. due to browning by Maillard reactions was not confirmed by changes in color. Oppositely, CD increased darkness of both *F. vesiculosus* and *Ulva* sp. but this did not compromise the proteins. Silva et al. (2019) found that convective drying at 60 °C would lead to color changes for *F. vesiculosus* whereas 25 and 40 °C would not.

## Sensory differences

The radar charts in Fig. 2 show the results of the sensory assessment. The attributes for *F. vesiculosus* (A) show a similar pattern for the three different drying methods. The firmness and crispiness have a lower intensity for the FD compared to the other two dried *F. vesiculosus*. For *Ulva* sp. (B) however, the patterns were not similar. In terms of texture, the CD dried *Ulva* sp. showed to be crispier and firmer, which was also seen by the qualitative visual inspection (Fig. 1). This is r with the possible case-hardening as casehardening often lead a quality decrease having a hard surface and a rubbery inner. More studies should be made to confirm this. The odors: seaweed, sea, and fresh fish had a higher intensity in the FD, possibly explained by the retention of flavor compounds due to the lower drying temperature.

## Changes in bioactive compounds

Once the changes in macronutrients, physicochemical and sensory differences for *F. vesiculosus* and *Ulva* sp. after drying using CD, FD and MVD were evaluated, the changes in the bioactive compounds were studied. Table 4 shows the composition of the following bioactive compounds in *F. vesiculosus* and *Ulva* sp.; Omega-3 and -6 fatty acids (18:2 n-6, 20:5 n-3), EAA ratio, pigments, and antioxidant capacity (only *F. vesiculosus*).

The most abundant essential amino acids (EAA) found in the two seaweed types were phenylalanine, leucine and tryptophane (only *Ulva* sp.). *Ulva* sp. had in general a 2-5 times higher content of EAAs compared to *F. vesiculosus*

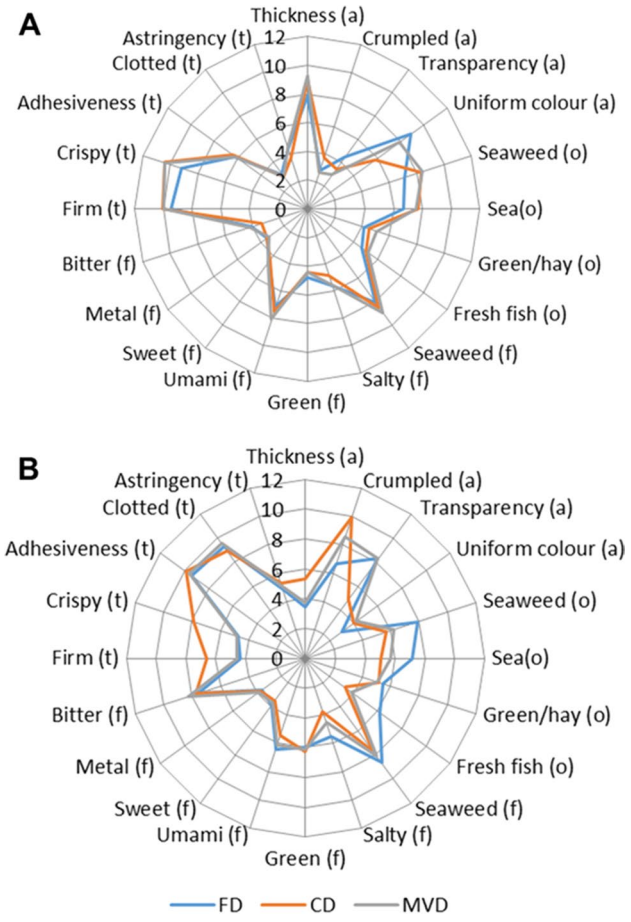


**Fig. 2** Intensity of the appearance (a), odor (o), flavor (f) and texture (t) measured by sensory profile (scaling 0 to 12) of *F. vesiculosus* (**A**) and *Ulva* sp. (**B**) after drying by convection (CD), freeze-drying (FD) or microwave-vacuum drying (MVD)

(data not shown). The essential amino acid ratio was also higher for *Ulva* sp. (46.8-47.3 %), compared to *F. vesiculosus* (35.3-37.8 %). The EAA ratios were comparable to animal-based proteins (whey 43 %, milk 39 %, casein 34 %, and egg 32 %), and higher than plant-based protein isolates such as oat (21 %), lupin (21 %), and wheat (22 %) (Gorissen et al. 2018).

Free aspartic and glutamic acid are associated with the taste of umami. In *F. vesiculosus,* the sum of these were 0.274 mg $g^{-1}$d.w. with no significant difference among the three drying methods ($p = 0.19$). This fits with the results from the sensory panel. For *Ulva* sp. the sum of the two free amino acids was significantly different (ANOVA: $F(2,6) = 25$, $p = 0.0012$), with the CD treated samples (0.177 ± 0.078 mg $g^{-1}$d.w.), being lower than the two others (0.496 - 0.539 mg $g^{-1}$d.w.), where the sensory panel also detected.

Whereas *F. vesiculosus* contained both eicosapentaenoic acid (22:5, n-3) (EPA) (4.5-4.9 % of the total lipids) and linolenic acid (18:2, n-6) (LA) (7.4-8.2 % of the total lipids), only LA was found in *Ulva* sp. (1.7-3.4 % of the total lipids). The results correlate with the review by Catarino et al. (2018), showing a LA content of 7.5-10.0 % of total lipids and an EPA content of 3.7-7.5 % in *F. vesiculosus*. For *F. vesiculosus*, the EPA content was not affected by CD or MVD compared to FD, however LA (18:2, n-6) content was significantly lower when CD or MVD were applied ($p < 0.05$). For *Ulva* sp. LA content was significantly higher ($p < 0.05$) using MVD compared to FD, but CD was similar ($p > 0.05$) to both FD and MVD.

Different types of carotenoids were found in the seaweeds. Beta-carotene was found in both with similar concentrations (20.3-28.8 µg $g^{-1}$ d.w.), whereas other carotenoids were specific for the species. *Fucus vesiculosus* showed high content of fucoxanthin, a xanthophyll associated with brown seaweed. Moreover, *Ulva* sp. contained lutein (11.1-22.0 µg $g^{-1}$ d.w.). Convection drying significantly decreased the content of beta-caroten of *Ulva* sp. (from 28.8 to 20.3 µg $g^{-1}$ d.w.) compared to FD, where MVD to a higher extend preserved this pigment (25.0 µg $g^{-1}$ d.w.). A similar trend was found for lutein in *Ulva* sp. On the other hand, both beta-carotene and fucoxanthin were highest in the samples dried by CD and MVD compared to FD, however only significantly for fucoxanthin. Uribe et al. (2019) described the effect of different drying methods (freeze-, vacuum-, solar-, and convective drying) on the quality of *Ulva* sp. (color, pigments, amino acids, and fatty acids among other). Color was not affected by any drying method and total flavonoid content (TFC), total carotenoids and antioxidant capacity (DPPH and ORAC) were higher in convective drying, which conflicts with our finding since only *F. vesiculosus* showed this.

The total phenolic content and DPPH radical scavenging capacity of *F. vesiculosus* was not affected by the drying. Silva et al. (2019) found that air-drying increased extraction of pigments but was negative for extraction of phenolic compounds.

To summarize how the different drying methods influenced the final quality of the two seaweed species, principal component analyses (PCA) are visualized in score plots in Fig. 3.

In the PCA score plot, it was observed that the quality of *F. vesiculosus* was affected differently by the three drying methods. The between-groups variance was larger than the within-group variance, as evidenced by both PC1 and PC2. In contrast, for *Ulva* sp., the differences in between-groups variances were not as pronounced as the within-group variance, especially for MVD and FD, indicating that these methods resulted in more similar products in terms of quality. On the other hand, CD differed from both MVD and FD.

**Table 4** Composition of the most abundant bioactive compounds in *Ulva* sp. and *Fucus vesiculosus*; Omega-3 and -6 (18:2 n-6, 20:5 n-3), EAA score, pigments, and antioxidant capacity (only *F. vesiculosus*). The drying methods abbreviated by: convection drying (CD), freeze drying (FD) or microwave-vacuum drying (MVD)

| | | Fatty acid, omega 3 and 6 (% of total FA) | | Essential amino acid ratio (%) | Carotenoids (μg $g^{-1}$ dw) | | Antioxidant capacity | |
|---|---|---|---|---|---|---|---|---|
| | | Linolenic acid (18:2, n-6) | Eicosapentaenoic acid (20:5, *n*-3) | EAA ratio | Fucoxanthin | Beta-carotene | TPC (μg GAE $g^{-1}$dw) | DPPH radical scavenging $EC_{50}$ (mg $mL^{-1}$) |
| ***F. vesiculosus*** | CD | 7.6±0.1[a] | 4.9±0.4[a] | 36.6±5.8 | 209±20.8 [a] | 24.0±3.4 [a] | 20.3±3.0[a] | 0.7±0.0[a] |
| | FD | 8.2±0.3 [b] | 4.5±0.2[a] | 35.3±1.0 | 117±4.0 [b] | 22.5±2.8 [a] | 22.8±1.0[a] | 0.4±0.0[a] |
| | MVD | 7.4±0.2[a] | 4.9±0.2[a] | 37.8±0.4 | 228±1.7 [a] | 26.2±1.0 [a] | 24.3±4.9[a] | 0.3±0.0[a] |
| | | | | | Lutein | Beta-carotene | | |
| ***Ulva* sp.** | CD | 1.7±0.2 [xy] | *nd* | 47.1±0.7 | 11.1±0.8 [x] | 20.3±1.9 [x] | | |
| | FD | 2.7±0.8 [x] | *nd* | 46.8±0.7 | 22.0±1.9 [y] | 28.8±3.9 [y] | | |
| | MVD | 3.4±0.3 [y] | *nd* | 47.3±1.6 | 19.7±3.9 [y] | 25.0±3.7 [xy] | | |

The superscript letters indicate statistically significance (ANOVA; $p < 0.05$; $n$=3) within column and seaweed species. *nd* = not detected

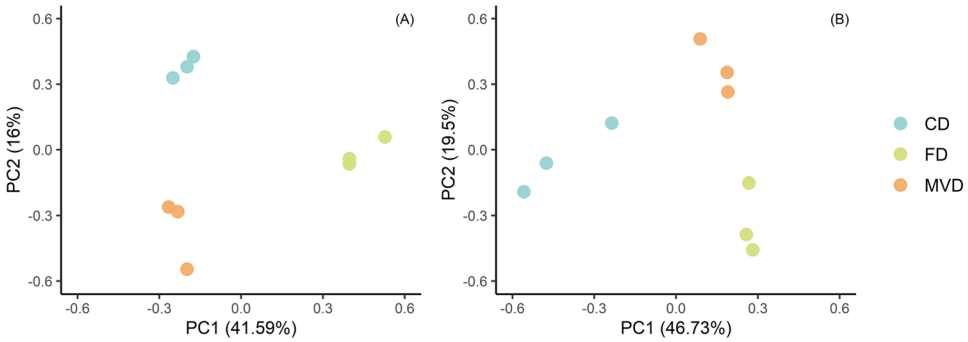


**Fig. 3** PCA score plots based on all variables (free and total amino acids, pigments, sensory, and fatty acids) for *F. vesiculosus* (**A**), and *Ulva* sp. (**B**). The colors represent the different drying methods. CD: convective drying, FD: freeze drying, and MVD: microwave-vacuum drying

## Conclusion

Overall *Ulva* sp. and *F. vesiculosus* were affected differently by the drying methods, indicating that differentiation in the drying method between seaweed species is necessary to obtain the optimal quality of the final product regarding sensory, nutritional, physicochemical properties and bioactive compounds. According to the summary (Fig. 3), it can be concluded that drying methods have a species-dependent influence on the quality. For *Ulva* sp., FD and MVD are similar and can be chosen based on factors such as energy consumption, while for *F. vesiculosus*, the selection of a drying method should be based on the desired food quality due to significant variations between drying methods.

**Acknowledgements** We thank the laboratory technicians Rie Sørensen and Inge Holmberg for their assistance and contributions to our research. Moreover, we thank the companies Hansen & Lindstrøm Tang ApS and Dansk Tang ApS for providing us with seaweed material and performing drying.

**Authors' contributions** All authors were involved in the idea-making and design of the study. Cecilie, Susan and Grethe performed sample preparation, the detailed planning, and the experiment with the drying company. Ditte, Cecilie and Grethe performed analyses of the data. All authors performed data evaluation and writing of report.

**Funding** Open access funding provided by Technical University of Denmark. This research was funded through the Innobooster program (Innovation Fund Denmark, 0172-00899B). Funding received by the Hansen & Lindstrøm Tang ApS, and Joint Alliance PhD program, Technical University of Denmark (DTU), and Norwegian University of Science and Technology (NTNU).

**Data availability** Datasets used in the manuscript can be accessed by written request to the corresponding author.

## Declarations

**Competing interests** The authors declare no competing interests.

## References

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

AOAC 938.08 (1990) AOAC Method 938.08 Official methods of analysis (Issue 16). Association of Official Analytical Chemists, Washington, DC

Arab-Tehrany E, Jacquot M, Gaiani C, Imran M, Desobry S, Linder M (2012) Beneficial effects and oxidative stability of omega-3 long-chain polyunsaturated fatty acids. Trends Food Sci Technol 25:24–33

Bak UG, Nielsen CW, Marinho GS, Gregersen Ó, Jónsdóttir R, Holdt SL (2019) The seasonal variation in nitrogen, amino acid, protein and nitrogen-to-protein conversion factors of commercially cultivated Faroese *Saccharina latissima*. Algal Res 42(101576)

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem 37:911–917

Boateng ID, Yang X-M (2021) Effect of different drying methods on product quality, bioactive and toxic components of *Ginkgo biloba* L. seed. J Sci Food Agric 101:3290–3297

Burtin P (2003) Nutritional value of seaweeds. Electron J Environ Agric Food Chem 2:498–503

Catarino M, Silva A, Cardoso S (2018) Phycochemical constituents and biological activities of *Fucus* spp. Mar Drugs 16:249

Claussen IC, Ustad TS, Strømmen I, Walde PM (2007) Atmospheric freeze drying - A review. Drying Technol 25:947–957

Diniz GS, Barbarino E, Oiano-Neto J, Pacheco S, Lourenco SO (2011) Gross chemical profile and calculation of nitrogen-to-protein conversion factors for five tropical seaweeds. Am J Plant Sci 2:287–296

Farvin KHS, Jacobsen C (2013) Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. Food Chem 138:1670–1681

Figueroa V, Bunger A, Ortiz J, Aguilera JM (2022) Sensory descriptors for three edible Chilean seaweeds and their relations to umami components and instrumental texture. J Appl Phycol 34:3141–3156

Fleurence J (1999) Seaweed proteins: Biochemical, nutritional aspects and potential uses. Trends Food Sci Technol 10:25–28

Gorissen SHM, Crombag JJR, Senden JMG, Waterval WAH, Bierau J, Verdijk LB, van Loon LJC (2018) Protein content and amino acid composition of commercially available plant-based protein isolates. Amino Acids 50:1685–1695

Haugan JA, Liaaen-Jensen S (1994) Algal carotenoids 54. Carotenoids of brown algae (Phaeophyceae). Biochem Syst Ecol 22:31–41

Hermund DB, Plaza M, Turner C, Jónsdóttir R, Kristinsson HG, Jacobsen C, Nielsen KF (2018) Structure dependent antioxidant capacity of phlorotannins from Icelandic *Fucus vesiculosus* by UHPLC-DAD-ECD-QTOFMS. Food Chem 240:904–909

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

ISO 8586 (2012) Sensory analysis — General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors https://www.iso.org/standard/45352.html

ISO 8589 (2007) Sensory analysis — General guidance for the design of test rooms https://www.iso.org/standard/36385.html

ISO 13299 (2016) Sensory analysis — Methodology — General guidance for establishing a sensory profile https://www.iso.org/standard/58042.html

Jacobsen C, Warncke SA, Hansen SH, Sørensen A-DM (2022) Fish liver discards as a source of long-chain omega-3 polyunsaturated fatty acids. Foods 11:905

Jannat-Alipour H, Rezaei M, Shabanpour B, Tabarsa M (2019) Edible green seaweed, *Ulva intestinalis* as an ingredient in surimi-based product: chemical composition and physicochemical properties. J Appl Phycol 31:2529–2539

Juul L, Stødkilde L, Ingerslev AK, Bruhn A, Jensen SK, Dalsgaard T K (2022) Digestibility of seaweed protein from *Ulva* sp. and *Saccharina latissima* in rats. Algal Res 63:102644

Karthik R, Manigandan V, Sheeba R, Saravanan R, Rajesh PR (2016) Structural characterization and comparative biomedical properties of phloroglucinol from Indian brown seaweeds. J Appl Phycol 28:3561–3573

Li Y, Qian Z-J, Ryu B, Lee S-H, Kim M-M, Kim S-K (2009) Chemical components and its antioxidant properties in vitro: An edible marine brown alga *Ecklonia cava*. Bioorg Med Chem 17:1963–1973

Mohamed S, Hashim SN, Rahman HA (2012) Seaweeds: A sustainable functional food for complementary and alternative therapy. Trends Food Sci Technol 23:83–96

Mujumdar AS (2014) Handbook of industrial drying, 4th edn. CRC Press, Boca Raton

Murata M, Nakazoe J (2001) Production and use of marine aigae in Japan. Jpn Agric Res Q: JARQ 35:281–290

NMKL Procedure No. 6 (2023) General guidelines for quality assuring sensory laboratories. NMKL Procedure No. 6, 2023. NMKL Bergen Norway

R-Core-Team (2022) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://www.r-project.org/

Rioux L-E, Turgeon SL (2015) Seaweed carbohydrates. In: Tiwari BK, Troy DJ (Eds) Seaweed Sustainability. Food and non-food applications, Elsevier, Amsterdam, pp 141–192

Safafar H, Van Wagenen J, Møller P, Jacobsen C (2015) Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. Mar Drugs 13:7339–7356

Sappati PK, Nayak B, van Walsum GP (2017) Effect of glass transition on the shrinkage of sugar kelp ( *Saccharina latissima* ) during hot air convective drying. J Food Eng 210:50–61

Scaman CH, Durance TD, Drummond L, Sun D-W (2014) Combined microwave vacuum drying. Emerg Technol Food Proc 427–445

Sikorski ZE, Pokorny J, Damodaran S (2007) Physical and chemical interactions of components in food systems. In: Fennema OR, Damodaran S, Parkin K (eds) Fennema's Food Chemistry. CRC Press, Boca Raton, pp 849–884

Silva AFR, Abreu H, Silva AMS, Cardoso SM (2019) Effect of oven-drying on the recovery of valuable compounds from *Ulva rigida*, *Gracilaria* sp. and *Fucus vesiculosus*. Mar Drugs 17:90

Stahl W, Sies H (2003) Antioxidant activity of carotenoids. Mol Aspects Med 24:345–351

Uribe E, Vega-Gálvez A, García V, Pastén A, López J, Goñi G (2019) Effect of different drying methods on phytochemical content and amino acid and fatty acid profiles of the green seaweed *Ulva* spp. J Appl Phycol 31:1967–1979

Wirenfeldt CB, Sørensen JS, Kreissig KJ, Hyldig G, Holdt SL, Hansen LT (2022) Post-harvest quality changes and shelf-life

determination of washed and blanched sugar kelp (*Saccharina latissima*). Front Food Sci Technol 2:1030229

Xiang S, Zou H, Liu Y, Ruan R (2020) Effects of microwave heating on the protein structure, digestion properties and Maillard products of gluten. J Food Sci Technol 57:2139–2149

Yamaguchi S (1991) Basic properties of umami and effects on humans. Physiol Behav 49:833–841

Yang J, Guo J, Yuan J (2008) In vitro antioxidant properties of rutin. LWT - Food Sci Technol 41:1060–1066

Zhou X, Ding W, Jin W (2021) Microwave-assisted extraction of lipids, carotenoids, and other compounds from marine resources. In: Garcia-Vaquero M, Rajauria G (Eds) Innovative and Emerging Technologies in the Bio-marine Food Sector. Applications, regulations, and prospects. Elsevier, Amsterdam, pp 375–394