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RESEARCH

The effects of freezing and thawing on *Alaria esculenta*

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Abstract
Seaweeds must be stabilised shortly after harvesting to avoid rapid deterioration. To handle large amounts harvested during a short period, freezing and frozen storage until utilisation or further processing is one of the methods used industrially. The aim of this study was to assess the effects of different freezing and thawing procedures on *Alaria esculenta* by analysis of the chemical composition of the seaweed and the drip loss expelled during thawing. Thawing of industrially frozen *A. esculenta* resulted in a drip loss of 57% of wet weight. The drip loss had a dry matter content of 7% of wet weight, of which 71% was mineral content. Analysis showed that, of the dry matter excluding ash, alanine, aspartic acid, and mannitol were the main components lost to the drip loss. Experiments with a second batch of *A. esculenta* looking at quick and slow freezing and thawing showed that quick freezing resulted in a significantly lower drip loss than slow freezing; 20% compared to up to 42% of wet weight. Dry matter and mineral content of the drip loss of these samples were all around 6% of wet weight and 31% of dry weight. For some applications it might be of interest to reduce the concentration of potentially toxic elements such as iodine and heavy metals, but due to a high loss of other biomass this was not very effectively done by freezing and thawing. For preservation purposes, quick freezing is the best alternative to retain seaweed biomass.

Keywords Winged kelp · *Alaria esculenta* · Phaeophyceae · Freezing · Drip loss · Minerals

Introduction

Increased production of nutritious and sustainable food to support a growing world population is important (FAO et al. 2022). Cultivation of seaweeds requires no land area, fresh water, or fertilisers, making their production more sustainable than the production of terrestrial plants (Kraan 2013). The biochemical composition of seaweeds varies significantly with species, season, and habitat. Nevertheless, there is an increasing interest in seaweeds as food due to their nutritional value (FAO 2018). Seaweeds contain important nutrients such as proteins, minerals, lipids, and carbohydrates. These nutritional components have been shown to be of high quality with essential amino acids, minerals, and vitamins, polyunsaturated fatty acids such as omega-3 and omega-6, and dietary fibre (Healy et al. 2023). Additionally, seaweeds have been shown to contain numerous bioactive compounds beneficial for human health (Holdt and Kraan 2011). However, despite their beneficial nutritional content, there are some challenges on the road from cultivated seaweed to finished product.

Seaweeds can accumulate harmful and persistent contaminants from surrounding waters. Such contaminants include inorganic arsenic, lead, cadmium, and mercury. Consumption of these metals can be harmful even at trace levels (Sá Monteiro et al. 2019). Additionally, some seaweeds, especially the kelps, have been shown to accumulate considerable amounts of iodine. Among these high-iodine kelps, are the two main cultivated seaweed species in Norway; *Saccharina latissima* and *Alaria esculenta* (Duinker et al. 2020). Although iodine is an essential micronutrient in the human diet, necessary for the synthesis of thyroid hormones, exaggerated exposure to iodine can also impair thyroid

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function (Sá Monteiro et al. 2019). Therefore, the high iodine content of these species poses a potential health risk for the consumer. Studies have shown that blanching and pulsed electric fields (PEF) treatment can reduce the iodine content of *S. latissima* by up to 88% and by 40%, respectively (Nielsen et al. 2020; Blikra, Henjum et al. 2022a; Blikra, Skipnes et al. 2022b). However, other dry matter is also lost during processing which can affect the nutritional value and flavour.

Additionally, the high water content of fresh seaweeds causes a rapid decomposition after harvest (Enríquez et al. 1993; Holdt and Kraan 2011). Because of this, seaweed raw material must be preserved after harvest to ensure biomass quality and product safety (Stévant et al. 2017a, b). Freezing is a popular method for long term food preservation which generally preserves taste, texture, and nutritional value (Choi et al. 2012). However, freezing and subsequent thawing of seaweed has been shown to result in a high loss of liquid from the raw material (Stévant 2019; Nielsen et al. 2020). Stévant (2019) reported a drip loss of over 40% of original sample weight from thawing of frozen *S. latissima*. The drip loss was described as viscous and brown, indicating loss of phytochemical compounds. This could affect the nutritional value and flavour of the seaweed. Fast freezing rates are known to cause the formation of smaller ice crystals which can limit cell membrane damage and consequently limit drip loss during thawing (James et al. 2015). Therefore, freezing method and rate could affect the amount of drip loss and its biochemical composition.

The aim of this study was to assess the effects of freezing and thawing procedure on *Alaria esculenta* by analysis of the chemical composition of the seaweed and the drip loss expelled during thawing. These results were used to discuss the suitability of the different freezing and thawing procedures for stabilisation of *A. esculenta* biomass.

Methods and materials

This study combines two different experiments that evaluate the effects of freezing and thawing of *Alaria esculenta*. The initial experiment was conducted in 2020 and looked at the biochemical composition of the original and thawed *A. esculenta*, as well as the drip loss from thawing. The supplemental experiment was conducted in 2022 and looked on the effects of different freezing and thawing methods on the amount of drip loss and the mineral content.

Sample preparation

The first batch of *Alaria esculenta* raw material was harvested on 06 May 2019 from the sea farm of Seaweed Solutions AS outside Frøya, Norway (N63° 42.279' E8° 52.232'). The seaweed was delivered by Seaweed Solutions as whole blades with stipes frozen in blocks of 20 kg packaged in plastic within carboard boxes and was kept frozen at -26°C until this study was started in January 2020. Pieces were cut from a frozen block and placed in a funnel with mesh attached to the bottom to separate the drip loss from the seaweed during thawing. The drip loss was collected in a beaker. In total, the pieces weighed just above 600 g and were thawed for approximately 21 hours at 4°C. There were no processing replicates of the thawing process. Before and after thawing, seaweed raw material was put aside for analysis. The collected drip loss, thawed seaweed, and original frozen seaweed samples were analysed for dry matter and ash content as well as freeze died in a Christ Alpha 1-4 LO plus freeze dryer (Germany) before analysis of carbohydrate and amino acid content. A process flow chart of this initial experiment is shown in Fig. 1.

The *A. esculenta* for the supplemental experiment was collected at Arctic Seaweed's location at Misje outside of Bergen, Norway (N60° 27.403' E4° 57.913'). Uncultivated samples were collected on 04 April 2022 and stored in seawater in a large tank outside at an average temperature of 3.1°C. On 05 April 2022, the seaweed was transferred to barrels with fresh seawater and kept refrigerated using ice packs during transport to Nofima Stavanger, Norway. Upon arrival at Nofima Stavanger on 06 April 2022 the barrels were topped up with fresh seawater and stored at 2°C until 07 April 2022. The seaweed was dewatered by hanging over a pole for at least five minutes. Batches of about 500 g of seaweed were packed in vacuum bags in layers between 10-13 mm thick. After packing, the samples were frozen in either a quick or slow manner. The quick freeze (QF) samples were frozen individually on pre-cooled metal trays in a -30°C freezer room with a fan for extra air circulation. The slow freeze (SF) samples were put in a Styrofoam box and into a freezer set to -18°C. When frozen, all samples were

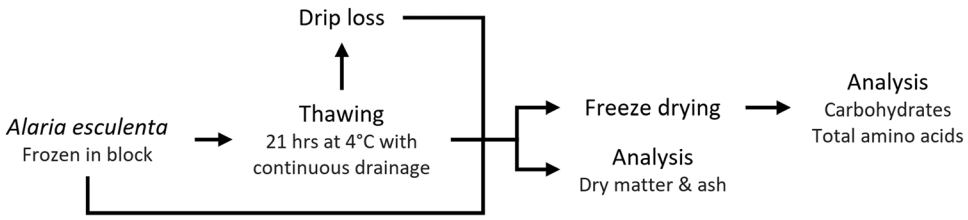

Fig. 1 Process flow chart for initial experiment: Thawing of industrially frozen *Alaria esculenta*

stored at -30°C until October 2022. Thawing of the samples was also done quickly and slowly. The quick thaw (QT) samples were thawed for 10 minutes submerged in a water bath with a temperature of 10°C. The slow thaw (ST) samples were thawed in a Styrofoam box at 4°C for approximately 48 h. After thawing, the drip loss was separated from the seaweed by draining in a colander for 10 min and dry matter content was determined for both drip loss and thawed seaweed. Each of the four processing combinations (QF-QT, QF-ST, SF-QT, and SF-ST) were conducted in triplicates. The thawed seaweed was then separated into two equal batches of between 142-202 g, where one was dried in a cabinet at 70°C for 3 h in a Metos System Rational oven (MSCC 61, Kerava, Finland) and the other freeze dried at 0.05 mbar for 72 h in a Christ freeze drier (Christ Gamma 2-16 LSC Plus, Martin Christ GmbH, Germany). The samples dried in the cabinet were evenly distributed in a thin layer across perforated oven trays (inner dimensions 29x48 cm) to ensure rapid drying. The drip loss was freeze dried. The dried drip loss and seaweed samples were then analysed for carbohydrate and mineral content. A process flow chart of this experiment is shown in Fig. 2.

In both sample preparations the amount of drip loss was calculated from the difference in *A. esculenta* raw material weight before and after thawing. This was done to limit uncertainties connected to the collection of the drip loss.

Dry matter and ash analysis

Dry matter analysis was conducted by drying at 105°C for 18-24 h to constant weight. The ash content was determined from the dried samples by combustion in a muffle furnace at 550°C overnight.

Mineral analysis

Analysis of iodine, macro minerals, and metals was done by the Institute of Marine Research (IMR). The iodine content was quantified by inductively coupled plasma-mass spectrometry (ICP-MS) according to the method described by Dahl et al. (2020). Samples were mixed with 1 mL tetramethylammonium hydroxide (TMAH) and 5 mL deionized water before extraction at 90 ± 3 °C for 3 h. The samples were then diluted, centrifuged, and filtered through 0.45 µm syringe and disposal filter. Tellurium was used as an internal standard. The macro minerals Na, Mg, K, and Ca, as well as the metals As, Cd, Hg, Pb, and Cu, were determined by the ISO accredited methods described by Moxness Reksten et al. (2020). The analysis was done by ICP-MS after acid wet digestion in a microwave oven according to the method described by Julshamn et al. (2007), using an external calibration curve.

Carbohydrate analysis

The carbohydrate content of the samples was analysed by high performance anion-exchange chromatography with pulsed amperiometric detection (HPAEC-PAD) on an ICS 5000+ system equipped with a pulsed amperometric detector (carboquad waveform) using a 4×50 mm CarboPac PA1 guard column and a 4×250 mm main column. Three parallels of approximately 20 mg sample were degraded in 5 mL of 1 M trifluoroacetic acid at 100°C for 24 h and then dried in a vacuum concentrator (Savant SC250EXP SpeedVac, Thermo Scientific, USA) at 40°C and 5 Torr for 4 h. The samples were then diluted in Milli-Q water to a total volume of 1 mL. The samples were run 5 times diluted and 50 times diluted. For the 5 times diluted samples, two analytical parallels were created after the acid hydrolysis, the average of the result from these analytical parallels was used in

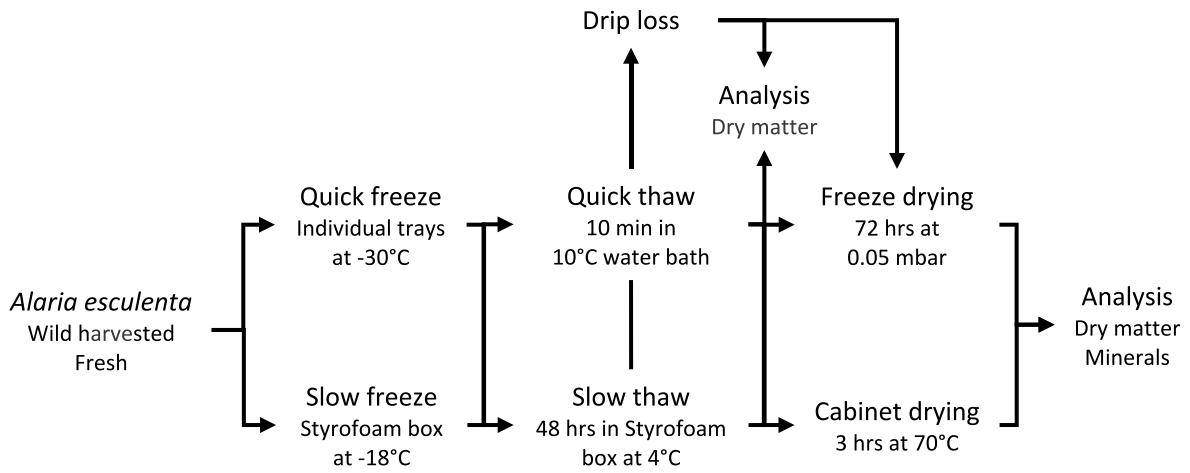

Fig. 2 Process flow chart for supplemental experiment: Quick and slow freezing and thawing of *Alaria esculenta*

further calculations. The method was based on the method described by Zhang et al. (2012).

Standards containing inositol, mannitol, fucose, glucose, xylose, rhamnose, galactose, and mannose were run at concentrations between 0.1-10.0 mg L^{-1} to create standard curves. In this method, the retention times of mannose and xylose are very close and difficult to separate in the calculation of the results. Therefore, an average standard curve for these two compounds was created using the standard concentrations and peaks areas, and a combined mannose and xylose concentration was calculated for the samples.

Amino acid analysis

The protein content was estimated by analysis of total amino acid content. This was done as described by Blackburn (1978). Samples were hydrolysed in 6 M hydrochloric acid (HCl) for 22 h at 105°C in airtight vials to limit oxidation, neutralised with sodium hydroxide and filtered through Whatman glass microfiber filter GF/C by vacuum. The samples were then diluted with de-ionised water and filtered through 0.22 µm syringe filter. Analysis was done by reverse phase HPLC (UltiMate 3000 HPLC, Thermo Scientific, USA) using a Nova-Pak C18 column on a with a Dionex RF 2000 fluorescence detector with OPA derivatisation.

The amino acids cysteine, proline, and tryptophan were not measured. Additionally, glutamine and asparagine are completely hydrolysed to glutamic acid and aspartic acid, respectively. Threonine and serine are partially hydrolysed, tyrosine is partially destroyed, and it has been shown that methionine suffers transformation during the acid hydrolysis (Fountoulakis and Lahm 1998; Mustățea et al. 2019).

Statistical analysis

Statistical analysis was performed using Minitab 21.41. One-way ANOVA with 95% confidence interval and Tukey post-hoc test was performed for analysis of significant difference.

Results

Drip loss, dry matter, and minerals

In the initial experiment, when thawing the industrially frozen *A. esculenta*, only 43% of the original raw material was left after thawing, indicating a drip loss of 57% of the original sample wet weight. The drip loss had a light-yellow colour and was slightly viscous. The dry matter and ash contents of the original frozen and the thawed *A. esculenta* and the drip loss are shown in Table 1. In total, 17% of the dry matter excluding ash and 60% of the ash content of the *A. esculenta* raw material sample was lost to the drip loss.

Table 1 Dry matter (% of WW) and ash content (% of DW) of drip loss, thawed *Alaria esculenta*, and original raw material. The values are listed with standard deviations, n=3

	Dry matter	Ash
Drip loss	6.55±0.04	70.8±1.0
Thawed *A. esculenta*	18.0±2.3	24.1±0.7
Original *A. esculenta*	10.9±1.4	40.7±2.2

Table 2 Drip loss (% of WW of original raw material) and dry matter content (% of WW) and mineral content (% of DW) of the drip loss and thawed *Alaria esculenta* (AE). The names of the samples indicate the freezing and thawing method (QF=Quick freeze, SF=Slow freeze, QT=Quick thaw, ST=Slow thaw). Mineral content was analysed on freeze dried material. The values are listed with standard deviation, n=3

Sample	Drip loss	Dry matter content		Mineral content	
		Drip loss	Thawed AE	Drip loss	Thawed AE
QF-QT	20.8±1.1^{a}	5.30±0.10^{a}	15.8±0.9^{a}	32±0^{a}	16±1^{a}
QF-ST	20.2±1.9^{a}	6.01±0.16^{a}	16.3±0.5^{a}	29±6^{a}	15±0^{a}
SF-QT	42.1±0.9^{b}	5.17±0.05^{a}	21.8±1.4^{b}	33±1^{a}	13±1^{a}
SF-ST	33.8±1.1^{c}	6.17±0.03^{a}	19.3±0.7^{c}	29±0^{a}	14±1^{a}

Letters in superscript indicate statistically significant differences within columns

The drip loss from the four different freezing and thawing methods tested on the second batch of *A. esculenta*, as well as the dry matter contents and mineral contents of the drip loss and the thawed seaweed are shown in Table 2. The samples were dried both by conventional cabinet drying and freeze drying. Drying method had no significant effect on the mineral content. Therefore, the results shown in the following section are for the freeze-dried samples.

The distribution of the total content of iodine (I), arsenic (As), and cadmium (Cd) between the thawed *A. esculenta* and the drip loss is presented in Fig. 3.

As seen in Fig. 3 the amount of potentially toxic elements (PTEs) lost to the drip loss varied with freezing and thawing method. However, the final concentration of these PTEs in the thawed *A. esculenta* was affected by the loss of other dry matter components. The concentrations of the PTEs in the fresh and thawed seaweed are presented in Table 3 together with the corresponding reduction. The concentrations of minerals of the fresh *A. esculenta* are calculated from the sum of the contents determined for the thawed *A. esculenta* and the drip loss.

The macro mineral content of the fresh and thawed *A. esculenta* is shown in Table 4. All the thawed *A. esculenta* samples had a Na/K-ratio between 1.0-1.1, with no significant differences between treatments.

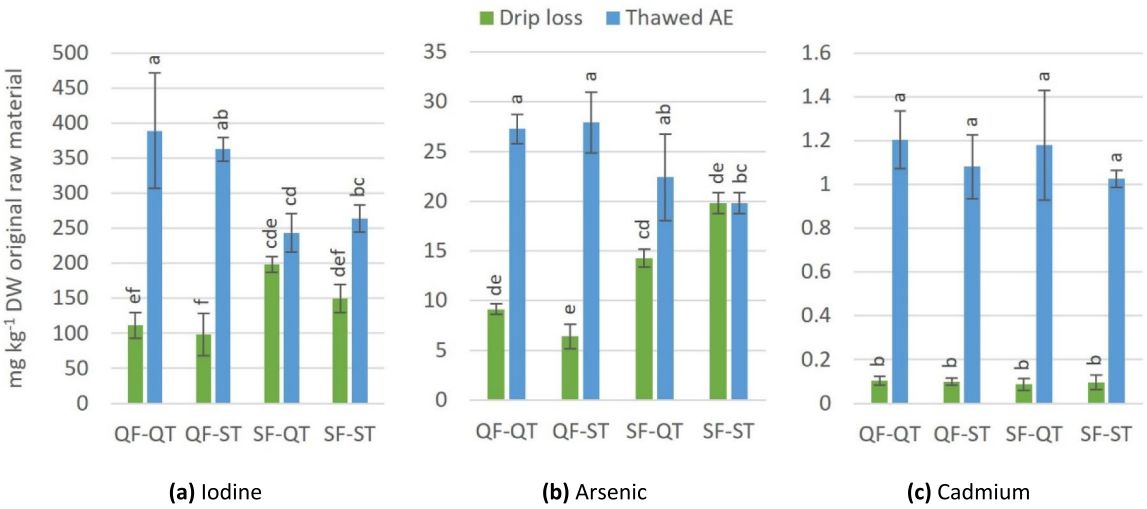

Fig. 3 Distribution of total iodine (**a**), arsenic (**b**), and cadmium (**c**) content (mg kg^{-1} DW of original raw material) between the thawed *Alaria esculenta* (AE) and the drip loss. The names of the samples indicate the freezing and thawing method (QF=Quick freeze, SF=Slow freeze, QT=Quick thaw, ST=Slow thaw). Lower case letters above the bars indicate statistically significant differences within the charts. Error bars show standard deviation, n=3

Table 3 Total iodine (I), arsenic (As), and cadmium (Cd) content in the fresh and thawed *Alaria esculenta* (mg kg^{-1} DW) and the reduction (%) of these for the different freezing and thawing combinations. The names of the samples indicate the freezing and thawing method (QF=Quick freeze, SF=Slow freeze, QT=Quick thaw, ST=Slow thaw). The values are listed with standard deviation, n=3

Sample		Iodine		Arsenic		Cadmium	
		Conc.	Reduction	Conc.	Reduction	Conc.	Reduction
QF-QT	Fresh	500±100^{a}	15±1	36±2^{a}	18±1	1.3±0.1^{a}	-0.2±1.2
	Thawed	420±90 ^{abc}		30±2 ^{abc}		1.3±0.1^{a}	
QF-ST	Fresh	460±20^{a}	14±6	34±4^{ab}	11±2	1.2±0.2^{a}	-0.16±0.35
	Thawed	400±20 ^{abc}		31±3 ^{abc}		1.2±0.2^{a}	
SF-QT	Fresh	440±40^{ab}	35±2	37±4^{a}	29±7	1.3±0.3^{a}	-9.1±1.5
	Thawed	290±30^{c}		26±5 ^{bc}		1.4±0.3^{a}	
SF-ST	Fresh	410±40 ^{abc}	26±2	31±1 ^{abc}	24±2	1.1±0.1^{a}	-6.5±2.6
	Thawed	310±20 ^{bc}		23±1^{c}		1.2±0.1^{a}	

Letters in superscript indicate statistically significant differences within columns

Table 4 Macro mineral content (mg kg^{-1} DW) of the fresh and thawed *Alaria esculenta*. The names of the samples indicate the freezing and thawing method (QF=Quick freeze, SF=Slow freeze, QT=Quick thaw, ST=Slow thaw). The values are listed with standard deviation, n=3

Sample		Ca	K	Mg	Na	P
QF-QT	Fresh	22000±1000 ^{a}	64000±2000 ^{a}	12000±1000^{a}	66000±4000^{a}	5600±200^{a}
	Thawed	24000±1000 ^{a}	58000±2000^{ab}	12000±1000^{ab}	59000±4000^{ab}	5300±200 ^{abc}
QF-ST	Fresh	22000±0 ^{a}	60000±3000^{ab}	12000±0^{ab}	65000±3000^{a}	5200±200 ^{abcd}
	Thawed	23000±0 ^{a}	55000±1000 ^{bc}	11000±0^{ab}	59000±1000^{ab}	4800±100 ^{bcd}
SF-QT	Fresh	22000±1000 ^{a}	60000±4000^{ab}	12000±1000^{ab}	63000±2000^{a}	5300±200 ^{abc}
	Thawed	24000±1000 ^{a}	46000±3000^{d}	11000±1000^{b}	47000±2000^{c}	4600±300^{d}
SF-ST	Fresh	21000±2000 ^{a}	58000±2000^{ab}	12000±1000^{ab}	63000±4000^{a}	5400±300^{ab}
	Thawed	23000±2000 ^{a}	49000±2000^{cd}	11000±1000^{b}	51000±4000 ^{bc}	4700±300^{cd}

Letters in superscript indicate statistically significant differences within columns

The concentrations of trace elements for the fresh and thawed *A. esculenta* are shown in Table 5. Mercury (Hg), silver (Ag), cobalt (Co), molybdenum (Mo), and nickel (Ni) are not included in the table as they were not detected above the limit of quantification (LOQ) in any of the samples. Furthermore, the concentration of copper (Cu), lead (Pb), chromium (Cr), and selenium (Se) was below the LOQ in several of the samples. The empty cells in the table below represent samples where all parallels were below the LOQ. When Cu, Pb, Cr, and Se were detected in one parallel or more of a sample the remaining parallels below the LOQ were included in further calculations as the LOQ. The LOQ for Cu, Pb, Cr, and Se was 1, 0.2, 0.2, and 0.1 mg kg^{-1}, respectively.

Carbohydrates

The concentrations of mannitol, fucose, glucose, galactose, rhamnose, mannose and xylose after hydrolysis in the industrially frozen *A. esculenta* and the corresponding thawed raw material and drip loss were determined. The results are shown as percent of dry weight of original raw material in Fig. 4. The total concentration of these in the three fractions showed that 62% of the carbohydrate content related to the

Table 5 Trace element content (mg kg^{-1} DW) of the fresh and thawed *Alaria esculenta*. The names of the samples indicate the freezing and thawing method (QF=Quick freeze, SF=Slow freeze, QT=Quick thaw, ST=Slow thaw). No significant differences were found within columns. The values are listed with standard deviation, n=3

Sample		Fe	Mn	V	Zn	Cu	Pb	Cr	Se
QF-QT	Fresh	35±3	5.4±0.3	0.25±0.04	55±6	1.6±0.6		0.22±0.02	0.12±0.01
	Thawed	36±3	5.1±0.3	0.23±0.04	56±6	1.3±0.5			0.12±0.01
QF-ST	Fresh	34±2	5.2±0.3	0.26±0.02	48±5	1.1±0.0	0.21±0.01		0.11±0.01
	Thawed	33±2	5.1±0.3	0.24±0.01	50±6		0.21±0.01		0.11±0.01
SF-QT	Fresh	33±7	5.1±0.7	0.22±0.05	54±12	1.4±0.3	0.67±0.81		0.10±0.00
	Thawed	36±9	4.6±0.9	0.20±0.06	53±14	1.2±0.3	0.75±0.95		
SF-ST	Fresh	38±4	4.7±0.1	0.21±0.01	48±6	1.0±0.0	0.53±0.50	0.21±0.01	0.10±0.00
	Thawed	39±4	4.3±0.2	0.18±0.02	49±6		0.58±0.57	0.21±0.01	

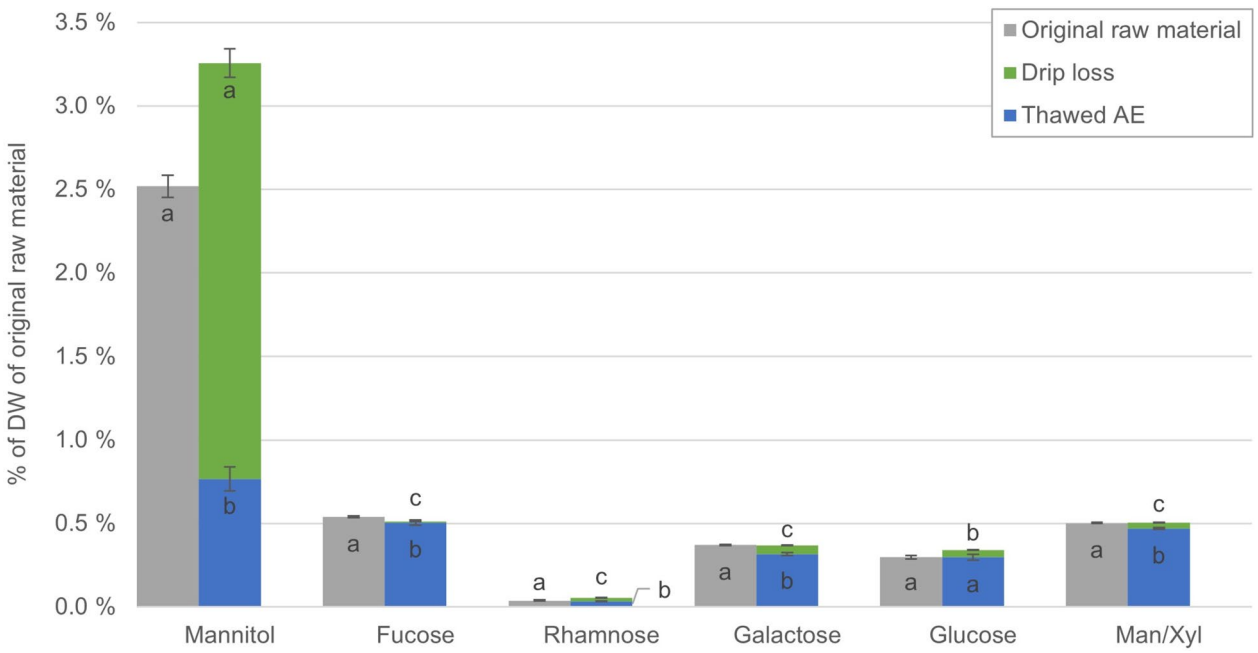

Fig. 4 Mannitol, fucose, glucose, galactose, and combined mannose and xylose content (% of DW of original raw material) of original raw material, thawed *Alaria esculenta* (AE), and drip loss. Lower case letters indicate statistically significant differences within each compound. Error bars show standard deviation, n=3

analysed compounds in the original raw material was lost to the drip loss.

Protein content

The total amino acid content was determined for the industrially frozen *A. esculenta* and the corresponding thawed raw material, and drip loss of the initial experiment. The determined total amino acid distributions are shown in Fig. 5. The sum of total amino acids showed that the original *A. esculenta* raw material had a protein content of 8.0±0.4% of DW where 6.3% of this total amino acid content was lost to the drip loss. The main amino acid lost to the drip loss samples during thawing was alanine, where 29% of the total content was lost to the drip loss. Some aspartic acid/asparagine was also lost to the drip loss, although this was just 3.2% of the total content. Additionally, there seemed to be some glutamic acid/glutamine lost to the drip loss, but the difference between the original and thawed *A. esculenta* was not significant.

Discussion

Thawing of frozen seaweed has been shown to cause a substantial loss of liquid (Stévant 2019; Obluchinskaya and Daurtseva 2020). However, the liquid fraction lost has, to the authors' knowledge, not previously been characterised. In this study, two experiments were conducted to assess the effects of freezing and thawing on *Alaria esculenta*. The initial experiment was executed with industrially frozen *A. esculenta* in block and thawing at 4°C for 21 h. The drip loss in the initial experiment equalled 57% of the original raw material, which was slightly higher than the 40% reported for *Saccharina latissima* in Stévant (2019). However, in Stévant (2019) the seaweed was thawed in bags, while it in the initial experiment of this study was thawed with continuous drainage of the drip loss. In the supplemental experiment of this study a second batch of *A. esculenta* was packed in thin layers in bags and frozen quickly or slowly, before being thawed in bags either quickly or slowly. This led to a lower drip loss more comparable to the results from Stévant (2019). The quickly frozen (QF) samples lost a smaller amount of drip loss during thawing than the slowly frozen (SF) samples. Thawing method did not significantly affect the amount of drip loss from the QF samples. However, quick thawing (QT) of the SF samples resulted in a higher amount of drip loss than slow thawing (ST) of these samples. The SF samples had a drip loss more like the one in Stévant (2019), while the QF samples had a substantially lower drip loss despite the samples in Stévant (2019) being frozen quickly using impingement technology. The differences in the drip loss could be due to other differences in the freezing and thawing process or the biological differences between the two seaweed species.

In Stévant (2019) the drip loss from *S. latissima* was mentioned to be viscous and brown. The drip loss samples

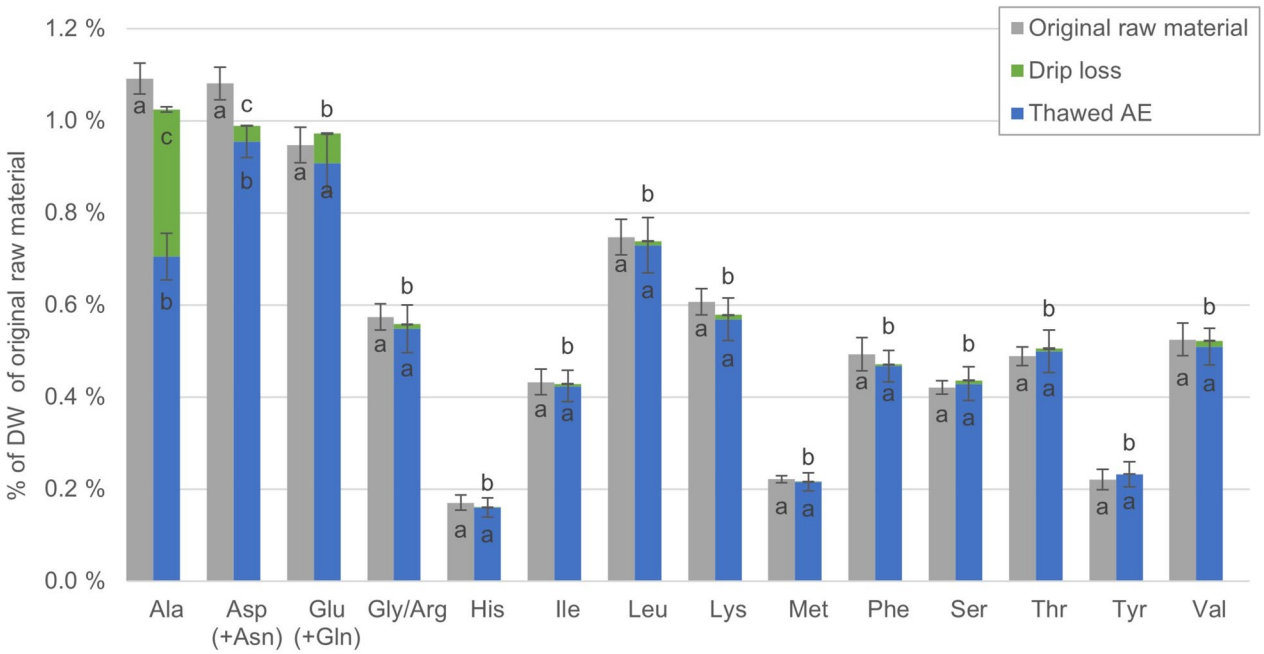

Fig. 5 Mass balances for total amino acid composition (% of DW of original raw material) of original raw material, thawed *Alaria esculenta* (AE), and drip loss. Lower case letters indicate statistically significant differences within the results for each compound. Error bars show standard deviation, n=3

from *A. esculenta* in this study were slightly viscous and light-yellow. These characteristics indicated the presence of biochemical components from the seaweed. When thawing the industrially frozen *A. esculenta* of this study, the dry matter and ash analysis showed that substantial amounts of the dry matter of the seaweed was lost to the drip loss. The majority (70.8 ± 1.0 %) of this dry matter loss was ash content, which reflects the mineral content. When using the quick and slow freezing and thawing procedures on the second batch of *A. esculenta*, the mineral content in percent of dry weight was much lower (up to 33±1%). This could be related to the thawing method as the industrially frozen seaweed was thawed with continuous drainage of the drip loss, while the second batch in the supplemental experiment was thawed in bags. However, there may be several other explanations, such as biological variations, storage temperature effects, and autolytic reactions.

Brown seaweeds have been shown to contain very high levels of iodine as well as some metal content (Lüning and Mortensen 2015; Blikra et al. 2021; Stévant et al. 2021; Blikra, Skipnes et al. 2022b). The second batch of *A. esculenta* that was frozen and thawed slowly and quickly was analysed for mineral content to determine the content of minerals of nutritional significance and potentially toxic elements (PTEs), and the effects the different freezing and thawing procedures had on this content.

The European Food Safety Authority (EFSA) has established a tolerable upper intake limit for iodine intake of 600 µg day^{-1} for adults and shown that replacing part of the diet with kelp might result in an intake well above these recommendations (EFSA et al. 2023). Reported concentrations of iodine in *A. esculenta* range from about 200 to 700 mg kg^{-1} DW, depending on geographic location and several other factors (Stévant, et al. 2017a, b; Roleda et al. 2018). The concentrations of iodine reported in this study were all within the range reported in literature. Substantial amounts of iodine were lost to the drip loss during thawing. However, the difference in iodine concentration of the fresh and thawed *A. esculenta* was only significant for the SF-QT samples, reaching a reduction of 35±2%. As high iodine contents are a food safety issue and market barrier for kelps, a reduction in iodine content is favourable (Blikra, Henjum et al. 2022a; Blikra, Skipnes et al. 2022b). Nevertheless, despite a 35% reduction, one would reach the tolerable upper intake limit by consumption 9.5 g WW of the SF-QT raw material. Additionally, as can be seen from the other analyses done in this study, freezing and thawing not only led to a reduction in iodine, but several other nutritional components were also lost. Therefore, slow freezing does not seem to be a viable option for iodine reduction.

Due to their ability to bioaccumulate metals from their environment, concerns have been raised around the consumption of seaweeds being a route of dietary exposure to toxic metals such as inorganic arsenic (iAs), cadmium (Cd), lead (Pb), and mercury (Hg) (EFSA et al. 2023). Of these metals, total arsenic (As) was quantified at the highest concentration, followed by Cd. Pb was only detected in 4 out of 12 samples. Pb contents of *A. esculenta* in literature range from not detected to 1.1 mg kg^{-1} DW (Ometto et al. 2018; Roleda et al. 2019; Afonso et al. 2021). The values reported in this study were consistent with this range.

As for iodine, the difference in the As concentration in the fresh and thawed *A. esculenta* was only significant for the SF-QT samples, reaching a 29±7% reduction. However, this was at the cost of loss of other dry matter compounds which limits the potential for freezing and thawing as a method of reducing As content. The total As contents reported in this study were, for both the original and thawed samples, below the concentrations reported in literature for *A. esculenta* (46-79.8 mg kg^{-1} DW) (Mæhre et al. 2014; Ometto et al. 2018; Roleda et al. 2019; Afonso et al. 2021). It is important to note here that the As content quantified in this study was total As, as no speciation between organic and iAs was done. Studies have shown that most of the As content in seaweeds is in organic forms and not the inorganic forms which are known to be carcinogenic and more harmful to human health (Díaz et al. 2011; Krook et al. 2023).

Only a small amount of the Cd content was lost to the drip loss during thawing, and despite this loss, the concentration of Cd in the thawed *A. esculenta* was not significantly different to the concentration in the original raw material in any of the samples. Although these differences were not significant, the calculated reduction was negative. This indicates that the Cd content was slightly concentrated in the thawed samples due to loss of other dry matter. Reported Cd contents for *A. esculenta* span from 1.33 up to 3.4 mg kg^{-1} DW (Mæhre et al. 2014; Stévant, et al. 2017a, b; Ometto et al. 2018; Roleda et al. 2019; Afonso et al. 2021). The Cd contents of both the original and thawed raw material in this study were all in the lower end of these reported values. According to the literature, Cd concentration in *A. esculenta* can vary by location and season and has often been shown to be significantly higher than in *S. latissima* from the same location, although not in all studies (Ometto et al. 2018; Stévant et al. 2018; Roleda et al. 2019; Afonso et al. 2021). This could imply that Cd accumulation could be a bigger issue in *A. esculenta* than in other species.

Diets with high sodium-to-potassium (Na/K) ratios have been shown to be associated with high blood pressure and cardiovascular diseases and previous studies have shown that seaweeds can be used to lower the Na/K-ratio of food products (López-López et al. 2009; Perez and Chang 2014). Previous studies have reported *A. esculenta* Na/K-ratios ranging from 0.61-0.94 (Tibbetts et al. 2016; Stévant, et al. 2017a, b; Blanco et al. 2023; Wegeberg et al. 2023). In this study, the

original *A. esculenta* raw material had a Na/K-ratio around 1. This was slightly higher than literature values. In Stévant, et al. (2017a, b), the Na/K-ratio was shown to increase to 1.22 after 22 h of storage in seawater. The seaweed in this study was kept stored in seawater for three days before freezing, which could have increased its Na/K-ratio. The slightly elevated Na/K-ratio could also be due to some remaining seawater on the surface of the seaweed as it was not rinsed with fresh water. Freezing and thawing of the seaweed led to loss of Na and K in similar amounts, resulting in an unaltered Na/K-ratio of the seaweed.

A carbohydrate analysis was executed on the industrially frozen *A. esculenta* and the corresponding thawed seaweed and drip loss. Analysis of the content of mannitol, fucose, glucose, galactose, rhamnose, mannose, and xylose after hydrolysis showed that the seaweed lost 62% of its carbohydrate content relevant to the analysed compounds. However, one of the main cell wall polysaccharides of brown algae, alginate, is not included in this analysis, as the content of its components mannuronic and guluronic acid was not determined (Rioux and Turgeon 2015). The drip loss was slightly viscous indicating a loss of some polysaccharides which might include some water-soluble alginate. However, this requires further investigation. There was observed some mismatch in the mass balance for the compounds analysed which is believed to be related to an observation of lower signals and bigger variations between samples than normally expected, which might be caused by the gold electrode used in the HPLC being old and worn. However, major trends were still discernible.

Mannitol was the main carbohydrate present in all the samples. A majority of the mannitol content present in the raw material was lost to the drip loss during thawing. Of the other compounds analysed, nearly everything was retained in the thawed *A. esculenta*. Mannitol is a storage carbohydrate in brown seaweeds that has a sweet flavour, often used as a low-calorie sweetener in foods (Rioux and Turgeon 2015). Loss of mannitol from the seaweed raw material can therefore affect the flavour and sweetness of the seaweed. Free mannitol is water soluble and has been shown to be positively correlated with water salinity as it acts as an osmoprotectant in seaweeds (Zhang and Thomsen 2019; Obluchinskaya et al. 2024). However, mannitol can also be bound to the end of M-chains of the polysaccharide laminarin, which acts as a carbohydrate reserve for the seaweed. Laminarin consists of glucose chains with branching containing glucosyl units, and depending on the degree of branching, laminarin can be soluble in cold water (Rioux and Turgeon 2015). Considering that a small amount of glucose was detected in the drip loss it is plausible to believe that some of the soluble laminarin content was lost to the drip loss. However, considering that there was no significant difference between the glucose content determined in the original and thawed *A. esculenta* this fraction of lost laminarin is most likely negligible.

A very low fucose content in the drip loss and only a small difference in the fucose content before and after thawing suggested that most of the fucoidan was left in the seaweed. Fucoidans are sulphated fucose-rich polysaccharides with varying amounts of other monosaccharides including galactose, mannose, xylose, rhamnose, and glucose. They are found in the cell walls of brown algae and have been shown to have bioactivities such as antioxidant, anti-inflammatory, and anticoagulant (Holdt and Kraan 2011; Bruhn et al. 2017). Therefore, retaining the fucoidan content in the seaweed after thawing is beneficial for it use as food.

The sum of total amino acid content showed that the majority of the protein content was retained in the seaweed during thawing. This is beneficial for the use of the seaweed for consumption. However, a substantial amount of the alanine content as well as some aspartic and glutamic acid was lost to the drip loss. It is plausible to believe that these amino acids were mostly in the free form as free amino acids have high water solubility and have been shown to be released from the raw material by boiling (Mæhre et al. 2016). Free alanine is known to have a sweet taste and free glutamic and aspartic acid are known to provide umami flavour (Holdt and Kraan 2011; Suess et al. 2015). Therefore, the loss of these amino acids could affect the flavour of the seaweeds.

Conclusion

Thawing of industrially frozen *Alaria esculenta* with continuous drainage led to a drip loss of 57% of wet weight. Controlled quick and slow freezing and subsequent thawing in bags showed that quick freezing limited the drip loss amount to less than 21% of wet weight, compared to slow freezing which led to a drip loss of up to 42% of wet weight. For these samples thawed in bags, the biochemical composition of the drip loss did not change significantly with freezing or thawing method. Therefore, quick freezing better retained dry matter content. Quick or slow thawing did not affect the amount of drip loss lost from the quickly frozen samples. However, slowly frozen samples had a significantly higher drip loss when thawed quickly. Analysis of the industrially frozen samples showed a loss of alanine, aspartic acid, glutamic acid, and mannitol during thawing, which could affect the flavour of the seaweed. Only a small change in fucose content of the seaweed before and after thawing indicated that the fucoidan content was not affected by freezing and thawing. Slow freezing and quick thawing led to a significant reduction in iodine (35±2%) and total arsenic (29±7%), but at the cost of high biomass loss. Quick freezing and thawing without continuous drainage is a good alternative for stabilisation of biomass due to better retention of nutrients and

flavour components. However, when it comes to reduction of PTEs, freezing is not efficient. Other methods for reducing PTE content in *Alaria esculenta* and other seaweeds should be investigated.

Authors' contribution Collection of raw material, sample preparation, data collection and analysis for the initial experiment was performed by Randi Sund and Turid Rustad. In the supplemental experiment the same tasks were performed by Randi Sund, Dagbjørn Skipnes, and Arne Duinker. The first draft of the manuscript was written by Randi Sund. Editing and reviewing of the manuscript was done by Turid Rustad, Dagbjørn Skipnes, and Arne Duinker. All authors have read and approved the final manuscript.

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Data availability All data generated or analysed during this study are included in the published article.

Declarations

Competing interests The authors declare no competing interests for this work.

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