Food Control 162 (2024) 110461

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

A case study on Norwegian commercial harvesting and production of *Saccharina latissima* (Part 1): Effect of processing seaweed on chemical hazards, allergens, and microbiological hazards

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ARTICLE INFO

Keywords:
Food safety
Seaweed
Blanch
Ferment
Heavy metals
Iodine

ABSTRACT

When producing seaweed for human consumption, incorporating a "Food Safety-by-Design approach" can help protect human health and ensure the safety of future consumers. Post-harvest processing of seaweed is one strategy to reduce undesirable compounds. The study aimed to determine the effects of a commercial processing strategy (blanching and fermentation) of Norwegian-harvested *Saccharina latissima* (sugar kelp) on the presence of prioritized food safety hazards. We compiled and prioritized food safety hazards to be analyzed based on the current impact of the hazard, the analytical feasibility, and the fit of the hazard to the case study site. Twelve prioritized hazards were included in the case study analyses: arsenic (total and inorganic), iodine, cadmium, lead, mercury, copper, zinc, the allergen tropomyosin, *Salmonella* spp., *Bacillus* spp., norovirus, and *Vibrio* spp. We found that blanching was very effective in reducing iodine concentrations but mildly increased the zinc concentration. Fermentation of blanched seaweed further decreased iodine concentrations and had similar effects on inorganic arsenic, cadmium, and zinc. An unintentional increase in copper was observed. *Bacillus cereus* was detected in a blanched sample and fermented samples. *Vibrio alginolyticus* seemed to be introduced during blanching, yet fermentation greatly reduced the detection frequency. Our study illustrates the importance of clearly defining the goals of seaweed processing and optimizing the process for food safety hazards. Meanwhile, it is advised to monitor for unintended effects that may occur during processing, such as, in our case, the introduction of copper and pathogens like *B. cereus* and *Vibrio* spp. This study serves as an example for seaweed stakeholders on how to consider a Food Safety-by-Design concept and a risk-based approach during seaweed harvest and post-harvest processing.

1. Introduction

Large-scale seaweed cultivation in European waters is gaining attention (Directorate-General for Maritime Affairs and Fisheries, 2022; Gercama et al., 2022). The potential for seaweeds as biomaterials, nutraceuticals, feed, and food is also taking shape in the European market (CBI, 2022). In order to yield the beneficial effects of farming seaweed, one should include safety early on during the innovation process. Incorporating a "Safe-by-Design" concept can help decrease uncertainty and increase the potential for health (Dekkers et al., 2020). When producing seaweed for human consumption, incorporating a "Food Safety-by-Design approach" can help protect human health and ensure the safety of future consumers.

A report from FAO and WHO (2022) identifies the need to include food safety implications of seaweed that are used for food consumption. For example, concerns about the bioaccumulation of hazardous substances in seaweed raise questions about its consumption (FAO and WHO, 2022). A risk-based approach to monitoring can help governments, national authorities, and food business operators (FBOs) determine the risk to public health from consuming seaweed. Thus, incorporating awareness of the potential food safety hazards and risks that may occur during seaweed farming and post-harvesting is an important first step to protecting human health.

Seaweed production in Europe has the potential for sustainable development if economic, environmental, and social challenges are addressed (Araújo et al., 2021). *Saccharina latissima* (sugar kelp) is commercially farmed in European countries like the United Kingdom, France, Spain, and Norway and is reported to be used for human

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https://doi.org/10.1016/j.foodcont.2024.110461
Received 21 December 2023; Received in revised form 12 March 2024; Accepted 16 March 2024
Available online 18 March 2024
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consumption (CBI, 2022; Netalgae, 2012). Besides having the highest biomass production among European countries, Norway has the highest number of seaweed aquaculture companies (Araújo et al., 2021). Norwegian seaweed aquaculture has focused on large-scale cultivation of kelp species, in particular, *S. latissima* (Stévant et al., 2017), and production is mostly by mechanical harvesting (Araújo et al., 2021).

A recent Norwegian study concluded that food safety hazards for seaweed grown in Nordic countries include iodine, cadmium, and inorganic arsenic, albeit the concentration of heavy metals varies, based on, i.a., the seaweed species, age, growing conditions, and processing of seaweed (Hogstad et al., 2023). Research on Norwegian-grown *S. latissima* has focused on processing seaweed to reduce iodine (Blikra et al., 2021; Nielsen et al., 2020; Stévant et al., 2018; Wirenfeldt et al., in preparation) as well as other undesirable compounds like cadmium and inorganic arsenic (Blikra et al., 2021; Stévant et al., 2018).

Post-harvest processing of seaweed is one strategy to reduce the concentration of undesirable compounds. Strategies have focused on drying, blanching, or fermenting *S. latissima* to reduce concentrations of some heavy metals and iodine. Nevertheless, data on the effects of processing on other chemical hazards and other food safety hazards, like microbiological pathogens in edible seaweeds, are more limited (Vorse et al., 2023). Also, few studies with data on the prevalence of algal allergenicity, let alone on the effects of processing on allergens, are reported (James et al., 2022). Results on these topics will help national authorities and FBOs design risk-based approaches (monitoring, risk assessments, etc.) and optimize food safety control when considering the effects of post-harvest seaweed processing.

Our study aims to determine the effects of a commercial post-harvest processing strategy of the brown algae, *S. latissima,* on the presence of prioritized food safety hazards. We used the commercial harvesting and production site of Norwegian seaweed producer Arctic Seaweed as a case study. Our results provide knowledge to seaweed stakeholders – governmental authorities and FBOs – on the effects of processing and provide input on future Safe-by-Design and risk-based approaches to ensure food safety.

2. Materials and methods

2.1. Demarcation of the study

This study focused on Norwegian farmed, harvested, and processed *S. latissima* intended for human consumption. Safety from other scientific disciplines was not the focus of this study. Given the case-specific nature of this study, we included insights from the seaweed producer, Arctic Seaweed (see section 2.2), to prioritize food safety hazards. Thus, trend observations (i.e., historical data) of previous food safety analyses and specific information on the cultivation site and processes that are applied to prevent or mitigate hazards were also considered. The study reports the effects of processing on prioritized food safety hazards. The list of prioritized hazards reflects the commercial cultivation, harvesting, and production according to Arctic Seaweed as intended to occur from November 2021–May 2022. The study also intends to illustrate how a Food Safety-by-Design concept, together with a risk-based approach, can be interpreted. To this end, hazards were prioritized and selected, and sampling was done for these hazards. The sampling was performed with two objectives: to determine the effects of processing on chemical and microbiological contaminants (this paper) and to determine effective sampling strategies. The latter is reported in a separate paper (Faassen et al., in preparation). For the research on sampling schemes, some batches and processing steps were oversampled compared to others. In order to make the most use of the data, all pre-processed samples (see Faassen et al., in preparation) were used in the current study (Table 1).

Table 1
Number of samples taken from each batch and at each processing step (I: after harvest; II, after blanching; and III after fermentation) that were used for this study.

	Iodine and metals			Tropomyosin			Pathogens		
Processing step	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**
Batch A	4	1	8	4	1	12	1	1	8
Batch B	1	1	1	1	1	1	1	1	1
Batch C	4	1	1	4	1	1	1	1	1
Batch D	1	1	8	1	1	12	1	1	8
Batch E	4	1	8	4	1	12	1	1	8

2.2. Selection and prioritization of food safety hazards

Three parameters were considered when selecting and prioritizing food safety hazards relevant to the intended cultivation of *S. latissima*. Firstly, an article on stakeholder perspectives in the seaweed value chain, which comprised a literature review, survey, and stakeholder elicitation on food safety hazards in seaweed, was consulted (Banach et al., 2022) and evaluated based on a survey of respondents with academic and/or industrial knowledge (n = 22). This served as input to determine the first parameter (i) the current impact of the hazard, i.e., where a threat indicates how big a hazard is considering its likelihood and severity. This parameter was scored from 1 to 5, where 1 represented a "very small threat" and 5 "a very large threat." Respondents could indicate, "I don't know," resulting in a score of 0. These unknowns were then subtracted from the overall total of respondents when averages were calculated in the analysis. Next, two practical considerations served as parameters; these were (ii) the analytical feasibility of the hazard and (iii) the relevance of the hazard to the case study site (e.g., historical data on results of previous food safety analyses and knowledge on the site were considered). These second and third parameters were based on expert judgment from researchers and the seaweed producer and were scored from 1 to 3. For (ii) the analytical feasibility, 1 represented a hazard that is currently "not possible to measure," and 3 represented a hazard that is "easily measurable." For (iii) relevance to the case study site, 1 represented "not relevant" and 3 "very relevant." This 3-parameter selection and prioritization resulted in a refined list of hazards to be investigated.

2.3. Cultivation site and seaweed

The effect of processing on hazards was tested during an industrial-scale (0.9 ha farm) *S. latissima* harvest in Norway. *S. latissima* was commercially farmed between November 2021 and May 2022. Seaweed was harvested on May 2, 2022, near the aquaculture site "Brattøyna" by Skjerjehamn, Norway. The farm is owned and managed by Arctic Seaweed A.S.. The water depth at the farm was about 40 m.

2.4. Seaweed harvesting and processing operations

The seaweed harvest and processing operations are part of Arctic Seaweed's intellectual property, meaning details are limitedly reported. In brief, seaweed was harvested with a vessel. Ropes with attached seaweed were brought on board and pre-washed with pressurized seawater. Next, seaweed was cut from the rope at about 7–10 cm ("Harvest" in Fig. 1). The next processing step consisted of blanching in seawater ("Blanching" in Fig. 1). Blanched seaweed was directly transferred into containers lined with food-grade material bags (PP or PE, circa 200 kg of wet seaweed biomass per container), to which a fermentation inoculum was added. The containers were then transported to the land-based facility under ambient temperatures and without intentional shaking or stirring ("Fermentation" in Fig. 1). Three days after harvest, the liquid in the containers was drained into a larger container. The drained seaweed was re-packed in lined containers and was ready to be sent to the customer ("Repacking" in Fig. 1).

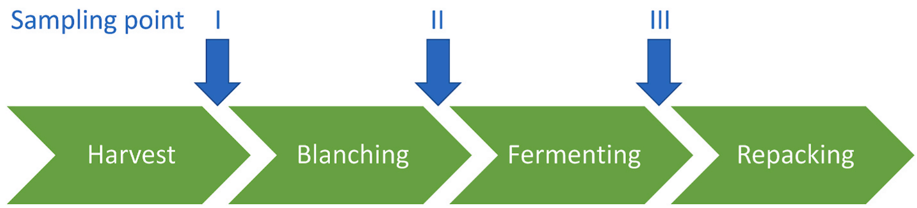

Fig. 1. Steps from harvest to re-packing of seaweed with the three sampling points. At each sampling point, five batches (A–E) were followed.

2.5. Sampling

During this study, five seaweed batches (batches A-E) were followed from harvest to re-packing. Samples were taken at three sampling points: (I) on the harvesting vessel after cutting from the ropes on May 2, 2022; (II) on the harvesting vessel after blanching on May 2, 2022; and (III) after fermentation and draining at the land-based facility on May 5, 2022 (Fig. 1). Samples were prepared for chemical analysis on the day after sampling (sampling points I and II) or on sampling day (sampling point III). Samples were analyzed for iodine (I), selenium (Se), the metals copper (Cu), zinc (Zn), arsenic (As, with speciation), cadmium (Cd), mercury (Hg), lead (Pb), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), molybdenum (Mo), and silver (Ag). In addition, the crustacean allergen tropomyosin and the pathogens *Salmonella* spp., *Bacillus cereus*, *Vibrio* spp., and norovirus were analyzed. Details on sample preparation and analysis can be found in the Supplementary materials. Se, Ni, V, Cr, Mn, Fe, Co, and Mo were part of the analytical package but were considered less relevant for this study (see section 3.1). Therefore, the data for these elements were not evaluated.

2.6. Data processing and statistical analysis

Data were processed in Excel, and statistical analyses were performed in SigmaPlot 14.5.

The laboratories expressed analytical results on a wet-weight basis. These results were transformed to dry weight (dw) concentrations by correcting for the moisture content of the individual samples. For 12 tropomyosin samples, moisture content was not analyzed. The wet weight-based concentrations of these samples were transformed into dry weight-based concentrations by correcting for the average moisture content of all other samples. One sample had a tropomyosin concentration above the method's upper quantification limit of 0.30 mg/kg wet weight. The laboratory estimated the concentration to be 0.33 mg/kg wet weight. As this concentration was close to the quantification limit, the tropomyosin concentration in this sample was assumed to be 0.33 mg/kg wet weight.

The effect of processing was determined by repeated measures (RM) ANOVA with the processing steps as treatment and the batches as experimental units (n = 5). Before running these tests, concentrations of samples from batches that were sampled repeatedly (Table 1) were averaged per processing step. A Bonferroni post hoc test was performed to determine the differences between the processing steps ($p < 0.05$).

Nine of the 57 tropomyosin samples had a concentration < limit of quantification (LOQ). A concentration of 0.5*LOQ was used to evaluate these samples, which is an acceptable approach for analyzing datasets with few non-detects (Croghan & Egeghy, 2003).

3. Results and discussion

3.1. Prioritized food safety hazards

The prioritized list of food safety hazards to be investigated in the case study is shown in Table 2. Twenty-one were studied and prioritized, with 12 included in the case study analyses (7 chemical hazards, 1 allergen, and 4 microbiological hazards).

Iodine and As were selected because these are taken up to a great extent by several edible seaweeds (Banach et al., 2020). The speciation between organic and inorganic As is important to analyze, as the latter is more toxic and a public health concern (FAO and WHO, 2022); hence, it was included in our study. Other heavy metals like Cd, Pb, and Hg were not scored in the survey, although these are recognized as potential food safety hazards in seaweed (Banach et al., 2020; FAO and WHO, 2022;

Table 2
Prioritization and inclusion of food safety hazards based on 3 parameters: current impact, analytical feasibility, and fit to the case study.

Food safety hazards	Hazard group	Current impact (1-5)	Analytical feasibility (1-3)	Fit to case study site (1-3)	Sum of parameters	Included in the case study analyses
Arsenic	Chemical	3.4	3	3	9.4	Yes
Iodine	Chemical	2.8	3	3	8.8	Yes
Salmonella spp.	Microbiological	2.3	3	3	8.3	Yes
Bacillus spp.	Microbiological	2.1	3	3	8.1	Yes
Plastics	Physical	2.4	2	3	7.4	No
Polychlorinated biphenyls	Chemical	2.2	3	1	6.2	No
Norovirus	Microbiological	2.1	2	2	6.1	Yes
Dioxins	Chemical	2.1	3	1	6.1	No
Pesticide residues	Chemical	2.1	3	1	6.1	No
Marine toxins	Chemical	2.1	3	1	6.1	No
Polycyclic aromatic hydrocarbons	Chemical	2.0	3	1	6.0	No
Pharmaceutical active compounds	Chemical	1.8	3	1	5.8	No
Radionuclides	Chemical	1.8	3	1	5.8	No
Endocrine-disrupting compounds	Chemical	2.1	2	1	5.1	No
Allergens (crustaceans, mollusks)	Allergen	[a]	3	3	6	Yes
Cadmium	Chemical	[a]	3	3	6	Yes
Copper	Chemical	[a]	3	3	6	Yes
Lead	Chemical	[a]	3	3	6	Yes
Mercury	Chemical	[a]	3	3	6	Yes
Zinc	Chemical	[a]	3	3	6	Yes
Vibrio spp.	Microbiological	[a]	3	2	5	Yes

[a] Not evaluated in the survey.

Hogstad et al., 2023) and were included in the case study analyses. Along with these, heavy metals like Cu and Zn were also not scored in the survey but could be analyzed in the same analytical package as I and As and are reported in our study.

The allergenic protein tropomyosin was selected because of its analytical feasibility (with an enzyme-linked immunosorbent assay) and relevance to the case study site. The seaweed producer has previously measured tropomyosin, given the visible presence of crustaceans (shrimp) on the seaweed during harvest. Although not scored during the survey, the presence of allergens in seaweed has shown to be a data gap (Banach et al., 2020), with our study aiming to try to close this gap.

Salmonella spp. and *Bacillus* spp., particularly *Bacillus cereus*, were selected because they scored high for all three parameters. *Salmonella* spp. is also reported to be a major hazard in seaweed (Banach et al., 2020; FAO and WHO, 2022), while *Bacillus* spp. (a spore-former) can be resistant to heat processing, and the need to control growth has been reported (Banach et al., 2020). *Vibrio* spp. were also selected for the case study analyses because of analytical feasibility and possible fit to the case study site. *Vibrio* spp., like *Vibrio parahaemolyticus,* are reported in marine waters, and their concern as a food safety hazard has been acknowledged (Banach et al., 2020; Løvdal et al., 2021). Its resistance to food processing (Løvdal et al., 2021) also makes it an interesting candidate for the case study. Norovirus was also selected for the case study analyses. It is an identified food safety hazard in seaweed (Banach et al., 2020; Løvdal et al., 2021). The methods available to analyze norovirus in seaweed are limited, although it is an important hazard to investigate during processing as it can occur, for instance, due to recontamination.

Nine hazards were excluded from the case study. Given the limited fit to the case study site (scored a 1), polychlorinated biphenyls (PCBs), dioxins, pesticide residues, marine toxins, polycyclic aromatic hydrocarbons (PAHs), pharmaceutically active compounds, radionuclides, and endocrine-disrupting compounds were excluded. This is because they may be location-specific hazards (e.g., PCBs, dioxins, PAHs, pharmaceutically active compounds, radionuclides, and endocrine-disrupting compounds) or not identified as a concern at the case study site (pesticide residues and marine toxins). In addition, plastics were identified as a hazard but excluded. This is due to limitations in analyzing macroplastics and microplastics in seaweed.

3.2. Chemical hazards

Of the selected chemical hazards, Hg and Pb were not detected in any samples. I, total and inorganic As, Cd, and Zn were detected in all samples. Cu was detected in only some replicates (Table 3).

Processing had a strong effect on I. Blanching reduced the initial concentration of 7,000 mg/kg dw by 66%, while fermentation caused an additional 42% reduction (averaged 1,400 mg/kg dw). The total reduction from harvest to after fermentation was 81% (Table 4, Fig. 2). Our results coincide with those of other Norwegian cultivated and processed *S. latissima*. Blikra et al. (2021) reported a similar I iodine content after processing, averaging an 85% reduction after rinsing (three times) and soaking (15 min) *S. latissima* (4,100–600 mg/kg dw). Samples were harvested in June 2020 from several harvest depths, although the effects of the different cultivation depths tested were not apparent after rinsing and boiling (Blikra et al., 2021). Stévant et al. (2018) reported samples of *S. latissima* averaging 4,900 and 6,600 mg/kg dw, respectively, in May and June 2015. Our samples were taken in early May 2022, and then initial concentrations exceeding 7,700 mg/kg dw were already observed (Fig. 2). For Stévant et al. (2018), soaking showed a decrease in I to less than 2,000 mg/kg dw for their hot freshwater rinse (the recommended maximum level in France is 2,000 mg/kg dw (AFSSA, 2009; ANSES, 2018; CEVA, 2014)), yet our results, with a seawater rinse and blanching showed that average concentrations still exceeded the 2,000 mg/kg dw recommendation, although a reduction to less than 2,000 mg/kg dw was regularly observed after fermentation (Table 3). Moreover, Nielsen et al. (2020) investigated several blanching temperature-time combinations for *S. latissima*, finding the highest reduction with water blanching at 80 °C for 120 s (94% reduction; 4,600 to 300 mg/kg dw). More recently, Wirenfeldt et al. (2022) reported even lower I content in untreated *S. latissima* (2, 000 mg/kg dw) and found a significant reduction when washed or blanched. Overall, the reduction of I content in sugar kelp during processing with rinsing and blanching steps is reported in the literature. Our results further illustrate that initial concentrations of >7,000 mg/kg dw can potentially occur in sugar kelp. The additional effects of processing, e.g., by fermentation, to reduce I content in sugar kelp may be required to reach recommended values for iodine. Processing steps like rinsing, soaking, blanching, and fermentation, including combinations of these, can reduce iodine content in sugar kelp. Factors like the temperature-time combinations of processing, order of processing, and initial concentration of iodine in seaweed should also be considered when deducing the overall efficacy and food safety risk.

The reduction of total arsenic (tAs) was less pronounced than for I (Fig. 3). The individual processing steps caused no significant reduction, but in total, a 46% reduction was achieved (Table 4). Inorganic arsenic (iAs) concentrations decreased similarly, with a 39% total reduction (Table 4). This did not lead to changes in the fraction of iAs, which remained stable during processing (RM ANOVA, $p = 0.374$). The fraction of inorganic arsenic was 0.24% (SD 0.06, $n = 45$). Blikra et al.

Table 3
Results for chemical hazards, allergens, and microbiological hazards per processing step. Average concentrations and standard deviations (SD) are calculated for each processing step from all samples with a concentration > limit of quantification (LOQ)[a] to show the variation between subsamples. Averages are calculated differently and, therefore, deviate from those presented in Table 4. Averages are expressed as mg/kg dry weight, except for *Bacillus* spp. (colony forming units (CFU)/g wet weight). '-' means not applicable or not detected. n = the number of samples analyzed.

Processing step	I - After harvest				II - After blanching				III - After fermentation			
	Average	SD	n	n > LOQ[a]	Average	SD	n	n > LOQ[a]	Average	SD	n	n > LOQ[a]
Iodine	7,100	370	14	14	2,300	370	5	5	1,500	440	26	26
Total arsenic	54	8.0	14	14	39	4.4	5	5	28	7.2	26	26
Inorganic arsenic	0.11	0.01	14	14	0.10	0.02	5	5	0.07	0.02	26	26
Cadmium	0.46	0.06	14	14	0.49	0.05	5	5	0.31	0.09	26	26
Copper	1.8	–	14	1	–	–	5	0	1.7	1.1	26	18
Mercury	–	–	14	0	–	–	5	0	–	–	26	0
Lead	–	–	14	0	–	–	5	0	–	–	26	0
Zinc	27	2.7	14	14	33	2.2	5	5	25	6.2	26	26
Tropomyosin	0.86	0.92	14	10	1.7	–	5	2	1.3	0.73	38	36
Salmonella spp.	–	–	5	0	–	–	5	0	–	–	26	0
Bacillus cereus	–	–	5	0	20	–	5	1	10	–	26	2
Vibrio spp.	–	–	5	2	–	–	5	5	–	–	26	1
Norovirus	–	–	5	0	–	–	5	0	–	–	26	0

[a] The limits of detection for *Salmonella* spp., *Bacillus cereus*, *Vibrio* spp., and Norovirus are based on the limits shown in Table S1.

Table 4
Reduction of chemical hazards and tropomyosin in *S. latissima* during different processing steps. The processing effect is determined by repeated measures (RM) ANOVA, n = 5. Differences between individual processing steps (I-II, II-III, and I-III) are determined by the Bonferroni *t*-test, $p < 0.05$. Non-significant reductions are indicated as '-'. The averages in this table are calculated from the averages of each batch at each processing step to enable RM ANOVA analysis and, therefore, differ from the values in Table 3.

Processing steps	Blanching (I-II)	Fermentation (II-III)	Blanching and fermentation (I-III)	Blanching (I-II)	Fermentation (II-III)	Blanching and fermentation (I-III)	Processing effect
	Average concentration (mg/kg dw)	*Average concentration (mg/kg dw)*	*Average concentration (mg/kg dw)*	*Reduction (%)*	*Reduction (%)*	*Reduction (%)*	*RM ANOVA (p)*
Iodine	7,000	2,300	1,400	66	42	81	<0.001
Total arsenic	55	39	30	–	–	46	0.006
Inorganic arsenic	0.11	0.10	0.07	–	35	39	<0.001
% Inorganic arsenic	0.20	0.27	0.26	–	–	–	0.374
Cadmium	0.47	0.49	0.32	–	35	32	<0.001
Zinc	28	33	24	−20	28	–	0.001
Tropomyosin[a]	1.1	0.7	1.5	–	−106	–	0.027

[a] For tropomyosin concentrations < limit of quantification (LOQ), a value of 0.5* LOQ was assumed.

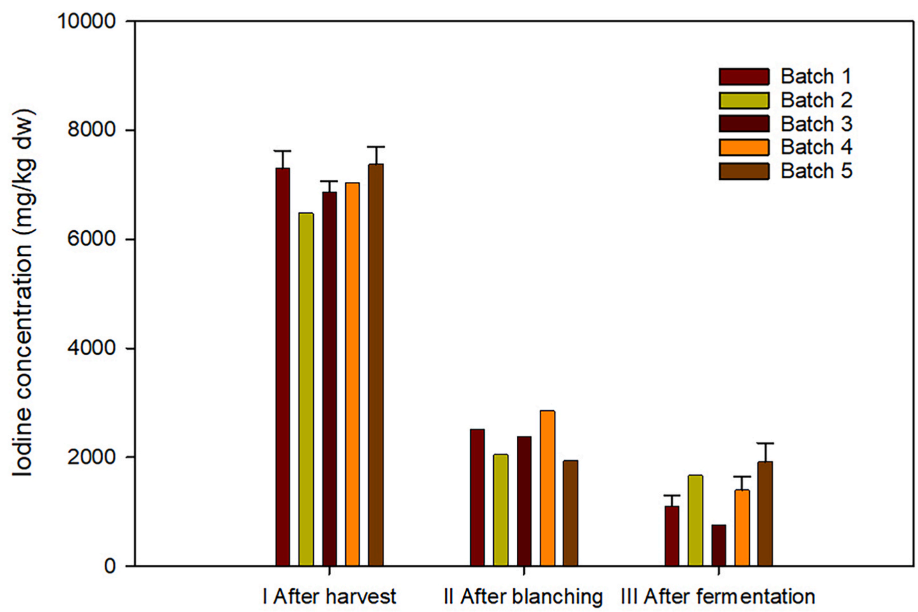

Fig. 2. Iodine concentrations per processing step and batch. The number of replicates used is shown in Table 1. Error bars represent standard deviations. dw = dry weight.

(2021) also reported similar concentrations of tAs in sugar kelp (62.7, SD 4.3 mg/kg dw) compared to our study (54, SD 8.0 mg/kg dw, Table 3). However, Blikra et al. (2021) found a lower total reduction of tAs of 43% (to 36, SD 3.1 mg/kg dw) compared to our study. Stévant et al. (2018) reported the average iAs concentrations in sugar kelp, ranging from 0.16 to 0.23 mg/kg dw. Our finding of iAs was lower, with an average initial concentration of 0.11 mg/kg dw (SD 0.01, Table 3), and is below the French recommended maximum level for iAS in edible seaweeds of 3 mg/kg dw (AFSSA, 2009; ANSES, 2018; CEVA, 2014). More recently, Trigo et al. (2023) reported a similar average initial concentration of iAs of 0.119 mg/kg dw (n = 3) in *S. latissima* and that the iAs made up 0.1–0.2% of the tAs, which is lower than observed in our study. Overall, a reduction in total As and iAs was observed for processed sugar kelp, although the extent of the reduction is less pronounced than that of I.

Cd concentrations did not change after blanching but were reduced by 35% following fermentation to an average concentration of 0.32 mg/kg dw (Table 4). In comparison, Stévant et al. (2018) reported a maximum Cd concentration of 0.27 mg/kg dw in *S. latissima*. Roleda et al. (2019) reported higher Cd concentrations in *S. latissima*, averaging 0.56 mg/kg dw (n = 9) in 2015 and 0.62 mg/kg dw (n = 14) in 2016.

Several factors may influence the concentration of Cd in seaweeds, like the country of origin and seasonal variation (Banach et al., 2020). Bruhn et al. (2019) reported the concentrations of Cd in sugar kelp and observed an increase between fresh (3.0, SD 0.08 mg/kg dw) and heat-treated sugar kelp (3.6, SD 0.12 mg/kg dw), followed by a reduction in fermented sugar kelp (2.0, SD 0.04 mg/kg dw), with a significant difference ($p < 0.05$) between fresh and fermented seaweed. We observed a similar reduction after fermentation in our study (reduction from initial to fermented of 32%), although, unlike the study of Bruhn et al. (2019), our results did not show a significant difference between the Cd concentrations in initial and fermented sugar kelp. Overall, the effect of processing on Cd in sugar kelp was not significantly different, albeit lower after fermentation, and concentrations were comparable to those reported in Norway for *S. latissima*.

Cu was detected in 1 of the 14 samples that were taken directly after harvest (1.8 mg/kg dw) and in 0 of the 5 samples taken after blanching. In 18 of the 26 samples taken after fermentation, a Cu concentration > LOQ was found. Cu was, therefore, more frequently detected in fermented samples than in fresh ($p < 0.001$) and blanched ($p = 0.008$) samples (Fisher's exact test). The average concentration in the 18 fermented samples was 1.7 mg/kg dw, SD 1.1 mg/kg dw (Table 3, Fig. 3).

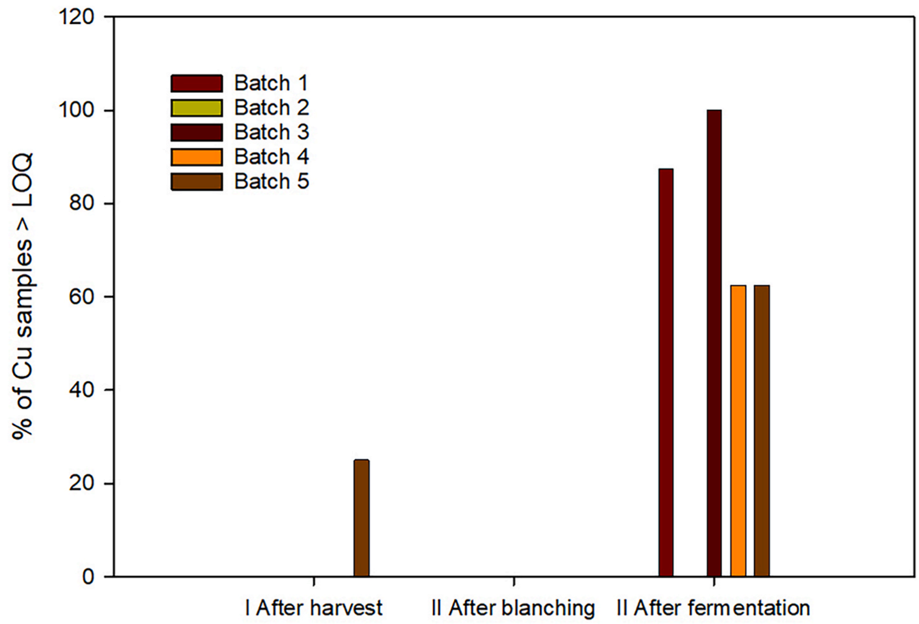

Fig. 3. Percentage of samples in which copper (Cu) was detected. The number of replicates used is shown in Table 1. LOQ = limit of quantification.

The reason why copper was detected more frequently in blanched seaweed is unclear. According to the seaweed company, the inoculum used for fermentation did not contain Cu (personal communication with the Arctic Seaweed Operations Manager). Another possible source may be Cu leaching from the fermentation container. The cause of the observed increase in Cu detection in fermented seaweed cannot be determined in hindsight and requires additional investigation.

Zn concentrations mildly increased by 20% after blanching (Table 3). Fermentation caused a 28% reduction, so the concentration in the final product (24 mg/kg dw) was similar to the concentration directly after harvest (28 mg/kg dw, Table 4). Trigo et al. (2023) reported a significant increase in essential elements like Cr, Cu, Mn, and Zn in *S. latissima* after blanching at 80 °C ($p < 0.05$). This significance was not found in our study and may be related to the blanching conditions applied. Bruhn et al. (2019) reported the concentrations of Zn in sugar kelp and observed an increase between fresh (58, SD 8.0 mg/kg dw) and heat-treated sugar kelp (100, SD 15 mg/kg dw), followed by a reduction in fermented sugar kelp (74, SD 4.1 mg/kg dw). Overall, the effect of processing on Zn in sugar kelp is limitedly reported in the literature, although it appears to fluctuate during processing. Zn concentrations were not significantly different during processing, albeit higher during blanching and lower after fermentation. The effects of sugar kelp processing with blanching and fermentation steps on Zn and some other essential elements (like Cu), given processing changes (e.g., in temperature or time applied), warrant further investigation on the cumulative effects on food safety.

3.3. Allergens

The crustacean allergen tropomyosin showed a large variation within and between batches. The three highest concentrations (3.3, 3.0, and 2.7 mg/kg dw) were found in the only three samples taken from Batch 2 (Fig. 4). The percentage of samples in which tropomyosin was detected increased after fermentation, as did the within-batch variation (Fig. 4), but the average tropomyosin concentration directly after harvest was similar to the concentration in the final product when for the samples < LOQ a concentration of 0.5*LOQ was assumed (Table 3). A significant 106% increase was observed after fermentation (Table 4), but as only two of the five samples taken after blanching had a concentration > LOQ, this effect should be interpreted with caution. Currently, there are still many uncertainties on whether the endogenic proteins of seaweed elicit an allergic reaction (Garciarena et al., 2022). Nonetheless, since organisms like crustaceans and mollusks naturally occur in the same environment of seaweed cultivation, there is a chance

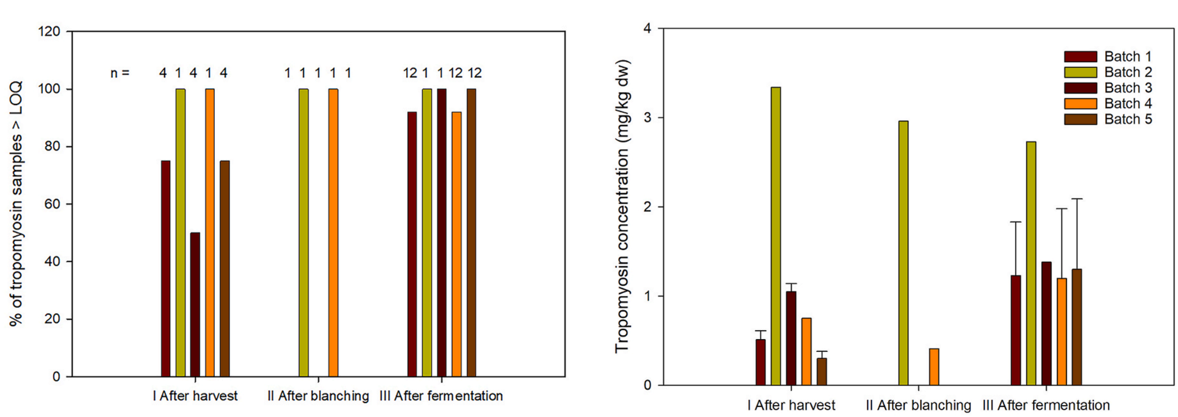

Fig. 4. Detection of tropomyosin per batch and processing step (left); the number of replicates are indicated. The right panel depicts the average concentration of all the samples > limit of quantification (LOQ). dw = dry weight.

that seaweed contains the allergen tropomyosin (Mildenberger et al., 2022).

3.4. Microbiological hazards

Salmonella spp. and norovirus were not detected in any of the samples. *Bacillus cereus* was detected once in a blanched sample and twice in fermented samples (Table 3); no effects of processing on *B. cereus* could be determined. It was not clear how and when *B. cereus* was introduced in the samples; this might have happened at any stage from growing up to analysis. *Bacillus* species are known spore-formers that are highly resistant to food processing (e.g., heat, acidity, dehydration). Pathogenic strains of *Bacillus*, namely *B. licheniformis* and *B. pumilus,* have been isolated from sugar kelp (Blikra et al., 2019). More recently, *B. cereus* has been detected in ready-to-eat dehydrated algae samples (Martelli et al., 2021), although our study appears to be the first, to our knowledge, to report *B. cereus* in sugar kelp. Further experiments that look into the behavior of *Bacillus* spp. in sugar kelp during additional processing (including blanching and fermentation) are important to realize so as to ensure that growth during further handling and storage is controlled.

Vibrio alginolyticus was detected in 2 samples taken directly after harvest and all 5 samples taken after blanching (Table 3, limit of detection (LOD): 1 colony forming unit (CFU)/20 g wet weight). After fermentation, the detection frequency was strongly reduced, with *V. alginolyticus* detected in only 1 of the 26 samples. If in the marine environment or introduced during processing (equipment, water supplies, or infected food handlers), then pathogens like *Vibrio* spp. can contaminate seaweed (Cressey et al., 2023). Løvdal et al. (2021) have also noted that vibrios are sensitive to food processing, especially thermal treatment, and are occasionally found in the environment and seafood from temperate waters. Water temperature is illustrated to affect *Vibrio* spp., with Sheikh et al. (2022) finding that results are multifaceted regarding its growth and pathogenicity. Factors besides temperature that affect *Vibrio* spp. are its growth and survival and can include physicochemical parameters like salinity and pH (Sheikh et al., 2022). The presence of *Vibrio* spp., including that of *V. alginolyticus*, in *S. latissima* cultivated in the northeast of the USA, has been reported, although at low levels for *V. alginolyticus* "below 0.4 ± 1 CFU/100 mL" (Barberi et al., 2020). In our study, *Vibrio* spp. may have been introduced during sample processing; however, much care was taken to rinse and disinfect the tables, hands, and equipment used with 96% ethanol between each sample (see Supplementary materials: Sample processing and analysis). Therefore, it is very unlikely that all the same samples from one processing step were contaminated. It may be possible that the water used during the blanching step contained *V. alginolyticus*, meaning it was introduced at this step. *V. alginolyticus* has a broad temperature and salinity tolerance with viable growth from "24–40 °C and 1–7% (w/v) NaCl with optimal growth at 35 ± 2 °C and 2–4%" (Norfolk et al., 2023). The observed decrease of *V. alginolyticus* in fermented sugar kelp may be due to the changed pH during fermentation, although further investigation on the effects of this fermentation step is warranted.

The occurrence of human foodborne pathogenic microbes in seaweed depends on the environment in which the seaweed is cultivated, as well as the processing and storage of seaweed samples. When these pathogens are present in the environment, it is likely they can be present in the seaweed. Besides the contamination of seaweed with pathogenic microbes during the cultivation phase, seaweed can also be contaminated during the processing phase. Cross-contamination during post-harvest handling and processing can be mitigated by implementing good hygiene practices; however, some contaminants cannot be lowered or eliminated by further processing (soaking, boiling, cooking, etc.) (BAFS, 2017; Banach et al., 2022; Løvdal et al., 2021). Overall, efforts to avoid or minimize the occurrence of food safety hazards should be made throughout the whole seaweed chain from early in the cultivation through the processing stage.

4. Conclusions

Post-harvest processing of seaweed can serve multiple purposes, such as prolonging shelf life, influencing texture, taste, or nutritional content, or reducing the presence of certain food safety hazards. Our study aimed to determine the effects of a commercial harvest and processing strategy, a combination of blanching and fermentation, on the brown algae, *S. latissima,* on the presence of prioritized food safety hazards. Twelve prioritized hazards were included in the case study analyses: arsenic (total and inorganic), iodine, cadmium, lead, mercury, copper, zinc, the allergen tropomyosin, *Salmonella* spp., *Bacillus* spp., norovirus, and *Vibrio* spp.

Blanching was very effective in reducing the iodine concentration but mildly increased the zinc concentration. Fermentation of blanched seaweed further decreased iodine concentrations and had similar effects on inorganic arsenic, cadmium, and zinc. An unintentional increase in copper was observed. *Bacillus cereus* was detected in a blanched sample and fermented sugar kelp samples. Given the presence of this spore-former, it is important to ensure that the growth of this pathogen during further processing, handling, and storage of seaweed is controlled. The pathogen *Vibrio alginolyticus* seemed to be introduced during blanching, which may result from contamination during processing or possibly as a result of the water used during blanching. On the other hand, fermentation greatly reduced the detection frequency of *V. alginolyticus*. The presence of pathogenic bacteria or viruses should be controlled during the harvesting and processing of seaweed.

Our study shows that it is important to clearly define the goals of seaweed processing and to optimize the process for the most relevant hazards. As deduced from the company's information, the studied process was optimized for the reduction of iodine. With an observed 81% reduction, the process seemed to be effective in that respect. A 32–46% reduction in (inorganic) arsenic and cadmium was also realized. It is advised to monitor and control for effects of processing on other food safety hazards. In our case study on sugar kelp, these are the introduction of copper and pathogens like *Bacillus cereus* and *Vibrio alginolyticus*.

With the transition from small to large-scale seaweed cultivation for food purposes, understanding the effects of processing on food safety hazards and risks is important. Using a Food Safety-by-Design concept together with a risk-based approach brings value to the seaweed sector by helping them to diminish uncertainty and potential harm to human health and focus on necessary controls to ensure safe seaweed consumption.

Funding

This work was co-funded by Lloyd's Register Foundation under grant no. GA\100395 (Safe Seaweed by Design). The Foundation helps to protect life and property by supporting engineering-related education, public engagement, and the application of research.

CRediT authorship contribution statement

J.L. Banach: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Y. Hoffmans:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **E.J. Faassen:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kim Kristensen (owner), Stefan Post (employee), Arctic Seaweed

Operations Manager (employee), and Marjoleine Hoefsloot (previous student) of Arctic Seaweed A.S. were consulted in this research when designing the experiment and/or participated in the commercial harvest; however, they did not influence the data interpretation, nor the results presented in this research.

Data availability

Data will be made available on request.

Acknowledgments

The authors would like to acknowledge Rosa Safitri (WFSR) as well as Kim Kristensen, Stefan Post, and the Arctic Seaweed Operations Manager (Arctic Seaweed A.S.) for their support of the research study. Marjoleine Hoefsloot (Wageningen University, Arctic Seaweed A.S.) is also acknowledged for her assistance during the sampling. Sophie Koch (Wageningen Economic Research) is kindly thanked for her contribution to the prioritization method and administrative assistance. Sander van de Burg (Wageningen Economic Research) is also thanked for his project coordination.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2024.110461.

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