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Effects of drying on the nutrient content, physico-chemical and sensory characteristics of the edible kelp Saccharina latissima

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Effects of drying on the nutrient content, physico-chemical and sensory characteristics of the edible kelp *Saccharina latissima*

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Abstract

The effects of convective air-drying at 25, 40 and 70°C and freeze-drying on the quality of the edible kelp *Saccharina latissima* to be used for food was investigated. Based on the analysis of the carbohydrate and amino acid profiles, as well as polyphenol, fucoxanthin and ash contents, no significant differences were detected among sample groups and air-drying up to 70°C results in equally nutritious products at shorter processing times. Only the iodine content was found lower in freeze-dried compared to air-dried samples. The swelling capacity of the air-dried samples was significantly lower than in freeze-dried samples, particularly at high temperatures (40 and 70°C), reflecting alteration of the physico-chemical properties of the seaweed during air-drying (attributed to product shrinkage) and reduced capacity of the final product to rehydrate. Structural differences between air-dried products at 25 and 70°C may explain the differences in mouthfeel perception (dissolving rate) among the two sample groups observed during a sensory evaluation. Overall the drying temperature within this range did not alter the aroma (i.e. odor) nor the flavor intensity of the product. In food applications where the product's mechanical properties (e.g. porosity) are essential, freeze-drying, and to a lesser extent air-drying at low temperatures, will result in higher quality products than air-drying at higher temperatures.

Key words: air-drying; freeze-drying; nutrients; physico-chemical properties; seaweed; sensory

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Introduction

Seaweeds have been used for centuries in Asian cuisine for their nutritional properties as well as for their rich and unique flavors. In Western countries, macroalgae have not been a significant food source throughout history and industrial applications have long been limited to the extraction of phycocolloids (alginate, agar, carrageenan) for the food industry. Seaweeds belong to a diverse group of photosynthetic marine plants, with a variable chemical composition depending on species, season and habitat, and the nutritional value of several species along with their health benefits have been reviewed (Holdt and Kraan 2011; Déléris et al. 2016). Most species are characterized by high levels of dietary fibers and minerals, and low lipid levels (MacArtain et al. 2007; Dawczynski et al. 2007). Their protein composition (Fleurence 2004; Dawczynski et al. 2007; Mæhre et al. 2014) and antioxidant activities, associated to their content in polyphenolic compounds (Wang et al. 2012) and pigments (e.g. fucoxanthin) (Fung et al. 2013) make seaweed an attractive raw material for the provision of bioactive substances with a broad range of applications, especially in human and animal nutrition. In addition to their nutritional benefits, edible seaweeds, including common species along the coast of Europe, have both flavor enhancing (Mouritsen et al. 2012; Chapman et al. 2015; Mouritsen 2016) and physico-chemical properties (texture, water- and fat binding properties, color) (Cofrades et al. 2008; Chapman et al. 2015) that can be applied to the field of gastronomy and to the food industry. Hence, seaweeds can be included in a wide range of foodstuffs and are increasingly recognized as versatile and delicious whole foods, promoted by health food trends and the use of locally available natural ingredients.

In Europe where the potential of seaweeds in various industrial applications has triggered the interest to cultivate biomass, a number of commercial initiatives have emerged in recent years (Stévant et al. 2017c). Large-scale seaweed cultivation largely focuses on kelp species, especially *Saccharina latissima*, due to its phytochemical content and ability to achieve high biomass yields in short time. Moreover, this species, which is closely related to the Japanese *konbu* (*Saccharina japonica*), is prized for its flavor as well as high levels of potassium compared to sodium salts, with potential as salt replacing ingredient in the food industry resulting in healthier mineral profiles in manufactured food products (Rioux et al. 2017). On the other hand, the particularly high iodine content of *S. latissima* (Stévant et al. 2017a; Roleda et al. 2018), could have negative consequences on human health, especially in sensitive individuals, if large amounts of this seaweed are ingested regularly over an extended period (Miyai et al. 2008). However, these levels can be reduced by processing in the perspective of an extensive use in the food industry (Lüning and Mortensen 2015; Stévant et al. 2017a). Although product development from cultivated seaweeds is still limited, products with relatively high market value such as foods and food ingredients, are predicted to play an important role in creating value from farmed seaweeds (Stévant et al. 2017c).

Kelp species are characterized by a high moisture content and rapid microbial decomposition once harvested (Enríquez et al. 1993) thus require adequate pre-treatments to maintain product quality and ensure consumer safety. Although several alternatives are available (e.g. salting, freezing), drying is the preferred method for stabilizing seaweed biomass for long-term storage. However, the effects of preservation treatments including drying, on the quality parameters of seaweed biomass is a major question which has only partially been studied. Previous studies on the pre-treatment of the brown macroalgae *Sargassum* spp. suggest that freeze-drying is the most appropriate drying method providing products with higher nutrient content when compared to convective air-drying methods (Chan et al. 1997; Wong and Cheung 2001a). Generally, due to the absence of liquid water and to the low temperatures during the process of freeze-drying biomaterials, the rate of most reactions responsible for the product deterioration are very low, resulting in high quality products (Bonazzi and Dumoulin 2011). On the other hand, freeze-drying is associated with high equipment and operation costs, along with slow drying rates, making this technology less attractive than conventional convective air-drying in commercial settings. The effects of certain drying conditions, e.g. temperature, on specific compounds or characteristics of some seaweed species are reported. Generally, higher drying temperatures lead to a reduction in phytochemical substances such as phenolic compounds (Moreira et al. 2016) and pigments (Tello-Ireland et al. 2011) together with modifications of the physico-chemical (Tello-Ireland et al. 2011; Sappati et al. 2017) and sensory properties (Michel et al. 1997) of the seaweed products. However, systematic knowledge on the effects of drying treatments on the overall quality of edible kelps of commercial importance, including *S. latissima*, is still missing.

The objective of this study is to characterize and compare the quality of *S. latissima* stabilized by different drying methods, i.e. convective air-drying (referred to as air-drying) at different temperatures compared to freeze-drying. Quality was defined as the nutrient content determined by the analysis of bioactive substances including proteins and amino acids, mineral fraction, carbohydrates, polyphenol and fucoxanthin pigment in the dried products. In food applications, the sensory and physico-chemical characteristics (i.e. water and fat binding properties, swelling) along with the product's appearance are important factors determining consumer acceptance. Hence, these parameters are included in the comparative quality assessment of *S. latissima* following different drying treatments. Understanding the behavior of the seaweed biomaterial is a key to develop processing strategies that will maximize the quality of the products to be used as food ingredients and as raw material for the provision of valuable compounds.

Materials and methods

Biomass harvest and drying treatment

Samples of *S. latissima* were harvested from SINTEF's cultivation site, off the coast of Hitra in Norway on May 18 and 19, 2016. Batches of 25 kg seaweed biomass were stored in airtight and refrigerated containers during transport to the laboratory where simultaneous drying experiments were conducted, i.e. air-drying at 25, 40 and 70°C and freeze-drying.

Air-drying treatments were performed in shelf dryers where 25 to 30 kg of seaweed (mature adult thalli) were scattered as monolayers to avoid uneven dying of the material (case hardening). The initial stocking density was approximately 1.4 kg m^2, with a shelf area of 0.4 m^2. Drying at 40 and 70°C was achieved by indirect heating of the air by liquefied propane gas, while a heat pump system was used to produce drying air at 25°C. The air velocity between the shelves was in the range of 1.5 to 3.0 m sec^{-1}. The temperature and relative humidity (RH) were monitored during the drying process (see fig. S1, online resource 1). The samples' weight was measured at regular intervals until equilibrium moisture content (EMC) was reached and no further variations were observed. Simultaneously, samples were vacuum-packed and frozen for subsequent vacuum freeze-drying (Alpha 2-4 LSC). All treatments were performed in three replicates. All dried samples were vacuum-packed and dispatched for further analyses.

Chemical Analyses

Moisture The moisture content in the dried samples was determined gravimetrically by drying at 105^0C until constant weight of the samples was achieved (typically 24 h). The subsequent results from chemical analyses were then expressed as part of the dry weight (DW) of the samples.

Ash content was determined after combustion of the dried samples at 590^0C for 12 h in a laboratory muffle furnace. The ashes were quantified as the residue from combustion expressed as percentage of the DW.

Iodine Dried seaweed samples were ground to 120 µm grain size using an electric grain miller and iodine (I) was extracted by dry alkaline incineration, a process where all inorganic and organic iodine species were converted to iodide (I^-) ions. Thereafter, the iodide in algal extracts, as a measure of the total iodine in algal samples, was quantified using a HPLC system (1200 Series, Agilent Technologies, Palo Alto, USA) according to Nitschke and Stengel (2015). An Acclaim Mixed-Mode WAX-1 column, protected by an Acclaim Mixed-Mode WAX-1 guard column (Dionex Corporation, Sunnyvale, USA) was used to separate iodide ions from interfering compounds. The mobile phase was 50/50 (v/v) methanol/phosphate buffer. The iodide eluted was detected by a diode array detector at 223 nm, identified via retention time and absorption characteristics, and quantified by peak area. The

HPLC method used has a limit of detection (LOD) and a limit of quantification (LOQ) of ~0.2 ng μL^{-1} and 1 ng μL^{-1}, respectively. Iodine contents were expressed in mg g^{-1} DW.

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, from pure solutions). 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 the laboratory. 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. The laminaran content of the samples was quantified by the glucose levels measured in the hydrolysates. Results were expressed as % of the DW.

Total nitrogen (N) was determined in ground samples using a CHNS-O elemental combustion system (Costech Instruments ECS 4010) at a temperature of approximately 1000°C, where the N of the samples is converted to N gas/oxides. The measurements were performed in 4 parallels. Results were expressed as % N of the DW.

Amino acid analysis The amino acid profiles were analyzed from ground samples by a HPLC system (Agilent Infinity 1260, Agilent Technologies) coupled to an on-line post-column derivatization module (Pinnacle PCX, Pickering laboratories, Mountain View, CA, USA), using ninhydrin (Trione) as a reagent and a Na^+-ion exchange column (4.6 x 110 mm, 5 mm). 18 standard amino acids and taurin were quantified from standard curves measured with amino acid standards. Prior to the analysis, the samples were hydrolyzed in 6 M HCl containing 0.4% mercaptoethanol for 24 h at 110°C (HCl hydrolysis). Glutamine (Gln) and asparagine (Asn) were converted to glutamic (Glu) and aspartic acid (Asp), respectively. Cystein (Cys) was quantified as cystin (Cys-Cys). The samples were filtered using a micro-filter, the pH was adjusted to 2.2 and the samples were further diluted with a citrate buffer (pH 2.2) for the HPLC analysis. All buffers, reagents, amino acid standards and the column were obtained from Pickering laboratories (Mountain View, CA, USA). HCl and mercaptoethanol were obtained from Sigma-Aldrich.

Free amino acid analysis The free amino acid content of the samples was determined using the method of Osnes and Mohr (1985). The proteins were extracted by agitating 100 mg of ground dried sample in 10 mL water for 1 h. The extract was centrifuged at 4°C and 2000 g for 20 min. 0.25 ml of 10% sulphosalisylic acid was added to 1 mL of the water-soluble extract in an Eppendorf tube. The mixture was then vigorously shaken and incubated at 4°C for 30 min prior to centrifugation at 7840 g for 10 min in order to precipitate the protein-bound amino acids. 1 mL of the supernatant was transferred to a new Eppendorf tube with 0.25 mL of 10% sulphosalisylic acid and the same operation as previously described was repeated until no protein precipitate was observed. The analysis was done in triplicate. Suitably diluted samples were filtered (0.2 µm) prior to analysis by HPLC (Dionex Ultimate 3000) using a Water Novapak C18 column (4.0 µm particle size) and a RF 2000 fluorescence detector (Dionex, Sunnyvale, CA, USA). The free amino acids were identified and quantified by comparison with pure amino acid standards purchased from Fluka (Buchs, Switzerland). Both cysteine and proline were excluded from the analysis, cysteine being unstable during the acid hydrolysis of the samples and proline cannot be detected following the o-phtalaldehyde (OPA) pre-column derivatization during the HPLC analysis. The results were expressed in mg g^{-1} DW of the seaweed samples.

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 phloroglucinol equivalent in % of the DW. Analyses were performed in duplicate with 10% relative uncertainty of measure.

Fucoxanthin content The extraction of fucoxanthin from air-dried samples at 25 and 70°C and freeze-dried 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 (250 x 4.6 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.

Color and physico-chemical properties

Surface color analysis The surface color of seaweed samples was analyzed by a computerized image technique as described by Girolami et al. (2013), using a digital camera (Canon EOS 60D) and a 35 mm lens (Canon EF 35 mm 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 6500 K (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 2017, 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).

Water and oil binding capacity (WBC and OBC) was determined as described by Rupérez and Saura-Calixto (2001) where 30 mL of either distilled water or a commercial soya oil was added to 0.5 g ground samples (particle size 0.8 mm) in a 50-mL centrifuge tube. The samples were then stirred and left at room temperature for 1 h. After centrifugation at 3000 g for 20 min, the supernatant was discarded and the residue weighed. WBC and OBC were expressed as g water per g of dried sample.

Swelling capacity (SC) was assessed following the method described by Rupérez and Saura-Calixto (2001) and slightly modified, where 1 to 2 g ground samples was added to a 50-mL measuring cylinder. 30 mL of distilled water was added under agitation using a vortex mixer to eliminated trapped air bubbles. The samples were covered and left overnight then SC was determined as the volume occupied by the sample (in mL) per g of dry sample initially added. The analysis WBC, OBC and SC of each sample was performed in 3 parallels.

Sensory analysis

A descriptive test (ISO:13299, 2003) was used to characterize the sensory profile of *S. latissima* samples air-dried at 25 and 70°C. The panel consisted of eight judges, ranging from 31 to 60 years of age, all of which had some experience with descriptive analysis but were not familiar with testing seaweeds. Assessors were trained according to the guidelines in ISO:8586:1 (2012).

The seaweed samples were pulverized using a blender and presented to the assessors in small beakers (1-2 g per assessor). During a first training phase, the assessors developed a vocabulary describing the samples' odor (aroma), flavor and texture characteristics, and agreed upon a total of 13 attributes listed and described in table 1. Samples of *S. latissima* produced from different pre-treatments (4 in total) were used in this sensory evaluation

although only the results concerning the air-dried samples at 25 and 70°C are relevant to this study and will be discussed. Several pretest sessions were conducted as described by Lawless and Heymann (2010), in which the panel members were trained in the evaluation of the attributes by testing samples that were characteristic. A continuous non-structured scale was used for the evaluation, ranging from lowest to highest intensity, corresponding to the range of intensity of the tested samples with regard to each attribute. The results from panelists were transformed to numbers from 0 to 100 (lowest to highest intensity) for the data analysis. The evaluation followed detailed instructions in which the panel members evaluated the aroma of the samples by smelling prior to evaluating their flavor and texture attributes. The training was conducted during two days before the main test and resulted in a calibrated panel.

During the main evaluation phase, each assessor performed a monadic assessment of the seaweed samples using a computerized system (surveymonkey.com) for direct recording of the data. The evaluation was performed in two replicates. Panel performance was monitored using PanelCheck Software (version 1.3.2, Nofima, Tromsø, Norway).

Statistical analysis

All statistical analyses were performed on R (version 3.4.1, R Development Core Team 2017). Raw data were pre-processed for descriptive statistics and the results expressed as mean ± standard error (n = 3) unless stated otherwise. A one-way analysis of variance (ANOVA, R function aov) was used to detect significant differences among treatment groups regarding individual quality parameters, after testing for the homogeneity of variances (Levene test). A Tukey's honest significant difference (HSD) test (R function TukeyHSD) was used for post-hoc comparisons of significant ANOVA results. A principal component analysis (PCA, R function prcomp) based on covariance matrix was applied to visualize differences in the amino acid and free amino acid compositions among treatment groups. A PCA based on correlation matrix, in which variables of different scales are standardized, was used to detect differences in color characteristics.

Results and discussion

Experimental drying

Freshly harvested biomass of *S. latissima* initially containing 89.5 ± 0.4% (n = 10) water was air-dried at 3 different temperatures (25, 40 and 70°C) and freeze-dried (used as reference treatment). The experimental drying kinetics of air-dried samples is shown in figure 1. EMC at 25, 40 and 70°C was achieved at 420, 270 and 100 min respectively. In comparison, freeze-drying of fresh *S. latissima* samples was achieved during a 20 h cycle. The

levels of residual moisture were significantly different among samples (ANOVA: $F(3, 8) = 10.69$, $p = 0.004$; table 2) with higher levels found in air-dried samples at 25°C, compared to other groups. This result can be explained by higher RH levels measured at this temperature using the heat pump drying system compared to air-drying at 40 and 70°C using a classical indirect air heating system (see fig. S1, online resource 1). Increasing RH decreases the drying rate due to lower mass transfer coefficient (Sappati et al. 2017). For an accurate comparison of the quality of the samples obtained from different treatments, the following results from the chemical analyses were adjusted to the residual moisture of the samples and expressed on a DW basis.

Nutrient content

The effects of drying treatments on the nutrient content of the raw material cultivated in Norway and harvested in May, were assessed by chemical composition. Table 2 summarizes the results from the chemical composition of the samples from 4 drying treatment groups, including residual moisture, ash and iodine content, carbohydrate composition, polyphenols and fucoxanthin contents. The lipid content was not analyzed in this study but is reported to be low in brown macroalgae in general (MacArtain et al. 2007; Dawczynski et al. 2007) and ranging from 0.8 to 2% DW in *S. latissima* (Gómez-Ordóñez et al. 2010; Sappati et al. 2017).

The chemical composition of the samples is dominated by their ash content (ca. 45% DW, table 2), directly reflecting the high mineral content of the samples, followed by carbohydrates (ca. 25% DW). Substantially higher carbohydrate and lower ash contents in freeze-dried *S. latissima* samples also harvested in May are reported in the literature (Schiener et al. 2015; Stévant et al. 2017b) highlighting the variability in the chemical composition of this kelp species among geographical regions. There were no significant differences in ash content among air- and freeze-dried samples. Particularly high levels of iodine in *S. latissima* are reported in the literature (Stévant et al. 2017a; Roleda et al. 2018), with a potentially negative impact on its nutritional value since excessive iodine intakes can be associated with clinical symptoms in sensitive individuals (Miyai et al. 2008). The iodine content was significantly lower in freeze-dried compared to air-dried samples (ANOVA: $F(3, 7) = 17.17$, $p = 0.002$). In kelp species, the iodine accumulates naturally in the extracellular matrix in the form of iodide (I^-) which readily scavenges a variety of reactive oxygen species (ROS) from both aqueous and gaseous oxidants (Küpper et al. 2008). A study from Hou et al. (1998) reported reduced recovery of iodide in aqueous solutions following freeze-drying (30 to 40%) compared to air-drying (100%) although a similar effect of freeze-drying on the recovery of iodide directly from seaweed material was not observed. However, the chemical species of iodine are known to

differ among seaweeds and no mention is made of the species used in this study. The mean iodine content of freeze-dried *S. latissima* from a large-scale sampling program (4.6 mg g^{-1} DW), including samples of the same biomass as used in this study (Roleda et al. 2018), is comparable to the values for air-dried samples presented in table 2. In the present study, the iodine level of freeze-dried samples is lower than in air-dried samples. However, it is in the lower range of the values (ranging 1.6 – 7.2 mg g^{-1} DW) reported by Roleda et al. (2018) across spatial and temporal variations of the biomass source. These results should be interpreted with caution due to the small sample size and contradictory findings from Nitschke and Stengel (2016) reporting no differences in iodine content between freeze-dried and air-dried samples, from the species *Alaria esculenta*, *Palmaria palmata* and *Ulva intestinalis*.

The total carbohydrate (TC) content, which was quantified as the sum of each individual sugar identified, did not significantly differ among samples (table 2). The carbohydrate fraction was mainly composed of alginate, reaching over 50% of the TC, followed by mannitol (approximately 25% of TC). The methanol-acid hydrolysis reaction (methanolysis) only allows for the detection of soluble sugars present in the samples. The insoluble fibers fraction, mainly found in kelps species within the cell walls in form of cellulose, cannot be quantified by this method. A study from Schiener et al. (2015) reports stable cellulose contents across seasons in *S. latissima* accounting for 11% of the DW. The fucose, mainly present in sulfated form in brown seaweeds, is indicative of the fucoidan content of the samples. Both laminaran and fucose are accounting for less than 10% of the TC. Galactose, mannose and glucuronic acid, which enter into the composition of fucoidans in *S. latissima* (Marfaing et al. 2009), were also detected in small amounts in all samples, at levels below 1% DW. The levels of individual sugars did not notably differ among sample groups suggesting no effects of the drying treatments on the carbohydrate composition of *S. latissima*.

Lower polyphenol contents are reported in *Sargassum* spp. following air-drying at 60°C compared to freeze-drying (Wong and Cheung 2001b) and decreasing levels of phenolic compounds as well as antioxidant activity in *Fucus vesiculosus* were observed from increasing drying temperature (from 35 to 75°C, Moreira et al. 2016). In contrast, Gupta et al. (2011) reported higher loss of total phenolic content in *Himanthalia elongata* following air-drying at 25°C compared to 40°C, which can be explained by higher enzymatic oxidative activity in the material dried at lower temperature. The polyphenol levels of dried *S. latissima* samples measured in this study were low and did not significantly differ among drying treatments (table 2). Relatively lower fucoxanthin contents were measured in the samples air-dried at 25°C compared to 70°C and freeze-dried although this trend was not

300 significant (ANOVA: $F(2, 6) = 4.88$, $p = 0.07$) due to the variability observed within treatment groups. These
301 results are contradictory to those obtained from a similar experiment conducted on *A. esculenta* in which air-
302 drying at 70°C produced samples with the lowest fucoxanthin content (Stévant, unpublished results). Moreover,
303 the sensitivity of this carotenoid pigment to high temperatures has previously been reported (Indrawati et al. 2015).
304 On the other hand, the longer drying time at 25°C may result in increased oxidation of the pigment. Low drying
305 temperatures may also fail to inactivate oxidative enzymes responsible for pigment degradation, although further
306 work on the fucoxanthin stability following preservation treatments of brown seaweeds is needed in order to better
307 understand the behavior of this compound in the raw material.
308
309 The protein content of the samples, reflected by the sum of all amino acids analyzed, ranged from 7.2 to 7.4%
310 DW (table 3) which is comparable to values reported in the literature for *S. latissima* (Schiener et al. 2015; Stévant
311 et al. 2017b). These levels did not vary among drying treatments, in contrast with the results obtained by Chan et
312 al. (1997) and Wong and Cheung (2001a) who detected lower levels of total amino acids in *Sargassum* spp.
313 samples air-dried at 60°C compared to freeze-dried samples. In both cases the protein loss was non-specific since
314 the relative amounts of individual amino acids remained constant. The amino acid composition of the *S. latissima*
315 samples in this study was dominated by glutamic acid, alanine and aspartic acid representing approximately one
316 third of the protein fraction in all sample groups. All essential amino acids (EAA) were detected in the samples
317 except tryptophan which can be destroyed during the acid hydrolysis of the samples. The PCA method only
318 explained 65.1% of the total variation (cumulated by the two principal components) in amino acid composition
319 among samples hence, was excluded from the analysis of the results. However, overlapping values of mean ±
320 standard error for individual amino acids across sample groups (table 3) suggest no effect of the drying treatments
321 on the protein quality of *S. latissima*.
322 Although the protein content of brown seaweeds is generally lower than those found in red and green species
323 (Fleurence 2004), the interest in large-scale cultivation of kelp, primarily *S. latissima*, is growing rapidly in Europe
324 (Stévant et al. 2017c) and biomass of this kelp species may be an alternative source of protein in food and feed
325 applications in the future. Not only the protein amount but also the protein quality is important when assessing
326 the nutritional value of a food product. The quality of a protein source is determined from its content in essential
327 amino acid (EAA, in mg amino acid g^{-1} protein) and compared to the EAA pattern of an ideal reference protein,
328 proposed by the WHO/FAO/UNU (2007). The tested protein is given a chemical score defined as the ratio between
329 each EAA of the protein source and the corresponding EAA level of the reference protein. Proteins from animal

sources generally have a chemical score of 100%, i.e. they contain all EAA in sufficient amount, while proteins from vegetal sources (i.e. cereals, legumes, beans and nuts) have lower values due to at least one limiting EAA (WHO/FAO/UNU, 2007). The levels of EAA in *S. latissima* samples in this study (tryptophan being excluded), exceeded the minimum values required in human nutrition, resulting in chemical scores of 100%. A comparable high chemical score (82%), with lysine as the first limiting EAA, was reported by Murata and Nakazoe (2001) for the same species originating from Japan. However, the protein quality of cultivated *S. latissima* samples from Denmark analyzed throughout a year was limited by low levels of histidine resulting in substantially lower chemical scores (16.7% to 68.9%, Marinho et al. 2015). Despite remarkable amino acid profiles, the protein digestibility of brown seaweeds is generally limited by the high content of dietary fibers and particularly the alginate fraction in kelp species (Horie et al. 1995) as well as phenolic compounds (Wong and Cheung 2001b). Lower levels of polyphenols were found in *Sargassum* spp. samples following air-drying at 60°C compared to freeze-drying, which also resulted in significantly higher protein extractability and digestibility of the protein concentrates in air-dried samples (Wong and Cheung 2001b). Although this aspect is not covered by the present study, the levels of anti-nutritional factors generally limiting the digestibility of seaweed protein fractions (i.e. alginate and polyphenols) were similar among treatment groups suggesting no effects of the tested drying treatments on the digestibility of proteins from *S. latissima*.

It should be noted that the N-to-protein ratio of *S. latissima* measured in this study (3.98 ± 0.03 across sample groups, n = 4) supports earlier results, reporting the inaccuracy of the commonly used conversion factor of N*6.25 to predict the protein content in brown macroalgae, due to the presence of non-protein N in the biomass (Angell et al. 2016).

A biomaterial may undergo multiple chemical reactions upon drying e.g. browning reactions, lipid oxidation, and protein denaturation, which can directly affect its quality. The present results did not reveal any major differences among drying treatments with regard to the phytochemical content of *S. latissima* samples, in contrast with previous studies (Chan et al. 1997; Wong and Cheung 2001a; Ling et al. 2015). However, losses of vitamins and other bioactive secondary metabolites may occur during processing and storage of seaweed biomass (Lage-Yusty et al. 2014), which were not estimated in the present study.

Mechanical alterations due to product shrinkage are also commonly observed from convective air-drying and typically result in changes in the product shape and structure (Bonazzi and Dumoulin 2011). These alterations may affect the extraction of phytochemical substances by influencing the factors governing solvent penetration in

360 the material e.g. capillarity, molecular diffusivity, which will ultimately affect their quantification. This should be
361 considered when studying the impact of a drying process on the chemical content of a biomaterial.
362
363 ***Color and physico-chemical properties***
364 The hydration-related properties of plant materials such as WBC and SC, as well as OBC are related to the
365 chemical structure of their polysaccharides (Rupérez and Saura-Calixto 2001). Therefore, the alteration of these
366 parameters during the drying process can be the result of tissue damage. No significant differences in the WBC
367 of the samples were observed among treatments (table 4), which may also result from large variations among
368 sample replicates, particularly in air-dried samples at 25°C and 70°C. However, the WBC from air-dried samples
369 at 25°C were lower compared to the other treatment groups. The SC of the samples tended to decrease following
370 increasing drying temperatures and freeze-dried samples showed significantly higher SC compared to air-dried
371 samples. This can be explained by alterations of the textural properties of the biomaterial upon air-drying,
372 predominantly shrinkage, which have been reported in brown seaweeds (Cox et al. 2012; Sappati et al. 2017).
373 These mechanical alterations are resulting in changes in the microstructure of the product i.e. fewer pores and less
374 open structure, affecting the ability of the material to entrap water during rehydration. Product shrinkage is highly
375 dependent on the physical state (rubbery or glassy) of the material during the process. The effect of drying
376 temperature on the shrinkage of *S. latissima* was studied by Sappati et al. (2017) who measured greater rates of
377 shrinkage during air-drying at 70°C compared to 40°C. This was explained by a higher mobility of the solid matrix
378 during the process following higher temperature above the glass transition temperature (T_g). The drying
379 temperature during freeze-drying is typically below or close to T_g, maintaining the product in the glassy state
380 hence, minimizing the mobility of the matrix and subsequent shrinkage. The OBC measured in the *S. latissima*
381 samples was relatively high compared to the values reported by Rupérez and Saura-Calixto (2001) for other brown
382 seaweed species. Freeze-dried samples were also characterized by higher OBC values than air-dried samples. The
383 OBC of food products can be related to the levels of non-polar residues in the protein fractions (Chel-Guerrero et
384 al. 2002) and the nature of their polysaccharides (Fleury and Lahaye 1991) but also depend on other factors such
385 as the porosity of the material. In this study, no differences could be detected among samples neither regarding
386 their polysaccharides nor on their levels of non-polar amino acids (i.e. Gly, Ala, Val, Leu, Ile, Met, Phe and Pro,
387 Trp being excluded from the analysis). Hence, higher OBC of the freeze-dried samples is likely the result of a
388 more porous microstructure compared to air-dried samples.
389

The variations in the surface color among samples (defined by the coordinates *L**, *a** and *b**) were recorded using computerized image analysis. The results, listed in table 4 were analyzed by PCA. The first two components of the PCA biplot explained 97.6% of the variance (63.4% and 34.2% by PC-1 and -2 respectively, fig. 2) among the data set. The variance in *b** (yellow/blue) explained by the first axis (PC-1), accounts for the largest part of the total variance among samples, followed by *L** (lightness) and *a** (red/green). Graphically, sample groups can be distinguished according to the *a** coordinate. Both samples air-dried at 25°C and freeze-dried exhibited a predominant red hue ($a^* > 0$) while green ($a^* < 0$) was dominating in samples air-dried at 40 and 70°C (fig. 2 and fig. S2 from online resource). Trends were also observed along *L** and *b**, i.e. lighter freeze-dried samples and increasing yellowness (increasing *b** values) with increasing drying temperatures, although the variability within groups is high. Fucoxanthin (an orange pigment) is an important compound responsible for the coloration of brown macroalgae but kelp species including *S. latissima* also contains other pigments such as violaxanthin and β-carotene along with chlorophylls (Chl *a*, Chl *c*) (Haugan and Liaaen-Jensen 1994). Variations in color characteristics among treatments may be the result of different reactions involving these pigments, leading to their degradation or the formation of secondary colored substances. Similar results, i.e. decrease in *a** and increase in *b** with increasing drying temperatures, were also reported by Moreira et al. (2016) in dried powder of *F. vesiculosus*. These observations were explained by the authors by the leaching of the chlorophyll during the drying process, resulting in increasing greenness, and its degradation, maximal within the range of 60 to 82°C, leading to the yellowing of the material as carotenoids become more exposed.

Sensory properties and free amino acids

The sensory characteristics including aroma, flavor, and texture qualities of *S. latissima* samples air-dried at 25˚C and 70˚C were evaluated by eight trained panel members. No major differences were detected between the two groups, based on the 13 selected sensory attributes listed in table 1 (fig. 3). The saltiness of the samples was described as intense, which can be correlated with their particularly high ash levels. The samples were also characterized by intense "fresh sea" aroma and flavor notes, while the umami flavor was only perceived as moderate. The texture (i.e. mouthfeel) from both samples was neither perceived as cohesive nor crispy, neither thin nor viscous and was rather tender. However, air-dried samples at 25°C dissolved more easily compared to those dried at 70°C. This can be explained by the reduced porosity of the latter sample group, illustrated earlier by lower SC due to product shrinkage. Although the intense perception of saltiness from the samples may have affected the evaluation of flavor and texture, the results from this sensory evaluation are quite similar to those of

Chapman et al. (2015) who reported preliminary data from the sensory description of four edible seaweed species including *S. latissima*.

Whereas there are numerous reports on the nutrient content of a wide range of seaweed species (see Holdt and Kraan 2011, and references therein), few scientific studies have attempted to characterize the sensory profile of relevant edible species. Some exceptions to this are studies describing the kelp *konbu* (*S. japonica*) as a rich source of umami flavor, which is directly related to high levels of free glutamate, in its monovalent sodium-salt form (monosodium glutamate, MSG) (Ikeda 2002; Ninomiya 2002). Generally, amino acids in their free form are identified as major taste-active compounds in various foodstuffs. The free amino acid (FAA) composition of *S. latissima* samples obtained from different drying treatments was analyzed and the results are summarized in table 5. The total amount of FAA represented approximately 8% of the total amino acids of the samples and did not vary significantly among drying treatments. The samples contained high levels of alanine (perceived as sweet), glutamate and aspartate in their free form (both eliciting umami sensation), relatively to other FAAs. The analysis of the data by PCA only explained 77.6% (cumulated by PC-1 and -2) of the total variation in FAA and did not provide an accurate comparison of the FAA profiles following different drying treatments. However, higher levels of free glutamate were measured in freeze-dried and air-dried samples at 40°C (table 5). Although differences were expected between samples air-dried at low (25°C) and high temperature (70°C) with regard to their composition in aroma-active compounds, both groups displayed very similar FAA profiles correlating to their similarity in flavor and aroma characteristics perceived during the sensory evaluation.

The sugar kelp *S. latissima* belongs to the same genus as Japanese *konbu*, however, the levels of glutamate measured in this study, ranging from 1.03 to 1.52 mg g^{-1} DW, are far below the value reported by Ninomiya (2002) for *konbu* (22.40 mg g^{-1} DW). Similarly, Mouritsen et al (2012) reported substantially lower amounts of free glutamate, aspartate and alanine in broth extracted from *S. latissima* when compared to those from different variants of Japanese *konbu*. After harvest, *konbu* is typically sun-dried and aged for several years in order to develop characteristic flavors. The high content of free glutamate from aged *konbu* may result from the enzymatic degradation of proteins during this maturation process. Sun-dried seaweeds typically contain higher moisture contents compared to air- or freeze-dried material (Chan et al. 1997), which is an important factor governing enzymatic activity. Endogenous enzymatic hydrolysis of proteins may occur during the storage of *konbu* leading to high glutamate levels and characteristic umami flavor. Although Mouritsen et al. (2012) did not measure any

discernible effect of maturation (i.e. ageing of the dried product) in the glutamate content of *S. latissima* extracts, no mention is made of the drying technique used nor of the storage conditions (e.g. temperature, moisture content of the material) during the process. Optimizing storage conditions e.g. temperature and moisture, can be a key to develop preferable sensory profiles in edible seaweeds and future studies on this topic are envisaged. However, the sensory characteristics of seaweeds cannot be reduced to their FAA content since a wide range of molecules including peptides, minerals, low-molecular-weight carbohydrates and volatile compounds contribute to the sensory characteristics of foods (Lindsay 2008). The analysis of volatile oils from the steam distillation of several fresh edible kelp species from Japan identified a sesquiterpene alcohol, namely cubenol, as an important contributor to the kelp flavor (Kajiwara et al. 1988). López-Pérez et al (2017) identified 137 different volatile compounds in dried *S. latissima*, mainly consisting of (in decreasing order) carboxylic acids, hydrocarbons, alcohols, aldehydes, ketones and esters. In this comparative study on the aroma characteristics of 7 edible seaweed species in dehydrated form, a positive relationship could be established between the detected levels of volatile esters and hay aroma which was prominent in *S. latissima*. As reported by Michel et al (1997) high drying temperatures (i.e. 150°C), produce drastic changes in the composition of volatile compounds of dried *Ulva* sp. and *P. palmata* samples, as opposed to drying at lower temperatures (60°C), when compared to fresh samples.

Conclusion

Convective air-drying, especially at high temperatures affected the physico-chemical characteristics of *S. latissima*, compared to freeze-drying, used as a reference treatment in this study. Alterations were attributed to product shrinkage resulting in reduced porosity and rehydration capacity, potentially decreasing the quality and market value of the seaweed to be used as a functional ingredient by the food industry, or directly by the consumer in a rehydrated form. Aside from the iodine content which was significantly lower in freeze-dried samples, air-drying in the temperature range of 25 to 70°C resulted in equally nutritious products with similar flavor and aroma properties.

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626

627 **Table 1:** Sensory attributes, and their definitions, associated to the *S. latissima* samples.

Sensory attribute	Label	Scale anchors	Definition
Aroma			
Fresh sea	A – fresh sea	none || much	Fresh sea odor
Fermented	A – fermented	none || much	Fermented odor, pungent, marmite, matured cheese, cured
Hay	A – hay	none || much	Dry hay, green tea
Flavor			
Salty	F – salty	none || much	Salty taste
Fresh sea	F – fresh sea	none || much	Sea flavor
Fermented	F – fermented	none || much	Fermented flavor, matured cheese, marmite, cured
Hay	F – hay	none || much	Fresh hay, green tea
Umami	F – umami	none || much	Umami, meat stock, brown crab meat
Bitter	F – bitter	none || much	Bitter taste
Texture			
Crispy	T – crispy	cohesive || crispy	During first bites, how crispy is the sample
Chewy	T – chewy	tender || chewy	While chewing, chewy: difficult to disintegrate
Viscous	T – viscous	thin || viscous	Viscous, slimy, porridge like
Dissolves	T – dissolves	None || much	Dissolves or melts easily in mouth while chewing

Table 2: Chemical composition of *S. latissima* samples air-dried at 25, 40, 70˚C and freeze-dried (FD). Values are given as mean ± standard error (n = 3, unless stated otherwise). Different subscript letters in the same row indicate significant differences (ANOVA, Tukey HSD, $p < 0.05$) among drying treatments.

	25˚C	**40˚C**	**70˚C**	**FD**
Residual moisture (%)	5.9 ± 0.2 [a]	4.4 ± 0.2 [ab]	4.0 ± 0.5 [b]	3.0 ± 0.4 [b]
Minerals				
Ash (% DW)	45.4 ± 1.7 [a]	45.2 ± 1.7 [a]	44.0 ± 2.2 [a]	43.8 ± 1.3 [a]
I (mg g^{-1} DW)	5.9 ± 0.3 [a]	5.7 ± 0.3 [a]	4.9 ± 0.2 [1 a]	3.0 ± 0.4 [b]
Carbohydrates (% Σ carb.)				
Alginate	54.6 ± 2.9	50.8 ± 2.3	53.6 ± 2.8 [1]	53.1 ± 1.4
Mannitol	25.5 ± 1.5	30.9 ± 2.0	28.0 ± 3.5 [1]	25.3 ± 3.5
Laminaran	7.1 ± 2.0	5.2 ± 1.4	4.9 ± 0.6 [1]	6.8 ± 2.5
Fucose (fucoidan)	4.7 ± 0.4	5.5 ± 0.5	5.4 ± 0.7 [1]	5.3 ± 0.2
Σ carbohydrates (% DW)	27.1 ± 0.5 [a]	27.8 ± 1.0 [a]	26.9 ± 0.2 [1 a]	23.9 ± 1.5 [a]
Polyphenols (% DW)	0.56 ± 0.03 [a]	0.67 ± 0.04 [a]	0.75 ± 0.02 [1 a]	0.63 ± 0.08 [a]
Fucoxanthin (mg kg^{-1} DW)	319 ± 49 [a]	na	737 ± 101 [1 a]	565 ± 115 [a]

[1] n = 2

Table 3: Amino acid composition (in mg amino acid g^{-1} protein), total amino acid (Σ AA, % DW), total essential amino acid (Σ EAA, in mg amino acid g^{-1} protein), essential amino acid ratio (EAA/AA, dimensionless), and chemical score (in %) of *S. latissima* samples air-dried at 25, 40, 70˚C and freeze-dried (FD). Values are given as mean ± standard error (n = 3). Different subscript letters in the same row indicate significant differences (ANOVA, Tukey HSD, $p < 0.05$) among drying treatments.

	25˚C	**40˚C**	**70˚C**	**FD**	**Pattern [2]**
Essential amino acids (EAA)					
Leu	89.3 ± 0.8	88.3 ± 0.5	87.4 ± 1.4	87.8 ± 1.8	63.0
Phe	63.7 ± 1.0	61.9 ± 0.8	59.9 ± 1.3	64.1 ± 1.5	46.0 [3]
Lys	59.2 ± 0.3	59.2 ± 1.1	59.2 ± 0.6	60.6 ± 0.5	52.0
Val	53.1 ± 0.4	53.0 ± 0.3	53.1 ± 0.5	52.6 ± 0.2	42.0
Ile	49.8 ± 0.5	49.6 ± 0.3	49.2 ± 0.5	48.9 ± 0.2	31.0
Thr	40.9 ± 1.5	39.9 ± 0.6	41.4 ± 1.7	40.7 ± 1.6	27.0
Met	24.1 ± 0.2	25.0 ± 0.3	24.7 ± 0.6	24.7 ± 0.4	26.0 [4]
His	21.6 ± 0.6	22.4 ± 0.3	20.8 ± 0.6	22.9 ± 0.5	18.0
Σ EAA	405.4 ± 1.0 [a]	403.0 ± 2.3 [a]	399.5 ± 6.9 [a]	405.3 ± 5.7 [a]	305.0
Chemical score (%)	100	100	100	100	

Non-essential amino acids (NEAA)				
Glu + Gln	120.0 ± 1.4	129.3 ± 2.8	125.6 ± 5.8	118.8 ± 2.6
Ala	117.9 ± 0.9	111.6 ± 4.6	120.5 ± 4.2	115.1 ± 3.0
Asp + Asn	90.0 ± 0.5	91.1 ± 4.1	87.4 ± 2.6	90.6 ± 1.9
Ser	58.9 ± 0.6	58.9 ± 3.2	53.9 ± 1.6	53.8 ± 2.6
Arg	51.8 ± 0.2	51.7 ± 0.5	50.9 ± 0.7	55.0 ± 0.4
Gly	50.8 ± 0.2	51.7 ± 0.4	50.5 ± 1.0	50.2 ± 0.3
Pro	46.8 ± 1.2	44.6 ± 1.3	49.2 ± 2.1	47.7 ± 1.4
Cys [1]	29.7 ± 0.4	27.7 ± 0.7	33.1 ± 2.2	31.3 ± 1.0
Tyr	27.2 ± 1.1	27.6 ± 0.9	26.8 ± 0.8	28.8 ± 0.6
Tau	1.5 ± 1.5	2.9 ± 1.5	2.7 ± 1.5	3.4 ± 3.4
Σ AA (% DW)	7.2 ± 0.3 [a]	7.3 ± 0.3 [a]	7.4 ± 0.3 [a]	7.2 ± 0.1 [a]
EAA/AA	0.4 ± 0.0 [a]	0.4 ± 0.0 [a]	0.4 ± 0.0 [a]	0.4 ± 0.0 [a]
Total N (% DW)	1.87 ± 0.10 [a]	1.85 ± 0.09 [a]	1.94 ± 0.05 [a]	1.82 ± 0.05 [a]
N-to-protein ratio	3.94 ± 0.07 [a]	4.03 ± 0.04 [a]	3.87 ± 0.03 [a]	4.03 ± 0.07 [a]

[1] quantified as cysteine, [2] EAA requirement pattern (WHO/FAO/UNU 2007), [3] Phe + Tyr, [4] Met + Cys.

Table 4: Physico-chemical properties and color parameters of *S. latissima* samples air-dried at 25, 40, 70˚C and freeze-dried (FD). WBC and OBC are expressed in g water and g oil per g dried sample respectively, and SC is expressed in ml per g dried sample. Values are given as mean ± standard error (n = 3). Different subscript letters in the same row indicate significant differences (ANOVA, Tukey HSD, $p < 0.05$) among drying treatments.

	25˚C	**40˚C**	**70˚C**	**FD**
Physico-chemical parameters				
WBC	6.7 ± 1.6 [a]	8.3 ± 0.6 [a]	7.3 ± 1.4 [a]	7.2 ± 0.2 [a]
OBC	4.1 ± 0.4 [a]	4.1 ± 0.1 [a]	4.4 ± 0.5 [a]	6.1 ± 0.1 [b]
SC	6.3 ± 0.5 [a]	5.0 ± 0.2 [a]	4.9 ±0.1 [a]	10.2 ± 0.4 [b]
Color				
*L**	46.0 ± 3.5	46.2 ± 3.9	52.3 ± 3.1	59.7 ± 2.5
*a**	2.1 ± 0.5	-3.4 ± 0.9	-4.6 ± 1.8	2.1 ± 0.8
*b**	34.0 ± 3.4	36.7 ± 4.8	44.3 ± 3.6	39.8 ± 3.5

Table 5: Total free amino acid content and free amino acid composition (in mg g^{-1} DW) of *S. latissima* samples air-dried at 25, 40, 70˚C and freeze-dried (FD). Values are given as mean ± standard error (n = 3). Different subscript letters in the same row (Σ FAA) indicate significant differences (ANOVA, Tukey HSD, $p < 0.05$) among drying treatments.

	25˚C	**40˚C**	**70˚C**	**FD**

Free amino acids (FAA)				
Ala	2.07 ± 0.07	1.86 ± 0.13	2.07 ± 0.08	2.06 ± 0.15
Glu	1.03 ± 0.08	1.51 ± 0.03	1.22 ± 0.15	1.52 ± 0.06
Asp	1.02 ± 0.02	0.95 ± 0.16	1.04 ± 0.01	1.18 ± 0.08
Gln	0.32 ± 0.01	0.22 ± 0.02	0.33 ± 0.07	0.26 ± 0.02
Thr	0.32 ± 0.00	0.16 ± 0.02	0.20 ± 0.04	0.30 ± 0.04
Phe	0.17 ± 0.01	0.19 ± 0.01	0.24 ± 0.00	0.18 ± 0.01
Aba	0.12 ± 0.01	0.21 ± 0.01	0.23 ± 0.02	0.10 ± 0.02
Ser	0.13 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.11 ± 0.00
Asn	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.10 ± 0.01
Val	0.09 ± 0.01	0.10 ± 0.02	0.13 ± 0.03	0.08 ± 0.01
Lys	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.07 ± 0.01
Tyr	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.00	0.06 ± 0.01
Gly + Arg	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.06 ± 0.01
Met	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00
Leu	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.00
Ile	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.00
His	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.02 ± 0.00
Σ FAA	5.8 ± 0.1 [a]	5.9 ± 0.1 [a]	6.2 ± 0.1 [a]	6.2 ± 0.2 [a]

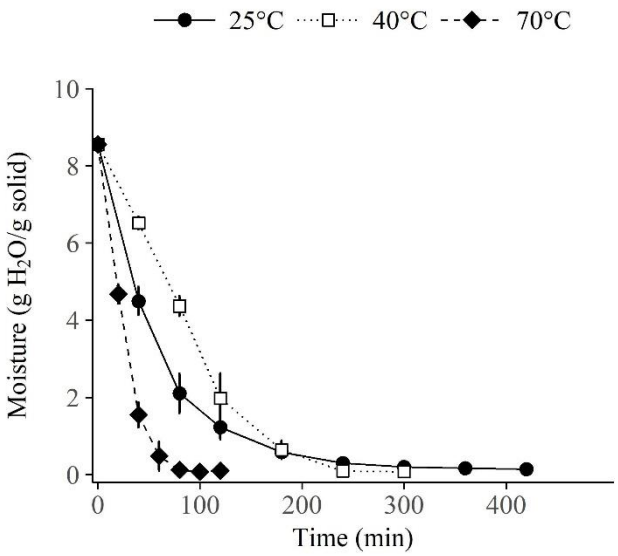

Fig. 1: Experimental drying curves of *S. latissima* at 25, 40 and 70˚C. Values are given as mean ± standard error (n = 3).

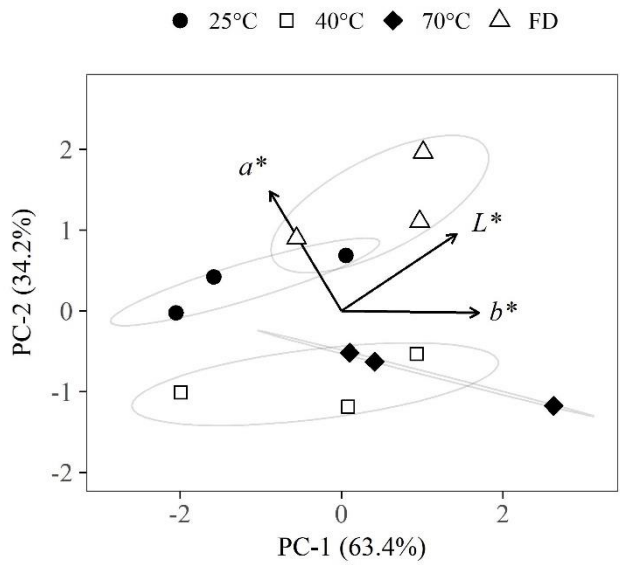

Fig. 2: PCA biplot (1st and 2nd principal component axis) obtained from the color analysis of *S. latissima* samples air-dried at 25, 40, 70˚C and freeze-dried (FD). Vectors indicate loadings representing the variation in individual color coordinates (*L**, *a** and *b**) among all samples.

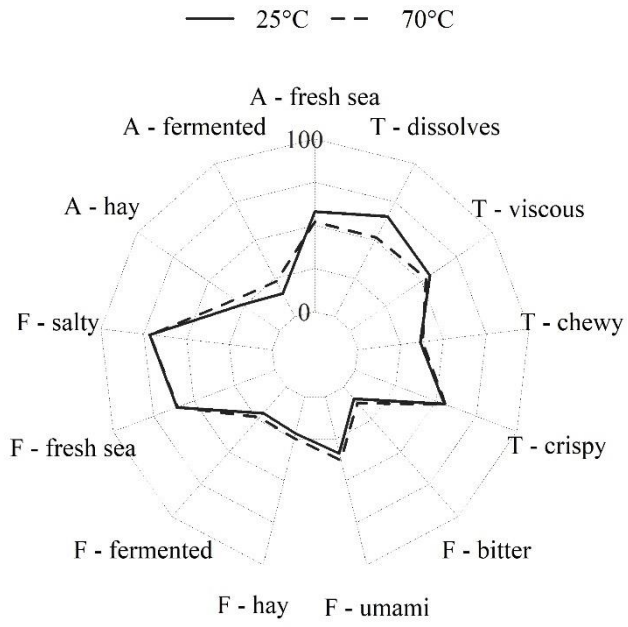

Fig. 3: Mean values from descriptive sensory analysis of *S. latissima*, air-dried at 25 and 70˚C, including aroma (A), flavor (F), and texture (T) characteristics.

Comments to reviewers and editor

Reviewer #1 & #2

Load density and sample mass in the dryer

Information on sample mass and density was added in lines 96-98.

Reviewer #2

Why using the monolayer configuration during drying?

By experience, stacking several seaweed fronds over each other may lead to uneven drying and case hardening. Although this configuration may not be appropriate in an industrial setting, it serves our purpose of studying the effects of drying temperature on the quality of the seaweed.

Reviewer #2

The indicated drying times (these were corrected in the new version of the manuscript) corresponded to the constant weight of the product. This detail was clarified in line 102 and drying times are mentioned in the first paragraph of the results and discussion (R&D) section.

Reviewer #2

Some additional data are interesting to add in order to improve the comprehension of the results.

Due to the amount of data from this experiment, the authors decided on splitting the results into two separate publications i.e. the present manuscript focusing on quality aspects of the material and a second on drying kinetics and technical aspects of the process. As the drying kinetics is an important factor influencing the quality of the final product, additional information was added to the manuscript in the first paragraph of the R&D section and in figure 1.

Reviewer #2

Relative humidity was constant, but what is the value?

This was corrected: RH was not constant upon drying and varied across temperatures. However, it was monitored during the process, added as supplementary online material to the manuscript and used in the discussion of the results (R&D section).

Reviewer #2

How long was the freeze-drying experiment?

line 239: “Freeze-drying of fresh *S. latissima* samples was achieved during a 20 h cycle.”

Reviewer #2

How were the samples milled? Why 120 µm?

Biomass was milled using an electric grain miller. The 120µm powder grain size was chosen for the analysis to be comparable with that of Roleda et al. 2018 (see full reference in the manuscript’s reference list)

Reviewer #2

"This result is expected due to lower drying capacities at lower temperatures". I consider that the result is more related to hygroscopic properties because at the same water activity the moisture content decreases increasing temperature.

An alternative explanation of this result based on the observed difference in RH between treatments was proposed in the first paragraph of the results and discussion section.

Reviewer #2

The comments about iodine content are interesting. Would be a vacuum drying an adequate technique to reduce iodine content for dried samples?

Although similar results were reported in the literature, other studies did not observe any effect of freeze-drying on the iodine content of seaweeds (see Nitschke & Stengel, 2016). As emphasized in this version of the manuscript these results should be interpreted with caution due to the small sample size and the reactive nature of iodide. Other thermal processes e.g. blanching will more effectively reduce the iodine content of kelps.

Reviewer #2

First paragraph of "color and physico-chemical properties": Perhaps a discussion based on processing time of seaweed elapsed at high temperature simultaneously at high moisture content would be interesting. Many reactions need simultaneously moisture content (plastifying effect) and temperature.

The possibility of longer drying times at 25°C resulting in increased pigment oxidation is already mentioned in the R&D section (line 295-304). Although the plastifying effect of water (i.e. even slight amount of water may considerably reduce T_g, increasing the rate of reactions in the matrix) is likely to affect the product quality upon drying, T_g was not measured in this study. An extensive discussion of the results from this perspective would be quite speculative.

Reviewer #2

First paragraph of "Sensory properties and free amino acids". Why were samples dried at 25 and 70ºC selected? Freeze dried samples would be useful as control…

The sensory evaluation of freeze-dried samples would definitely be useful and relevant. As mentioned in line 208 samples of *S. latissima* produced from different pre-treatments (4 in total) were evaluated in this sensory analysis and only the results from two of them (air-drying at 25 and 70°C) were discussed in this study. Since the evaluation of drying treatments on the organoleptic properties of *S. latissima* was not the main purpose of this session (the main purpose was the training of a sensory panel to the evaluation of seaweeds) the freeze-dried samples were left out of the analysis. Larger difference were expected between these two drying treatments but consistent results from the panel members demonstrated the opposite.

Reviewer #2

Second paragraph of this chapter: "The total amount of FAA represented… 8 and 9%...". Really? Are these values right? Please, verify, I have doubts analysing the results shown in Tables

This result was obtained from calculating:

Σ FAA / Σ AA * 100

Where Σ FAA is expressed in mg g^{-1} DW and Σ AA in % DW

From the analysis of the samples air-dried at 25ºC (values from tables 3 and 5), Σ FAA = 5.8 mg g^{-1} DW and Σ AA = 72 mg g^{-1} DW:

5.8/72*100 = 8.05%

Regarding the protein content (Σ AA) the results presented in this study are in the range of values reported in the literature (Schiener et al. 2016; Stévant et al. 2017b; see reference list in the manuscript). Although no reports of the FAA composition of *S. latissima* was found in the literature, the results from this study for Σ FAA are in the range of

those found earlier at the Norwegian University of Science and Technology (NTNU) for the same species (3 – 8.5 mg g^{-1} DW) and comparable to values found in other species i.e. *Alaria esculenta* (13 mg g^{-1} DW) and *Palmaria palmata* (20 mg g^{-1} DW) (unpublished results)

Reviewer #2

The conclusions section must be rewritten. Only conclusions based on the experiments must be written and no new ideas or discussions are necessary.

The conclusion was modified accordingly

Reviewer #2

Finally, the number of Figures and tables is too high (10). I suggest removing the first two Figures. The information given in the text is enough.

The PCA plots from AA composition and FAA composition were removed since they only partially explained the total variation among samples. The PCA plot from the carbohydrate composition was also removed as suggested.

Editor

Please reformat the references to the journal style (see attached style guidelines)

The manuscript (including the reference list) was adapted to the journal's guidelines. Supplementary online material was also added.

Table 1: Sensory attributes, and their definitions, associated to the *S. latissima* samples.

Sensory attribute	Label	Scale anchors	Definition
Aroma			
Fresh sea	A – fresh sea	none || much	Fresh sea odor
Fermented	A – fermented	none || much	Fermented odor, pungent, marmite, matured cheese, cured
Hay	A – hay	none || much	Dry hay, green tea
Flavor			
Salty	F – salty	none || much	Salty taste
Fresh sea	F – fresh sea	none || much	Sea flavor
Fermented	F – fermented	none || much	Fermented flavor, matured cheese, marmite, cured
Hay	F – hay	none || much	Fresh hay, green tea
Umami	F – umami	none || much	Umami, meat stock, brown crab meat
Bitter	F – bitter	none || much	Bitter taste
Texture			
Crispy	T – crispy	cohesive || crispy	During first bites, how crispy is the sample
Chewy	T – chewy	tender || chewy	While chewing, chewy: difficult to disintegrate
Viscous	T – viscous	thin || viscous	Viscous, slimy, porridge like
Dissolves	T – dissolves	None || much	Dissolves or melts easily in mouth while chewing

Table 2: Chemical composition of *S. latissima* samples air-dried at 25, 40, 70˚C and freeze-dried (FD). Values are given as mean ± standard error (n = 3, unless stated otherwise). Different subscript letters in the same row indicate significant differences (ANOVA, Tukey HSD, $p < 0.05$) among drying treatments.

	25˚C	**40˚C**	**70˚C**	**FD**
Residual moisture (%)	5.9 ± 0.2 [a]	4.4 ± 0.2 [ab]	4.0 ± 0.5 [b]	3.0 ± 0.4 [b]
Minerals				
Ash (% DW)	45.4 ± 1.7 [a]	45.2 ± 1.7 [a]	44.0 ± 2.2 [a]	43.8 ± 1.3 [a]
I (mg g^{-1} DW)	5.9 ± 0.3 [a]	5.7 ± 0.3 [a]	4.9 ± 0.2 [1 a]	3.0 ± 0.4 [b]
Carbohydrates (% Σ carb.)				
Alginate	54.6 ± 2.9	50.8 ± 2.3	53.6 ± 2.8 [1]	53.1 ± 1.4
Mannitol	25.5 ± 1.5	30.9 ± 2.0	28.0 ± 3.5 [1]	25.3 ± 3.5
Laminaran	7.1 ± 2.0	5.2 ± 1.4	4.9 ± 0.6 [1]	6.8 ± 2.5
Fucose (fucoidan)	4.7 ± 0.4	5.5 ± 0.5	5.4 ± 0.7 [1]	5.3 ± 0.2
Σ carbohydrates (% DW)	27.1 ± 0.5 [a]	27.8 ± 1.0 [a]	26.9 ± 0.2 [1 a]	23.9 ± 1.5 [a]
Polyphenols (% DW)	0.56 ± 0.03 [a]	0.67 ± 0.04 [a]	0.75 ± 0.02 [1 a]	0.63 ± 0.08 [a]
Fucoxanthin (mg kg^{-1} DW)	319 ± 49 [a]	na	737 ± 101 [1 a]	565 ± 115 [a]

[1] n = 2

Table 3: Amino acid composition (in mg amino acid g^{-1} protein), total amino acid (Σ AA, % DW), total essential amino acid (Σ EAA, in mg amino acid g^{-1} protein), essential amino acid ratio (EAA/AA, dimensionless), and chemical score (in %) of *S. latissima* samples air-dried at 25, 40, 70˚C and freeze-dried (FD). Values are given as mean ± standard error (n = 3). Different subscript letters in the same row indicate significant differences (ANOVA, Tukey HSD, $p < 0.05$) among drying treatments.

	25˚C	**40˚C**	**70˚C**	**FD**	**Pattern [2]**
Essential amino acids (EAA)					
Leu	89.3 ± 0.8	88.3 ± 0.5	87.4 ± 1.4	87.8 ± 1.8	63.0
Phe	63.7 ± 1.0	61.9 ± 0.8	59.9 ± 1.3	64.1 ± 1.5	46.0 [3]
Lys	59.2 ± 0.3	59.2 ± 1.1	59.2 ± 0.6	60.6 ± 0.5	52.0
Val	53.1 ± 0.4	53.0 ± 0.3	53.1 ± 0.5	52.6 ± 0.2	42.0
Ile	49.8 ± 0.5	49.6 ± 0.3	49.2 ± 0.5	48.9 ± 0.2	31.0
Thr	40.9 ± 1.5	39.9 ± 0.6	41.4 ± 1.7	40.7 ± 1.6	27.0
Met	24.1 ± 0.2	25.0 ± 0.3	24.7 ± 0.6	24.7 ± 0.4	26.0 [4]
His	21.6 ± 0.6	22.4 ± 0.3	20.8 ± 0.6	22.9 ± 0.5	18.0
Σ EAA	405.4 ± 1.0 [a]	403.0 ± 2.3 [a]	399.5 ± 6.9 [a]	405.3 ± 5.7 [a]	305.0
Chemical score (%)	100	100	100	100	
Non-essential amino acids (NEAA)					
Glu + Gln	120.0 ± 1.4	129.3 ± 2.8	125.6 ± 5.8	118.8 ± 2.6	
Ala	117.9 ± 0.9	111.6 ± 4.6	120.5 ± 4.2	115.1 ± 3.0	
Asp + Asn	90.0 ± 0.5	91.1 ± 4.1	87.4 ± 2.6	90.6 ± 1.9	
Ser	58.9 ± 0.6	58.9 ± 3.2	53.9 ± 1.6	53.8 ± 2.6	
Arg	51.8 ± 0.2	51.7 ± 0.5	50.9 ± 0.7	55.0 ± 0.4	
Gly	50.8 ± 0.2	51.7 ± 0.4	50.5 ± 1.0	50.2 ± 0.3	
Pro	46.8 ± 1.2	44.6 ± 1.3	49.2 ± 2.1	47.7 ± 1.4	
Cys [1]	29.7 ± 0.4	27.7 ± 0.7	33.1 ± 2.2	31.3 ± 1.0	
Tyr	27.2 ± 1.1	27.6 ± 0.9	26.8 ± 0.8	28.8 ± 0.6	
Tau	1.5 ± 1.5	2.9 ± 1.5	2.7 ± 1.5	3.4 ± 3.4	
Σ AA (% DW)	7.2 ± 0.3 [a]	7.3 ± 0.3 [a]	7.4 ± 0.3 [a]	7.2 ± 0.1 [a]	
EAA/AA	0.4 ± 0.0 [a]	0.4 ± 0.0 [a]	0.4 ± 0.0 [a]	0.4 ± 0.0 [a]	
Total N (% DW)	1.87 ± 0.10 [a]	1.85 ± 0.09 [a]	1.94 ± 0.05 [a]	1.82 ± 0.05 [a]	
N-to-protein ratio	3.94 ± 0.07 [a]	4.03 ± 0.04 [a]	3.87 ± 0.03 [a]	4.03 ± 0.07 [a]	

[1] quantified as cysteine, [2] EAA requirement pattern (WHO/FAO/UNU 2007), [3] Phe + Tyr, [4] Met + Cys.

Table 4: Physico-chemical properties and color parameters of *S. latissima* samples air-dried at 25, 40, 70˚C and freeze-dried (FD). WBC and OBC are expressed in g water and g oil per g dried sample respectively, and SC is expressed in ml per g dried sample. Values are given as mean ± standard error (n = 3). Different subscript letters in the same row indicate significant differences (ANOVA, Tukey HSD, $p < 0.05$) among drying treatments.

	25˚C	**40˚C**	**70˚C**	**FD**
Physico-chemical parameters				
WBC	6.7 ± 1.6 [a]	8.3 ± 0.6 [a]	7.3 ± 1.4 [a]	7.2 ± 0.2 [a]
OBC	4.1 ± 0.4 [a]	4.1 ± 0.1 [a]	4.4 ± 0.5 [a]	6.1 ± 0.1 [b]
SC	6.3 ± 0.5 [a]	5.0 ± 0.2 [a]	4.9 ±0.1 [a]	10.2 ± 0.4 [b]
Color				
*L**	46.0 ± 3.5	46.2 ± 3.9	52.3 ± 3.1	59.7 ± 2.5
*a**	2.1 ± 0.5	-3.4 ± 0.9	-4.6 ± 1.8	2.1 ± 0.8
*b**	34.0 ± 3.4	36.7 ± 4.8	44.3 ± 3.6	39.8 ± 3.5

Table 5: Total free amino acid content and free amino acid composition (in mg g^{-1} DW) of *S. latissima* samples air-dried at 25, 40, 70˚C and freeze-dried (FD). Values are given as mean ± standard error (n = 3). Different subscript letters in the same row (Σ FAA) indicate significant differences (ANOVA, Tukey HSD, $p < 0.05$) among drying treatments.

	25˚C	**40˚C**	**70˚C**	**FD**
Free amino acids (FAA)				
Ala	2.07 ± 0.07	1.86 ± 0.13	2.07 ± 0.08	2.06 ± 0.15
Glu	1.03 ± 0.08	1.51 ± 0.03	1.22 ± 0.15	1.52 ± 0.06
Asp	1.02 ± 0.02	0.95 ± 0.16	1.04 ± 0.01	1.18 ± 0.08
Gln	0.32 ± 0.01	0.22 ± 0.02	0.33 ± 0.07	0.26 ± 0.02
Thr	0.32 ± 0.00	0.16 ± 0.02	0.20 ± 0.04	0.30 ± 0.04
Phe	0.17 ± 0.01	0.19 ± 0.01	0.24 ± 0.00	0.18 ± 0.01
Aba	0.12 ± 0.01	0.21 ± 0.01	0.23 ± 0.02	0.10 ± 0.02
Ser	0.13 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.11 ± 0.00
Asn	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.10 ± 0.01
Val	0.09 ± 0.01	0.10 ± 0.02	0.13 ± 0.03	0.08 ± 0.01
Lys	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.07 ± 0.01
Tyr	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.00	0.06 ± 0.01
Gly + Arg	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.06 ± 0.01
Met	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00
Leu	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.00
Ile	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.00
His	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.02 ± 0.00
Σ FAA	5.8 ± 0.1 [a]	5.9 ± 0.1 [a]	6.2 ± 0.1 [a]	6.2 ± 0.2 [a]

Figure 1

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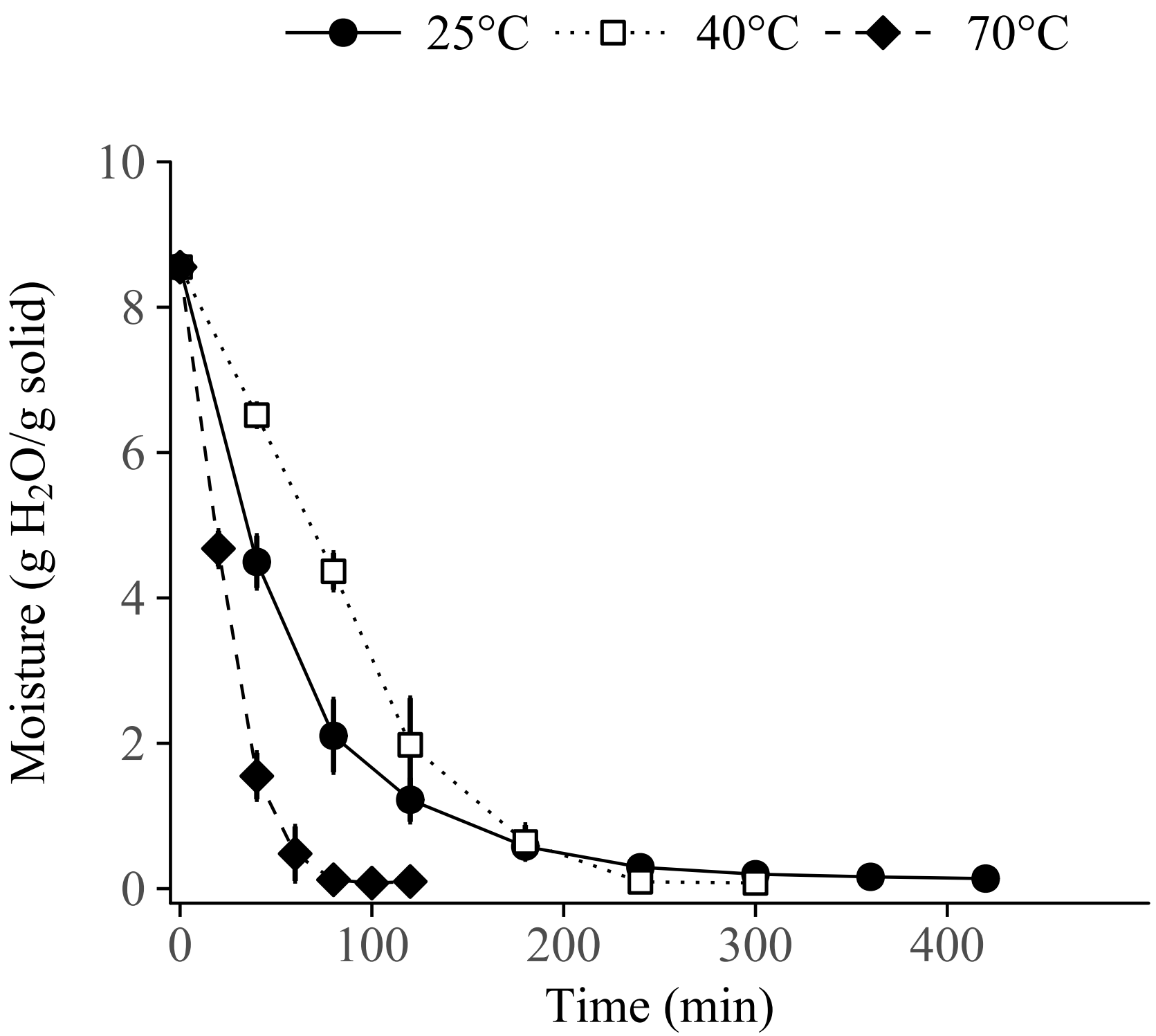

Figure 2

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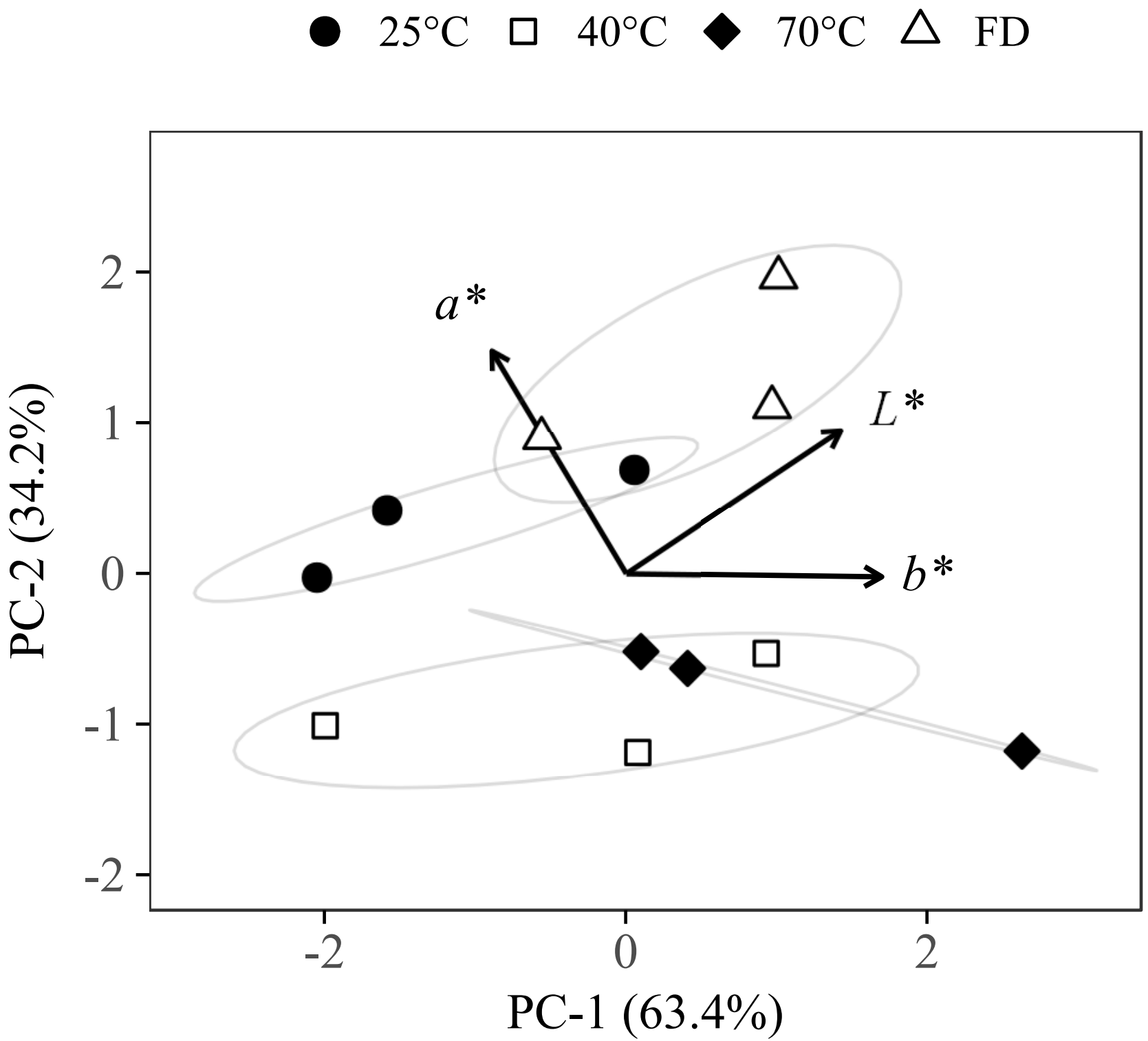

Figure 3

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