ASSESSMENT OF FOOD QUALITY AND MICROBIAL SAFETY OF BROWN MACROALGAE (*ALARIA ESCULENTA* AND *SACCHARINA LATISSIMA*)

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ABSTRACT

BACKGROUND

There is a fast increase in the number of seaweed farms in the Western World, and it is crucial for these companies and their customers to have standardized methods for quality assessment and optimization. The aim of this study was to adapt known methods for food quality determination for analysis of seaweed quality, including color, texture and microbiology, and discuss optimal heat treatments for the popular macroalgae *Saccharina latissima* and *Alaria esculenta*.

RESULTS

The development of a desirable, green color during heating was highly specific to species, freezing history and part of the thallus. The resilience and its thermostability was also species dependent. Low microbial numbers (1 – 3 log cfu/g) for total aerobic count, psychrotrophic bacteria, and spore-forming bacteria were found, but *Bacillus* spp. were isolated. No enterococci, coliforms, pathogenic vibrios, nor *Listeria monocytogenes* were detected.

CONCLUSION

The employed methods were able to describe clearly the physical and microbial quality of *A. esculenta* and *S. latissima*, as well as the quality change during processing. Based on the results, optimal cooking for a minimum of 15 minutes at 95 °C was suggested for *S. latissima*. Fresh and frozen *A. esculenta* showed the greenest color after heating for a few seconds (5 – 9 s) at a high temperature (> 85 °C). If a higher heat load is needed for

achieving safe and stable food products, using fresh and not frozen *A. esculenta* is highly recommended, as fresh specimens remain green even after 15 minutes at 95 °C.

KEY WORDS:

Seaweed, Quality, Heat treatment, Color, Texture, Microbiology

INTRODUCTION

The utilization of brown macroalgae, also known as kelp, is becoming increasingly popular in the Western World.[1-3] As a part of the green bioeconomical shift, their applications for direct human consumption, as well as food ingredient, have gained a growing interest both in Europe (Conlon L (http://www.foodinnovationsolutions.com/seaweed-as-a-food-ingredient/); Holland J (http://www.seafoodsource.com/commentary/seaweed-fleeting-trend-or-realistic-future-food)) and Northern America (Zeiber B (http://seagrant.noaa.gov/News/Article/ArtMID/1660/ArticleID/594); Fehrenbacher K (https://www.theguardian.com/sustainable-business/2017/jun/29/seaweed-farms-us-california-food-fuel)). While there are long traditions for use of macroalgae in Asia, the European and Northern American market for macroalgae as food is less developed. Optimization of processing of local species is a necessary step in developing a place for macroalgae in the Western cuisine.

As thoroughly reviewed by Holdt and Kraan (2012), brown algae are known to contain 73 – 94 % water, and of the dry weight, 36 – 61 % and 15 – 45 % are polysaccharides and ash, respectively.[4] Brown algae are considered high in health promoting compounds,

such as antioxidants,[5, 6] and have therefore been considered a functional food and feed ingredient.

The color of brown algae change to a desirable green color upon heating. The color transformation occur immediately upon exposure to water at high temperatures, making the seaweed visually resemble what is seen for commercially available green vegetables. As the majority of the European population is still unfamiliar with seaweeds as a part of the diet, a bright, vegetable-associated color may lower the threshold for tasting seaweeds. The color of Asian kelp has previously been assessed by visual evaluation by Yamanaka and Akiyama (1993),[7] but evaluation of color changes in Nordic seaweeds are not found in the literature.

The color of raw brown algae is attributed to fucoxanthin, an unstable orange carotenoid.[8, 9] In addition to this pigment, kelp also contain chlorophyll *a*, c_1 and c_2, as well as β-carotene.[6, 9, 10] Fucoxanthin is readily broken down upon cooking, which reveals the green color of the chlorophylls. Using CIE *L**, *a**, *b**, the green color can easily be measured by the red hues (*a**), which expands from green to red.[11] The changes in chlorophyll pigments in broccoli and green peas has previously been monitored using the ratio of $-a^*/b^*$.[12]

Brown algae often have tough cell walls, making them difficult to chew. The major textural component in the cell wall is alginate, which is a polysaccharide specific to kelps. The chemical composition of alginate is known to vary with species, specimen age and part of the thallus, and these factors are therefore crucial for determining the physical

properties.[13-15] As a result, different species of brown algae are expected to react differently to cooking at specific time and temperature combinations with regards to elasticity, strength, and heat stability. It is also expected that farmed seaweeds, which have a uniform age, will show greater homogeneity than their wild counterpart.

Seaweed texture has been previously described,[16-19] however, these studies were aiming to investigate the strength of seaweed against factors in the habitat, not food properties. For assessing salads, the elasticity has been analysed using a stretch test to obtain the elastic modulus.[20, 21] To ease the performability of the test, a notch can be applied to the specimen, controlling the mechanism of the fracture.[21] The elastic modulus (E, Equation 1) can be defined as the amount a material is stretched when a specific force is applied. In Equation 1, L and t are the length and thickness of the sample, and the total width is represented by the width of the sample subtracted by the length of the notch. The position where the gradient, $\Delta F/\Delta d$, in the elastic region of the force-displacement curve was measured is indicated in Figure 2.

$$E = \frac{L\,(w-n)}{t}\frac{\Delta F}{\Delta d} \qquad \text{Equation 1}$$

The ultimate tensile strength (σ, Equation 2) describes the relative force needed to break the material, and is calculated from the sample dimensions and the peak force (F) in the force-deformation curve.

$$\sigma = \frac{F}{t(w-n)} \qquad \text{Equation 2}$$

Microbiological safety is a basic constraint for all foods, and the applied analytical methods for assessment of most foods are aiming to detect pathogens commonly associated with the food in question. Heat treatment generally reduces the microbial load of a product, and the heat load chosen is usually a compromise between avoiding undesirable quality changes and inactivation of bacteria. The groups of bacteria currently associated with the highest health risk in kelp in Norway are spore-forming bacteria. Spores are able to survive higher heat loads than vegetative bacterial cells, and many spore-forming species are known to cause disease.[22-24] Identifying the number of spore-forming bacteria, as well as the species, is therefore necessary before proposing a microbiologically safe preparation of the food in question. Bacteria belonging to the genus *Vibrio* are Gram-negative, oxidase-positive, non spore-forming and typically appear as slightly curved rods. In contrast to most other bacteria of importance for seafood safety, members of this group have the marine and estuarine environment as the main natural habitat.[25-27] The incidence and density of human pathogenic vibrios in the environment, and consequently in seafood products, are highly dependent on the ambient water temperatures. Vibrios may be detected in seawater, bivalve mussels and marine sediments in Norway during the summer months,[28, 29] however the isolates are not of the pandemic types (O1, O139), nor carry pathogenicity genes (*tdh*, *trh*). Even though vibrios would likely be found on brown algae after harvest, these bacteria are sensitive to desiccation and heat, and would be decimated during production of the algae as described in this work. However, if in contact with non-treated seawater after heat treatment, a recontamination may occur.

Dehydration is the most common preservation method for seaweed as food today, although freezing and refrigeration are used for some species and applications.[30] Drying and freezing enable longer shelf life – which is important due to a so-far limited circulation of the kelp products in the market. Both processes decrease the water activity of the food, which limits the activity of several microorganisms.[23] Freezing also has a range of further functions on microbiota, such as sudden death for some bacterial species and growth inhibition for surviving organisms.[23]

For an increasing number of seaweed farms in the Western World, safety and recommended processing are of critical importance to their market success. It is crucial to the seaweed farming industry to define the quality and safety of their products and, thus, they need documented reference methods of analysis. Some safety issues may potentially limit the use of macroalgae as food, such as the occasional high levels of inorganic arsenic and cadmium found in brown algae.[31-33] Methods to reduce the level of undesirable metals in seaweed is a very critical issue which require focus in further studies. In this study we have presented reference methods for determining quality and microbiological safety for the most popular macroalgae in Northern-Europe.

MATERIALS AND METHODS

Preparation of samples

All *Alaria esculenta* (winged kelp) and *Saccharina latissima* (sugar kelp) used in the described experiments were cultivated at Værlandet-Bulandet (61 °N), Norway, by Seaweed AS.

Preparation of frozen seaweeds

A. esculenta and *S. latissima* were harvested in April 2015, washed in seawater, sorted, and packed in vacuum pouches of approximately 2.5 kg before freezing. After freezing, the contents were cut into thin slices, vacuum-packed, and stored again at -40 °C.

Preparation of fresh, live seaweeds

A. esculenta and *S. latissima* were harvested in March and April 2016, washed in seawater, sorted, and transported to Stavanger, Norway, in seawater at 0 °C in a solid container. The seaweeds were analyzed directly from the container, within 5 days of harvesting.

Heat treatments of the specimen

For analysis of how color and texture of the kelp changed during cooking, specimen were by immersion in a water bath (Lauda Ecoline E300 Star Edition and Grant -4 GD150 S12) directly before measurements, at the temperatures and durations described in Table 1.

Preparation of samples for microbiological analysis

For microbiological analysis, seaweeds were harvested in April 2015, washed, sorted, cut, centrifuged, vacuum-packed in pouches of approximately 150 g, and subsequently heat-treated as described in Table 1 or frozen raw.

Color analysis

Color analysis of frozen seaweeds

Frozen samples were collected from the -40 °C storage, and kept in a dark container at 0 °C until analysis within 0 to 6 hours. Pieces of seaweed (n = 6) were put single layered on a white surface, and the color of thawed specimen was measured using CIE *L**, *a**, *b**. A photo chamber from VeriVide (Version 3.1. with Grey Mask, Leicester, UK) with day-light lamps (6500K) coupled to a digital camera (Nikon D80, 35 mm lens, Tokyo, Japan) and DigiEye software (Version 2.4.7 (revision e)) was used. Raw and heat-treated (Table 1) specimen were analyzed for color within 3 minutes of cutting and heating, respectively. For collection of data, three separate spots of 4.0 x 4.8 mm from each seaweed sample were chosen. The chosen spots were judged to be representatives for the overall color, without holes or tears, and levelled with the surface underneath.

Color analysis of fresh seaweeds

Proximal, middle, and distal pieces of seaweeds were cut, and analyzed as described in the previous section. The proximal and distal samples were cut from seaweeds collected in March 2016, and were used for analysis of biological variation within and between specimens. The data were collected using flexible large circular measuring points covering the seaweed, as demonstrated in Figure 1. Middle samples cut from seaweeds collected in April 2016 were used for analysis of the effect of selected heat treatments on the seaweed. The average color values from whole middle area was collected (Figure 1).

Texture analysis

Texture analysis of frozen seaweeds

Frozen samples were collected from the -40 °C storage, and kept at 0 °C until analysis. Rectangular specimen of 10 x 60 – 70 mm, with notches of 2 – 4 mm, were cut from thawed pieces of seaweed at a random position and orientation. The dimensions were measured accurately using a calliper. For raw seaweed samples, the specimen were analyzed for texture directly after cutting. The heat-treated samples were cooked directly after cutting, followed by the tensile test. An adjacent piece of seaweed was used for measurement of thickness. The samples were heated as described in Table 1. The rectangular specimens were monitored between two Tensile grips (A/TG) of a Texture Analyzer (Stable Micro Systems, Godalming, UK) with 40 mm apart. The tensile test was performed at 16 mm/s.

Texture analysis of fresh seaweeds

Specimen were cut from the distal, proximal and middle part of *S. latissima* and *A. esculenta* as described above (Figure 1). From fresh seaweeds collected in March, samples from the distal and proximal parts of the kelp were used to examine the biological variation within the organisms. To examine the effect of heat treatment on the samples, specimens from the middle part of seaweed collected in April were used (Figure 1).

Microbiology

Assessment of microbial numbers

Preparation of samples for microbial analysis

Each parallel was prepared from 19 – 25 g seaweed taken from a separate vacuum-packed bag and mixed with autoclaved saline solution (0.9 % NaCl) at a ratio 1:10.[34] The samples were then homogenized in a stomacher machine (Star-Blender™ LB400, VWR) for 180 seconds. For the analysis of spore-forming bacteria, 5 mL were placed into glass tubes with a plastic cork and heat-treated at 80 °C for 12 minutes in a water bath (Lauda Ecoline E300 Star Edition) before further plating.

Total aerobic count

Pour plates with 1 ml sample and Marine Agar (MA, 18.7 g Marine Broth 2216, Difco™, with 8.0 g Agar-Agar, Merck KGaA in 500 ml distilled water) were made for raw (n = 3) and heat-treated *A. esculenta* (n = 9) and *S. latissima* (n = 8).[35] This method was simplified and modified after Bengtsson *et al* (2011).[34] The plates were incubated aerobically at 25 °C for 72 hours.

Psychrotrophic bacteria

Pour plates with 1 ml sample and MA, Plate Count Agar (PCA, ISO4833 GranuCult™ Merck KGaA), or PCA with 1.0 % added salt (NaCL, EMSURE® Merck KGaA) were made to characterize psychrotrophic bacteria in raw seaweeds (n = 3). For seaweeds, pour plates with MA (n = 9) were made. All plates were incubated at 17.0 °C for 20 hours, followed by 7.0 °C for 72 hours, or, alternatively, 6.5 °C for 10 days, as outlined in NMKL

method no. 86 (2013).[36] Due to practical issues, pour plates with PCA were incubated at 8 °C for 7 days as previously done by Rotabakk *et al* (2009).[37]

Spore-forming bacteria

Aerobic and anaerobic spore-forming bacteria from raw and heat-treated *A. esculenta* and raw *S. latissima* were characterized by spreading 100 or 200 µl of the heat-treated sample onto Blood Agar plates (BA; Oxoid CM271+B; n = 3), according to NMKL method no. 189 (2008).[38] Due to low bacterial numbers, spore-forming bacteria were also examined using pour plates with 1 ml heat-treated sample and tryptone soy agar with yeast (TSAY; 20 g Tryptose soy agar, Oxoid, CMO131 with 3.0 g Yeast extract, granulated, Merck KGaA in 500 ml distilled water). The latter method was performed for heat-treated *A. esculenta* (aerobic n = 9, anaerobic n = 3) and *S. latissima* (n = 9), as well as raw seaweeds (n = 3). All plates used for examining germination of spores were incubated at 30 °C for 8 days. Morphology was examined using a light microscope (Leica DM1000, Wetzlar, Germany).

Identification of bacterial species

BA and TSAY plates used to enumerate the spore-forming bacteria in *A. esculenta* and *S. latissima* were further analyzed to identify bacterial strains. All colonies of medium to good growth were isolated, namely six colonies from raw and two with *A. esculenta*, and eight colonies from heat-treated *S. latissima*. The colonies were then spread on BA and MA plates for both aerobic and anaerobic incubation at 30 °C for 7 days. Bacterial species were identified using Analytical profile index (API) ® 50 CHB/E combined with 20 E for identification of *Bacillus* related genera,[39] as described by the producer.

Microbial safety parameters

Analyses for microbial safety parameters were performed on both fresh and frozen seaweed samples (n = 1). To reveal possible faecal contamination, the presence of indicator organisms was examined. Analyses for enterococci were performed according to NMKL method no. 68 (2011).[40] Results were reported as log cfu/g, and the detection limit was 2.0 log cfu/g. Analyses for coliforms and thermo-tolerant coliforms were performed after homogenization in a stomacher (Seward 400 Circulator, UK) for 2 minutes of 10 g seaweed in peptone water. Volumes of 0.1 and 1.0 ml were examined on Petrifilm™ (3M™ coliform Count Plates), according to the protocol supplied by the producer. The results were reported as log cfu/g and the detection limit was 1.0 log cfu/g. For coliforms, m-Endo agar LES (Difco) was incubated at 37 °C for 24 h. For thermo-tolerant coliforms, m–FC– agar (Difco) was incubated at 44.5 °C for 24 h. Blue colonies on m–FC– agar were to be confirmed as *E. coli* by inoculation and incubation in EC-broth, prior to indole testing.

Analyses for pathogenic *Vibrio* species were performed according to NMKL method no. 156 (1997).[41] Results were reported as detected/not detected in 20 g of sample.

For detection of *L. monocytogenes*, 25 g of seaweed was homogenized with 225 ml Half-Frazer broth (BioRad), incubated at 30 °C for 24 h, followed by analyses using chromogenic agar RAPID'L.mono (Bio-Rad), performed according to the protocol supplied by the producer. Results were reported as detected/not detected in 25 g of sample.

Other analyses

Thickness

Thickness was measured by placing a seaweed sample between two objective glasses followed by placement between the probes of a Sylvac Metop System 2001 micrometer. The algal thickness was obtained by subtracting the thickness of the glass.

pH

Frozen, vacuum-packed bags of *A. esculenta* (n = 6) and *S. latissima* (n = 6) were thawed. Half of the bags (n = 3) were subsequently cooked sous vide at 95 °C for 15 minutes, followed by cooling in a water bath. The contents of each bag, including drip loss, were homogenized, and a KCl solution (0.15 M) was added in a 1:1 ratio. The pH was then measured at 21.5 – 23.0 °C, using a Mettler Toledo Five Easy Plus FEP20 (Zürich, Switzerland) pH-meter, with a LE438 (Mettler Toledo, Zürich, Switzerland) electrode calibrated with pH 4 and 7 buffer solutions.

Statistical analysis

The results from the color and texture analyses were processed using one-way analysis of variation (ANOVA) with 95 % confidence interval in Minitab© 18, with Tukey post-hoc test.

RESULTS AND DISCUSSION

Color

Trends in color development in frozen-thawed seaweeds

S. latissima treated at different time and temperature combinations seemed to follow some clear trends with regard to its green color development (Figure 3). The green color, expressed as *–a*/b**, increased in a two-step manner upon heat treatment. The first step was an initial increase after only 1 second, which was visible at all tested temperatures (60 – 95 °C). This increase was attributed to the breakdown of the pigment fucoxanthin. The color remained relatively constant from 1 seconds up to 15 minutes. After this time, the second step in rising green color was observed up to endpoint color. For temperatures 60, 75 and 95 °C shown in Figure 3, the measured green color did not differ between 45 and 60 minutes, indicating that a plateau was reached. The end-point plateau was higher the higher the temperature, thus both long time intervals (45 – 60 min) and higher temperatures (90 – 95 °C) were necessary in order to reach the greenest colors. This indicates a non-Arrhenius type temperature dependency in the tested temperature and time interval for *S. latissima*.

For *A. esculenta*, the first step of increasing green color occurred after 1 second and for all temperatures (60 – 95 °C), just like for *S. latissima*. The color remained relatively constant and at a greener color-value than *S. latissima* during the first 9 seconds, and thereafter the

greenness started to decrease from 5 – 60 minutes at what seems to be a temperature dependent rate. This observation held true for temperatures 60, 75 and 90 °C portrayed in Figure 3, and it is likely to be caused by a break-down of chlorophyll and other coloring compounds in the alga. The temperature development at 95 °C seems to reach a low plateau with unchanged average values at 30 and 45 minutes. At 60 minutes there was an unexpected observation of a large increase in green color, that was also accompanied with a significantly smaller standard deviation than that of the observations after 30 and 45 minutes (n = 18). The sudden greenness after 1 hour is worth re-examining if cooking times above 45 minutes will be applied, but may also be an expression of the large variation present in the samples, as discussed in the next section.

The difference in color development in *A. esculenta* and *S. latissima* was very unexpected based on the similarity in the type of pigments present. It was hypothesized that breakdown of the brown pigment fucoxanthin, which masked the green color of the chlorophylls in the raw condition, would lead to initial increasing green color during heating. Thereafter, the chlorophylls would break down, and the color would again become brown-ish due to formation of chlorophyll breakdown products, including pheophytin. This hypothesis fits well with observations for *A. esculenta*, where green color rise and fall again during heating. In *S. latissima*, however, the color rise and remain on top after cooking for one hour at 95 °C. The latter observation suggests differences in cell biology or metabolism that we encourage other research facilities to explore.

Effect of biological variation on the color of fresh seaweeds

For *A. esculenta*, both red-green hue (*a**) and yellow-blue hues (*b**) were significantly higher for proximal parts compared to distal. As the examined specimen were grown vertically on ropes, this difference could indicate locally optimized pigment composition in the light harvesting complexes due to varying distance to the ocean surface. No such difference was found in *S. latissima*. Both *A. esculenta* and *S. latissima* showed increased lightness (*L**) in proximal parts compared to the distal parts, despite significantly thicker structures ($P \leq 0.13$), perhaps as a result of photodamage. Thus, the large deviations associated with the values in Figure 3 may in part be due to large biological differences within seaweed specimen.

Comparison of color of fresh and frozen-thawed seaweeds

Heat treatment of fresh and frozen-thawed seaweeds resulted in varying greenness as shown in Table 3. The time- and temperature combinations were carefully selected based on results from experiments with frozen seaweeds. The blanching temperature at 54 °C was chosen based on differential scanning calorimetry (DSC) performed on *A. esculenta* (not shown). The DSC analysis showed a distinct change within the range 50 – 55 °C. The blanching temperature at 85 °C was chosen based on a bright green color when analyzing frozen *A. esculenta*, and the cooking time at 95 °C was chosen based on high microbiological quality (Table 5).

For all heat treatments and both species, the greenness (*a** and *-a*/b**) was significantly higher for fresh than frozen-thawed seaweed (Table 3). For instance, a bright green color was seen in fresh *S. latissima* after 15 minutes cooking at 95 °C, while a bright greenness was not observed before after 30 minutes for frozen-thawed *S. latissima*. Similarly, fresh *A. esculenta*

blanched for 5 seconds at 85 °C showed an average red hue (*a**) of -9.64, which was significantly greener than frozen-thawed *A. esculenta*, which had a measured red hue (a*) of -1.66. Destruction of tissue, freeze denaturation, altered enzymatic reactions, and perhaps microbiological effects, are likely to play a role in altering the color upon block freezing and subsequent thawing. It should also be noted that differences between the harvest years 2015 and 2016 may have an effect.

Texture of fresh and frozen-thawed seaweed

The texture of raw and heat-treated *A. esculenta* and *S. latissima* was measured using a tensile test. For *A. esculenta*, both fresh and frozen-thawed samples were assessed, whereas only fresh samples of *S. latissima* were tested. Texture results, expressed as Elastic modulus and ultimate tensile strength, were associated with very high standard deviations, and few significant differences between the tested treatments (Table 4). The average results were in the order 1.92 – 9.10 MPa, compared to values of 1.10 – 4.40 MPa reported for salad.[21] For *S. latissima*, there were significant differences between the proximal and middle versus the distal part of the raw thallus. For *A. esculenta*, no significant differences between the parts or between fresh and frozen seaweeds were observed. Blanching and cooking the middle part of *S. latissima* did not result in a significant different texture than the raw middle part. For *A. esculenta*, blanching decreased the Elastic modulus significantly. Cooking for 15 minutes at 95 °C resulted in an increase in Elastic modulus to the same order as raw kelp. The exception was frozen-thawed *A. esculenta*, which had a significantly higher Elastic modulus after cooking for 15 minutes at 95 °C than the other treatments. This suggests that the freezing method (block-freezing) and storage temperature (-30 °C) used in this study resulted in a toughening of the

kelp thallus. Toughening during freezing is a well-documented phenomenon in fish muscle, and is correlated with crystal formation and disruption of cells,[42] which can be minimized by optimization of freezing conditions.[43] This result was so far only detected in heat-treated specimens, and not in raw kelp (*A. esculenta* only). To gain a better understanding, freezing strategies for kelp should be further investigated.

Microbiology

Microbial quality and safety of raw seaweeds

Low microbial numbers, between 1 and 3 log cfu/g, were found for all measured parameters (Table 5). In raw kelp, a lower total microbial count and lower numbers of spore-forming bacteria were found in *S. latissima* than in *A. esculenta*. Low numbers of psychrotrophic bacteria were detected in raw kelp.

Coliform bacteria mainly originate from the intestines of warm-blooded animals including faeces of humans. Hence, assays for coliforms are often used as an indicator of the hygienic standards during food production. Such indicator organisms of faecal contamination include coliforms, thermo-tolerant coliforms, *E. coli*, and enterococci.[44] There is no documented correlation between the occurrence of vibrios and indicator bacteria of faecal origin, thus commonly applied indicator organisms as coliforms do not give information on presence of potentially pathogenic vibrios. Vibrios are not considered robust bacteria, and are therefore relatively sensitive to heating, freezing, drying and several other preservation techniques, as well as the low pH in the stomach of humans. Regarding microbial safety parameters, no

144 enterococci, coliforms, pathogenic vibrios, nor *Listeria monocytogenes* were detected in any
145 of the seaweed samples analysed.

146 *Microbial quality of vacuum-packed, cooked seaweeds*

147 There did not seem to be a difference in any of the bacterial numbers on *A. esculenta* and *S.*
148 *latissima* compared to the raw (Table 5). There were still significant numbers of heat tolerant
149 bacteria on cooked kelp, indicating that the applied heat treatments – 80 °C for 15 minutes
150 for sugar kelp, and 30 minutes for winged kelp – was not sufficient to eliminate the number
151 of viable spore-forming bacteria on the kelp.

152 *Identification of bacterial species*

153 From the BA and TSAY plates used to count spore-forming bacteria, 11 out of 16 colonies
154 continued to grow when reinoculated. Of these, eight showed aerobic growth, whereas four
155 showed anaerobic growth. Of the seven colonies identified with API 50 CHB/E from *A.*
156 *esculenta*, six were *Bacillus licheniformis* (99.9 % ID). The remaining colony was identified as
157 *Bacillus pumilus* (95 % ID). Of the four colonies identified from *S. latissima*, two were *Bacillus*
158 *subtilis*/*Bacillus amyliquefaciens* (≥ 99.5 % ID), one was *B. licheniformis* (99.9 % ID), and one
159 *Bacillus pumilus* (96.4 % ID). Results from Gram staining, catalase and oxidase tests
160 corroborated API analysis. *B. pumilus* and *B. licheniformis* has previously been associated with
161 food poisoning,[22, 24] and therefore the growth of these organisms should be controlled or
162 eliminated.

pH

The pH of raw and cooked *S. latissima* and *A. esculenta* were, in all cases, between 6.1 and 6.4. This pH is not sufficiently low to have a major effect on the growth of most microorganisms.[23] Since there was no significant change in pH upon cooking it is not likely that pH will have an effect on color transformation during heating.

Optimization of heat treatment

Within defined outer and inner conditions, the D-value can be described as the time necessary to reduce the number of bacteria with one logarithm.[23] D-values for *B. licheniformis* and *B. cereus* at 95 °C in foods with neutral pH, have been previously estimated to 7.2 and 2.4.[45, 46] Based on this, a heat treatment of 15 minutes at 95 °C can be proposed for a 3-log reduction of spore-forming bacteria in *S. latissima* and *A. esculenta* from Værlandet-Bulandet.

Frozen-thawed *S. latissima* reached its greenest color after a heat treatment of 45 – 60 minutes at 95 °C, which is likely to give a more than adequate microbial reduction. The color of fresh *S. latissima* was bright after 15 minutes at the same temperature, and based on the results for frozen-thawed seaweeds it is likely to stay green during longer cooking times. The texture of blanched sugar kelp was not significantly different from that of kelp cooked at 95 °C for 15 minutes, suggesting that the elastic properties of this kelp require tougher treatments to change. Frozen-thawed *A. esculenta*, had a bright green color after blanching, but after heating at higher temperatures (80 – 95 °C), the greenness decreased when 5 minutes or longer cooking times were used. In contrast to Frozen-thawed kelp, fresh *A. esculenta* remained green after 15 minutes at 95 °C, and a greener color value was measured

also for blanching treatments. Thus, when processing kelp that has already been frozen and thawed, the color can be expected to be much less bright than for fresh kelp. It can also be expected, based on results for frozen-thawed kelp, that fresh *A. esculenta* will fade when exposed to longer heating durations than 15 minutes at 95 °C. In order to maintain greenness, short-time heating can be combined with other processing methods, to achieve an adequate shelf-life. The textural resilience of *A. esculenta* seemed to decrease with a more thorough heat treatment, thus making the kelp easier to chew. Cooking at 95 °C for 15 minutes resulted in a less resilient texture than blanching. However, sensoric analysis of the taste, texture and color of both species cooked at 95 °C for 15 minutes, as well as experimental determination of the D-value of *B. licheniformis* and *B. pumilus* isolated from the kelp, should be carried out to ensure that this product is both sensorically appealing and safe.

CONCLUSION

Saccharina latissima reached its lowest red hue (*a**), which corresponds to the greenest color, after thorough boiling for 30 – 60 minutes at 95 °C. Shorter heating durations were necessary for fresh kelp. The texture of *S. latissima*, measured as Elastic modulus and ultimate tensile strength, did not significantly differ between blanched and boiled specimen, indicating that the chewiness remained unchanged. *A. esculenta* reached its greenest color after blanching treatments for up to 9 seconds. Higher heat loads, such as boiling at 95 °C for 15 minutes, reduced the green color of the algae. The color reduction was much greater if the specimen had been previously frozen, and thus using fresh algae and not frozen is recommended if a higher heat load is needed to attain a more storage-stable product. Boiling at 95 °C for 15 minutes also led to a significant increase in Elastic modulus, corresponding to less resilience, than blanched *A. esculenta*. Toughening of the thallus as a result of freezing was also observed in heat-treated specimen.

There were low total bacterial counts on the algae, between 1 – 3 log cfu/g both for unprocessed as well as for the examined heat loads. Regarding microbial safety parameters, no enterococci, coliforms, pathogenic vibrios, nor *Listeria monocytogenes* were detected in any of the seaweed samples analysed. Due to isolation of potentially toxin producing spore-forming bacteria (*Bacillus pumilus* and *B. licheniformis*), measures need to be taken to control the growth of these species in the food during handling and storage.

The methods presented were successfully used to describe the quality of *S. latissima* and *A. esculenta*. The methods described for color and texture analysis are also applicable for other

flat-bladed macroalga, but require some adjustments for analysis of more irregular shaped alga. Differences between fresh and frozen produce, as well as biological differences, should be considered during analysis.

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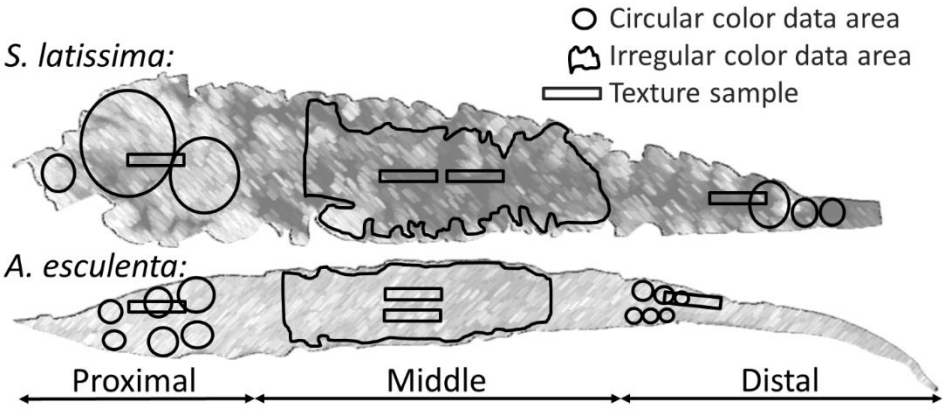
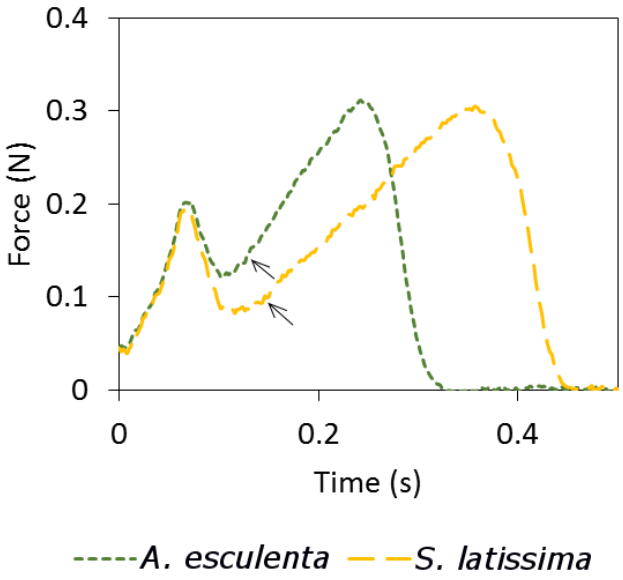


336

337 **Figure 1.** The areas selected for color and texture analysis of fresh *Saccharina latissima* and *Alaria*
338 *esculenta*. The proximal and distal parts were used to evaluate the biological variations within and
339 between specimens, whereas the middle part was used to analyze quality change upon heat
340 treatments.

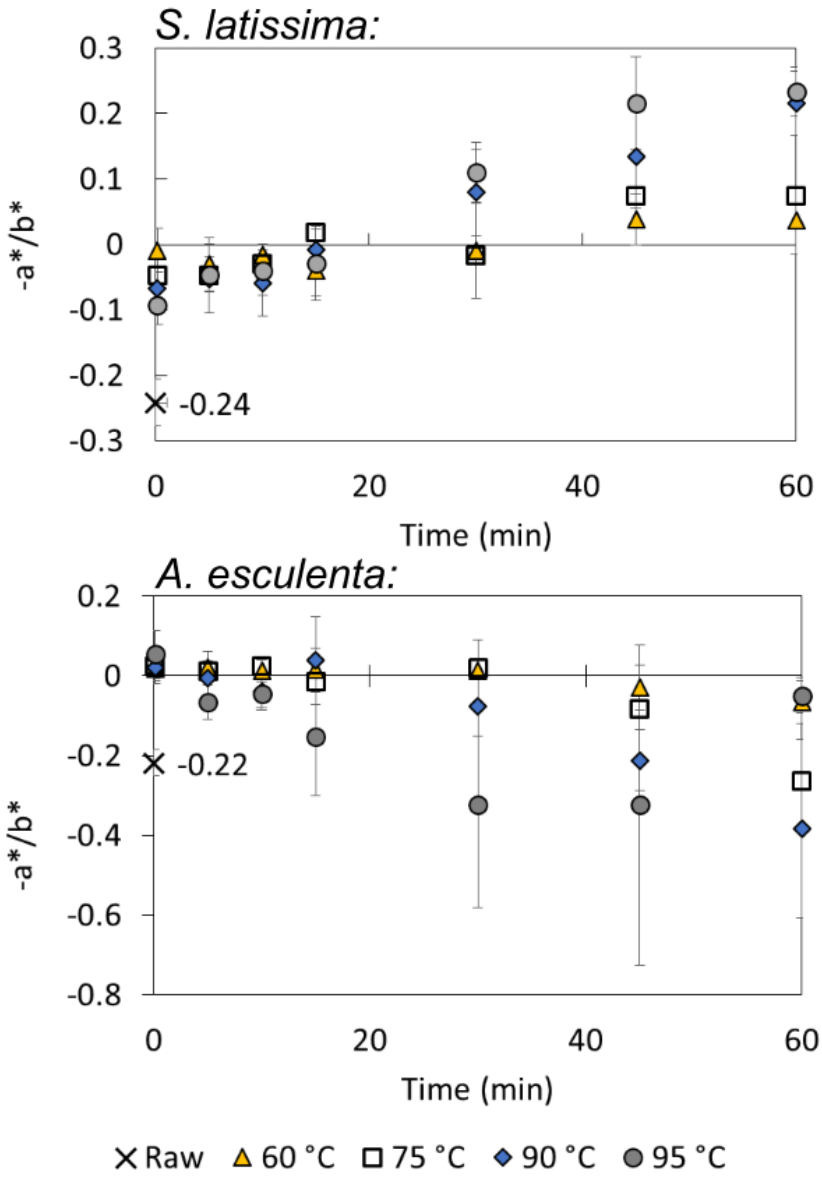
341



342

343 **Figure 2.** Typical force-displacement curves resulting from tensile tests of notched specimen of *Alaria*

344 *esculenta* and *Saccharina latissima*. The arrows indicate where the initial gradient was measured.



345

346 **Figure 3.** Green color development ($-a^*/b^*$) during blanching of kelp at selected temperatures. Mean

347 values ($\pm$ SD) are shown for raw *Saccharina latissima* (n = 282) and *Alaria esculenta* (n = 231), as well

348 as heat-treated kelp (n = 18) at 60, 75, 90, and 95 °C for 9 s, 5, 10, 15, 30, 45, and 60 min.

349

Table 1. Overview of storage conditions and heat treatments (HT) used in the analyses.

Analyses	Storage	Samples and HT	
		S. latissima	*A. esculenta*
Color	Frozen	Raw and HT at 60, 65, 70, 75, 80, 85, 90, 95 °C for 1, 3, 5, 7, 9, 300, 900, 1800, 2700, 3600 s	Raw and HT at 60, 65, 70, 75, 80, 85, 90, 95 °C for 1, 3, 5, 7, 9, 300, 900, 1800, 2700, 3600 s
	Fresh	Raw, 85 °C/5 s, 95 °C/15 min	Raw, 54 °C/2 min, 85 °C/5 s, 95 °C/15 min
Texture	Frozen	NA	Raw and 95 °C/15 min
	Fresh	Raw, 85 °C/5 s, and 95 °C/15 min	Raw, 54 °C/2 min, 85 °C/5 s, and 95 °C/15 min
TMC, PB, SB	Frozen	150 g vacuum packed, raw	150 g vacuum packed, raw
		150 g vacuum packed, 80 °C/15 min	150 g vacuum packed, 80 °C/30 min
Microbial safety	Frozen*	150 g vacuum packed, 80 °C/15 min	150 g vacuum packed, 80 °C/30 min
	Fresh**	Raw	Raw

Frozen storage at -40 °C VP, ≤ 1 year, and fresh storage at 0 °C SW, ≤ 5 days, unless stated otherwise. For analysis of color and texture, all heat treatments were performed by direct immersion in water bath. TMC, Total microbial count; PB, psychotropic bacteria; SB, spore-forming bacteria; VP, vacuum packed; SW, in sea water; NA, not analyzed. *) Storage at -20 °C VP ≤ 1 month; **) Storage at <4 °C in plastic bags, ≤ 5 days.

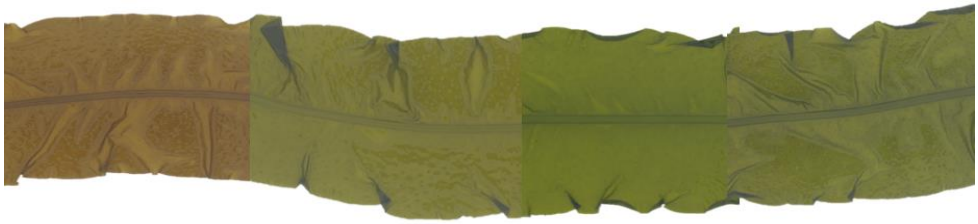
Table 2. CIE L*, a*, b* color data – lightness (L*), red hues (a*) and yellow hues (b*) – for proximal (near connecting organ) and distal (far from connecting organ) of *Alaria esculenta* and *Saccharina latissima*.

		L*	a*	b*
A. esculenta	Distal (n = 60)	40.6 ± 3.50^{a}	11.9 ± 1.24^{a}	35.2 ± 8.24^{a}
	Proximal (n = 60)	45.7 ± 7.58^{b}	13.7 ± 2.38^{b}	41.0 ± 8.25^{b}
S. latissima	Distal (n = 26)	49.2 ± 2.71^{c}	13.9 ± 0.783^{b}	46.3 ± 4.74^{c}
	Proximal (n = 26)	56.9 ± 1.54^{d}	13.0 ± 0.807^{b}	$42.7 \pm 3.27^{b,c}$

Values are expressed as mean ± SD. Values in a column not sharing a common footnote are significantly different ($P < 0.05$).

Table 3. Greenness ($a*$ and $-a^*/b^*$) of raw fresh (n = 15) and frozen-thawed *Alaria esculenta* (n = 231), raw fresh (n = 10) and frozen-thawed *Saccharina latissima* (n = 282), and heat-treated fresh (n = 5) and thawed (n = 18) samples of both species.

		A. esculenta			
		Raw	54 °C, 2 min.	85 °C, 5 s.	95 °C, 15 min.
Thawed	a*	8.64 ± 1.32^{a}	–	-1.66 ± 1.53^{b}	4.78 ± 4.94^{c}
	-a*/b*	-0.218 ± 0.0323^{a}	–	0.0466 ± 0.0233^{b}	-0.151 ± 0.150^{c}
Fresh	a*	12.2 ± 0.458^{a}	-3.55 ± 1.22^{b}	-9.64 ± 1.41^{c}	-5.18 ± 0.859^{d}
	-a*/b*	-0.421 ± 0.0439^{a}	0.087 ± 0.0295^{b}	0.228 ± 0.0315	0.177 ± 0.0433^{c}



		S. latissima		
		Raw	85 °C, 5 s.	95 °C, 15 min.
Thawed	a*	9.76 ± 1.52^{a}	-0.0350 ± 0.703^{b}	1.24 ± 1.91^{c}
	-a*/b*	-0.242 ± 0.0356^{a}	0.00108 ± 0.0175^{b}	-0.029 ± 0.0457^{c}
Fresh	a*	11.3 ± 3.39a	-4.34 ± 2.53b	-5.66 ± 1.15b
	-a*/b*	-0.305 ± 0.0380^{a}	0.097 ± 0.0482^{b}	0.126 ± 0.0253^{b}

Values are expressed as mean ± SD. Values in a row not sharing a footnote are significantly different ($P < 0.018$), in addition to significant differences between values for fresh and frozen seaweed for both species and all treatments ($P = 0.000 – 0.003$).

370 **Table 4.** Elastic modulus (*E*) and ultimate tensile strength (σ) of raw and heat-treated (HT) *Alaria*
371 *esculenta* and *Saccharina latissima*.

Specimen description			Blade position	HT	*E* (MPa)	σ (MPa)
A. esculenta	Raw	Fresh	D (n = 9)	–	4.77 ± 1.49^{a}	1.14 ± 0.336^{abc}
			P (n = 9)	–	2.26 ± 1.02^{a}	0.737 ± 0.263^{bc}
		Thawed	R (n = 46)	–	4.87 ± 4.87^{a}	1.39 ± 0.521^{a}
	H. T.	Fresh	M (n = 9)	54 °C/2 min	1.92 ± 0.951^{b}	0.587 ± 0.208^{c}
			M (n = 9)	85 °C/5 s	2.04 ± 0.595^{b}	0.717 ± 0.154^{bc}
			M (n = 11)	95 °C/15 min	4.29 ± 1.94^{ab}	0.849 ± 0.257^{bc}
		Thawed	R (n = 11)	95 °C/15 min	9.10 ± 1.49^{c}	1.23 ± 0.453^{ab}
S. latissima	Raw	Fresh	D (n = 5)	–	8.03 ± 1.80^{a}	0.712 ± 0.171^{a}
			M (n = 7)	–	3.58 ± 1.82^{bc}	0.567 ± 0.174^{a}
			P (n = 8)	–	3.91 ± 103^{b}	0.572 ± 0.126^{a}
	HT	Fresh	M (n = 9)	85 °C/5 s	2.57 ± 0.742^{bc}	0.756 ± 0.224^{a}
			M (n = 9)	95 °C/15 min	1.98 ± 0.626^{c}	0.786 ± 0.218^{a}

372 Values are expressed as mean ± SD. Values in a column within each species not sharing a footnote are
373 significantly different. Blade position is given as D, distal (far from connecting organ); P, proximal (near
374 connecting organ); R, random; M, middle.

375

376 **Table 5.** Microbial numbers (log cfu/g) found on raw and heat-treated (HT) *Alaria esculenta* and
377 *Saccharina latissima*.

		No. of colonies			
		Raw		HT	
Parameter	Medium	*A. esculenta*	*S. latissima*	*A. esculenta*	*S. latissima*
TMC	MA	2.01 ± 0.39	1.10 ± 0.14	1.20 ± 0.25	1.13 ± 0.18
PB	MA	1.10 ± 0.14	1.01 ± 0.01	1.00 ± 0.01	1.00 ± 0.01
	PCA	1.00 ± 0.01	1.16 ± 0.22	–	–
	PCA+	1.00 ± 0.01	1.01 ± 0.01	–	–
SB, Aerob.	TSAY	2.28 ± 1.14*	1.01 ± 0.01	1.00 ± 0.01**	1.11 ± 0.31
	BA	1.70 ± 0.01	ND	2.90 ± 1.28	–
SB, Anaerob.	TSAY	1.47 ± 0.82*	1.01 ± 0.01	1.00 ± 0.01**	1.08 ± 0.20***
	BA	1.70 ± 0.01	1.70 ± 0.01	2.33 ± 0.47	–

378

379 Values of TMC, total microbial count; PB, psychotropic bacteria; SB, spore-forming bacteria are
380 expressed as mean ± SD on MA, marine agar; PCA, plate count agar; PCA+, PCA with added 1 % NaCl;
381 TSAY, tryptose soy agar with yeast; and BA, Blood Agar. ND, not detected, < 1.70 log cfu/g. *) 2/3 plate
382 were difficult to count due to swarming. **) 5/9 plates not included due to swarming; ***) 1/9 plates
383 not included due to swarming.