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Impact of post-harvest processing on *Alaria esculenta* and its application in a model fish-based product

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ABSTRACT

Alaria esculenta is a promising seaweed species for food applications due to its nutritional composition. However, its high content of iodine as well as its short shelf life after harvesting, makes post-harvest processing necessary. Processing of biomass may affect its functional properties, flavour, and nutritional contribution. This study evaluated the effects of four post-harvest processing methods: pulsed electric field (PEF), ultrasound (US), warm water treatment (WWT), and WWT combined with freezing and thawing (WWT-FT) on the nutritional value and safety of cultivated Norwegian A. esculenta. Processed seaweed was analysed for dry matter, ash, free and total amino acids, minerals and metals, and then incorporated into fish patties to assess sensory, textural, and nutritional impacts. Processing significantly reduced ash and iodine concentrations while increasing protein content and essential amino acid ratios. Free amino acids, including flavour-enhancing compounds such as glutamate and alanine, were reduced. Iodine concentrations were reduced by 68-85 %. The relative concentration of other PTEs incl. cadmium, mercury, and lead levels increased in most treatments, though remaining within acceptable limits. The inclusion of processed A. esculenta in fish patties had minimal impact on flavour, odour, and overall sensory profile, though WWT and US treatments increased rubbery texture. The sodium-topotassium (Na/K) ratio in seaweed was reduced to below 0.60 in most treatments, supporting its potential use as a salt replacer. These findings suggest that post-harvest processing can improve the safety and nutritional quality of A. esculenta, enabling its integration into commonly consumed foods like fish patties.

1. Introduction

Sustainable production methods, potential for carbon sequestration and other ecosystem services, nutritional composition, and bioactive compound content have led to an increased interest in the production and utilisation of seaweeds as food in Western countries (FAO, 2024). Seaweeds are generally rich in carbohydrates and minerals, with lower levels of proteins, lipids, and certain vitamins. These nutritional compounds have been shown to be of high quality with elevated levels of dietary fibres, essential minerals, essential amino acids (EEAs), and polyunsaturated fatty acids (PUFAs) such as omega-3 and omega-6. However, seaweeds are also known to accumulate potentially toxic elements (PTEs) from the surrounding waters, which can impact their suitability as food or feed (Holdt & Kraan, 2011).

The PTEs present in seaweeds of highest concern include inorganic

arsenic (iAs), cadmium (Cd), mercury (Hg), lead (Pb), and iodine (I). Exposure to iAs, Cd, Hg, and Pb can potentially lead to adverse health effects, even at low levels. Meanwhile, iodine is an essential nutrient required for the synthesis of thyroid hormones, but of which excessive intake can lead to adverse health effects. Some seaweed species, especially kelp species, have been shown to have very high iodine content. Therefore, consumption of seaweeds with high levels can lead to excessive iodine intake and consequently increase the risk of the related adverse health effects (FAO & WHO, 2022). The presence of high levels of PTEs, and especially the high I concentrations, has been identified as one of the main market barriers for increased use of seaweeds in food applications (Blikra et al., 2021; Blikra, Henjum et al., 2022). Different post-harvest processing methods aiming at removing or reducing the PTE content in seaweeds have been investigated. These include blanching, warm water treatment (WWT), soaking, steam treatment,

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ultrasound (US) treatment, and pulsed electric field (PEF) processing (Blikra et al., 2024; Blikra, Skipnes et al., 2022; Jönsson & Nordberg Karlsson, 2024; Krook et al., 2023; Nielsen et al., 2020; Stévant et al., 2024).

Incorporating seaweeds into food products can fortify the food with dietary fibres, essential minerals, protein with high essential amino acids (EAA) content, and PUFAs, and consequently increase the nutritional value. It can also work as a salt replacer, contributing to a salty flavour while reducing the sodium-to-potassium (Na/K) ratio of the food (López-López et al., 2009). Excessive Na intake has been shown to be correlated to high blood pressure and non-communicable diseases (NCDs) such as cardiovascular disease and stroke. However, adequate K consumption has been shown to mitigate the risk of these NCDs. Therefore, the World Health Organisation recommends a diet with a Na/K ratio close to 1.0 (WHO, 2003). Na/K ratios of seaweeds are often reported to be below 1.0 (Blanco et al., 2023; Krook et al., 2023; Stévant et al., 2025), making them suitable for use as salt replacers (Gullón et al., 2021). Pindi et al. (2024) showed that addition of a seaweed gel in chicken patties significantly increased patty moisture, ash, and dietary fibre, while the protein content was not significantly impacted. They also observed a decrease in hardness of the patties. Additionally, Nagai et al. (2022) showed that the incorporation of a seaweed powder into pork burgers led to increased antioxidant capacity as well as increased hardness and chewiness of the burgers.

Fish patties were chosen as a model food as it is a popular food in Norway where seaweed can be easily integrated. It is also expected to be an application of seaweed that can easily be accepted by consumers as a similar product, a fish burger with seaweed, is available on the Norwegian market and won a prize for the best healthy fast food in Norway in 2018 (Fiskeribladet, 2018).

This study aimed to evaluate the effects of PEF processing, US treatment, WWT, and WWT combined with freezing and thawing on cultivated Norwegian *Alaria esculenta* and whether the inclusion of the differently processed seaweed into fish patties could alter their nutritional composition, sensory properties, or textural properties. This study will give insight into the efficiency of different processing methods as well as the impact of including seaweed in a common food product. This could aid kelp and food producers in producing safe and nutritious food and food products from cultivated *A. esculenta*.

The effect of processing of the seaweed was evaluated by changes in the concentrations of dry matter, ash, PTEs, minerals, trace elements, and total and free amino acids (AA) in the unprocessed and processed *A. esculenta* biomass. The fish patties were subjected to sensory profiling, textural analysis, and iodine analysis. The effect of the seaweed inclusion on the concentrations of other PTEs and nutrients in the fish patties was estimated based on their concentrations in the seaweed and the concentration of seaweed in the fish patties.

2. Methods and materials

2.1. Sample preparation and processing

Cultivated *A. esculenta* was obtained from Arctic Seaweed (Misje, Vestland, Norway). The seaweed was harvested on the 24th of April and was transported to Nofima (Stavanger, Rogaland, Norway) by car (approx. 5 h) in barrels filled with seawater. Before processing, seawater was drained from the seaweed by placing approximately 1 kg of seaweed in a large sieve for 10 min and shaking by hand halfway and at the end. The seaweed was then homogenised using a grinder from T. Myhrvold AS, Oslo, Norway, equipped with a hole disc with 10×20 mm openings. Homogenised seaweed was then portioned for processing by WWT, PEF, and US. The processing conditions are described in the following sections. All processing was performed in triplicate in a water-to-seaweed ratio of 20:1. The samples were separated from the processing water by draining in sieves for 10 min, shaking halfway. PEF processed and US treated samples were dried in a Metos System Rational Oven (Finland) at

 $70~^{\circ}\text{C}$ for 150 min. The WWT samples were split into two after WWT, where one part was dried in the same way as the PEF and US samples, while the other half was frozen.

2.1.1. Warm water treatment and freezing

Three filter bags were filled with subsamples of ground seaweed biomass with amounts just below 500 g (total 1496 g) and were subjected to WWT together in a Jøni Foodline Multi-Mix kettle (Denmark) filled with 30 litres of tap water. The starting temperature of the WWT was 45.5 °C, and the end temperature after 120 s was 44.2 °C. The freezing of the seaweed was done by dividing each parallel into two vacuum bags in thin layers. The bags were vacuum sealed at 95 % vacuum before freezing individually at $-30\,^{\circ}\text{C}$ to ensure rapid freezing. The frozen seaweed was thawed in 10 °C water for 5 min and mixed thoroughly before being added to the fish mince. The seaweed was not drained, and any drip loss was included in the mince.

2.1.2. Pulsed electric field processing

A PEF Pilot Dual (Elea GmbH, Quakenbrück, Germany) equipped with a 10 L batch treatment chamber with parallel electrode plates and an electrode distance of 24 cm was used for the PEF processing. The PEF settings were set as follows: Electrode voltage at 24 kV (i.e. 1 kV cm $^{-1}$), frequency at 30 Hz, pulse width at 6 μs , and pulse count at 800 with a rectangular pulse shape. Three batches of about 250 g seaweed were PEF processed separately in about 5000 g of water. The temperature before PEF processing was on average 18.9 \pm 0.1 $^{\circ} C$ and after on average 21.1 \pm 0.1 $^{\circ} C$. Treatment time was 26 s. The energy applied to the samples was on average 9.15 \pm 0.19 J g $^{-1}$.

2.1.3. Ultrasound treatment

The US treatment was done in a US bath from Ultrasonic Power Corporation (Illinois, US) with the following settings: Frequency at 40+170 kHz, energy at 1000 W, and temperature at 45 $^{\circ}$ C. The treatment time was 30 min. The treatment was done similarly as the WWT with three filter bags filled with just under 500 g of seaweed and the US bath filled with 30 L of water. The temperature before running the US treatment was 43.5 °C and the temperature after was 50.2 °C. To increase the water level in the US bath to the minimum required level without increasing the water-to-seaweed ratio, the US bath was filled with glass jars filled with water, adding weight of 10,848 g. The specific energy applied to the samples was 44.0 J g⁻¹. The specific energy was calculated by multiplying the effect (W) by treatment time and dividing by the total weight of water, seaweed, and additional weight in the US bath. However, the true energy applied is likely lower due to energy losses through heat and noise in the generator. Temperature increases measured in the water and seaweed were used to calculate the thermal energy generated by the US. This was used to estimate the energy loss during treatment, resulting in an estimated loss of 38 %.

2.2. Analysis of processed seaweed

2.2.1. Iodine

Iodine content of processed and dried seaweed and freeze-dried fish patties were determined using the analytical method described in Jerše et al. (2023). Briefly, samples were dried and homogenised before with milli-Q water (Millipore) and tetra-methyl-ammonium-hydroxide (TMAH) (Alfa Aesar). Extraction of I from the samples was done in a conventional oven (Memmert UF 30, Memmert GmbH, Germany) at 90 °C for 3 h. After this, samples were diluted with milli-Q water and centrifuged at 15,000 g at 20 °C for 5 min. Supernatants were further diluted with milli-Q water before analysis by inductively coupled plasma mass spectrometry (ICPMS) (ICAP TQ Triple Quadrupole, Thermo Scientific, USA) using tellurium as an internal standard. The certified reference materials NIST 3232 Kelp Powder (NIST, Gaithersburg, MA, USA) and ERM-CD200 Bladder Wrack (IRMM, Geel, Belgium) were used for quality assurance. The A-parallel

of each seaweed sample was analysed in duplicate for quality assurance. The average of the duplicates was used in further calculations.

2.2.2. Minerals and metals

The content of calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), sulphur (S), and zinc (Zn) was determined by inductively coupled plasma optical emission spectroscopy (Aglient 5800 ICP-OES, Agilent Technologies, USA) and quantified by external calibration with internal standardisation using Y as internal standard. The content of aluminium (Al), chromium (Cr), cobalt (Co), nickel (Ni), copper (Cu), arsenic (As), selenium (Se), strontium (Sr), cadmium (Cd), tin (Sn), barium (Ba), mercury (Hg), and lead (Pb) were determined by ICPMS (ICAP TQ ICP-MS) and quantified by external calibration using rhodium as an internal standard. The analytical approach followed the principles described in the European standards EN 13805:2014, EN 17851:2023 and EN 16943:2017 on trace element determination in foodstuffs (European Committee for Standardization (CEN), 2014, 2017, 2023). All standards were prepared from certified stock solutions (SPS Science, Contrabeuf, France). Samples were prepared by mixing dried and homogenised sample matter with concentrated nitric acid (SPS Science, Paris, France) before microwave-assisted digestion (Multiwave 7000, Anton Paar, Graz, Austria). After digestion, samples were diluted with milli-Q water before analysis. The certified reference materials DORM-5 fish protein and BCR218 Rye grass were used for quality assurance purposes. The A-parallel of each seaweed sample was analysed in duplicate for quality assurance. The average of the duplicates was used in further calculations.

2.2.3. Inorganic arsenic

The inorganic arsenic (iAs) content was determined using high-performance liquid chromatography (HPLC) coupled with ICPMS (HPLC 1260; ICP-MS 7900, both Agilent Technologies, USA). The method was based on the principles of the fully validated European standard method EN 16,802:2016 (CEN, 2016). Dried and homogenised samples were mixed with an extraction solution of 0.1 M nitric acid (HNO3) and 3 % hydrogen peroxide (H2O2) before being placed in a water bath at 90 °C for 1 h. Extracts were centrifuged for 10 min at 3803 g, and the supernatant was transferred to sample vials before analysis. Certified reference materials Hijiki 7405a Algae and Rice Flour ERM CE211 were used for quality assurance purposes. The A-parallel of each seaweed sample was analysed in duplicate for quality assurance. The average of the duplicates was used in further calculations.

2.2.4. Total and free amino acids

The content of total and free amino acids was determined by liquid chromatography-mass spectrometry (Agilent 6120 Quadrupole LC/MS, USA). Data was treated in Masshunter version 10.2. For the determination of total amino acids, samples were prepared by mixing dried and homogenised sample with 6 M hydrochloric acid (HCl) before hydrolysis at 110 °C for 18 h. After hydrolysis, samples were filtered into vials using 0.2 μm cellulose acetate syringe filters (Fisher Scientific, USA). The pH was adjusted by the addition of 0.2 M sodium carbonate (Na₂CO₃) and 100 mM ammonium formate. For the determination of free amino acids, samples were prepared by storing dried and homogenised sample matter mixed with 0.1 M HCl at 4 °C for 18 h, before centrifuging for 5 min at 4193 g (4K15 Sigma Laboratory Centrifuges). The supernatant was collected and mixed with 100 mM ammonium formate. The A-parallel of each seaweed sample was analysed in duplicate for quality assurance. The average of the duplicates was used in further calculations.

The acid hydrolysis necessary for determining total amino acid content completely degrades cysteine and tryptophan. It also converts asparagine and glutamine to aspartic and glutamic acid, respectively. Therefore, cysteine, tryptophan, asparagine, and glutamine are not included in this analysis. For the calculation of total amino acid concentrations, the molecular weight of water (18 g mol⁻¹) was subtracted from each amino acid's molecular weight to compensate for the

integration of water during the acid hydrolysis (Lourenço et al., 2002). Protein content was determined by summing the concentrations of all amino acids from the total amino acids analysis.

2.2.5. Dry matter and ash

Dry matter was determined by drying samples at $105\,^{\circ}\text{C}$ for $18\text{--}21\,\text{h}$ until constant weight. Subsequent combustion of the samples in a combustion oven at $600\,^{\circ}\text{C}$ for $20\text{--}24\,\text{h}$ was done to determine ash content (Liu, 2019). Before weighing after drying and combustion, samples were cooled in a desiccator. Dry matter was calculated as percentage of wet weight (WW) by dividing the remaining sample mass after drying by the initial sample mass. Ash content was calculated as percentage of dry weight (DW) by dividing the remaining sample mass after combustion by the dried sample mass. Each seaweed sample was analysed in duplicate for quality assurance. The average of the duplicates was used in further calculations.

2.3. Preparation of fish patties

The fish patties were prepared at Nofima Stavanger. Five different fish patties varieties were made: A control with dried parsley instead of seaweed, three different varieties with dried seaweed processed by PEF, US, or WWT, and a last variety with WWT, frozen, and thawed seaweed. Dried parsley was added to the control patties to camouflage the lack of seaweed in the control. It was chosen as it has a green hue similar to the processed seaweed and is quite flavour-neutral. The parallels (n=3) of the processed seaweed were combined and mixed thoroughly before integration into the fish patties to avoid process replicate differences in the fish patty study. Three different recipes were created to make these five different varieties. All ingredients and their relative amount in the three different recipes are listed in

Table 1. The first two recipes were the control patties and the patties using dried kelp. These recipes were identical except that the control patties had dried parsley instead of dried kelp. The third recipe uses thawed kelp. The relative content of thawed kelp in this recipe was adjusted so that all recipes contained approximately the same amount of kelp dry matter. To keep the total moisture concentration in all recipes equal, the moisture added to the mince via the thawed kelp was calculated, and the combined whole milk and heavy cream concentration was reduced accordingly. Salt concentration in the different recipes was adjusted according to the Na concentration in the seaweed sample, so that all fish minces contained the same amount of Na.

The fish mince was prepared using cooled equipment and cold ingredients. First, fish and salt were combined, and then whole milk and heavy cream were added slowly. Potato starch and nutmeg were added after the liquid, and kelp was added at the very end to avoid chopping the kelp into very small pieces. Around 85 g of mince was piped into moulds and cooled before removing the moulds and cooking in an oven

Table 1
The composition of the fish mince, with the amount of ingredients listed in % (w/w) of the total fish mince weight. Three different recipes were used: One for the control fish patties with dried parsley, one using the dried kelp processed by pulsed electric field (PEF), ultrasound (US), or warm water treated (WWT), and a last one using thawed kelp pretreated by WWT and freezing.

	Control	PEF/US/WWT	WWT-FT
Haddock filet	55.2	55.2	55.2
Whole milk	30.7	30.7	26.2
Heavy cream	9.20	9.20	7.87
Potato starch	3.07	3.07	3.07
Salt	1.10	1.08 (US: 1.09)	1.08
White pepper	0.18	0.18	0.18
Ground nutmeg	0.11	0.11	0.11
Dried parsley	0.50		
Dried kelp		0.50	
Thawed kelp			6.25

at 170 °C until their core temperature was 80 °C. This core temperature was reached after cooking for 15–18 min. After cooking, the fish patties were cooled by placing them in 0 °C cold storage before vacuum packing at 95 % vacuum and freezing at -30 °C.

2.4. Sensory evaluation

Vacuum-packed and frozen fish patties were sent to the Technical University of Denmark (DTU) for objective sensory evaluation by the tested and trained sensory panel at DTU Food. Upon arrival, they were frozen at $-80~^{\circ}$ C. Before evaluation, the fish patties were moved from $-80~^{\circ}$ C to $-18~^{\circ}$ C three days before analysis. They were then thawed in cold water for 20 min and heated in ceramic containers with lids in an oven at $100~^{\circ}$ C for 20 min. The core temperature of the fish patties was then between $62-65~^{\circ}$ C. This was chosen to mimic the consumer behaviour when preparing the fish patties in the oven, and to ensuring control of the conditions so all samples were heated equally.

The trained sensory panel at DTU Food fulfils the ISO standard ISO 8589 and NMKL procedure 6. Before the evaluation session, there were three training sessions. The first session was used to develop a vocabulary for describing attributes of the odour, appearance, flavour, and texture of the samples. The next two sessions were used to develop the vocabulary further and to train the panel in measuring the intensity of the attributes on an unstructured 15 cm line scale with anchor points. During the sensory evaluation, each sample was tested in duplicates and served to assessors in a randomised order. There was a break between each serving where the assessors cleansed their palates with water and a neutral crisp bread. The attributes developed and assessed are presented in Table 2. Most attributes were placed on an intensity scale with increasing intensity, from low to high. Some attributes were measured on an attribute-specific scale. For those attributes, the scale is presented after the attribute in Table 2.

2.5. Texture analysis

Vacuum-packed and frozen fish patties were moved from $-80~^{\circ}\text{C}$ to $-18~^{\circ}\text{C}$ 24 h before analysis. They were thawed in cold water for 20 min before being heated in a water bath at 65 $^{\circ}\text{C}$ for 1 h to obtain a core temperature around 65 $^{\circ}\text{C}$, as in the sensory evaluation. The edges of each fish patty were removed and the core was cut into 6 cubes. The cubes were placed in plastic containers with cling film on top and placed in a heating cabinet at 65 $^{\circ}\text{C}$ until analysis. An illustration of the cut cubes of the fish patties and a picture of 3 cubes cut from a fish patty are presented in Fig. 1.

The texture of the fish patties was measured using a Texture Analyser TA.XT Plus (Stable Microsystems Ltd., Surrey, UK) equipped with Thermal cabinet (Stable Microsystems Ltd., Surrey, UK). Fish patties were analysed using texture profile analysis (TPA) method. Cubes of fish patties from each group, were placed (same side up) in the thermal cabinet preheated to 65 $^{\circ}\text{C}$ and compressed twice to 50 % of their height

Table 2Sensory attributes of fish patties developed and assessed by a sensory panel at DTU Food.

Odour	Appearance	Flavour	Texture
Sweet	Airy (Dense to airy)	Salty	Rubbery (Little to much)
Acidic	Green areas (% of total area)	Sweet	Firmness (Soft to firm)
Ocean/ Seaweed		Green	Chewing resistance (Soft to firm)
Fishy		Pepper/ spicy	Juicy (Dry to juicy)
		Fishy	Noticeable pieces (Few to many)
			Chews before swallowing (Few to many)

using a compression plate probe of 75 mm diameter (type P/75). The pause between compression cycles was set to 3 s. The properties evaluated are the hardness, chewiness, cohesiveness and springiness. Texture analysis was used to evaluate the effect of freezing by analysing the freshly made control fish patties stored in the fridge for 24 h. The preparation and analysis was the same as described above.

2.6. Statistical analysis

The duplicates were used for quality checking the analyses, and then the average was used as one replicate (out of three) in further calculations. During the sensory evaluation, each type of fish patty was evaluated in duplicates by all seven assessors. Texture analysis was performed on three fish patties of each kind, each cut into six pieces as described above, giving a total of 18 replicates of each fish patty type.

Statistical analysis of concentrations of biochemical compounds and the TPA texture analysis were performed using Minitab® 21.4.3 (64-bit) (Minitab, LLC). Statistically significant differences were determined using one-way ANOVA with a 95 % confidence interval, with a Tukey post hoc test. Statistical analysis of results from the sensory evaluation was done using R (version 4.2.2 (R Development Core Team, 2022)) by running a mixed model ANOVA (R function lmer (Bates et al., 2015)) treating the assessors as a random factor. The Benjamini-Hochberg method (Benjamini & Hochberg, 1995) was used to control false discovery rate and adjust *p*-values. Different lowercase letters indicate statistically significant differences within Tables and figures.

3. Results

3.1. Effect of processing

Table 3 presents the dry matter, ash, and protein concentrations, as well as the percentage of essential amino acids (EAA) of total amino acids (TAA) of unprocessed and processed A. esculenta samples. The results showed that all tested processes led to significantly reduced dry matter and ash concentrations. Calculations of TR values showed that 55–60 % of the original dry matter content was retained during processing, with no significant differences between processing methods. TR values also showed that 26–28 % of the original ash content was retained in the PEF, WWT, and WWT-FT seaweed, while significantly less of the ash content was retained in the US seaweed (19 \pm 2 %). TR for protein showed high retentions of 76–77 % following all processing.

Concentrations of each of the amino acids measured in total and free form are given in Tables S1 and S2 in the supplementary material, respectively. For the total amino acid content, Arg was below the limit of quantification (LOQ) in 11 out of 15 samples, and C-C was below the LOQ in all samples. In the unprocessed A. esculenta, Ala was present in the highest concentration, followed by Asp, Glu and Gly, while Tyr, Met, and Phe were present in the lowest concentrations. Processing significantly reduced Ala concentrations in all samples, while Asp, Glu and Gly concentrations were significantly increased, resulting in the three main AAs present in the PEF, WWT, and WWT-FT samples being Asp>Glu>Ala>Gly, and in the US sample Asp>Glu>Gly>Ala. The AAs present in the lowest concentrations remained unchanged from the unprocessed samples for the US, WWT, and WWT-FT samples, while for the PEF sample the AAs present in the lowest concentrations were Met>His>Phe. Except for Ala and for His in the PEF sample, concentrations of all total AAs were significantly increased after processing.

In the analysis of free AAs, arginine was below the LOQ in 10 out of 15 samples, and histidine and lysine were below the LOQ in all samples. For methionine, some parallels were below the LOQ; these were included as zero in further calculations. The free AAs present in the highest concentrations were identical for the control, PEF, WWT, and WWT-FT, following the order Ala>Glu>Asp>Thr. While for the US sample, Thr was present at a higher concentration than Asp. The free AAs present at the lowest concentrations were Ile and Met for the

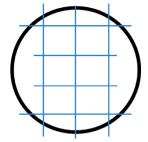




Fig. 1. Illustration of the cutting of the fish patties for texture analysis and a picture of three cubes cut from a fish patty. The black circle illustrates the fish patty and the blue lines cutting points. Each blue square represents a samples cube that was analysed.

Table 3 Dry matter (% of WW), ash (% of DW), protein (% of DW), and essential amino acids (EAA) to total amino acids (TAA) ratio (%) of unprocessed and processed Alaria esculenta samples. Alaria esculenta samples were processed by pulsed electric field (PEF), ultrasound (US), and warm water treated (WWT) with and without freezing and thawing (FT). Values are listed as mean with standard deviation, n = 3. Different superscript letters in the same row indicate statisti-

cally significant differences between samples (Tukey HSD, p < 0.05).

	Control	PEF	US	WWT	WWT-FT
Dry matter	$9.2\pm0.2^{\text{a}}$	5.6 ± 0.2^{c}	6.1 ± 0.2^{c}	6.9 ± 0.2^{b}	$7.1\pm0.3^{\rm b}$
Ash	$\begin{array}{l} 35.5 \pm \\ 0.8^a \end{array}$	$\begin{array}{c} 16.5 \pm \\ 0.4^{b} \end{array}$	$\begin{array}{c} \textbf{12.7} \pm\\ \textbf{0.1}^{c} \end{array}$	$\begin{array}{c} 16.0 \; \pm \\ 0.7^b \end{array}$	$\begin{array}{c} \textbf{15.6} \pm \\ \textbf{0.5}^{b} \end{array}$
Protein	9.17 ± 0.12^{c}	$\begin{array}{l} 11.7~\pm\\ 0.4^{\rm b}\end{array}$	$12.7\ \pm \\0.2^a$	$\begin{array}{c} \textbf{12.0} \pm \\ \textbf{0.1}^{b} \end{array}$	$\begin{array}{c} \textbf{11.7} \pm \\ \textbf{0.3}^{\text{b}} \end{array}$
EAA/TAA	$\begin{array}{l} 27.5 \pm \\ 0.3^d \end{array}$	$\begin{array}{c} \textbf{31.2} \pm \\ \textbf{0.1}^c \end{array}$	$\begin{array}{c} 33.0 \; \pm \\ 0.1^a \end{array}$	$\begin{array}{l} \textbf{32.6} \pm \\ \textbf{0.3}^{ab} \end{array}$	$\begin{array}{l} \textbf{32.1} \pm\\ \textbf{0.2}^{\text{b}} \end{array}$

control, PEF, WWT, and WWT-FT samples. In the US sample these had the lowest concentrations of those that were above the LOQ. However, Ser and Tyr were reduced to below the LOQ in the US sample. Except for Gly and Met and for Leu and Ile in the PEF sample, concentrations of all free AAs were significantly reduced after processing.

The concentration of bound Ala was calculated by subtracting the concentration of free Ala from the total Ala concentration (molar concentrations). This showed that the concentration of bound Ala was significantly increased in all samples after processing despite the reduction in the total Ala concentration. The concentrations of bound Ala were 7.3 \pm 0.2, 10.0 \pm 0.5, 11.9 \pm 0.4, 10.7 \pm 0.3, and 10.8 \pm 0.1 mg g $^{-1}$ DW in the control, PEF, US, WWT, and WWT-FT samples, respectively. Calculations showed that free Ala comprised 64±1 % of the total Ala content in the control sample, while in decreased to 36±2 %, 5.7 \pm 0.1 %, 24±4 %, and 23±3 % in the PEF, US, WWT, and WWT-FT samples, respectively.

The concentrations and reductions in concentration of the PTEs I, As, iAs, Cd, Hg, and Pb are presented in Table 4. Processing significantly reduced concentrations of I, tAs, and iAs in all samples, while it significantly increased concentrations of Cd in the PEF, US, and WWT-FT samples, Hg in US and WWT-FT samples, and Pb in all samples. The iAs content was very low compared to the tAs content, resulting in iAs to tAs ratios of 0.24 ± 0.02 %, 0.18 ± 0.00 %, 0.21 ± 0.02 %, 0.19 ± 0.01 %, and 0.16 ± 0.02 % of tAs, for the control, PEF, US, WWT, and WWT-FT samples, respectively. The iAs/tAs ratios of the PEF, WWT, and WWT-FT samples were significantly lower than the control sample.

Concentrations of the remaining elements analysed can be found in Table S3 in the supplementary material. The major minerals present in the control sample were Na>K>Mg>Ca. Processing significantly reduced the Na, K, and Mg concentrations in all samples, while Ca concentrations significantly increased. In the PEF, WWT, and WWT-FT samples, the order of the major minerals was then changed to K>Na>Ca>Mg, while in the US sample it was changed to Ca>Na>K>Mg. Sodium-to-potassium (Na/K) ratios of the samples were

Table 4

Initial Concentrations (mg kg $^{-1}$ DW; conc.) and reductions in concentrations (%; red.) of iodine (I), total arsenic (tAs), inorganic arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) in unprocessed (control) and processed Alaria esculenta. Samples were processed by pulsed electric field (PEF), ultrasound (US), and warm water treated (WWT) with and without freezing and thawing (FT). Values are listed as mean with standard deviation, n=3. Different superscript letters in the same row indicate statistically significant differences between samples (Tukey HSD, p<0.05).

		Control	PEF	US	WWT	WWT-FT
I	Conc.	1060 ±60 ^a	$337{\pm}51^b$	163±19 ^c	$209{\pm}80^{bc}$	181±39 ^c
	Red.		68 ± 5^{b}	85 ± 2^a	$80{\pm}8^{ab}$	83 ± 4^a
tAs	Conc.	47.8 \pm	34.2 \pm	14.4 \pm	25.8 \pm	26.5 \pm
		1.7 ^a	0.8^{b}	0.2^{d}	2.4 ^c	1.6 ^c
	Red.		29 ± 2^{c}	70 ± 0^a	46±5 ^ь	$45\pm3^{\mathrm{b}}$
iAs	Conc.	0.11	0.061	0.031	0.050	0.043
		$\pm 0.01^{a}$	$\pm 0.000^{\rm b}$	$\pm 0.003^{d}$	$\pm 0.003^{c}$	$\pm 0.006^{c}$
	Red.		$46\pm1^{\rm b}$	58 ± 0^a	$46\pm2^{\mathrm{b}}$	47±2 ^b
Cd	Conc.	1.16	1.47	1.62	1.27	1.37
		$\pm 0.04^{ m d}$	$\pm 0.08^{ab}$	$\pm 0.10^{a}$	$\pm 0.03^{\mathrm{cd}}$	$\pm 0.03^{\rm bc}$
	Red.		$-26\pm7^{\mathrm{bc}}$	-40 ± 9^{c}	-10 ± 2^a	$-18\pm3^{\mathrm{ab}}$
Hg	Conc.	0.0055	0.0074	0.0089	0.0070	0.0077
		$\pm 0.0007^{b}$	$\pm 0.0013^{ab}$	$\pm 0.0004^{a}$	$\pm 0.0008^{ab}$	$\pm 0.0005^{a}$
	Red.		-34 ± 24^{a}	-62 ± 8^{a}	$-28{\pm}15^{a}$	-41 ± 9^{a}
Pb	Conc.	0.12	0.15	0.22	0.17	0.17
		$\pm 0.01^{\rm c}$	$\pm 0.01^{\mathrm{b}}$	$\pm 0.02^{a}$	$\pm 0.01^{\mathrm{b}}$	$\pm 0.00^{\mathrm{b}}$
	Red.		-34 ± 7^{a}	-93 ± 13^{b}	-50 ± 8^{a}	-48 ± 4^{a}

 $1.05\pm0.1,~0.59\pm0.02,~1.04\pm0.04,~0.57\pm0.01,~and~0.56\pm0.01$ for the control, PEF, US, WWT, and WWT-FT, respectively. The Na/K ratios of the control and US samples were significantly higher than the others. The elements present at the lowest concentrations in the control sample were Hg<Se<Pb<iAs. Significantly increased Pb concentrations and significantly decreased iAs concentrations after processing led to the following order: Hg<iAs<Se<Pb in the processed samples.

3.2. Fish patties

The amount of I, As, iAs, Cd, Hg, and Pb added to one fish patty of approximately 85 g was calculated and multiplied by to estimate the dietary exposure to these PTEs from seaweed in a meal consisting of two patties. These results are presented in Table 5. The total I concentration in the fish patties was also determined, showing that a portion of two fish patties of the control, PEF, US, WWT, and WWT-FT varieties contained 240, 467, 329, 417, and 387 µg I, respectively.

In addition to PTEs the seaweed will contribute to the total nutritional content of the fish patties by the addition of protein and minerals. As with the PTEs, the amount of the protein and nutritionally relevant minerals and trace elements in the seaweed consumed through the consumption of two fish patties of approximately 85 g was calculated. These amounts were then compared to the recommended intake (RI), adequate intake (AI) and upper intake level (UL) of the individual nutrients given in the Nordic Nutrition Recommendations from 2023

Table 5

The amount (µg) of iodine (I), arsenic (As), inorganic arsenic (iAs), cadmium (Hg), and lead (Pb) in a serving of two fish patties (2×85 g) supplied by the addition of processed Alaria esculenta. Samples were processed by pulsed electric field (PEF), ultrasound (US), and warm water treated (WWT) with and without freezing and thawing (FT).

	PEF	US	WWT	WWT-FT
I	250	120	154	133
As	25.4	10.6	19.3	19.9
iAs	0.0456	0.0226	0.0371	0.0320
Cd	1.09	1.19	0.95	1.03
Hg	0.00546	0.00652	0.00524	0.00579
Pb	0.115	0.164	0.130	0.129

(Blomhoff et al., 2023). These results are presented in Table 6.

Pictures of the fish patties (taken before sensory analysis) are presented in Fig. 2. The results from the sensory evaluation are presented in Fig. 3 in a principal component analysis (PCA) biplot. The PCA of the mean scores for each attribute explains 84.5 % of the variance in the dataset, 62.95 and 21.55 % by PC1 and PC2, respectively. The plot shows that the control and WWT-FT samples were different from the US and WWT samples, characterised by green areas. Meanwhile, the US and WWT samples were characterised by a rubbery texture. The attributes "F.Green" and "T.Noticeable pieces" are not very visible in the biplot due to overall low scores. The average ratings of all attributes are presented in Table S4 in the supplementary material.

Texture profile of fish patties is shown in the Table 7. There were no significant differences in hardness, chewiness, cohesiveness and springiness between the different fish patty varieties. The control patties were also measured before freezing to assess if the freezing and thawing impacted the texture. The results of this are presented in Table S5 in the supplementary material. The results showed that all the measured parameters were significantly increased after freezing.

4. Discussion

4.1. Effect of processing

Processing led to loss of just below half of the original dry matter (TR 55–60 %) and over 70 % of the original ash in the *A. esculenta* samples. The US treatment led to significantly higher ash reduction of the

Table 6

The amount of protein, calcium (Ca), iron (Fe), zinc (Zn), copper (Cu), phosphorous (P), potassium (K), magnesium (Mg), selenium (Se), manganese (Mn), and sodium (Na) in a serving of two fish patties (2×85 g) supplied by the addition of processed Alaria esculenta presented as percent of the recommended intake (RI), adequate intake (AI) and upper intake level (UL) of the individual nutrients given in the Nordic Nutrition Recommendations from 2023 (Blomhoff et al., 2023). The RI for protein is based on an adult weighing 70 kg. RI for Fe and Zn, and AI for Mg and Se were different for women and men and the amount was compared to both values. The results are presented separated by a slash (women/men). Seaweed samples were processed by pulsed electric field (PEF), ultrasound (US), and warm water treated (WWT) with and without freezing and thawing (FT).

		PEF	US	WWT	WWT-FT
% of RI	Protein	0.15	0.16	0.15	0.15
	Ca	1.17	1.37	1.15	1.18
	Fe	0.44 /0.73	0.52/0.87	0.51/0.85	0.50/0.83
	Zn	0.57/0.44	0.69/0.52	0.61/0.46	0.63/0.48
	Cu	0.20	0.18	0.31	0.79
% of AI	P	0.42	0.38	0.40	0.42
	K	0.75	0.32	0.78	0.75
	Mg	2.62/2.25	2.79/2.40	2.57/2.20	2.64/2.26
	Se	0.12/0.10	0.13/0.11	0.14/0.12	0.13/0.11
	Mn	0.22	0.21	0.22	0.22
% of UL	Na	0.68	0.51	0.67	0.64

seaweed biomass, this could be due to the different working mechanisms of the US treatment, the combination of US and high temperature (45 °C), the longer processing time (30 min), or a combination of all. The ash concentrations in the processed samples were less than half of the original ash concentration. Loss of dry matter during processing is generally an undesired effect seen from the producer's viewpoint as it results in a lower volume of the finished product. On the other hand, protein concentrations were significantly higher after processing, which is desirable if the product is meant for food or feed. TR calculations for protein content showed retentions over 75 % and the EAA/TAA ratio increased during processing, showing that the EAAs are retained.

Processing significantly increased the total concentrations of most AAs and significantly reduced the concentrations of most free AAs. This was expected as free AAs are smaller than larger peptides and proteins and can more easily be lost to the liquid phase during processing due to cell disruption and leaching. The total concentration of Ala was an exception for this as it was significantly reduced in all processed samples. Calculations separating bound and free Ala concentrations and calculating the total Ala concentration as a sum of these, showed that the free Ala content comprised more than half of the total Ala content in the control samples and that this fraction was significantly reduced after processing. It also showed that the concentration of bound Ala was significantly increased after processing. This further strengthens the hypothesis that it is mostly the free AAs that are lost during processing. Free Ala is known to have a sweet taste, and free Glu and Asp are part of the complex umami flavour (Mouritsen et al., 2019). Loss of these free AAs can therefore impact the flavour of the seaweed. Concentrations of free Ala, Glu, and Asp were significantly decreased during all processing. However, the concentrations of free Ala and Glu were significantly higher in the PEF processed sample than in the other processed samples, indicating that the PEF processing better retained these flavour compounds.

The concentration of I was significantly reduced in all samples. The highest reduction achieved was through US treatment, although it was not significantly higher than the reduction achieved by WWT. Since the US treatment was executed at 45 °C for 30 min and the WWT at the same temperature but only for 2 min these results indicate that neither the US treatment nor the longer treatment time had any additional effects on the I reductions. This is consistent with results in Nielsen et al. (2020), where it was shown that the I concentration of Saccharina latissima reached a constant level after 120 s of WWT at 45 °C. All samples had an I concentration below the proposed maximum level (ML) in seaweed food products of 2000 mg kg⁻¹ DW as recommended by the French food safety authority (ANSES, 2020). However, the I concentration in the unprocessed (control) sample in this study was higher than previously reported values for A. esculenta (213-707 mg kg-1 DW (Afonso et al., 2021; Jönsson & Nordberg Karlsson, 2024; Mæhre et al., 2014; Roleda et al., 2018; Stévant et al., 2018, 2025). PEF processing did not reduce the I concentration as efficiently as WWT. This is consistent with the results presented in Blikra et al. (2024) were WWT at 45 °C led to a 90 % reduction in the I concentration of S. latissima while PEF only reduced the I concentration by 50 %. Nevertheless, the I reduction achieved by PEF in this study was slightly higher (68 %) than that shown in Blikra et al. (2024). This might be due to the higher pulse count, and therefore higher energy, applied in this study, the higher water-to-seaweed ratio i. e. 20:1 used in this study vs 2:1, or structural differences between the two kelp species e.g. cell permeability. Previously reported results have shown that the lower water-to-seaweed ratios can lead to lower iodine reductions (Wirenfeldt, 2023). The tolerable upper intake level (UL) of I established by the European Food Safety Authority (EFSA) is 600 µg day-1 for adults (EFSA, 2006). Calculating the tolerable daily intake (TDI) of the seaweed in this study based on a maximum intake of 600 µg of I resulted in acceptable portion sizes between 0.62-3.7 g DW processed seaweed.

The concentrations of As and iAs were also significantly reduced in all samples. Additionally, the iAs/tAs ratios in the PEF, WWT, and WWT-

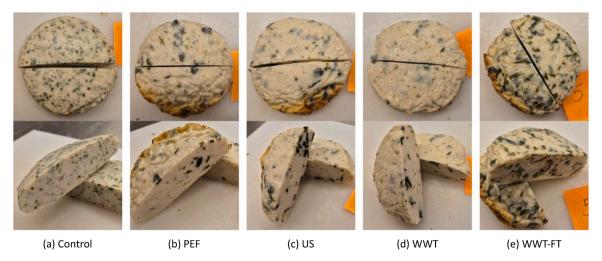


Fig. 2. Pictures of the surface and cross section of the fish patties with and without processed Alaria esculenta. The patties are named according to the processing the A. esculenta added to the fish patty was subjected to. The control contained dried parsley and no seaweed, while the others contained seaweed processed by pulsed electric field (PEF), ultrasound (US), and warm water treatment (WWT) with and without freezing and thawing (FT).

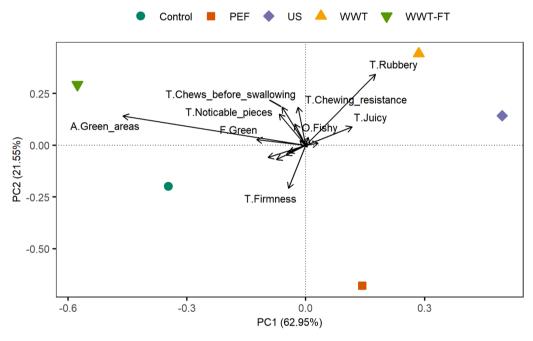


Fig. 3. Principal component analysis (PCA) biplot (1st and 2nd principal component) based on average scores from assessors (n = 7) in the sensory evaluation of fish patties with differently processed *Alaria esculenta*. The control contained dried parsley instead of seaweed. Seaweed samples were processed by pulsed electric field (PEF), ultrasound (US), and warm water treated (WWT) with and without freezing and thawing (FT). Variation in intensity of the different attributes are illustrated by vectors of different lengths.

Table 7 Hardness, Chewiness, Cohesiveness and Springiness of different variants of the fish patties. Values are listed as mean with standard deviation, n=18. Different superscript letters in the same row indicate statistically significant differences between samples (Tukey HSD, p < 0.05).

	Control	PEF	US	WWT	WWT-FT
Hardness	$1411.1 \pm \\210.0^{a}$	$1339.5 \pm \\386.3^{a}$	$1285.8 \pm \\204.8^{a}$	$1439.3 \pm \\ 433.8^{a}$	$1439.5 \pm \\190.7^{a}$
Chewiness	919.4 ± 140.5^{a}	847.0 ± 241.0^{a}	831.9 ± 125.3^{a}	$894.1 \pm \\282.1^{a}$	897.1 ± 119.3^{a}
Cohesion Springiness	$\begin{array}{l} 0.7 \pm 0.0^{a} \\ 89.1 \pm \\ 1.7^{a} \end{array}$	$0.7 \pm 0.0^{a} \ 84.4 \pm 10.0^{a}$	$\begin{array}{l} 0.7 \pm 0.0^{a} \\ 88.0 \pm \\ 2.0^{a} \end{array}$	$0.7 \pm 0.0^{a} \ 85.1 \pm 9.1^{a}$	$\begin{array}{l} 0.7 \pm 0.0^{a} \\ 86.1 \ \pm \\ 2.0^{a} \end{array}$

FT samples were significantly lower than in the control sample. This indicates that iAs was removed to a higher degree during PEF processing and WWT (both with and without freezing) than the total As content. This is beneficial for the use of the seaweed as food or feed, as iAs is known to be carcinogenic and can lead to adverse health effects for the consumer even at low levels (EFSA, 2024). The iAs concentrations of all samples were well below the proposed maximum level for seaweed food products recommended by ANSES (3 mg kg⁻¹ DW) (ANSES, 2020). The tAs concentration of the control sample reported in this study is in the lower end of previously reported tAs concentrations in A. esculenta (48–88 mg kg⁻¹ DW) (Afonso et al., 2021; Jönsson & Nordberg Karlsson, 2024; Mæhre et al., 2014; Schiener et al., 2015). The iAs concentration in the control sample in this study is lower than previously reported literature values for iAs in A. esculenta (0.22–4.19 mg kg⁻¹ DW) (Jönsson

& Nordberg Karlsson, 2024; Stévant et al., 2018).

Cd concentrations were significantly increased in PEF, US, and WWT-FT samples. The US sample showed the highest increase at 40 %. Nevertheless, all samples were above the ANSES recommended ML of 0.5 mg kg⁻¹ DW (ANSES, 2020), but below the ML of 3.0 mg kg⁻¹ DW Cd in seaweed food supplements established by the European Commission (EC) (European Commission, 2023). Consumption of Cd can lead to adverse health effects in the consumer at low levels. Therefore, EFSA has established a tolerable weekly intake (TWI) of 2.5 µg kg⁻¹ body weight (BW) For an adult weighing 70 kg this equals a weekly intake of 175 μ g. However, since Cd is present in many different foods, a European adult's estimated average weekly dietary intake of Cd is 1.77 µg kg⁻¹ BW (EFSA, 2012). Subtracting this average dietary Cd intake from the TWI and dividing it by 7 to get a daily intake, leaves us with a TDI of 7.3 µg of Cd for an adult weighing 70 kg. Using this to calculate TDI for the seaweed samples in this study resulted in portion sizes between 4.5-7.0 g DW. This was in all cases higher than the TDI determined to avoid excessive I intake, showing that Cd intake should not pose a health risk if the portion size is adjusted to the I concentration. The Cd concentration in the control sample in this study was in the lower end of previously reported Cd concentrations in A. esculenta ranging from 0.72–3.4 mg kg⁻¹ DW (Afonso et al., 2021; Blanco et al., 2023; Jönsson & Nordberg Karlsson, 2024; Mæhre et al., 2014; Stévant et al., 2018, 2025).

Hg concentrations were significantly increased in the US and WWT-FT samples and Pb concentrations were significantly increased in all samples. However, the concentrations of Hg and Pb were both far below the ANSES recommended MLs of 0.1 and 5 mg kg⁻¹ DW (ANSES, 2020) and the EC's MLs in food supplements of 0.1 and 3.0 mg kg⁻¹ DW for Hg and Pb (European Commission, 2023), respectively. Both were in the lower end of previously reported concentrations of Hg and Pb in A. esculenta, ranging from <LOQ-0.0107 (Afonso et al., 2021; Jönsson & Nordberg Karlsson, 2024; Schiener et al., 2015; Stévant et al., 2025) and 0.0698–1.1 mg kg⁻¹ DW (Afonso et al., 2021; Blanco et al., 2023; Jönsson & Nordberg Karlsson, 2024; Stévant et al., 2025), respectively.

WWT resulting in reduction of I, As, and iAs concentrations and increase of Cd, Hg, and Pb concentrations were also seen for *S. latissima* in Krook et al. (2023). The same trend was furthermore reported for *S. latissima* in Blikra et al. (2024), except for the decrease in Pb concentration (not significant). In Blikra et al. (2024), PEF processing did not lead to the reduction of tAs, but reduced I and increased Cd and Hg concentrations as seen in this study. In Blikra, Skipnes et al. (2022), PEF processing was also shown to reduce concentrations of I and tAs (not significant) in *S. latissima*. Many factors can influence the observed differences between the studies, including processing conditions, species differences, and water-to-seaweed ratios. However, the overall trends seem to agree.

Na was one of the major minerals present in all samples. Excessive Na consumption can lead to high blood pressure and several noncommunicable diseases (NCDS) such as cardiovascular disease and stroke. However, adequate intake of K has been shown to reduce blood pressure and the risk of these NCDs. Therefore, the World Health Organisation (WHO) recommends a dietary Na/K-ratio close to 1.0 (WHO, 2003). Despite the high Na content of seaweeds, reported Na/K-ratios are usually below 1.0 due to high K concentrations. Reported Na/K ratios of A. esculenta range from 0.25-0.96 (Blanco et al., 2023; Stévant et al., 2017, 2025; Wegeberg et al., 2023; Zhu et al., 2022). Seaweeds with low Na/K ratios have been investigated as salt replacers in food, where they can add salty flavour and other nutrients while reducing the total Na/K-ratio of a food product (López-López et al., 2009). The control and US samples in this study were just outside this range at 1.05 and 1.04, respectively. However, PEF processing and WWT reduced the Na/K-ratios significantly to just below 0.60, making them suitable as salt replacers.

4.2. Fish patties

The inclusion of seaweed in the fish patties contributed an additional I content of 120-250 µg to a portion of two fish patties. The highest I content was found in the fish patties with the PEF processed seaweed, and the lowest in the fish patties with the US treated seaweed. This shows that the inclusion of pretreated A. esculenta in dried form at a 0.50 % concentration or wet form at a 6.25 % concentration led to the addition of I content close to or above the AI of 150 µg I day⁻¹ established by EFSA. However, fish and milk are also sources of I and contribute to the total I content in the fish patties (EFSA, 2014). The total I content in a portion of two control fish patties was determined to be 240 μg , which shows that the fish patties would be a good source of I even without the seaweed. Despite the significant I content added to the fish patties by the seaweed, none of the varieties contained >600 µg I per two fish patties. However, increasing portion size to three fish patties shows that consumption of the US and WWT fish patties would lead to I intakes over 600 µg. To avoid I intake above 600 µg one could safely consume 5 patties of the control variety. Seaweed inclusion reduced this to 2.6, 3.6, 2.9, and 3.1 for the PEF, US, WWT, and WWT-FT patties, respectively. This shows that using seaweed as an ingredient in fish patties could lead to excessive I intake if the I concentration is not sufficiently reduced during post-harvest processing. However, as a normal portion size for an adult is 2-3 fish patties in one meal, the inclusion in this study did not necessarily restrict portion size. Additionally, this shows that seaweed could be used as an additive for increasing the I concentration in vegan food products. The Cd content added to two fish patties via the seaweed was low at around 1 µg, well below the previously estimated TDI of 7.3

The amount of protein, most minerals and all trace elements added via the seaweed in a portion of two fish patties constituted <1~% of the daily RI, AI, and UL presented in the Nordic Nutrition Recommendations from 2023 (Blomhoff et al., 2023). Ca and Mg were the only compounds added in amounts that constituted >1~% of the daily intake, at 1.16–1.37 % of the daily RI and 2.20–2.79 % of the daily AI, respectively. This shows that the seaweed concentration in the fish patties was not high enough to add nutritionally significant amounts of protein, macro minerals, and trace elements. This is consistent with other findings reported in literature (Jacobsen et al., 2023; Stévant et al., 2025).

Sensory analysis showed that there were few major differences between the different fish patties. The inclusion of the differently processed *A. esculenta* did not have any significant impact on the odour or flavour profile of the fish patties compared to the control with dried parsley. The fish patties with dried kelp (PEF, US, and WWT) resulted in fewer green areas in the fish patties than the inclusion of the frozen and thawed WWT *A. esculenta* or the dried parsley in the control. The inclusion of US-treated A. esculenta led to a significantly more rubbery texture than that of the control. Firmness, juiciness, and number of chews before swallowing were among the top five attributes for all fish patty varieties. These were not significantly impacted by the different kelp addition.

Texture results showed no significant changes in the hardness, chewiness, springiness and cohesiveness of the fish patties after the addition of differently processed *A. esculenta*. This is not consistent with the results shown in Pindi et al. (2024) and Nagai et al. (2022), where the addition of seaweed increased the hardness of chicken patties and pork burgers, respectively. However, this could be due to these studies using different seaweed species, differences in the formulation of the food product, or how and in what format the seaweed was added. It was observed that freezing of control fish patties significantly increased all the attributes evaluated. This indicates that freezing and thawing might not be the optimal preservation method for this product. The changes during freezing and thawing might also have diminished any potential effects of the seaweed.

5. Conclusion

Processing of *A. esculenta* by PEF, US, and WWT (with and without freezing) led to significant changes in the dry matter, ash, and protein concentrations and compositions. Dry matter and ash concentrations were decreased, while protein concentrations were increased. The EAA/TAA ratio was also increased, indicating a higher loss of non-essential AAs than of EAAs. For most AAs, the total concentration was increased, while the concentration of the free AA was decreased. Results indicated that all processing methods mostly affected the free AA content and not the AA content bound in protein. Concentrations of the flavour compounds free Ala, Glu, and Asp were reduced after processing, which might impact the flavour of the seaweed.

Analysis of PTEs showed reductions in I, tAs, and iAs concentrations after processing. The iAs/tAs ratios were low (<0.25 %) and were also decreased after processing, indicating an easier removal of iAs than tAs content. Concentrations of Cd, Hg, and Pb were increased in all processed samples, although not significantly in all. The concentrations of Hg and Pb were generally low. The Cd concentrations in all seaweed samples were above the ML recommended by ANSES. Nevertheless, the I content was the limiting factor deciding portion size. Na was one of the major minerals in all samples, but PEF and WWT (with and without freezing) reduced the Na content and the Na/K-ratios from just above 1.0 to just below 0.60.

Adding the processed A. esculenta to the fish patties added significant amounts of I. However, the total I content in a portion of two fish patties did not surpass 600 μg for any of the varieties. Other PTEs would not pose significant health risks if the portion size is determined to avoid excessive I intake. The kelp's contribution of other nutritional compounds was low. The inclusion of kelp in the fish patties did not affect their odour or flavour profiles. The texture analysis showed no significant differences in hardness, chewiness, cohesion, or springiness between the different fish patty varieties.

The results of this study show that kelp can be added in amounts large enough to significantly increase the I content of a food without changing flavour, odour, or texture. While higher I reductions can allow higher inclusion levels and higher contribution of other nutritional compounds via the kelp, the reduction here was sufficient to produce a safe product. This can especially be relevant for vegan products where I sources are scarce. Future research should continue working on optimising I reduction of kelps and preferably include proximate analysis to evaluate the impact of the differently processed kelp on the complete nutritional profile of the fish patties.

Ethical statement

All relevant ethical questions regarding the sensory analysis were covered through the panel judges' employment contract with DTU.

CRediT authorship contribution statement

Randi Sund: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. Gorana Drobac: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. Pierrick Stévant: Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. Susan Løvstad Holdt: Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. Claus Heiner Bang-Berthelsen: Writing – review & editing, Supervision, Methodology, Conceptualization. Grethe Hyldig: Writing – review & editing, Resources, Methodology, Funding acquisition. Dagbjørn Skipnes: Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.101309.

Data availability

Data will be made available on request.

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