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BRIEF REPORT

Live storage of kelp under stressful conditions led to higher iodine reductions during subsequent blanching

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Abstract
Kelp growers and the food industry, as well as food researchers, are currently finding methods for controlling the iodine content of kelp intended for food as this is one of the major obstacles to entering a profitable market. Kelps are rich sources of dietary iodine since iodine is up-concentrated in algal tissue and utilized as an inorganic antioxidant during exposure to stress. As kelp contains much more iodine than any other food source, it is warranted to reduce the amount of iodine in the biomass prior to consumption, since both iodine deficiency and excess can cause health problems. Iodine is typically removed post-harvest using traditional methods such as blanching. In the present work, we attempted to utilize inherent stressors, i.e., intermediate storage (3 days) with high light exposure and low turnover of water, to reduce the iodine content prior to processing. Furthermore, we assessed the effect on subsequent blanching, comparing samples stored in tanks and not stored samples. The iodine content was slightly reduced when comparing storage to no storage, but in most cases not significantly so. However, after subsequent blanching, there was a pronounced added reduction for stored samples (87 % reduction) compared to not stored samples (80 % reduction). Although the differences are smaller than we expected, our research shows that using post-harvest intermediate storage of kelp may alter the iodine content post-processing. Fine-tuning the stressors and conditions could lead to new possibilities for iodine reduction.

Keywords Sugar kelp · Phaeophyceae · Live storage · Inherent stressors · Iodine reduction · Processing · Blanching

Introduction

Iodine functions as an inorganic antioxidant in kelp (Küpper et al. 2008). Iodine in the form of both iodide (I^-) and iodate (IO_3^-) is taken from the surrounding seawater into the apoplast (extracellular space) and stored in the form of I^- (Verhaeghe et al. 2008; Fievet et al. 2023). Details regarding exact localization of iodide and mechanisms of uptake, storage and efflux are still lacking (Katsaros et al. 2021). The uptake of iodine is highly effective and kelp up-concentrate iodine up to 30,000-times compared to the iodine concentration in seawater (Gall et al. 2004). The contents of iodine in kelps range from 30 to 31,000 mg kg^{-1} dry weight (dw) (Blikra et al. 2022a, b) and vary depending on a range of factors including kelp species, blade size, harvesting time, geographic location, and cultivation depth (Gall et al. 2004; Sharma et al. 2018; Blikra et al. 2021). The commercially relevant species *Saccharina latissima* (sugar kelp), which is the most cultivated species in Europe, contains iodine ranging 670 to 10,000 mg kg^{-1} dw, with typical values (25 % quartiles) between 2600 and 4600 mg kg^{-1} dw (Duinker et al. 2020).

Kelp and other seaweeds are up-and-coming marine food sources, with new flavors and other warranted food properties (Mouritsen et al. 2012, 2019). As an example, the addition of kelp in recipes allows for total salt reduction without compromising the salt taste, due to taste enhancers naturally present in kelps (Jensen et al. 2022). When included in the diet, kelp provides a very rich source of iodine. The iodine status in ≥ 21 countries of the world, including ≥ 3 European, is still characterized as insufficient (Zimmermann & Andersson 2021). To maintain normal thyroid function, a dietary intake of 150 µg per day is sufficient for most adults, whereas pregnant and lactating women require somewhat more, and children require somewhat less (dependent on age; EFSA 2014). There are also health-risks associated with excessive iodine consumption (Laurberg et al. 2009), and

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therefore it is advised against exaggerated addition of kelp to food items. Eating 0.04 g of dried, unprocessed sugar kelp provides the daily requirement of iodine for adults (Blikra et al. 2021). Such low levels have limited commercial interest. However, if the iodine content could be reduced in an order of magnitude 90 %, the dried kelp would certainly be interesting as use for e.g., spice. The total production of cultivated seaweeds in Norway in 2022 was around 1 % of the wholesale market of spices which was 3.2 million kg (Norwegian ministry of health). Thus, substituting up to 1 % of spices with kelp, which would provide flavor enhancement and function as a salt replacer, e.g., in ready-made food products (Vilar et al. 2020; Gullón et al. 2021), could potentially be a valuable food application of the produce which, if done thoughtfully, could have added health benefits including improved iodine status and lowered sodium intake.

Both too low and too high dietary iodine intake can result in thyroid dysfunction and negative health outcomes, especially in susceptible population groups, including pregnant women, fetuses, newborns, and elderly people (Emder & Jack 2011; Lee et al. 2015; Farebrother et al. 2019; Zhao et al. 2019). Regarding iodine excess, persons having a history of low iodine intake and those with underlying, subclinical or clinically diagnosed thyroid dysfunction have higher associated risks of negative health outcomes, such as hypo- and hyperthyroidism (Lee et al. 2015). With increasing interest for kelps as ingredients in Western countries, some of which have populations with histories of mild to moderate iodine deficiency, including some European countries (Andersson et al. 2007), iodine reduction prior to consumption is necessary to reduce risk of iodine excess. Post-harvest processing methods, such as washing, blanching, boiling, fermentation, and pulsed electric field processing may yield high iodine reductions, reducing the content by up to 95 % (Nitschke & Stengel 2016; Bruhn et al. 2019; Nielsen et al. 2020; Blikra et al. 2021, 2022a, 2022b; Lafeuille et al. 2023). However, there may be yet another possibility if we consider the biochemistry of iodine in kelp.

As exemplified using *Laminaria digitata*, kelp release iodine into the surrounding seawater or air when they are stressed, as part of an oxidative stress response (Küpper et al. 2008). Potential stressors include high light levels, atmospheric ozone, and desiccation (Küpper & Carrano 2019). And by this, addition of stress factors should, in theory, reduce the iodine content in alive kelp. Moreover, sugar kelp cultivated in tanks with low turnover of seawater contained only 380 mg iodine kg^{-1} dry weight (Lüning & Mortensen 2015), proving that alive sugar kelp can contain low amounts of iodine. Higher turnover of seawater during cultivation resulted in higher iodine contents. Furthermore, this study boiled the kelp after harvesting, and 77 % of the iodine was removed, resulting in a final iodine content of 90 mg iodine kg^{-1} dry weight (Lüning & Mortensen 2015). To the best of our knowledge, this is the lowest value for iodine in processed sugar kelp found in scientific literature. The approach of tank cultivation is very energy demanding and may not be economically feasible on a large scale. It might, however, be possible to add a processing step post-harvest in which the seaweed was placed in stressful conditions in such a way that the iodine content of the harvested biomass was reduced significantly.

Our research question was therefore: is it possible to utilize inherent stressors in kelp for iodine reduction while the kelp is still alive? And would this potential reduction influence the iodine content in kelp after subsequent processing?

Materials and methods

Storage trial

Saccharina latissima sporophytes were harvested in Tromsø 20 June 2022, and kept in seawater in interior tanks irradiated by ~50 µmol photons m^{-2} s^{-1} light (from LED sources) in Tromsø until 22 of June, when they were packed in Styrofoam boxes and shipped overnight to Stavanger (with seawater-moistened tissue paper). The sporophyte lengths and weights were recorded to be 72±18 cm and 39±18 g, respectively. The sporophytes were then placed in three interior tanks in a refrigerated room, with different storage conditions (Table 1). Two of them (A and B) with low light intensity, µmol photons m^{-2} s^{-1} (approximating overcast day conditions), and one with a high light intensity, µmol photons m^{-2} s^{-1} (approximating clear day conditions; C). The light for tanks A and B was provided by a warm white (3000 K) LED ceiling light, whereas the light for tank C was provided by white LED light strips (~4000 K) attached to the lid of the tank (Fig. 1). For the negative control tank (A), seawater was used directly, while for the other two tanks (B and C), seawater was filtered through a coal filter of 5 µm. Tanks B and C also received a lower turnover of seawater. The kelp specimens were kept for 3 days and sampled on day 0 and 3. The tanks had dimensions 68 × 68 × 54 cm (roughly 250 L), and a maximum of 17 sporophytes were placed in each tank.

Table 1 Storage conditions for samples in tanks for negative control (A), positive control (B, filtration and low seawater turnover), and samples stored high light intensity (C)

	A	B	C
Irradiance (µmol photons m^{-2} s^{-1})	16	15	185
Filtration	No	Yes	Yes
Seawater turnover (L min^{-1})	3.5	<1	1.6

* The irradiance was estimated from the measured illuminance

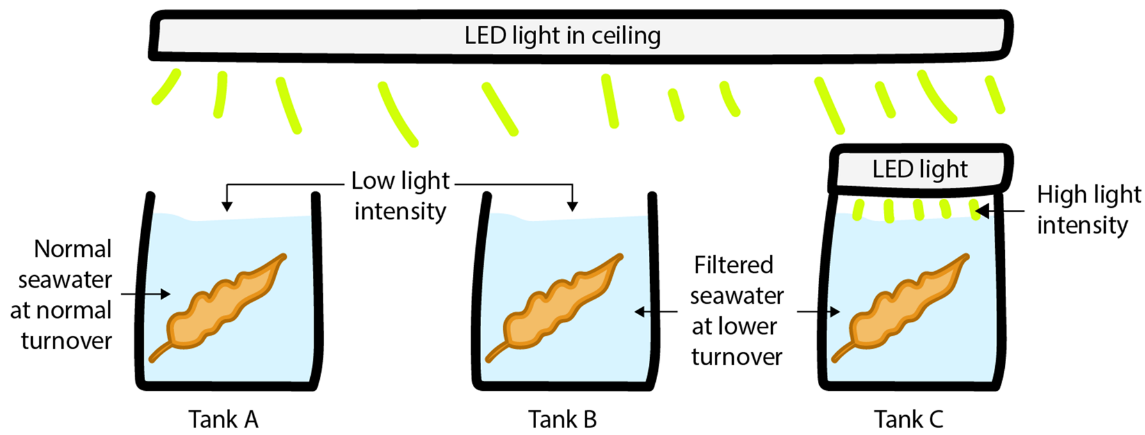

Fig. 1 The experimental set-up

Cooking trial

A cooking test was performed on kelp sporophytes directly upon arrival from Tromsø (day 0) and after intermediate storage (day 3). For this experiment, specimens were sampled from tank A and C. Kelp was blanched at boiling temperature for 2 mins, three specimens at a time in plentiful fresh water (5 L). The seaweed to water ratio (wt:wt) was roughly 1:45. The samples were not immersed in cold fresh water after blanching.

Drying

The samples were placed on top of baking paper on a large, perforated steel shelf. The samples were subsequently dried in a Bastramat C1500 drying/smoking cabinet equipped with a MC700 Microprocessor (Bastra GmbH, Germany) at 25 °C and low humidity until constant weight (3 days).

Iodine content

Elemental iodine analysis was performed by Mikroanalytisches Labor Kolbe, Oberhausen, Germany, as previously described (Blikra et al. 2021). Briefly, ground seaweed samples (n=3) were crushed and taken through a 0.5 mm sieve. The digestion was performed in a special combustion unit at 1100 °C and burned in an argon/oxygen stream. The resulting gases were measured on a Metrohm Model 883 Plus ion chromatograph. The lower limit of detection was 1 ppm. Two analytical replicates were taken from each sample. Results are reported on a dry matter basis, i.e., accounting for any differences in humidity content of the dried samples.

Dry matter analysis

The dry matter content in dried kelp samples was determined based on previously described methodology (NMKL 1991), but using a sample weight of 1.03±0.25 g and drying at 100 °C for 18 h prior to reweighing. The residual water content was calculated as a percentage of the samples' initial mass.

Statistical analysis

Analysis of variance (ANOVA) was performed to test for significant differences between sample groups, using Minitab version 19.2020.1 and a 95% confidence interval. A Tukey post hoc test was applied when more than two sample groups were present. The results are given as average ± sample standard deviation.

Results

No significant changes in total iodine during stressful storage.

The iodine contents after drying were lower in stored samples compared to unstored samples, but no statistically significant differences were found (Table 2). The lowest iodine content (2100±700 mg kg^{-1} dw) was found in samples from tank B, which were stored under low light intensity (~15 µmol photons m^{-2} s^{-1}) with low turnover of seawater (Table 1). The highest iodine content was found in samples which were not stored (3300±1400 mg kg^{-1} dw).

Light stress prior to processing could reduce the subsequent iodine content

Samples which were boiled after storage for 3 days (tank A and C) contained less iodine after processing than samples

Table 2 Iodine and residual water content in samples after storage and processing. Results are reported as average ± standard deviation

	Not stored (n=6×2)	Stored in tank (n=3×2)		
		A	B	C
Unprocessed				
I (mg kg^{-1} dw)	3300±1400^{A}	3200±500^{A}	2100±700^{A}	2600±300^{A}
water (%)	8.1±0.4^{B}	8.7±0.7^{AB}	8.8±0.1^{A}	9.2±0.5^{A}
Processed				
I (mg kg^{-1} dw)	600±100^{A}	500±100^{AB}	n.a.	400±100^{B}
water (%)	12.1±0.5^{B}	12.8±0.1^{A}	n.a.	11.9±0.4^{B}

Capital letters indicate significant differences within rows

dw: dry weight; n.a.: not analyzed

which were boiled at day 0, although the iodine levels were not different before processing. The difference was only found when comparing unstored samples to samples stored in tank C (tank B was not assessed for this part of the experiment). Unstored samples contained 600±100 mg kg^{-1} iodine post-processing, whereas samples stored in tank C contained 400±100 mg kg^{-1} iodine, constituting losses of 80 and 87 % iodine during processing, respectively (dw basis).

Discussion

Previous studies have found that kelp release iodine – an inorganic antioxidant – during exposure to stress (Küpper et al. 2008). However, this has until now not been exploited during processing of kelp for food or feed applications. In the present pilot study, we attempted to stress kelp samples with the aim of lowering the iodine content of processed biomass. The stressors we used were a high light intensity (tank C) and a low turnover of seawater (tank B). As outlined above, no significant changes between not stored samples and samples stored in tanks were found after storage in stressful conditions alone. This finding is in accordance with those of a recent master thesis, which found no efflux of iodine after exposure of kelp to light stress (Haughom 2022). However, after subsequent processing (boiling), the iodine content of stressed samples (tank C) was significantly lower than the iodine content of samples processed without intermediate storage. It thus seems that it is possible to apply inherent stressors to reduce the iodine content in kelp after processing.

Although the differences in iodine content of stressed versus unstressed kelp found in our experiment were small, it might be possible to design experimental set-ups where larger iodine reductions can be achieved. Moreover, we did not subject samples from tank B to a subsequent blanching treatment. Samples in tank B received a slightly lower turnover of seawater than tank C, and those samples also had the lowest iodine content after storage. Low turnover likely resulted in less available iodine for uptake by the kelp specimen, and might also add a type of oxidative stress, since less oxygen was supplied. Samples of tank B had, surprisingly, a lower (although not significant) iodine content than tank C and were not further processed. However, processing of samples stored at low turnover should be followed up in later experiments.

Possible mechanisms involved

As to the mechanism of improved iodine reduction during subsequent processing after storage in tank C, one might speculate that one of the following occurred:

1. The chemical speciation of iodine changed

The I^- content in *S. latissima* as a percentage of total iodine has been measured previously to be 93 %, both in fresh, frozen specimen and in fermented specimen, as outlined in a project report (Stévant et al. 2021). The same work reported that other iodine species were below the level of quantification. For its relative (same genus) *Saccharina japonica*, similar I^- fractions (88 and 94 %) have been documented (Hou et al. 1997; Shah et al. 2005), whereas 10 % was found to be organically bound (Hou et al. 1997). Our previous meta-analysis of I^- content and reduction of total iodine during processing showed a clear linear correlation (R^2=0.95) of increasing loss of total iodine with increasing relative I^- content (Blikra et al. 2022a). Thus, if the relative I^- content increased during exposure to stress, this could generate an increased efflux of I^- during processing.

2. The intermediate storage location of iodine changed

As for storage location, most of the iodine is stored in the apoplast in external tissue (the meridosterm) and thus should be available for release during stress (Verhaeghe et al. 2008). Some iodine has also been found in more internal tissue (the cortex and medulla). Thus, a possibility for a shift in iodine location also exists, and a shift to a location allowing more efficient (passive or active) transport of I^- could occur.

3. Components involved in the efflux of iodine were upregulated

A third option could be upregulation of enzymes involved in efflux of iodine, which in turn, would facilitate loss of iodine during processing. Haughom (2022) found that exposing *S. latissima* to light stress led to upregulation of proteins presumed to be vanadium-dependent iodoperoxidases (vIPO), which are enzymes involved in stress response in brown algae. These enzymes have also been previously identified in extracts from *S. latissima* (Almeida et al. 2001). Details regarding mechanisms of iodine efflux are still lacking (Katsaros et al. 2021), however, one proposed stress response involves catalysis by vIPO (Almeida et al. 2001). The activity of vIPO from *S. latissima* was found to be dependent on temperature, and was highest from 40-50 °C, markedly lower at 60 °C, and apparently not active above 70 °C (Almeida et al. 2001). Thus, it seems unlikely that upregulation of vIPOs was the primary mechanism involved in our study, where boiling temperatures were applied. However, a possibility of further iodine reduction during blanching at 40-50 °C after upregulation of these enzymes through stressors exists. Further research is needed to investigate the mechanisms at play, and following that, maximize their impact on iodine reduction.

Significance for future research

These findings should be kept in mind during the design of new experiments where storage of samples in tanks with seawater and artificial light is necessary, and where the iodine content is of importance. We suggest to randomize the experimental set-up as much as possible in such studies, and to collect control samples both at the beginning and end of the storage period, to observe for any effects.

Conclusion and further research

This study demonstrates that it is indeed possible to utilize inherent stressors to reduce the iodine content in the commercial kelp species *S. latissima*, although it is not as straight forward as one might imagine. Intermediate storage in high light intensities improved iodine reduction during a subsequent blanching treatment. The mechanisms remain unknown, although one might speculate that changes in iodine speciation or intra-/extracellular location play a role. Although scientifically interesting, and important for further planning of experiments where intermediate storage is necessary, it is not easy to extrapolate the findings to industrial practice. Further research should optimize the storage conditions for further reductions in iodine content, while simultaneously investigating the possibility of reducing the storage time and seaweed to water ratio, thus increasing both yield and efficiency of the method. If the mechanisms at play are elucidated, it could accelerate such studies. Furthermore, later studies should investigate whether adding an inherent stressor (e.g., high intensity light or storage in seawater with low turnover) can lead to reductions in either temperature, time, or water usage of subsequent processing, while achieving a sufficiently low iodine content, and by that allowing for reductions in total costs. If successful, live intermediate storage in stressful conditions could prove to be a possible solution for industry aiming to reduce the iodine content of their harvest, thus allowing for safe consumption of larger amounts of kelp. The impact of this would be an opportunity for the food industry to increase the use of kelp as ingredient from a permille level to a percent level and thus kelp farmers might ten-fold their production.

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Author contributions Both authors contributed to research ideas and method, as well as laboratory work and funding acquisition. M.J.B. wrote the main manuscript text, performed statistical analysis and prepared Tables 1 and 2, as well as Fig. 1. Both authors reviewed the manuscript.

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Data availability The data supporting the findings of this study are available upon request.

Declarations

Competing interests The authors declare no competing interests.

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