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Physicochemical- and bioactive properties of acid preserved *Alaria esculenta* and *Saccharina latissima* during storage

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A B S T R A C T

The short harvesting period of cultivated brown seaweed in Europe can make it difficult for cultivators to produce high quality seaweed biomass all year around. Hence there is a need for novel processing and preservation methods. Acid preservation is a well-known method to preserve food, where the aim is to reduce the pH below 4.5 to inhibit microbial growth. To evaluate the effectiveness of acid preservation, a shelf-life experiment was conducted with *Saccharina latissima* and *Alaria esculenta*. The biomass was either treated with lactic- or citric acid and stored for approximately seven months. Physicochemical (including proximate composition, trace minerals, total phenolic content (TPC), texture and pH), microbial-, sensory attributes, and antioxidant (ORAC, DPPH) analyses were performed on the preserved biomass during storage. The proximate composition, color, pH, and texture of the acid-preserved seaweed were relatively stable throughout the storage. However, a decrease was observed in TPC and antioxidant properties (assessed by DPPH) with the acid treatments. Acid preservation is, thus, a good method to stabilize the studied biomass for food and feed applications but less applicable if intended for antioxidant purposes. However, the acid treated biomass might be suitable as an ingredient for a wide range of value-added products.

1. Introduction

During the last decades, aquaculture has become the fastest-growing food processing technology sector (Garlock et al., 2020). The total aquaculture production in 2018 was 114.5 million tons, whereof seaweed contributed 32.4 million tons, or 28.3% of the total produced biomass. Approximately 97% of the seaweed production originates from cultivation, which is mainly practiced in Asia (FAO, 2020). However, seaweed cultivation has recently gained more interest in Europe where according to the report “Hidden champion of the ocean” the seaweed sector in Europe could grow from the 300.000 tons annual wet weight produced today up to 8 million tons in the future (Vincent et al., 2020). However, before starting scaling up production it is crucial to solve some of the main bottlenecks within the sector to ensure high quality of the harvested biomass.

One of the main bottlenecks in the seaweed cultivation sector in Europe lies in the preservation of seaweed biomass. When brown seaweed species, such as *Alaria esculenta* and *Saccharina latissima*, are cultivated, sporelings are deployed on ropes into the ocean in October to November (depending on locations) and allowed to grow over approximately 5–6 months. The seaweed is then harvested in late April throughout May, before biofouling of the biomass which starts in June–July. The short harvesting period makes it difficult for seaweed cultivators to provide their customers with high quality seaweed biomass all year around and makes them reliant on using various preservation methods to stabilize their products. The main methods used to preserve the biomass are drying, freezing, salting, and fermentation (Choi et al., 2012; Singh & Heldman, 2001; Stévant, et al., 2018a). Freezing of the biomass can increase the shelf-life of the seaweed for long periods, but the method is, however, both energy and infrastructure demanding and can lead to substantial nutritional loss and quality deterioration due to drip loss (Blikra et al., 2021). Dehydration or drying

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of seaweed biomass in northern countries is expensive due to its high energy consumption and infrastructure demands. Furthermore, the drying can effect nutritional content of the biomass (Singh & Heldman, 2001; Stévant et al., 2017b). With rising energy prices in Europe, seaweed cultivators in Europe cannot rely on drying and freezing processes for biomass preservation alone. Therefore, it is important to explore other alternatives to preserve seaweed biomass.

Acidification is a less energy-demanding preservation method than freezing or drying, and does not require expensive equipment (Blikra et al., 2021; Sandbakken et al., 2018). The acidification can be obtained by two different processes, by addition of acids, or fermentation. The addition of acids to foods, also called artificial acidification, is a widely employed method aimed at improving the safety, stability, and shelf-life of food products. This technique involves the use of acidifying agents, commonly organic acids like lactic, citric, malic, and acetic acids, or their salts (Dauthy, 1995; Theron & Lues, 2010). These acids serve various purposes within the food industry, and may function as antioxidants, flavor enhancers, acidulants, and pH adjusters. One of their key roles is to lower the pH of the food product below pH 4.5 to inhibit the growth of pathogenic bacteria, such as *Clostridium botulinum*, *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes*, that are known to cause foodborne illnesses (Adams, Moss, & Moss, 2000; CFR, 1979; Dauthy, 1995; Theron and Lues, 2007). Acid preservation does not only inhibit growth of pathogenic bacteria but is also known to affect the sensory attributes of foods, including the texture, odor, and taste, depending on both type of acid used and their concentrations (Dauthy, 1995).

Currently, the knowledge and literature on the effects of acid preservation of seaweed intended for human consumption and its effect on storage shelf-life is limited. Some studies have described acid preservation of seaweed biomass intended for biofuel purposes (Sandbakken et al., 2018), feed applications (Novoa-Garrido et al., 2020), quality of alginate and cellulose post acid treatment (Nøkling-Eide et al., 2023), and protein quality during short storage periods (Standal et al., 2023). Due to the gap in knowledge and the need to explore alternative methods for preservation of cultivated seaweed biomass, the objective of the current study was to evaluate the efficiency and stability of acid preserved seaweeds intended for human consumption, with focus on two of the most cultivated brown seaweeds in Europe, *Alaria esculenta* and *Saccharina latissima*.

2. Material and methods

2.1. Seaweed sampling, preparation, and acidification

Saccharina latissima and *Alaria esculenta* were cultivated and harvested by Seaweed Solutions AS (SES) in Norway. The seaweed spores were produced in Trondheim, Norway, where seedlings of *S. latissima* were deployed in the ocean in November 2021, and seedlings of *A. esculenta* were deployed in January 2022. The ropes were approximately 1–4 m below the surface at the SES farming sites in Frøya, Måsskjæra (N63′44, E8′53 N) in Norway, where the seaweed was allowed to grow over the winter and spring. Both species were harvested on the May 30, 2022. Following harvesting, the seaweed was landed at Hitra, Norway, and transported to the laboratory facilities of SINTEF in Trondheim, Norway, where the biomass was stored in a cold room (0–4 °C) until the following day. Each species was minced separately, and then either acid preserved or frozen (control). Due to the fast deterioration and long shipment of samples across countries for analysis, frozen seaweed biomass was chosen instead of fresh as a control to evaluate the physicochemical and sensory changes obtained in the acid treated biomass during acidification. Freezing is a widely employed and efficient preservation method to prevent deterioration while having minimal impact on the chemical composition of food products (Singh & Heldman, 2001). This especially applies if the freezing is fast, and the samples are kept under stable frozen conditions. The frozen samples were thus stored at −25 to −30 °C till they were analyzed (after approximately two months). The acid treatment was performed with either lactic or citric acid. Lactic acid was added as a 3 M solution, while citric acid was added dry. The acids were added until the pH reached approximately 3.7. The final lactic acid concentration in the samples were 106 mmol/kg for *A. esculenta* and 60 mmol/kg for *S. latissima*. The corresponding concentrations of citric acid were 28 and 17 mmol/kg. After adding the acids, the seaweed samples were divided into several 1 kg plastic bags, which were vacuum packed and stored at room temperature until used. The seaweed samples were transported to Matís, Iceland where physicochemical, bioactive, and sensorial properties of the samples were analyzed to assess the quality and storage stability of the biomass.

Sampling took place on three occasions during the 32-week storage time where the first sampling occurred 4 weeks (W4) post acid treatment, and the other two samplings were on week 16 (W16), and week 32 (W32) post acid treatment. One bag (1 kg) from each treatment was divided into three samples replicates (n = 3) per sampling point. At least 70 gr of each sample replicate was freeze-dried in a Genesis 25 SQ EL freeze dryer (SP industry, Philadelphia United States of America), and the rest of the biomass was used for chemical, microbial, and physiological analysis.

2.2. Proximate composition of seaweed samples

The water content of the seaweed samples was analyzed according to the ISO 6496:1999 method, where around 5 g of each sample were dried in an oven at 102°C–104 °C for at least 4 h. The weight of the evaporated water from the sample was used to assess the water content of sample. The protein content was determined with the Kjeldahl method as described by ISO 5983-1:2005 (2005) and ISO 5983-2:2009 (2009), where a conversion factor of 5 was used to calculate crude protein content as recommended for seaweed samples due to their high non-protein-nitrogen content (Angell et al., 2016). To determine the total lipid content of the seaweed samples, the Soxhlet extraction method was used according to the OCS Official Method Ba-3-38 (AOCS, 2009). Ash content was determined by weight loss of the sample after burning approximately 2 g of seaweed sample at 550 °C for 3 h (ISO 5984:2002). The Volhard titration method was used to determine the salt content (AOAC, 1990). The total carbohydrate (TC) content of seaweed samples was then calculated for each sample based on results from the chemical analysis of the abovementioned water, protein, lipid, and ash, measurements, using the following equation:

$$TC = 100 - (water + protein + ash + lipids)$$

2.3. Trace minerals

The iodine content was analyzed with inductively coupled plasma mass spectrometry (ICP-MS) with the method described by DIN EN 15111 (2007–06), with modifications according to CON-PV 01187 (2022–06). The contaminants lead, mercury, arsenic (inorganic and organic), and cadmium were analyzed in the control samples to assess the safety of the seaweed biomass for human consumption. The total arsenic content was analyzed with graphite furnace atomic absorption spectrometry (GF-AAS), performed according to ASU L 00.00–19/3 (2004–07), and modified for expansion of analytes according to the CON-PV 00508 (2020–01) method. Inorganic arsenic was analyzed according to the ASU L 25.06–1 (2008–12) method with hydride generation (HG-ASS) after acidic extraction with some modifications (CON-PV 01288 (2020–05). Cadmium, lead, and mercury were determined according to NMKL (2007), method 186 (NMKL, 2007b).

2.4. Physical parameters

The water activity of the samples was determined with an Aqua lab 4

T water activity meter (Decagon Devices, Pullman, WA, USA). About 2 gr of sample were put in a plastic cup, and placed in the water activity meter, and the water activity was read at a temperature between 24 °C and 25 °C. The pH value was analyzed by using a portable pH meter (Knick, Berlin, Germany). Color was analyzed by using Minolta Chroma Meter CR-300 (Minolta, Oska, Japan) with use of CIE Lab system. L-, a*- and b*-values are recorded by the instrument. L-values indicate lightness on the scale from 0 to 100 from black to white, respectively, a-values represent green color (−) to red (+), and the b-value blue (−) to yellow (+). Each sample (n = 3 per treatment) was measured in duplicate, or at two different points within each sample at each sampling point.

2.5. Total volatile basic nitrogen (TVB-N) and microbial analysis

TVB-N was determined during storage by steam distillation of a 7.5% trichloroacetic acid (TCA) extract (Billon & Tao, 1979). NaOH was added to the extract, and the blend was steam distilled with a Struers TVN steam distillatory and collected in a beaker glass containing 4% boric acid, along with methyl red and bromocresol green indicators. The solution was then titrated with 0.025 N H_2SO_4 to the equivalence point for steam distillation along with 10% NaOH.

Total aerobic microbial counts (TVC) were determined according to the NMKL (2013), 86 method, 5th ed., where the preparation of sample dilution series were performed according to general microbiological principles. Dilutions were pour plated on Petri dishes with unspecific agar medium, followed by incubation under aerobic conditions for 72 ± 6 h at 30.0 ± 1.0 °C. Lactic acid bacteria were determined according to NMKL 140, 2nd ed. 2007 method (NMKL, 2007a), where preparation of the dilution series was performed according to general microbiological principles, and dilutions spread with spiral plating on a surface of a De Man, Rogosa, and Sharpe (M.R.S.) agar. The plates were incubated in anaerobic jars for 72 h at 30 °C. For both methods, colonies were counted, and the number of viable microorganisms were calculated by multiplying the number of counted colonies by the appropriate dilution factor. The results are expressed as log of colony forming units/gram sample (log CFU/g) (n = 3 per each treatment) on each sampling occasion.

2.6. Sensory evaluation

Generic descriptive analysis (GDA) (Stone et al., 2020) was performed on the seaweed samples after 16 weeks storage by nine trained panelists, who were trained according to ISO. (1993) standard. The training sessions were two, where both acid-preserved and frozen-thawed samples of *A. esculenta* and *S. latissima* were used. The panel training involved the determination of appropriate descriptors for the seaweed products, including 17 sensory attributes in three categories, i.e., odor (5), flavor (9), and texture (3), which were chosen during the sensory training sessions. The tested sensory attributes for odor were sour, seaweed, extra odor, rancid, and spoilage, while the tested flavor attributed included sour, bitter, salt, sweet, seaweed, metal, extra flavor, rancid, and spoilage. The texture attributes examined included liquid, tender and astringent (Table S1, Supplementary material 1). The intensity of each sensory attribute was described by using an unstructured scale from 0 to 100. The two species were evaluated on separate sensory evaluation occasions.

Approximately 24 h prior to the sensory evaluation the frozen control *A. esculenta* samples were put in a refrigerator (4 °C) and allowed to thaw. In preparation prior to the sensory evaluation, approximately two tablespoons of each sample were put in each aluminum tray, which were each marked with random three-digit numbers. The seaweed samples were served to the sensory panelists at room temperature, and red lights were used to hide the appearance of the samples. Prior to sensory evaluation, the frozen-thawed *S. latissima* samples were evaluated as spoiled and were hence not included in the sensory evaluation. Each panelist (n = 9) evaluated all available samples, two for *S. latissima* and three samples of *A. esculenta*, which were each provided in duplicate.

2.7. Extract preparations, total phenolic content (TPC) and antioxidant properties

The total phenolic content (TPC) and antioxidant properties of water extracts of the seaweed samples were performed to evaluate potential bioactive properties of the seaweed samples. Approximately 4 g of freeze-dried seaweed sample were added to 40 ml of water, the samples were shaken on an IKA KS 130 basic plate shaker (IKA, Staufen, Germany) for 1 h, and then centrifuged in a TJ-25 centrifuge (Beckman coulter, California, United States) for 20 min at 5100 rotations per minute (RPM). The supernatant was collected and filtered through a Whatman filtration paper (no 4, CAT No. 1004–150). The extracts were freeze-dried in a Genesis 25 SQ EL freeze dryer (SP industry, Philadelphia United States of America) freeze dryer, and stored at −25 °C till analysis of antioxidant properties. One extract was made from each sample replicate, or three extracts per treatment for each species (n = 3) on each sampling occasion.

To evaluate the total phenolic content (TPC) of the seaweed samples, analysis was performed on the freeze-dried water extracts made from each seaweed sample. The Folin-Ciocalteu procedure (17) was used to determine TPC, with slight modifications as described by Hrólfsdóttir et al, 2022. Each extract (n = 3 per treatment) was measured in duplicate, and results expressed as grams phloroglucinol equivalent (PGE)/100 g freeze dried extract.

To determine the antioxidant properties of seaweed samples, 2,2-Diphenyl-1-picrylhydrazy radical scavenging activity (DPPH) and Oxygen radical absorbance capacities (ORAC) were analyzed. DPPH was determined with method according to Sharma and Bhat (2009), as described by Hrólfsdóttir et al. (2022). Results are expressed as %inhibition of DPPH radicals, and measurements were performed with multiple concentrations (from 0.1 to 10 mg/ml) of the seaweed extracts to calculate the half inhibitory effect (IC50 number). The ORAC measurements were performed according to the method described by and Huang et al. (2002) and Ganske and Dell (2006) with slight modifications as described by Hrólfsdóttir et al. (2022). The results are expressed as ORAC value (Ø), or μmol of trolox equivalent (TE)/gram freeze-dried extract. All measurements were performed in duplicate with three samples for each treatment (n = 3) on each sampling point.

2.8. Statistical analysis

Collection of data and calculation of averages and standard deviations (SD) were performed in Microsoft excel (Microsoft, Redmond, WA, USA). JMP pro 16 (SAS, Cary, NC, USA) was used to perform statistical analysis, e.g. one way ANOVA (analysis of variance) and Tukey's honest significant difference test for the chemical composition, trace minerals.

The FIZZ software (Version 2.51C, Biosystémes, Couternon, France) was used to collect data during sensory analysis and Panelcheck V1.4.0 (Nofima, Tromsø, Norway) was used to monitor the panelists performance. For statistical analysis of sensory data, a General linear model (GLM), corrected for use of scale by the panelist, was applied by using the NCSS software 2000 (NCSS, Utah, USA) coupled with Duncan's post hoc test. A significance level of $p \leq 0.05$ was used for all statistical analysis to determine significant difference between samples during storage and in sensory analysis.

3. Results and discussion

3.1. Proximate composition

Proximate composition of acid-preserved *A. esculenta* and *S. latissima* was assessed during storage (Table 1). As expected, the seaweed samples

Table 1
Nutritional composition of seaweed samples, untreated (control) and acid preserved (with lactic or citric acid) during storage of 28 weeks. Results are expressed as g/100 g sample written as average ± standard deviation (n = 3).

Specie	Sample type	Storage time	Water	Lipid	Protein	Ash	Carbohydrates	Salt
Saccharina latissima	Control	W4	90.8 ± 0.2	0.3 ± 0.1[a,b]	0.7 ± 0.0[a,b]	4.3 ± 0.0	3.9 ± 0.2[a,b]	3.0 ± 0.1[a,b]
	Lactic acid	W4	90.6 ± 0.6	0.2 ± 0.1[a,b]	0.7 ± 0.1 [a,b]	4.2 ± 0.0	4.3 ± 0.6 [a,b]	2.9 ± 0.0[b]
		W16	90.2 ± 0.4	0.1 ± 0.0[b]	0.8 ± 0.0 [a]	4.2 ± 0.1	4.7 ± 0.5 [a,b]	3.0 ± 0.1[a,b]
		W32	89.8 ± 0.9	0.1 ± 0.0[b]	0.6 ± 0.1 [b]	4.2 ± 0.0	5.2 ± 0.8 [a]	3.0 ± 0.0[a,b]
	Citric acid	W4	91.0 ± 0.5	0.4 ± 0.2[a]	0.7 ± 0.0 [a,b]	4.3 ± 0.0	3.6 ± 0.6 [b]	3.0 ± 0.0 [a,b]
		W16	90.6 ± 0.1	0.3 ± 0.1[a,b]	0.8 ± 0.0 [a]	4.3 ± 0.1	4.2 ± 0.3 [a,b]	3.0 ± 0.1 [a,b]
		W32	90.0 ± 0.2	0.1 ± 0.0[b]	0.7 ± 0.0 [a,b]	4.4 ± 0.0	4.9 ± 0.2 [a,b]	3.1 ± 0.0[a]
Alaria esculenta	Control	W4	84.7 ± 0.4	0.1 ± 0.0	1.9 ± 0.1[a,b]	4.1 ± 0.1[a,b]	9.3 ± 0.5	2.4 ± 0.1[c]
	Lactic acid	W4	85.7 ± 0.4	0.2 ± 0.1	1.5 ± 0.2[c]	4.0 ± 0.0[b]	8.6 ± 0.3	2.5 ± 0.0[b,c]
		W16	83.8 ± 0.5	<0.1	1.7 ± 0.0[a,b,c]	4.1 ± 0.1[a,b]	10.4 ± 0.6	2.5 ± 0.1[b,c]
		W32	86.0 ± 1.5	<0.1	1.5 ± 0.0[c]	4.0 ± 0.0[b]	8.5 ± 1.5	2.6 ± 0.0 [a,b]
	Citric acid	W4	84.7 ± 1.2	0.1 ± 0.0	1.9 ± 0.1[a]	4.2 ± 0.0[a]	9.0 ± 1.0	2.5 ± 0.0 [b,c]
		W16	86.1 ± 0.9	0.1 ± 0.0	1.6 ± 0.1[b,c]	4.1 ± 0.1[a,b]	8.2 ± 0.8	2.6 ± 0.1[a]
		W32	84.6 ± 0.1	<0.1	1.7 ± 0.0[a,b,c]	4.2 ± 0.0[a]	9.6 ± 0.2	2.6 ± 0.0[a]

Subscript letters (a-c) show significant differences ($p < 0.05$) in nutritional composition between samples for each species separately.
Storage time is written in weeks, where W4 is first sampling point, W16 sampling at 16 weeks storage and W32 sampling at week 32 storage.

had a high water content, and the main proportion of dry matter content originated from ash and carbohydrates for both species, while having lower content of protein and lipids (Schiener et al., 2015; Stévant et al., 2017a). Only minor changes were observed in the chemical composition of the acid preserved seaweed species throughout the 32-week storage duration, which can mainly be explained by natural variation in chemical composition of the biomass itself and partially due to methodological uncertainty during the analysis, rather than due to spoilage mechanisms. However, some changes were observed during storage of *S. latissima* samples in carbohydrate content, but none can be directly linked to each treatment. Overall, the result indicates that both lactic acid and citric acid treatments seem to be suitable to preserve the biomass for at least 32 weeks duration.

3.1.1. Trace elements

To assess the safety of seaweed as a food ingredient, some of the main trace elements that have been reported to be of concern in seaweed for food applications were analyzed in the frozen control samples (Table 2). These trace minerals are total arsenic (As), inorganic arsenic (IAs), mercury (Hg), lead (Pb), cadmium (Cd), and iodine (I). A significant difference was observed between species in arsenic, lead, cadmium, and iodine content, where the *A. esculenta* contained significantly lower amounts of iodine, while simultaneously containing significantly higher content of the other trace minerals.

While comprehensive regulations and guidance have been established for various fishery resources, food safety of seaweed has been overlooked. European Union (EU) legislations do only exist for maximum levels of lead, cadmium, and mercury in feed and supplement applications. With increased seaweed interest within Europe, the urgent need of legislation for seaweed as a food material must be highlighted to address this significant gap in regulatory aspects (WHO, 2022). Even though the European union has not yet set direct legislations for heavy metals and iodine for seaweed intended for food applications, some guidelines have been published both in Norway and France (ANSES, 2018, 2020; Hogstad et al., 2023). Seaweed producers in Norway have also established guidelines for cultivation and handling of *A. esculenta* and *S. latissima*, addressing food safety concerns, focusing on cadmium, inorganic arsenic, and iodine (Hogstad et al., 2023). The French authorities, ANSES (French Agency for Food; Environmental and Occupational Health & Safety), have set guidelines regarding above the mentioned trace minerals, where recommended maximum levels of cadmium are set to 0.5 mg/kg dry weight (dw), for lead maximum levels of 5 mg/kg dw, for mercury maximum levels of 0.1 mg/kg dw, for inorganic arsenic 3 mg/kg dw (ANSES, 2020), and for iodine maximum levels of 2000 mg/kg dw apply (ANSES, 2018). If results from analysis on dry matter basis are compared to values recommended from ANSES for seaweed intended for human consumption, the cadmium levels in both species were too high (1.0 ± 0.1 mg/kg dw in *S. latissima* and 2.4 ± 0.1 mg/kg dw in *A. esculenta*), and the same applies to iodine in the *S. latissima* samples (4462 ± 123 mg/kg dw). Furthermore, even though there are not any specific set limits of the total arsenic content in the ANSES recommendation, the total arsenic content of both species exceeded the allowed limits for seaweed used as animal feed, or <40.0 mg/kg dw (European Commission, 2002). It is important to highlight that Inorganic arsenic, which is the toxic form of arsenic, is below set limits by ANSES for both species. Several studies have assessed the efficiency of applying different processing methods for trace element reduction, including blanching, soaking in water, or ultrasound-assisted approaches (Correia et al., 2021; Nielsen et al., 2020; Noriega-Fernández et al., 2021; Stévant et al., 2018b). Nielsen et al. (2020) showed that the iodine content can be reduced up to 90% by blanching *S. latissima* or from approximately 4600 mg/kg dw to roughly 300 mg/kg dw. Furthermore, Noriega-Fernández et al. (2021) showed that by using mild heating and ultrasound assisted approaches, iodine,

Table 2
Trace elements of control samples (frozen, nontreated samples). Results are expressed as mg/kg of sample of wet weight and dry weight. Results are expressed as average ± standard deviation (n = 3).

	Sample type	Sample type	Total Arsenic	Inorganic Arsenic	Mercury	Lead	Cadmium	Iodine
Wet weight (ww)	*Saccharina latissima*	Control	5.47 ± 0.25[a]	<0.10	<0.005	0.01 ± 0.00[a]	0.09 ± 0.01[a]	410 ± 17.0[a]
	Alaria esculenta	Control	9.67 ± 0.49[b]	<0.10	<0.005	0.03 ± 0.00[b]	0.36 ± 0.01[b]	100 ± 0.0[b]
Dry weight (dw)	*Saccharina latissima*	Control	59.55 ± 3.4	<0.10	<0.005	0.07 ± 0.00 [b]	1.0 ± 0.1[b]	4462 ± 123[a]
	Alaria esculenta	Control	63.24 ± 4.10	<0.10	<0.005	0.2 ± 0.02[a]	2.4 ± 0.1[a]	654 ± 17[b]
	Max content[a] (mg/kg dw)			3	0.1	5	0.5	2000
	Maximum daily intake[b]							600 μg

Subscript letters (a-b) show significant differences ($p < 0.05$) in trace element composition between species, separately for wet weight and dry weight.
[a] As defined by French authorities ANSES (ANSES, 2018, 2020).
[b] Maximum levels of daily intake as defined by the Icelandic Directory of Health (Icelandic Director of Health, 2017).

cadmium, and arsenic content could be effectively reduced in *Laminaria hyperborea*. The result from the present study thus highlights the importance of such trace element reducing pre-treatments of the seaweed biomass prior to development of food applications thereof.

3.2. Physical properties

Both the pH and color were recorded of seaweed samples throughout the storage period (Table 3) as well as water activity (aw). The aw of the samples was stable throughout the storage time, where it ranged from 0.985 to 0.992. The untreated *S. latissima* and *A. esculenta* had pH of approximately 6.4–6.5, and the acid treated samples pH values between 3.4 and 3.9, showing that the acid preservation was performed efficiently since the achieved pH was lower than 4.6 (CFR, 1979; Dauthy, 1995). Furthermore, the pH was relatively stable, but statistically significant decrease was observed throughout the storage duration, for both the lactic acid and citric acid treated samples. Nøkling-Eide et al. (2023) observed similar trends in pH within their study on formic acid preserved *A. esculenta*, which showed a slight decrease in pH from week 4 post acid treatment to week 16 of storage. Furthermore, Sørensen et al, 2021 showed that both *A. esculenta* and *S. latissima* contain natural

Table 3
Physical properties of the seaweed samples untreated (control) and acid preserved (with lactic or citric acid) during storage of 32-weeks.

Specie	Sample type	Storage duration (weeks)	pH	L*-value	a*-value	b*-value
Saccharina latissima	Control	W4	6.47 ± 0.07^{a}	20.4 ± 1.2^{b}	4.6 ± 0.4^{a}	3.5 ± 1.1^{c}
	Lactic acid	W4	3.62 ± 0.02^{c}	26.8 ± 2.8^{a}	2.5 ± 0.9^{b,c}	8.5 ± 1.5^{a}
		W16	3.57 ± 0.01^{c}	26.2 ± 2.2^{a}	2.1 ± 0.6^{c}	7.0 ± 1.4^{a,b}
		W32	3.47 ± 0.01^{d}	26.1 ± 1.6^{a}	1.8 ± 0.4^{c}	7.2 ± 0.8^{a,b}
	Citric acid	W4	3.76 ± 0.01^{b}	26.1 ± 1.5^{a}	3.3 ± 0.6^{b}	8.7 ± 0.7^{a}
		W16	3.73 ± 0.01^{b}	25.2 ± 0.4^{a}	2.7 ± 0.1^{b,c}	7.2 ± 0.4^{a,b}
		W32	3.61 ± 0.01^{c}	26.3 ± 1.5^{a}	2.0 ± 0.3^{c}	6.6 ± 0.8^{b}
Alaria esculenta	Control	W4	6.38 ± 0.02^{a}	23.1 ± 1.3^{c}	3.9 ± 0.5^{a}	4.3 ± 0.8^{b}
	Lactic acid	W4	3.70 ± 0.00^{c,d}	30.5 ± 2.6^{b}	0.4 ± 0.5^{b,c}	8.9 ± 1.4^{a}
		W16	3.67 ± 0.02^{d}	34.6 ± 2.4^{a}	0.0 ± 0.6^{b,c}	10.0 ± 2.3^{a}
		W32	3.53 ± 0.00^{e}	30.7 ± 2.0^{b}	0.5 ± 0.4^{b}	9.7 ± 1.1^{a}
	Citric acid	W4	3.88 ± 0.01^{b}	33.0 ± 2.8^{a,b}	−0.7 ± 0.8^{c}	10.1 ± 1.5^{a}
		W16	3.86 ± 0.02^{b}	31.4 ± 0.8^{a,b}	0.5 ± 0.2^{b}	9.3 ± 0.7^{a}
		W32	3.73 ± 0.00^{c}	31.9 ± 1.0^{a,b}	0.5 ± 0.2^{b}	10.0 ± 0.9^{a}

Subscript letters (a-e) show significant differences ($p < 0.05$) in pH and color parameters (L*-,a*-, b*-values) between samples for each species separately.

microbiota that can contribute to natural fermentation of the biomass. The natural microbiota within the biomass might contribute to the pH decrease, since acids are produced during this natural fermentation process (Stanbury et al., 2013; Sørensen et al., 2021).

The CIE system was used to assess potential color changes of the seaweed samples during the storage period. Sensory aspects such as appearance and color are possible barriers for increased consumption of seaweed biomass within the European populations (Blikra et al., 2021). The color of the brown seaweed biomass can play an important role in increasing interest in seaweed as a food source and processing of the biomass can contribute to more appealing color of the seaweed. Blanching, where biomass is emerged in hot water for a short time is e.g., used in wakame processing to retrieve the bright green color that consumers prefer and are used to from vegetables as well as to reduce undesired compounds (Blikra et al., 2019; Yamanaka and Akiyama, 1993). In the current study higher L*- and b*-values and lower a*-values were obtained in both species after acid treatment compared to the frozen control samples. The results hence imply that when seaweed is treated with acid, the seaweed gets lighter, greener, and more yellow in color, which is considered a positive change rather than negative, especially if the final product is intended for human consumption. During storage a significant decrease was observed in the a*- and b*-values in the citric acid treated *S. latissima* between the first (week 4) and last sampling point (week 32). Furthermore, a significant increase was observed in the a*-value of the citric acid treated *A. esculenta* samples during storage. These changes were not observed in the lactic acid treated samples during storage, independent of species, which indicates that the citric acid has more effect on the color compared to lactic acid during storage. However, it is important to evaluate further how the color changes could change if the biomass is blanched prior to the acid treatment. Furthermore, it is important to note that the color of the biomass itself is not homogenous which could affect the results. Blikra et al. (2019) showed for example that the distal parts of *A. esculenta* were less red and yellow than the proximal parts of the plant, while the proximal parts of both *A. esculenta* and *S. latissima* were lighter in color compared to the distal parts.

3.3. Total viable count (TVC) and total volatile basic nitrogen (TVB-N)

To assess the effectiveness and safety of the treatment to stabilize the biomass and prevent microbial growth of the acid preserved seaweed samples, the total viable count (TVC) was analyzed. The method is often used as a quality indicator to assess efficiency of preservation method during storage and to determine the safety and shelf-life of food products. Significantly lower TVC were observed in the frozen control samples compared to the lactic acid and citric acid treated samples for all sampling points in the *S. latissima*, and for all except for week 4 of lactic acid treated *A. esculenta* samples (Fig. 1). Freezing is known to prevent or reduce the growth rate of microorganisms as well as reducing reaction rates, e.g. in enzyme and oxidative reactions, and additionally the freezing and thawing processes cause injuries and even death of some microorganisms (Archer, 2004; Singh & Heldman, 2001). The results hence show that the freezing of the seaweed biomass was efficient in inhibiting microbial growth. Furthermore, the TVC did not significantly differentiate over the storage time in the lactic acid and citric acid treated *S. latissima* samples, where TVC was ranging from 5.6 to 6.2 log cfu/g. Notably, in week 32, the TVC of the lactic acid treated *S. latissima* (6.2 ± 0.1 log cfu/g) was significantly higher compared to the citric acid treated samples (5.6 ± 0.0 log cfu/g). However, higher TVC was observed in the citric acid samples (4.9 ± 0.1 log cfu/g) compared to the lactic acid treated samples (3.7 ± 0.2 log cfu/g) in the *A. esculenta*. The results hence indicate that different acids might have different effects on different seaweed species. When the two species are compared, higher TVC was observed in the *S. latissima* samples compared to the *A. esculenta*.

Sánchez-García et al, (2021) suggested that TVB-N and

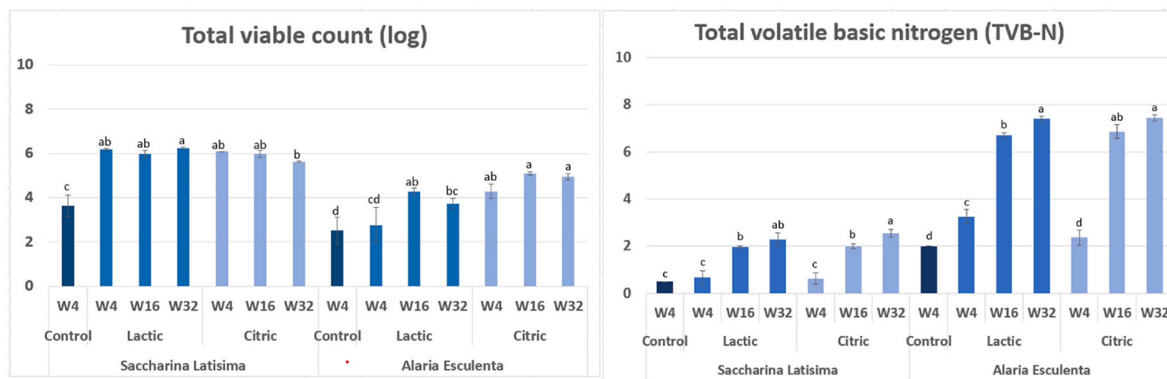

Fig. 1. Results of analysis of Total volatile basic nitrogen (TVB-N) and total viable count (TVC) during storage for 32 weeks. Results are expressed as mean ± standard deviation of three sample replicates. The results from TVB-N are expressed as mg nitrogen (N)/100 g sample, and the TVC as log colony-forming units per gram sample (log cfu/g). Different subscript letters indicate significant differences of samples of same species.

Trimethylamine nitrogen TMA-N might be useful quality parameters to determine degradation due to either microbial or enzyme activity in seaweed. Since TVB-N might be an indicator of these changes, total volatile basic nitrogen (TVB-N) was analyzed. The analysis in the present study showed that the TVB-N gradually increased throughout the storage period in both species, but the values were low, e.g. for fish products set limit for TVB-N is 25–35 mg N/100 g sample (European Commission, 2008). Higher TVB-N values were observed in *A. esculenta* samples compared to *S. latissima* samples, which might be due to *A. esculenta's* higher crude protein content, which is available for degradation through microbial or enzymatic processes. Furthermore, nitrogenous compounds such as biogenic amines can be produced by microorganisms during fermentation to survive (Ekici & Abdullah, 2020) Thus, the increased TVB-N during storage might relate to the fermentation of natural microbiota, but these changes require further research to evaluate possible causes.

3.4. Sensory analysis

To evaluate the acid preserved seaweed as an ingredient for products for human consumption, a sensory evaluation was performed (Table 4). Differences between the two species were mainly observed in the textural properties. Overall, the texture of the *A. esculenta* was more astringent and tough compared to the texture of *S. latissima.* Furthermore, citric acid treated *S. latissima* samples contained significantly more liquid assessed by texture compared to all *A. esculenta* samples. These results align with the proximate chemical composition (Table 1), which showed that the *S. latissima* had a higher water content than *A. esculenta*. Generally, sensory values above 20 indicate that the tested component should be detectable by all panelists, and values around and above 50 indicate strong properties. The results hence show that both the acid treated and frozen *A. esculenta* possess strong seaweed and sour odor and strong salt, seaweed, and sour flavor. Furthermore, results show values above 20 in bitter flavor that should hence be easily detectable by most. Due to the strong flavor and odor properties, the products tested might not be favored by consumers to consume directly but could be used as an ingredient. The results hence imply that the acid preserved biomass needs to be improved if intended for direct human consumption. Therefore, methods such as blanching prior acidification is a feasible option to explore, since the method is often performed on seaweed before it is added into food products, since e.g. the salt and iodine content of the seaweed biomass has been shown to decreases significantly during blanching (Nielsen et al., 2020), changing both the safety and flavor of the biomass. Overall, the sensory evaluation indicated that acid preservation of *A. esculenta* did not affect flavor, odor, and texture negatively.

3.5. Total phenolic content and antioxidant properties

To evaluate the antioxidant properties of the two seaweed species,

Table 4
Results from Generic descriptive analysis (GDA) of seaweed samples. Results are presented as mean values of the responses from nine trained sensory panelists.

	Sensory attribute	Alaria control	Alaria citric	Alaria lactic	Saccharina citric	Saccharina lactic	p-value
Odor							
	Sour	41	49	44	45	44	0.540
	Seaweed	58^{a}	49	52	46	40^{b}	**0.018**
	Extra smell	8	9	12	17	20	0.140
	Rancid	2	1	1	2	2	0.548
	Spoilage	2	1	1	1	2	0.927
Flavor							
	Sour	43	54	54	49	51	0.293
	Bitter	28	21	21	23	24	0.279
	Salt	47	40	36	45	48	0.046
	Sweet	9	11	11	8	8	0.362
	Seaweed	54^{a}	55	54^{a}	49	38^{b}	**0.040**
	Metal	10	7	8	8	7	0.500
	Extra taste	10	6^{b}	6	18	20^{a}	**0.017**
	Rancid	2	1	1	3	2	0.191
	Spoilage	2	1	1	6	2	0.271
Texture							
	Liquid	43^{c}	$43^{b,c}$	$42^{b,c}$	64^{a}	$58^{a,b}$	**0,002**
	Tough	51^{a}	46^{a}	43^{a}	26^{b}	24^{b}	**0,000**
	Astringent	49^{a}	41^{a}	39^{a}	21^{b}	24^{b}	**0,000**

Subscript letters (a-c) show significant differences ($p < 0.05$) in sensory attributes between samples as determined by general linear model (ANOVA) and Duncans test.

and how they are affected by acid preservation, analysis of DPPH radical scavenging activities and oxygen radical absorbance capacities (ORAC) were performed. The phenolic compounds in seaweed have often been associated to the antioxidant properties of seaweed and extracts retrieved from seaweed (Jiménez-Escrig et al., 2012; Sabeena Farvin and Jacobsen, 2013; Wang et al., 2009). Hence the total phenolic content (TPC) was evaluated to assess the relationship between ORAC, DPPH and TPC for the two seaweed species (Table 5).

The TPC was low in all cases in the *S. latissima* samples (around 0.5 g/100 g dw extract) where the small differences can also be linked to variation within the biomass itself rather than changes in the biomass due to storage (Table 5). Higher concentrations were, however, observed in the *A. esculenta* samples, where the content ranged from 1.59 ± 0.09 to 3.00 ± 0.12 g PGE/100 g dw extract. For the *A. esculenta* samples, the TPC in the control samples were significantly higher compared to all acid treated samples, for all sampling points, indicating that the acid treatment caused deterioration of the phenolic compounds (Table 5). The phenolic content of both species is, however, relatively low compared to the water extracts of other brown seaweed species, such as *Ascophyllum nodosum* and *Fucus vesiculosus,* which have 4–6 times higher phenolic content than observed in the present study. The TPC in Fucus species, however, may depend both on the harvesting time of the biomass and the extraction method used (Hrólfsdóttir et al., 2022; Wang et al., 2009). For future research, more accurate quantitative methods such as High-performance liquid chromatography (HPLC), might be useful to further estimate the deterioration of TPC of the acid treated seaweed biomass.

Table 5
Total phenolic content (TPC), Oxygen radical absorbance capacity (ORAC), and DPPH radical scavenging activities of dry weight (dw) extracts of acid preserved seaweed samples for 32 weeks storage. Results are expressed as mean ± standard deviation (n = 3), where TPC results are expressed as g phloroglucinol equivalent (PGE)/100 g dw extract, ORAC results expressed as ORAC value (Ø) (μmol of tocopherol equivalent (TE)/g dw extract), and DPPH results expressed as IC50 number (amount of active component required to reduce the absorbance of DPPH radicals by 50%).

Species	Sample type	Storage	TPC	ORAC	DPPH
Unit		Weeks	PGE/100 g dw extract	TE/g dw extract	IC50
Saccharina latissima	Control	W4	0.53 ± 0.01^{a}	$17.3 \pm 1.9^{a,b}$	$6.35 \pm 0.75^{a,b}$
	Lactic acid	W4	0.43 ± 0.02^{b}	10.7 ± 1.4^{b}	$6.55 \pm 0.17^{a,b}$
		W16	0.44 ± 0.01^{b}	19.7 ± 0.6^{a}	$6.13 \pm 0.23^{a,b}$
		W32	$0.48 \pm 0.01^{a,b}$	21.6 ± 0.9^{a}	7.08 ± 0.99^{a}
	Citric acid	W4	0.42 ± 0.03^{b}	$16.3 \pm 5.6^{a,b}$	$5.77 \pm 0.22^{a,b}$
		W16	0.47 ± 0.04^{b}	20.7 ± 1.6^{a}	5.17 ± 0.06^{b}
		W32	$0.50 \pm 0.04^{a,b}$	19.6 ± 1.8^{a}	$5.88 \pm 0.41^{a,b}$
Alaria esculenta	Control	W4	3.00 ± 0.12^{a}	$94.0 \pm 6.3^{a,b}$	0.35 ± 0.07^{c}
	Lactic acid	W4	1.87 ± 0.05^{b}	$68.7 \pm 7.9^{b,c}$	$0.70 \pm 0.25^{a,b}$
		W16	1.76 ± 0.04^{b}	$93.4 \pm 15.5^{a,b}$	$1.51 \pm 0.72^{a,b}$
		W32	1.59 ± 0.09^{b}	$92.2 \pm 7.6^{a,b}$	1.75 ± 0.38^{a}
	Citric acid	W4	1.77 ± 0.05^{b}	65.3 ± 3.6^{c}	$0.76 \pm 0.03^{a,b,c}$
		W16	1.94 ± 0.35^{b}	105.6 ± 14.3^{a}	$0.82 \pm 0.42^{a,b,c}$
		W32	1.72 ± 0.07^{b}	104.1 ± 2.3^{a}	1.62 ± 0.17^{a}

Subscript letters (a-c) show significant differences ($p < 0.05$) in TPC and antioxidant properties (ORAC, DPPH) between samples for each species separately. Storage time is written in weeks, where W4 is the first sampling point, W16 corresponds to sampling at 16 weeks storage and W32 to sampling at 32 weeks of storage.

Similar trends were observed in the DPPH radical scavenging activities as in the TPC, where the acid treatments appeared to decrease the activity of the *A. esculenta* when treated with acid (both lactic and citric acid samples). The IC50 of the *A. esculenta* control samples were significantly lower when compared to the *A. esculenta* acid preserved seaweed samples, but the IC50 value of the control samples was 0.35 ± 0.07 mg/ml, 0.70 ± 0.25 to 1.75 ± 0.38 mg/ml for the lactic acid treated samples and 0.76 ± 0.03 to 1.62 ± 0.17 mg/ml for the citric acid treated samples. The results hence indicate that antioxidant properties assessed by DPPH decrease with acid treatment. That is the opposite of what studies have shown when seaweed is fermented for short time periods (Lee et al., 2015; Sumardianto, PH, AD, & Rianingsih, 2021). Sumardianto et al. (2021) showed an increase in both polyphenols and antioxidant properties (assessed by DPPH) of extracts obtained from the red seaweed species *Gelidium* sp. and *Eucheuma cottonii* that had been fermented for 24 h with *Lactobacillus plantarum* and *Lactobacillus acidophilus* cultures when compared to the starting point of fermentation. Furthermore, Lee et al. (2015) showed an increase in DPPH scavenging activity with fermentation of *Sargassum siliquanstrum* biomass. Even though no significant difference was observed in the IC50 numbers within each species during the storage period of the acid treated samples, the extract quantities (mg/ml) needed to reduce the DPPH radicals decreased with storage time. For the *S. latissima* samples, no specific tendencies were observed in either the TPC or DPPH activities which might also be explained by the low overall antioxidant activity and low amounts of phenolic compounds present in the biomass.

ORAC values were low for both species, ranging from 10.7 ± 1.4 to 21.6 ± 0.9 μmol of TE/g dw extract in the *S. latissima* samples, and from 65.3 ± 3.6 to 105.6 ± 14.3 μmol of TE/g dw extract in the *A. esculenta*. No specific trends were observed in the ORAC values during storage or with acid treatment. The results correspond to earlier studies, where both *A. esculenta* and *S. latissima* have shown low antioxidant properties when assessed by ORAC (Mildenberger, Stangeland, & Rebours, 2022; Wang et al., 2009) compared to extracts from other brown seaweed species (e.g. Fucus species), which can have been reported to have ORAC values of 1500–3000 μmol of TE/g dw extract (Hrólfsdóttir et al., 2022; Wang et al., 2009). Therefore, *S. latissima* and *A. esculenta* might be more suitable for applications that do not rely on antioxidant properties, such as ingredients in food products post blanching treatments.

4. Conclusion

The results show that the proximate composition, color, pH, and microbial counts of the acid-preserved seaweed remained relatively stable for at least seven-month (32-week) storage at room temperature, with minimal differences observed between lactic acid and citric acid treated seaweed. However, acid preservation of seaweeds might cause protein deterioration or increase in biogenic amines as evidenced by increase in TVB-N over the storage period. Additionally, the study revealed that the seaweed biomass has relatively high cadmium, arsenic, and iodine content on a dry weight basis, along with high salt content that could limit the application of the biomass as it is for food and feed applications Furthermore, the strong salt and acid flavor detected in the samples might also influence the possible uses of the biomass as a food ingredient. Notably, a decrease was observed in the total phenolic content (TPC) and antioxidant properties (assessed by DPPH) during the storage of acid preserved biomass of *A. esculenta*. In conclusion, acid preservation using lactic acid or citric acid was effective in stabilizing the seaweed biomass and could possibly be used as a stabilization method for *A. esculenta* and *S. latissima* as well as other seaweed species. Low antioxidant characteristics of the tested *S. latissima* and *A. esculenta*, however, imply that these species might not be ideal if the seaweed is intended for applications for its antioxidant properties. Nevertheless, the

acid treated biomass might be suitable for seaweed as an ingredient in a wide range of value-added products.

CRediT authorship contribution statement

Anna Þóra Hrólfsdóttir: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sigurjón Arason:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Conceptualization. **Hildur Inga Sveinsdóttir:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Conceptualization. **Maren Sæther:** Writing – review & editing, Methodology, Conceptualization. **Inga Marie Aasen:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization. **María Gudjónsdóttir:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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