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Preface

- Seaweed is gaining popularity and economic importance as an ingredient in (novel) foods and is now fairly commonly consumed across all population strata in the Netherlands. Moreover seaweed may also provide a sustainable source of plant-based protein for human and animal provisioning.
- Food safety of seaweed is an important condition for bringing seaweed-containing food products on the market. However, previous studies have shown that seaweeds can accumulate high concentrations of iodine and heavy metals, although there is high variation among seaweed species, geographic location, harvesting season, seaweed metabolic activity, and cultivation method.
- Better understanding of the effect of processing steps during the food production on the fate of iodine and heavy metal concentrations in seaweed can help to reduce/remove the exposure of consumers to these contaminants due to seaweed consumption. Therefore, an assessment of the changes in iodine and heavy metal concentrations in response to sequential processing steps, such as washing, freezing, blanching, drying, and/or fermenting is necessary to identify those processing steps with the most potential to reduce contaminant concentrations in the product.
- The aim of this study (PROCESS I) was to assess several production chains of seaweed-containing food to identify the main processing steps and their potential to reduce concentrations of iodine and heavy metals in the product. This project does not cover other seaweed based health hazards nor the effect of processing steps on the beneficial qualities, taste and structure of the seaweed.
- The results of this study showcase processing steps with good potential to reduce iodine and heavy metal contaminant levels. Moreover, they help select the choice of processing steps and treatment ranges of the intended follow-up study (PROCESS II).

Summary

Seaweed is now relatively commonly consumed across all population strata in the Netherlands and may provide a sustainable, plant-based protein source for humans and animals. Previous studies have revealed that seaweeds can accumulate high concentrations of iodine and heavy metals, although with high variation among seaweed species, geographic location, harvesting season, seaweed metabolic activity, and cultivation method. Food safety of seaweed is an important condition for bringing seaweed food products to the market. Finding ways to reduce the concentrations of iodine and other contaminants in seaweed containing products is therefore desirable. The aim of this study was to identify commonly used processing steps in the seaweed value chain and their potential to reduce concentrations of iodine, arsenic and heavy metals (nickel, cadmium, mercury, lead, and bromine) in the intermediate or final product. We first provide a short literature review of the current knowledge on iodine and heavy metal contaminations in seaweed and how the concentrations of these contaminants are affected by commonly used processing steps. We then report the results of a pilot study assessing changes in iodine and heavy metal concentrations along a series of processing steps in seven food or feed production chains, utilizing the green seaweed *Ulva lactuca* (in three production chains) or the brown seaweed *Saccharina latissima* (in four production chains) as starting products. The sequential processing steps we cover fall into roughly two categories: washing (washing, blanching, rehydrating dried seaweed) and structural disintegration (fermenting, drying). Our literature search highlighted that concentrations of iodine and heavy metals in seaweeds vary widely with seaweed species, but also with season, growth conditions and age of the plant tissue. Overall, washing and related processing steps (washing, soaking, blanching, rehydrating) are generally the most promising procedures to reduce the mobile fraction of iodine and heavy metals in the biomass. Combining these washing steps with additional heat treatment or destruction of cell walls may increase the efficiency of contaminant removal further. The results of our pilot support these findings as we find large differences in elemental concentrations among the three species tested (*Ulva* sp., *Saccharina* sp., and *Palmaria* sp.). Moreover, rehydration of dried seaweed (observed in four processing chains) generally resulted in reduced elemental concentrations (expressed as mg kg^{-1} dry weight) in the rehydrated biomass. Unexpectedly, we also observed increases in elemental concentrations that may suggest methodological artefacts (when measuring close to the limit of quantification), or contaminations by other, added ingredients (e.g., in dough mixtures), or remain unexplained. The results of this study can be used to determine the most processing steps with most potential for contaminant reduction and, moreover, will inform the choices of processing steps and treatment ranges of the intended follow-up study (PROCESS II).

1 Introduction

In this report, we present a short overview on the current knowledge on the consumption of seaweed in the Netherlands, the commonly observed elemental contaminants in seaweeds and the effects of commonly used processing steps on the reduction of elemental contaminants such as Iodine, Arsenic and heavy metals. This overview is followed by seven case studies aimed at assessing processing steps and their effects on elemental concentrations in the seaweed, intermediate products and final products.

1.1 Seaweed and contaminants in seaweed

1.1.1 Seaweed and seaweed consumption in the Netherlands

Seaweed is an umbrella term that covers several taxonomic groups of marine, macroscopic photosynthetic algae. Based on their pigmentation, seaweeds are commonly classified into brown (Phaeophyceae), green (Chlorophyceae) and red (Rhodophyceae) seaweeds. However, there are considerable morphological and functional differences within and between these seaweed groups (Holdt & Kraan, 2011). There are more than 12000 species of seaweed, of which ca 220 are considered of commercial value (FAO, 2018). The supply of seaweed comes either from wild stocks or from aquaculture. Global seaweed production in aquaculture has increased steadily to 34.7 million tonnes in 2016 (including some microalgae production (FAO, 2018)), while harvesting of wild stocks has remained around 1.1 million tonnes (FAO & WHO, 2022). Only about 10 seaweed species are cultivated intensively, with the brown algae *Saccharina japonica* and the red algae *Eucheuma* sp accounting for 66% of the global production in 2015 (Campbell et al., 2019). The major producers of cultivated seaweeds are China, Indonesia and further Asian countries, while European production is still emerging and contributes ca 1% to the global production (FAO, 2018; FAO & WHO, 2022).

Human consumption of seaweed can either be direct (seaweeds or food products containing seaweeds) or indirect (food products containing seaweed-based extracts such as alginate or carrageenan). Direct consumption of seaweeds has historically been most relevant in Asian countries, but has been increasing world-wide in recent years (Dawczynski et al., 2007; Food Safety Authority of Ireland (FSAI), 2020). Also in the Netherlands, the direct consumption of seaweed is now quite common across all socioeconomic strata and age groups of the population (Dinnissen et al., 2021). A survey by the RIVM detected that almost half of the respondents occasionally consumed seaweed in the form of (novel) food products containing fresh or dried seaweed, and the median daily intake in seaweed users amounted to 0,05 (95%-CI 0,04-0,06) g wet weight kg^{-1} human bodyweight, resulting in about 4 g d^{-1} for an 80 kg person (Dinnissen et al., 2021). In comparison, daily per capita seaweed consumption is estimated at 4 g d^{-1} in Japan, 5.2 g d^{-1} in China, and 8.5 g d^{-1} in South Korea (Roleda et al., 2019). In the Netherlands, in addition to more established food products containing seaweed such as sushi, there is now a wide range of novel(ty) food products available that are based on or contain seaweed, ranging from highly visible examples such as seaweed meat replacers and seaweed pasta, to products with seaweeds as flavouring agent such as seaweed cheese, seaweed mayonnaise, seasoning mixes, and even beers and liquor with seaweed (Banach, Koch, et al., 2022; van den Burg et al., 2021).

Seaweeds also have a long history of use as livestock feed in the form of fodder of meal, particularly in coastal regions (FAO & WHO, 2022; Makkar et al., 2016). Generally, green and red seaweeds contain higher protein ratios but lower mineral ratios than brown seaweeds, although the specific composition strongly depends on time and location of harvest as well as on the environmental conditions during the growth phase (Banach et al., 2020). Due to their relatively high protein contents, complex carbohydrates and the presence of polyunsaturated lipids, seaweeds in animal feed can contribute to the nutrient, protein and energy demands of livestock (Makkar et al., 2016). Moreover, prebiotic compounds produced by seaweeds (complex carbohydrates) have been shown to contribute to gastro-intestinal health and improve immune status of monogastric livestock, including pigs, chicken and fish (FAO & WHO, 2022; Makkar et al., 2016).

Supplementation of livestock diet with red or brown seaweeds can substantially reduce methane emissions in cows (Lean et al., 2021). The red seaweed *Asparagopsis taxiformis* has been highlighted as beneficial in this regard (Kinley et al., 2020), although there are yet challenges to be overcome as, for example, the active compound (bromoform) is known to be toxic and may be transferred to milk in lactating cows (Muizelaar et al., 2021).

Given the need to feed a growing world population and to mitigate the effects of climate change on food production, seaweed is perceived as a sustainable crop with a potential to provide blue carbon and be part of the protein transition (Banach, van der Berg, et al., 2022; FAO & WHO, 2022). Generally, seaweed does not directly transfer carbon to the marine sediments, hence it remains unclear how large the contribution of increased seaweed farming can be on net carbon sequestration (Bindoff et al., 2019). However, seaweed farming or ocean afforestation may contribute to the mitigation of carbon emission when employed as alternative energy source, although full lifecycle analyses would be needed to assess upscaled, real-world net effects (Bindoff et al., 2019). Moreover, (offshore) seaweed cultivation can promote healthy ecosystems by taking up nutrients (and thereby reduce the eutrophication levels in e.g. the North Sea), by providing habitat for invertebrates and young fish (thereby increasing biodiversity) and by oxygenating water and increasing the pH (thereby counteracting acidification), although intensive farming can also have negative ecological impacts due to strong nutrient competition, increased traffic and risk to animals and traffic formed by the seaweed cultivation installations themselves (Tonk & Jansen, 2019). Protein content varies widely across seaweed species and ranges from 0.7 to 45% of its dry weight, with red seaweeds showing the highest protein contents (Banach, van der Berg, et al., 2022; Cherry et al., 2019). While these protein contents are comparable to those of beef, the typical amounts of seaweed consumption are comparatively rather small (Cherry et al., 2019). Moreover, the digestibility of proteins of raw seaweed is variable (Cherry et al., 2019), in some species it can be even be relatively low and pre-processing before consumption may be necessary to increase the bioavailability of these proteins (Juul et al., 2022).

1.1.2 Contaminants in seaweed

Seaweeds have a documented capacity to accumulate minerals and potentially harmful contaminants, among which also iodine, arsenic and heavy metals (FAO & WHO, 2022), warranting a need to better understand potential food and animal feed safety hazards of seaweeds (Banach et al., 2020). In seaweeds, the concentration of iodine and, to some extent of heavy metals, varies with seaweed species, geographic location, harvesting season, seaweed metabolic activity, and cultivation method (Cherry et al., 2019; Karthick et al., 2012). Due to their capacity to accumulate metals, seaweeds have also been used as sentinel species to assess metal pollution in estuaries and coast lines (Phillips, 2018) and are assessed as bioremediation agents to remove heavy metals from polluted locations (Znad et al., 2022). In general, heavy metal concentrations in seaweeds depend on the environmental concentrations of these elements and on the abiotic environmental conditions (e.g. salinity, light, temperature, etc), and on structural differences among seaweeds (Malea et al., 2015) that affect uptake capacity and storage of metals (Besada et al., 2009). Overall, green seaweeds have a lower metal-binding capacity than brown seaweeds; and metal concentrations in seaweed tissues are lower during the active growth phase in summer and higher in periods of low metabolism in winter (Besada et al., 2009).

While there is European Union legislation regulating the maximum amounts of some of the heavy metals in seaweeds used in or as feed (organic and total arsenic, cadmium, mercury and lead) and in food supplements made mainly of seaweed (cadmium, lead and mercury), there is no European or Dutch legislation regarding food safety of seaweeds or definition of threshold concentrations for consumption-safe seaweed (FAO & WHO, 2022).

1.1.2.1 Iodine

Seaweeds, in particular the brown ones, are known to accumulate the halogen iodine (FAO & WHO, 2022). In seaweeds, iodine is involved in several functions, among which it supports seaweed immunity, osmoregulation and acts as an antioxidant (Küpper et al., 2008). As antioxidant, it protects seaweeds from high light intensities during low water or during air exposed periods. Hence exposure to such stress conditions trigger an active efflux of iodine from the seaweed into the water or the air to detoxify reactive oxygen species (Nitschke et al., 2015).

Iodine is an essential element for human health, associated with the function of the thyroid hormones thyroxine and triiodothyronine. Iodine is mostly acquired through the consumption of foods naturally rich in iodine (e.g., seafood, dairy, eggs, grain products) or fortified with iodized salt (Fuge & Johnson, 2015). An iodine deficient diet can impact human health through dysfunction and/or enlargement of the thyroid gland. On the other hand, excess consumption of iodine can also affect human health, particularly so in persons with risk factors (e.g., pre-existing thyroid conditions) where it can cause thyroid dysfunction (Leung & Braverman, 2014; Miyai et al., 2008). However, the bioavailability of iodine differs across groups and species of seaweeds and can be as low as 2% in raw *Ulva* or 28% in raw *Laminaria* (Cherry et al., 2019). But in an in vitro study, about 49–82% of seaweed iodine appeared to be accessible for absorption by humans after gastrointestinal digestion (Domínguez-González, et al., 2017). These differences in bioavailability and bioaccessibility may be due to matrix effects with iodine bound to e.g., proteins, polysaccharides, or polyphenols which may result in limited liberation of iodine species, limited solubility or limited absorption of iodine (Cherry et al., 2019). Furthermore, there are speculations about the effect of goitrogenic foods that are traditionally consumed together with seaweeds in many Asian cuisines, although the reduction of iodine uptake due to the inhibitory effect of e.g. soy and cruciferous vegetables has not yet been documented successfully (Zava & Zava, 2011).

Iodine concentrations are generally higher in brown seaweeds than in red or green ones (FAO & WHO, 2022; Hou & Yan, 1998). While the measured iodine concentrations can reach a few thousand mg kg^{-1} dry weight in brown seaweeds (Roleda et al., 2018), they often remain below 200 mg kg^{-1} dry weight in red and green seaweeds (Banach et al., 2020). In a study on Irish seaweeds, iodine content in red seaweeds (56-1530 mg kg^{-1} dry weight) was about 10 times lower, and in green seaweeds (41-79 mg kg^{-1} dry weight) even about 100 times lower than in the brown seaweeds (1734-10203 mg kg^{-1} dry weight; (Nitschke & Stengel, 2015)). Moreover, the iodine content is highly variable even within one species. Moreover, iodine content of *Saccharina* depends on cultivation location and method, with *Saccharina* grown in lower salinity water showing also lower iodine concentrations (Lüning & Mortensen, 2015). Iodine content also varied with season, for example, iodine concentrations in the brown seaweed *Sargassum kjellmanianum* were about three times higher in March than in December or May (Hou & Yan, 1998). Also a study of Irish seaweeds documented pronounced seasonality in iodine contents with above average values in winter and below average values in summer (Nitschke et al., 2018). Moreover, iodine contents can additionally vary with physiological stress as well as with size and age of the specimen (summarised in (Nitschke & Stengel, 2015)), whereby oxidative stress induced by air exposure, high irradiance or bacterial infestation causes an efflux of iodine from the seaweed and thus can lead to a lower iodine content (Nitschke et al., 2015).

1.1.2.2 Total and inorganic arsenic (As)

Arsenic can occur in several forms, both organic and inorganic, whereby the inorganic form is generally considered as the greater public health concern. Some of the inorganic arsenic species are classified as a known human carcinogen by the EPA (Hughes, 2002) although the exact mechanism of arsenic carcinogenicity seems not yet to be understood. Arsenic species in the marine environment have natural as well as human sources. Anthropogenic arsenic sources include mining activities, and the use of pesticides and wood preservatives. In seawater, arsenic predominantly presents as arsenate and occurs generally in the range of a few µg L^{-1} (Lin et al., 2021; UNEP, 1988). Additionally low concentrations of the organic arsenic species monomethylarsonic acid (MMA) or dimethylarsinic acid are present through excretion from marine organisms (Lin et al., 2021). Arsenic can accumulate in seaweeds with concentrations up to 150 mg kg^{-1} in dry products (summarised in (Cheyns et al., 2017)) or up to concentrations 2000-5000 times greater than the surrounding seawater (UNEP, 1988). In the brown seaweed *Hizikia fusiformis*, in particular, high inorganic arsenic concentrations have been reported, ranging from 30 to 117 mg kg^{-1} dw (summarised in (Cheyns et al., 2017), see also (Besada et al., 2009)).

In higher plants, physiological studies have shown that arsenate and phosphate share the same transport pathway through strongly conserved, transmembrane phosphate transporters involved in the acquisition of inorganic phosphorus from the environment (Wang et al., 2017). To our knowledge, there are no physiological studies on the presence of these phosphate transporters in seaweeds, but given the highly conserved structure of PHT's across higher plants (Victor Roch et al., 2019), and their structural similarity to phosphate transporters in yeasts (Wang et al., 2017), it is likely that the phosphate, and hence also arsenate, uptake in seaweed also occurs through transporters of the PHT family. Uptake of arsenite occurs

mainly under anoxic conditions and therefore may play a smaller role in seaweeds cultivated in well oxygenated waters. In addition to phosphate-dependent uptake pathways, there are also reports of phosphate-independent uptake of arsenate, suggesting the presence of additional, other uptake mechanisms in (macro-) algae (al Mamun et al., 2019). In higher plants, once taken up, arsenate is reduced to arsenite, methylated to MMA and finally presents as (non-toxic) arsenosugars (Zhao et al., 2010). This detoxification pathway can, however, be disturbed at too high environmental arsenic concentrations or under low environmental phosphorus concentration conditions. Increased environmental phosphorus concentrations decrease accumulation of inorganic arsenic in seaweed although the underlying mechanism is not yet understood (Lin et al., 2021). Seaweeds can detoxify arsenate, but when exposed to high levels of inorganic arsenic, their biotransformation capacity may reach a limit and the inorganic arsenic accumulates in the organism (Cheyns et al., 2017). Arsenic disturbs ATP production and hence the plants' energy metabolism through multiple pathways. Amongst other pathways, arsenate competes with phosphate and uncouples the oxidative phosphorylation, inhibiting mitochondrial respiration and ATP synthesis, resulting in ATP depletion and cell necrosis (Hughes, 2002).

1.1.2.3 Nickel (Ni)

Nickel is a heavy metal and a significant contaminant in sediments of industrialised areas. In low concentrations, it is an essential trace element that is required in the plant nitrogen metabolism, however, excess nickel has adverse effects on key metabolic processes in plants, causing reduced plant health and population growth (Parwez et al., 2021). In animals and humans, nickel is not considered an essential micronutrient. Nickel is considered an immune system sensitiser and may lead to hypersensitivity reactions (EFSA, 2020). Short-term repeated dose toxicity tests in rodents and dogs showed decreasing bodyweight and changes in kidney and liver histopathology and consistent evidence of developmental toxicity in rats (EFSA, 2020). Some forms of nickel have been classified as a human and animal carcinogen by the IARC. Nickel concentrations in seaweeds are rarely measured, hence there is also not a lot of information available on the role, distribution or variability of this metal in seaweeds.

1.1.2.4 Cadmium (Cd)

Cadmium is a heavy metal which occurs as an environmental contaminant naturally but predominantly due to anthropogenic activities (Banach et al., 2020). Excess cadmium seems to damage and reduce the number of chloroplasts, leads to disintegration of cell walls and eventually to the death of algal cells resulting in reduced population growth (Lamaia et al., 2005). In humans and animals, long-term exposure to cadmium targets kidney function and the calcium metabolism. The IARC classifies cadmium and cadmium components, particularly when inhaled, as cancerogenic. Seaweeds have been shown to absorb cadmium fast and efficiently (Mouritsen, 2013) and several studies have observed cadmium in seaweeds. The observed concentrations range from below detection limit to 9.8 mg kg^{-1} dry weight (summarized in (Banach et al., 2020)). Cadmium contents vary among seaweed species, season of harvest and geographical origin, with potentially higher cadmium accumulation in red seaweeds than in brown ones (Banach et al., 2020; Chen et al., 2018).

1.1.2.5 Mercury (Hg)

Mercury is a metal that occurs as environmental contaminant with natural and anthropogenic sources. It presents in several forms in the marine and oceanic environments (Gworek et al., 2016), with methylmercury being a commonly found form of organic mercury in the food chain, particularly so in marine products (Banach et al., 2020). Mercury poisoning hampers the formation of antioxidants and therefore leads to oxidative damage in the central nervous system as well as that of the intestines and kidneys, resulting in morbidity, and in long and/or high exposure cases to mortality. Environmental methylmercury can be produced by abiotic as well as biotic processes. Methylation of mercury is favoured in, among others, low oxygen environments and in presence of sulphate-reducing bacteria (Gworek et al., 2016). Occurrence of mercury in seaweeds has been reported by several studies, whereby mercury seems to accumulate more in brown seaweeds than in red ones (Chen et al., 2018). Measured concentrations of total mercury range up to 0.05 mg kg^{-1} dw (summarised in (Banach et al., 2020)) and vary with seaweed group and species as well as with geographic origin of the seaweed (Banach et al., 2020).

1.1.2.6 Lead (Pb)

Lead is a heavy metal which is found in marine environments due to natural sources and human activities. Several studies report bioaccumulation of lead in seaweeds (summarised in (Banach et al., 2020)). Observed concentrations vary among seaweeds, the highest observation reported in Banach et al (2020) being 6.7 mg kg^{-1} dw in an *Ulva lactuca* sample. While the variation in lead concentrations is not conclusively linked with seaweed group, it seems to depend on season of harvest and geographic origin with higher metabolic activity in summer potentially leading to higher accumulation of lead (summarised in (Banach et al., 2020)). In algae, lead exposure seems to damage and reduce the number of chloroplasts and to disintegrate cell walls, resulting in death of algal cells and hence reduced population growth (Lamaia et al., 2005). In animals including humans, exposure to lead causes irreversible health effects. Although the mode of action is not fully understood yet, lead exposure leads to oxidative stress and affects the functioning of the central nervous system, kidney and liver as well as the formation of blood cells, leading to serious morbidity and in extreme cases to mortality (Flora et al., 2012).

1.1.2.7 Bromine (Br)

The halogen bromine is an essential trace element in animals where it is involved in the formation of collagen IV. It naturally occurs as Br- (bromide) in seawater. Human produced brominated chemicals (e.g. flame retardants, pesticides, fumigants) have been detected in seawater and sediments (Covaci et al., 2011). Seaweeds are known to accumulate bromine. They naturally produce a range of organohalogens and reduced forms (e.g. Br-) that serve as antioxidants or antibacterial agents (Leri et al., 2019). A study of total bromine contents in 10 seaweeds reported values from 12.4 - 972.1 mg kg^{-1} dry weight with the brown seaweed *Saccharina latissima* (*Laminaria saccharina)* showing the highest values, whereby the in-vitro digestible proportion ranged from 10-47% (Romarís–Hortas et al., 2011). Another study (Leri et al., 2019) reported between 120-350 mg kg^{-1} dry weight aromatic organobromine, again with a brown seaweed showing the highest value. However, Hou and Yan (1998) reported higher total bromine accumulation in red seaweeds than in brown ones.

1.2 Seaweed in food and feed products and as animal protein alternatives

1.2.1 Products and common processing steps

Seaweed consumption by humans has a long history. Traditionally, seaweeds were particularly prominent in the Asian cuisines. Humans consume seaweed both directly or indirectly. Indirect consumption occurs mainly through food products in which refined, seaweed-based products are used to improve the appearance and texture of the product. For example, red and brown seaweeds produce a range of phycocolloids (e.g., alginates, agar agar) that are used as stabilisers, texturing agents and emulsifiers in the food-processing industry (Bixler & Porse, 2011). Direct consumption includes consumption of the seaweed itself and as an ingredient in the food product such as dried seaweed snacks, sheets of dried nori for sushi, or seaweed flakes in cheese and burgers. Due to the high mineral and protein contents of seaweeds, many of these products also carry a health benefit claim (Lozano Muñoz & Díaz, 2020). Moreover, seaweeds are also used as feed additives in livestock and companion animals (Makkar et al., 2016). Products include refined proteins as well as seaweed based supplements for improving digestion and for supplementation of minerals.

Commonly used post-harvest processing steps can be roughly divided into two approaches: washing and changing the structural integrity. Washing includes all processing steps that (apart from other beneficial functions) clean the seaweed and dilute the water-soluble compounds / elements in the seaweed tissue. This includes washing and soaking in saltwater, freshwater or deionised (DI) water at room temperature; blanching in warm to hot water or steam often followed by rapid cooling to halt the cooking process; cooking; and rehydrating (soaking) of dried seaweed. Processing steps that change the structural integrity of the seaweed change the surface-to-volume ratio of seaweed pieces and/or change the tissue permeability for water mediated exchange of elements by, for example cutting, freezing, drying (including freeze-drying), fermenting as well as carbohydrase steps to break up the cell walls. Some washing related steps can also cause structural changes, for example, osmotic shocking is used to lyse cells through rapid dilution. All the

mentioned processing steps can serve multiple aims and are often combined sequentially to achieve the desired product texture and taste as well as to achieve a longer shelf life.

1.2.2 Effects of common processing steps on contaminant levels in seaweed

1.2.2.1 Washing related steps

Generally, washing related steps clean off periphyton and other organisms colonising the seaweed surface. Moreover, washing in water of a higher osmotic potential (fewer solutes in water) will lead to osmotic exchange whereby water soluble elements will leach out of the seaweed tissue into the surrounding water (e.g. (Hou et al., 1997)). While dilution of the iodine and heavy metal contaminants is obviously desired, washing can also have undesired effects if it also dilutes the water-soluble beneficial compounds in the seaweed or changes its tase. In the following, literature results for different washing processing steps are presented, and, depending on the available information, split into the here assessed elements.

Washing / soaking of fresh seaweed

Iodine: Washing and soaking of fresh seaweed seems to give variable outcomes. While one study reports that soaking *Saccharina* at 32 °C for 1-6 h reduces iodine content by 84 % to 88 % (Stévant et al., 2018), another study doesn't see significant reduction in iodine contents in three seaweed species after soaking for 10 min in deionised (DI) water (Nitschke & Stengel, 2015). Soaking in DI water and stirring combined and repeated three times, was shown to leach over 99 % of the water-soluble iodine from the brown seaweed *Laminaria japonica* and between 17-41 % from 6 other seaweed species (Hou et al., 1997).

Blanching / boiling

Iodine: Boiling and blanching (soaking of fresh seaweed in water at a temperature between 30-90°C) generally reduces iodine concentrations in seaweeds. For example, boiling *Saccharina* for 2-20 min reduces iodine by up to 33 %-75 % (Lüning & Mortensen, 2015; Nitschke & Stengel, 2015), and boiling kelp for 20 min reduced iodine by ~90 % (Chung et al., 2013). Similarly, a test of various combinations of blanching temperatures and durations found that blanching at ≥45 °C and ≥30 sec significantly reduced iodine without reducing protein contents and quality.

Arsenic: Boiling also affects arsenic concentrations, in particular water soluble arsenic species. Total arsenic was reduced by up to 75 % by blanching at 90 °C for 5min in dried brown seaweed *Hizikia fusiformis* (Park et al., 2019), while boiling dried *Hizikia fusiformis* 3-4 times reduced total arsenic concentrations by up to 92 % (Yamashita, 2014). Soaking and subsequently boiling *Hizikia fusiformis* reduced inorganic arsenic to 10-60 % of the original concentrations (summarised in (Ho & Redan, 2022)). Note that these examples didn't document whether dry weight or wet weight is reported. In some species, however, boiling didn't change the concentration of total arsenic, as for example boiling *Porphyra* did not affect total arsenic concentrations, although it did lower concentrations of other metals (Cheyns et al., 2017).

Rehydration of dry seaweed

Iodine: Soaking of previously dried or freeze dried seaweed seems to reduce iodine substantially. One study reported iodine reduction between 10 %-62 % following soaking of dried seaweed in DI water for 1-24 h, although the reduction depended on the species (Nitschke & Stengel, 2016). Unfortunately, this study didn't indicate the water to seaweed ratio or the water content of the seaweed after rehydration. Similarly, 5 min of rehydration in an abundance of water and subsequent separation of the seaweed biomass from the soaking liquid was shown to reduce the concentrations of a range of minerals including iodine, although the reduction efficiency depended on the seaweed species (Correia et al., 2021).

Arsenic: Soaking dried seaweed *Hizikia fusiformis (*i.e. *Sargassum fusiforme)* in 2 % NaCl water for 20 min reduces total arsenic up to 55 % (Park et al., 2019), whereas soaking in distilled water at 20 °C for 30 min reduced total arsenic up to 31 % or less (Yamashita, 2014). Note that these reductions are most likely reported in wet weight for the soaked samples.

1.2.2.2 Structural changes steps

Cutting
No specific information on the effect of cutting as a stand-alone procedure was found in the literature.

Drying / freeze-drying
Due to the water solubility of many iodine species, drying may evaporate part of the iodine content in seaweeds (Banach et al., 2020). Higher temperature during the drying process may result in incrementally higher iodine loss, with freeze-drying showing the largest effect in a study of limited sample size on *Saccharina latissima* (Stévant et al., 2018). However, this effect is not reported consistently. For example, air drying and freeze-drying doesn't seem to affect the iodine content of seaweeds (Nitschke & Stengel, 2016).

Freezing
Freezing and subsequent thawing showed variable effects. While some studies report no reduction of iodine after freezing and thawing (Nielsen et al., 2020), others reported substantial leaching of minerals into the drip loss liquid after freezing (Sund, 2020).

Fermentation
A combination of heat treatment and lactic acid bacteria fermentation reduced the mineral content in *Saccharina latissima*, notably it reduced cadmium by 35 % and mercury by 37 % in comparison to the fresh seaweed but increased lead by 11 % (Bruhn et al., 2019).

Carbohydrase
Carbohydrase or other enzyme assisted extraction methods aim to break the cell wall and allow better extraction of solid cell compounds while separating these from the liquid fraction of the cytosol. Hence the expectation is that carbohydrase can help to remove the leachable fraction of the minerals and improve the extractability of proteins or other compounds of interest (Shanura Fernando et al., 2018).

Electroporation
Electroporation of the cell wall causes leakage of intracellular liquids and other dissolved substances from the seaweed and is achieved by the relatively recently developed process called pulsed electric field processing (Blikra et al., 2022). This process has been shown to reduce concentrations of iodine by 40 % and of mercury by 19 % (Blikra et al., 2022).

Combining steps
Several studies show report changes in elemental composition of seaweeds along a chain of processing steps. For example, Nitschke and Stengel (2015) analysed iodine concentrations in freshly harvested green, red and brown seaweed species, as well as after washing, after drying for 72 h, after rehydration for 24 h and after boiling for 20 min. While washing and drying only showed insignificant reductions in iodine, rehydration and boiling reduced iodine concentrations by up to 75 %. While such evaluations of processing steps along the usual order of processing steps as used in the industry are obviously valuable, they do not allow assessing whether the effect of one step may be contingent on the previous step. For example, the effect of rehydration may be improved due the structural disintegration provided by the drying step and therefore make the drying step valuable even if it does not change the iodine concentrations by itself. To assess such contingency effects, assessments would need to be performed using a multifactorial experimental design rather than a scenario design.

1.3 Research question and approach used in this study

1.3.1 Research questions and approach

Here we followed up on the processing steps of six seaweed-containing food products and one animal feed product by Dutch producers to catalogue

a. which processing steps the seaweed undergoes from harvesting of the fresh plants to the final product, and

b. how these processing steps affect the concentrations of following contaminants: iodine, arsenic (total and inorganic), nickel, mercury, lead, cadmium, and bromine along the processing chain.

Ideally, this will allow highlighting which processing steps are particularly promising in reducing the concentrations of these contaminants. However, our assessment will not cover how these processing steps influence the nutritional quality, texture or taste of the seaweed.

2 Materials and Methods

2.1 Seaweed-containing products and processing chains

2.1.1 Choice of producers and products

Dutch producers of food and feed products were selected with the support of North Sea Farmers, a non-profit organisation for the seaweed sector working on joint investment projects and knowledge exchange. The first criterion for the selection of processing chains was their coverage of multiple processing steps in the process chain from fresh seaweed to consumer-ready product. Particular interest was in selecting chains that covered washing, fermenting, drying, and rehydration of dry seaweed. The second criterion related to the species of the processed seaweed. The preferred species was the brown seaweed *Saccharina latissima* as this is a widely used species in a variety of food and feed products but also is known for its high concentrations of iodine. The second choice was the green seaweed *Ulva lactuca*, which is known for relatively lower mineral concentrations and higher protein concentrations. Last but not least, the choice of producers and products was, of course, also determined by the producing companies' willingness and opportunity to cooperate. Altogether, this selection process resulted in the choice of seven processing chains, four based on *Saccharina* and three based on *Ulva*. For simplicity the seven processing chains will be called as follows, but they include more processing steps than their name may suggest.

Ulva: seaweed cheese
Ulva (and *Palmaria palmata*): plant-based sausages
Ulva: animal feed

Saccharina: plant-based burger
Saccharina: recreated seaweed cheese process
Saccharina: fermented and dried
Saccharina: dried and rehydrated

Each processing chain will be described in more detail in the following sections.

2.1.2 Sampling

Sampling was performed by employees of North Sea Farmers according to the sampling protocol drafted in earlier seaweed research projects for LNV and NVWA-BuRO. Since seaweed mineral contents can be highly heterogeneous across seaweed cultivation fields, care was taken to receive a large (750 grams) sample of well mixed seaweed in the case of freshly harvested material. Samples along the processing chains were smaller (typically 25 grams) as we assumed that the processed products would already be well homogenised. In total, 75 samples were analysed, originating from seven process chains. Where possible, two samples were taken per sampling moment. Also, where possible, samples were taken from the same batch of seaweed along the processing steps, however that was in some cases not possible, and in other cases not well documented. Samples that come from the same batch were highlighted in the process descriptions in the following sections.

Where possible, all sequential processing steps per chain were sampled (Table 1), however, in each chain some of the steps were not available for sampling (see "-" in Table 1). The most consistently sampled subsequent processing step is from "dried" to "rehydrated" (in four chains).

Table 1 *Processing chains, processing steps and sampled moments: green fields denote steps present in the processing chain, "yes" means the processing step was sampled, "-" means processing step was not sampled, and "n.a." means that this processing step was not part of the particular processing chain. The animal feed example does not fit with the other examples and therefore required its own set of headings, where "C" stands for carbohydrase and "P" for protease.*

seaweed	product	harvest	washed	centrifuged	Cut	frozen	fermented	dried	rehydrated	raw dough / curd	cooked dough	final product
Ulva	seaweed cheese	**yes**	-	**yes**	*n.a.*	*n.a.*	*n.a.*	**yes**	**yes**	-	*n.a.*	**yes**
Ulva	plant-based sausages	-	-	-	*n.a.*	*n.a.*	*n.a.*	**yes**	-	**yes**	**yes**	**yes**
Saccharina	plant-based burgers	-	**yes**	-	-	-	*n.a.*	*n.a.*	*n.a.*	**yes**	**yes**	**yes**
Saccharina	seaweed cheese process	-	**yes**	-	*n.a.*	*n.a.*	*n.a.*	**yes**	**yes**	*n.a.*	*n.a.*	*n.a.*
Saccharina	fermented and dried	-	-	**yes**	*n.a.*	*n.a.*	**yes**	**yes**	**yes**	*n.a.*	*n.a.*	*n.a.*
Saccharina	dried and rehydrated	-	-	**yes**	*n.a.*	*n.a.*	*n.a.*	**yes**	**yes**	*n.a.*	*n.a.*	*n.a.*
seaweed		**MARC1**	**C -pellet**	**C - supernatant**	**P - pellet**	**P - supernatant**						
Ulva	animal feed protein	**yes**	**yes**	**yes**	**yes**	**yes**						

2.1.3 Ulva lactuca

In the following, each food production process is shortly introduced with a flow diagram where green boxes show those steps at which samples were taken and arrows give additional explanation about intermediate actions and gains/losses of water.

2.1.3.1 Seaweed Cheese: Production process and sampled moments

The production process for the *Ulva* seaweed cheese begins with harvesting of *Ulva lactuca* on Vlieland. Either wild growing or line grown *Ulva* is harvested at one specific location, harvesting takes place every two weeks. Here we cover two harvesting days (first at end of June/ mid of July and, second mid of August) that yielded 10 kg fresh *Ulva* per batch. The fresh seaweed is first washed in fresh water until all sand is removed, this also cleans out any attached shrimps and epiphytes. The washed seaweed is centrifuged, then dried at 65 °C. The dried seaweed is sieved to remove large clumps / seaweed flakes and stored in the cheese factory (conditions unknown) until further processing. The dried seaweed is rehydrated by soaking it in just boiled water for half an hour. The volume of water is chosen so that all water is taken up by the seaweed and no liquid is left over. The rehydrated seaweed is finally added to the cheese curd, the added amount equals 1 kg of dried *Ulva* for 70 cheeses (which amounts to ca 250-300 kg cheese and ca 3.3-4 g dry *Ulva* per kg cheese). The cheeses then ripen for 6 weeks before they are sold to consumers.

A total of 18 samples were taken at following process steps (Fig 1):

- Fresh seaweed: at harvest: two batches sampled, 2 samples each from harvest day 1 and 2
- Centrifuged: After washing and centrifuging, 4 samples
- Dried: After drying, 4 samples
- Rehydrated: After rehydrating, 4 samples (whereby 2 samples are from the same batch as the dried sample)
- Cheese: In ripened, ready-to-sell cheese: 2 samples

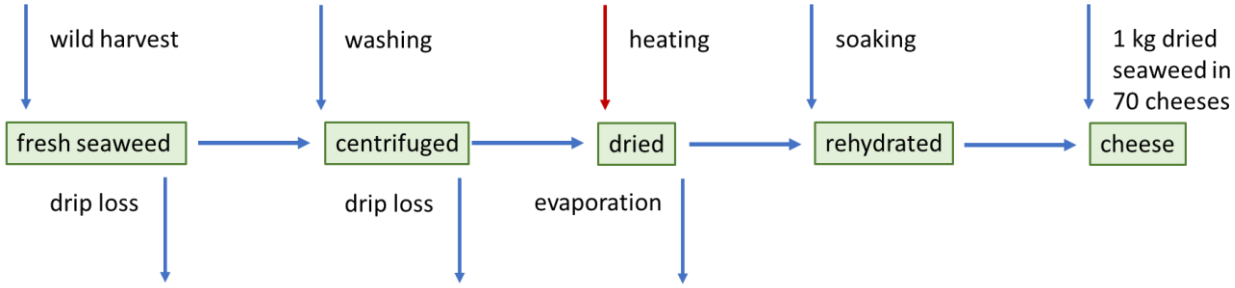

Figure 1 *Process steps in the seaweed cheese processing chain. Green squares are sampled moments.*

2.1.3.2 Plant-Based Sausages: Production process and sampled moments

The food production process for the plant-based sausages starts with dry *Ulva* and *Palmaria* (Dulse) bought from Portuguese sources. The dried seaweed is stored at the factory until further processing (conditions undocumented). The dried seaweed is rehydrated and cut (conditions undocumented, drip loss is assumed) before mixing it into the plant based sausage dough. The dough is enriched with 4.5 % of rehydrated *Ulva* and Dulse each. The shaped sausages are cooked, subsequently chilled to -4 °C and stored at the production facility until delivery to selling points. Lastly, the sausages are pan fried at the WFSR.

A total of 10 samples of 25 g each were taken at following process steps (Fig 2):

- Dry seaweed: dried Portuguese seaweed as bought: 2 samples each of *Ulva* and *Palmaria*
- Dough: after rehydration and adding mix of 4.5% rehydrated *Ulva* and *Palmaria* to raw dough, 2 samples
- Sausage cold: after shaping, cooking and chilling sausages: 2 samples
- Sausage hot: after pan heating: 2 samples

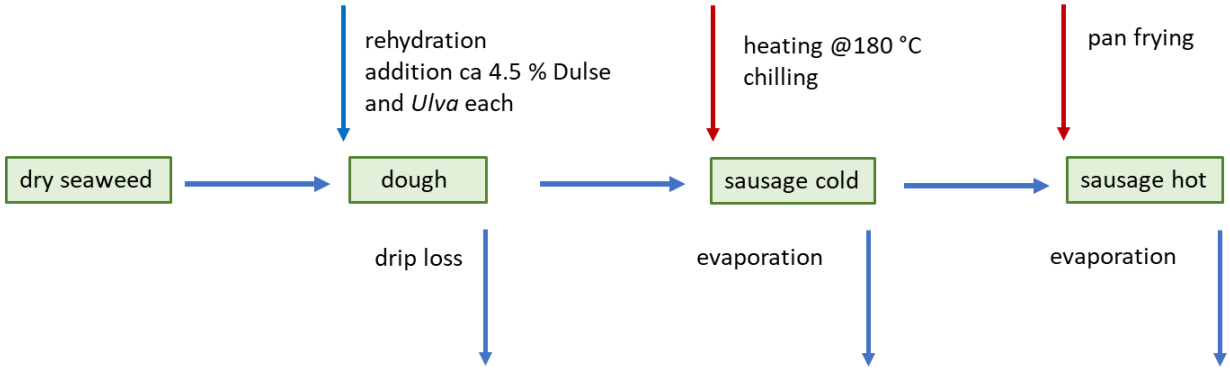

Figure 2 *Process steps in the plant-based sausages processing chain. Green squares are sampled moments.*

2.1.3.3 Plant based protein for animal feed: Production process and sampled moments

To refine proteins from *Ulva* for substitution in animal feed, the batch of seaweed first undergoes a carbohydrase step at 50 °C and pH 6 for 24 h, after which the solids are spun down into a pellet and the supernatant is removed. Subsequently, the pellet is further treated by a protease step at 60 °C for 20 h and the solids are again spun down. The pellet is then discarded and the proteins of interest are finally in the supernatant fraction. Here the process was repeated twice. In experiment 1 the pellets and supernatants were sampled separately, while in experiment 2 the samples contained pellet and supernatant combined per extraction step. Note that the mass balance of this process was not closed and hence recovery of minerals does not add up to 100%.

A total of 14 samples were taken (Fig 3):

- Marc1: starting material before extractions, 2 samples (same for experiment 1 and 2)
- Within experiment 1:
  - Pellet1: pellet material after carbohydrase, 2 samples (same batch as Marc1)
  - Supernatant 1: supernatant material after carbohydrase, 2 samples
  - Pellet 2: pellet material after protease, 2 samples (same batch as Pellet 1)
  - Supernatant 2: supernatant material after protease, 2 samples (same batch as Pellet 1)
- Within experiment 2
  - Carbohydrase: combined sample of pellet 1 and supernatant 1, 2 samples (same batch as Marc1)
  - Protease: combined sample of pellet 2 and supernatant 2, 2 samples (same batch as carbohydrase)

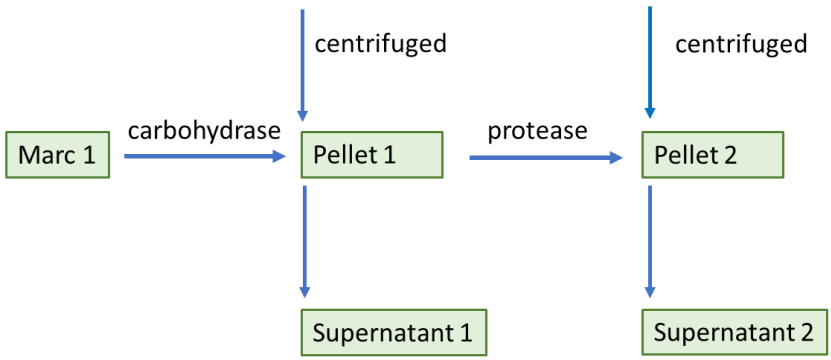

Figure 3 *Process steps in the animal feed protein processing chain. Green squares are sampled moments.*

2.1.4 Saccharina latissima

2.1.4.1 Plant-Based Burgers: Production process and sampled moments

The plant-based burger process starts with fresh *Saccharina* harvested from the Eastern Scheldt. The *Saccharina* is washed once in sea water and twice in fresh water by the harvester, and then again rinsed at the processing factory. Thereafter, it is cut and frozen until further processing. After raising the temperature to -4°C and further shredding, the frozen seaweed is added to the raw burger dough at 9% v/v. The dough is then shaped into burgers and cooked at 180°C, chilled to -4°C, and stored until delivery to the selling partners. Finally the burger is pan fried at the WFSR.

A total of 8 samples were taken at following process steps (Fig 4):

- Fresh seaweed: after harvesting and triple washing, 2 samples
- Dough: after rinsing, cutting, freezing and shredding, and mixing into raw dough, 2 samples
- Burger cold: after cooking and chilling burger, 2 samples
- Burger hot: after panfrying, 2 samples

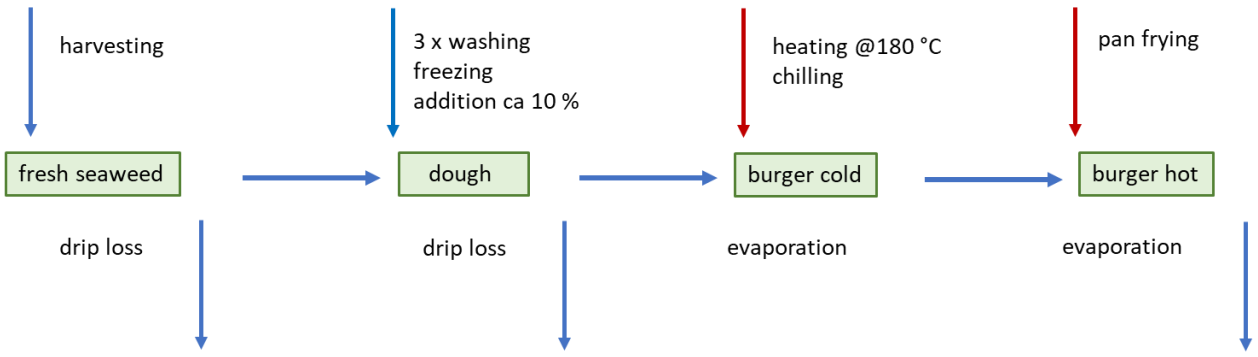

Figure 4 *Process steps in the plant-based burgers processing chain. Green squares are sampled moments.*

2.1.4.2 Recreated seaweed cheese process: Production process and sampled moments

To assess whether *Saccharina* shows the same response pattern as *Ulva* in the cheese making process, the same setup was repeated with fresh *Saccharina* up to and including the rehydration step. These procedures were performed at the cheese factory. Freshly harvested and washed *Saccharina* was sent to the cheese factory to be washed in fresh water, centrifuged, dried at 65 °C, and finally rehydrated in a low water to seaweed ratio so that all water is taken up. All steps were performed as in 2.1.3.1.

In total, 8 samples of 25 g each were taken at following steps (Fig 5):

- Fresh seaweed: after harvesting and maybe washing at harvesting location, 4 samples
- Dried: after washing, centrifuging and drying, 2 samples
- Rehydrated: after sieving and rehydration, 2 samples

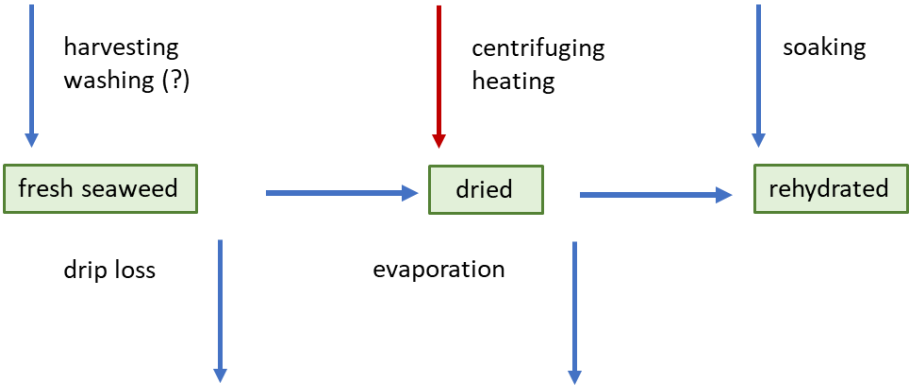

Figure 5 *Process steps in the recreated seaweed cheese processing chain. Green squares are sampled moments.*

2.1.4.3 Fermented and dried *Saccharina*: Production process and sampled moments

To simulate a typical production process of fermented to rehydrated *Saccharina*, North Sea Farmers recreated a fermentation process based on the ensiling protocol described in (Larsen et al., 2021). Freshly harvested *Saccharina* was freed from epiphytes by scrubbing with a scouring pad, washed in saltwater and centrifuged, cut in 10 cm pieces and mixed well before the start of the fermentation process. Fermentation was initiated by addition of lactic acid solution (9 g / kg fresh weight), mixing and storage in a vacuum bag at 20°C for 14 days. Thereafter, the fermented seaweed was dried at 60 °C for 48 h. Rehydration followed the Seaweed Cheese process, thus soaking for 30 min in just enough freshly boiled water so that all water is taken up. All steps up to and including the drying step were performed by North Sea Farmers, the rehydration was performed at the WFSR.

In total, 18 samples (15 solid, 3 liquid) of 25 g each were taken (Fig 6):

- Fresh seaweed: after harvesting and washing, 4 samples (same "fresh seaweed" samples as in 2.1.4.2)
- Fermented: after fermenting, 4 samples
- Dried: after separating solids from fluids, and drying, 4 solid samples and one liquid sample (from the same batch as "fermented")
- Rehydrated: after rehydration and separation of solids from liquids, 3 solid and 2 liquid samples (from the same batch as "dried")

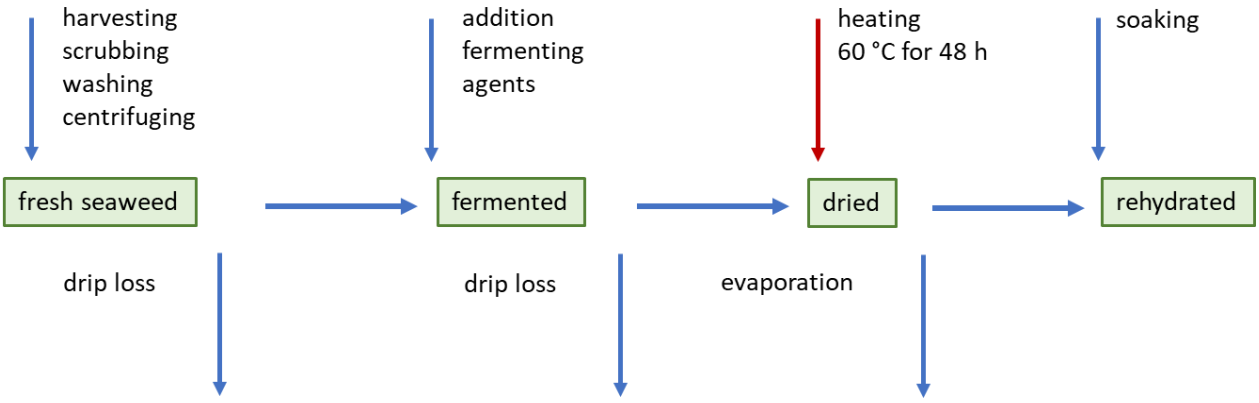

Figure 6 *Process steps in the fermented and dried Saccharina processing chain. Green squares are sampled moments.*

2.1.4.4 Dried and rehydrated *Saccharina*: Production process and sampled moments

Freshly harvested *Saccharina* was freed from epiphytes by scrubbing with a scouring pad, washed in saltwater and centrifuged. The seaweed was then dried at 60 °C for 48 h. Rehydration followed the Seaweed Cheese process, thus soaking for 30 min in just enough freshly boiled water so that all water is taken up. All steps up to and including the drying step were performed by North Sea Farmers, the rehydration was performed at the WFSR.

In total 10 samples were taken (Fig 7):

- Fresh seaweed: after harvesting and washing, 4 samples (same "fresh seaweed" samples as in 2.1.4.2)
- Dried: after heat drying, 4 samples
- Rehydrated: after rehydration, 2 samples (from same batch as "dried")

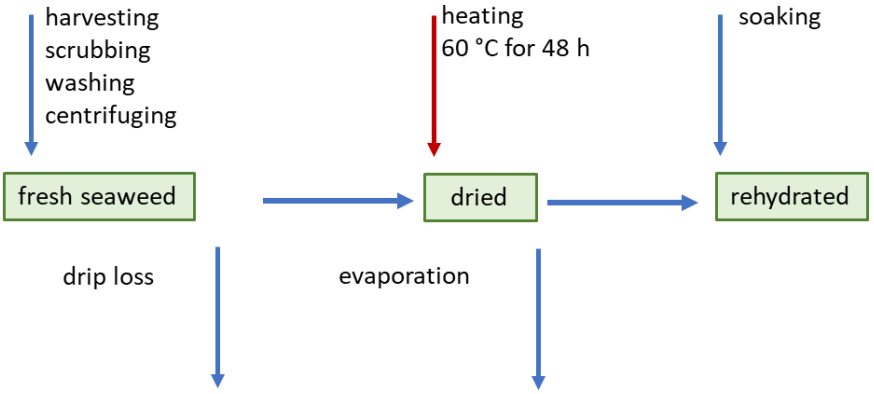

Figure 7 *Process steps in the dried and rehydrated Saccharina processing chain. Green squares are sampled moments.*

2.2 Sample and data analysis

All samples were processed in the state in which they were delivered or manufactured at the WFSR, meaning that dry as well as moist samples were analysed. The samples were measured for moisture content and analysed for the concentrations of iodine, nickel, cadmium, lead, mercury, bromide, and total as well as inorganic arsenic. Moisture was measured with a gravimetric method and drying at ca 85 °C under pressure for 4 h. Iodine and the heavy metals were analysed using a confirmatory ICP-MS (induced coupled plasma mass spectrometer) method at the WFSR. Based on the moisture content, the amount of sample used for analysis differs, which influences the actual limit of detection (LOD) and limit of quantification (LOQ). Moisture content was analysed following WFSR SOP-N-0272, element analysis was performed for Ni, total As, Cd, Hg, and Pb according to WFSR SOP-A-1331, inorganic arsenic according to WFSR SOP-A-1086, and the bromine and iodine concentrations according to WFSR SOP-A-1341.

Here we report all analysis results as dry weight (dw) to allow comparison of changes in elemental concentrations along different processing steps. Only those samples in which the measured concentrations were above LOQ were included in the further calculations. All elemental concentrations were calculated back to dry weight (dw) concentrations using following formula:

mg kg^{-1} dw = mg kg^{-1} moist sample/ (1-proportional moisture content in moist sample)

Analysis results below LOQ were excluded from all further calculations to avoid bias in the data. This means that missing values in the results represent samples below LOQ rather than the absence of these samples. Absolute concentrations are shown per process chain and per element in the Appendix 1. Changes in dw concentrations of each element (averaged per sampling moment) across the food production process are presented as proportional changes relative to the concentrations in the starting product (fresh or dry seaweed) (See Annex 2 for all data). Additionally, we compare proportional changes in elemental concentrations between specific process steps across (several) process chains. All calculations and plotting were performed in Excel.

3 Results

In total, 75 samples were analysed. The percentage of samples measured above LOQ varied per element, while 100% of all samples could be quantified for iodine, only 28 % could be quantified for mercury (Table 2). The respective LOQ's per element depend on the moisture of the sample and its fattiness, therefore different LOQ's can apply in the measurement of an element. Table 2 only shows the concentrations of samples quantified above LOQ, for all wet weight and dry weight concentrations per sample and indication of LOQ level in those samples that could not be quantified, see Annex 1.

Table 2 *The number and proportion out of a total of 75 samples in which an element could be quantified above LOQ. Ni is nickel, As is total arsenic, Cd is cadmium, Hg is mercury, I is iodine, Br is bromine, and iAs is inorganic arsenic.*

	Ni	As	Cd	Hg	Pb	I	Br	iAs
samples above LOQ	71	73	54	21	69	75	69	22
% above LOQ	95	97	72	28	92	100	92	29

Measured ranges across all chains and process steps varied widely within and among elements. Table 3 summarises the minimum and maximum measured concentrations (mg kg^{-1} dry weight) per element in all measurements >LOQ. The highest I, Br and total As concentrations are observed in dried *Saccharina* (see Annex 1 for concentrations per sample).

Table 3 *Minimum and maximum dry weight concentrations (mg kg^{-1}) per element in samples that were quantified above LOQ.*

	Ni	As	Cd	Hg	Pb	I	Br	iAs
min	0,08	0,11	0,01	0,004	0,01	0,29	5,13	0,16
max	6,20	87,01	0,33	0,12	8,14	6357	2630	1,48

3.1 Results per analysed processing chain

3.1.1 Ulva lactuca

3.1.1.1 Seaweed Cheese

Overall, washing and centrifugation reduced elemental concentrations to 22 % (Pb) – 78 % (total As) of the starting material, with some elements dropping below quantifiable concentrations (Hg, iAs, Cd). Further drying and rehydration in little water didn't lead to a further sustained reduction. Note that elements close to the limit of quantification (LOQ) may sometimes be detected just below or above the LOQ, which explains the patterns in some elements that are quantified in the fresh product and the final product but may just drop below LOQ in intermediate process steps.

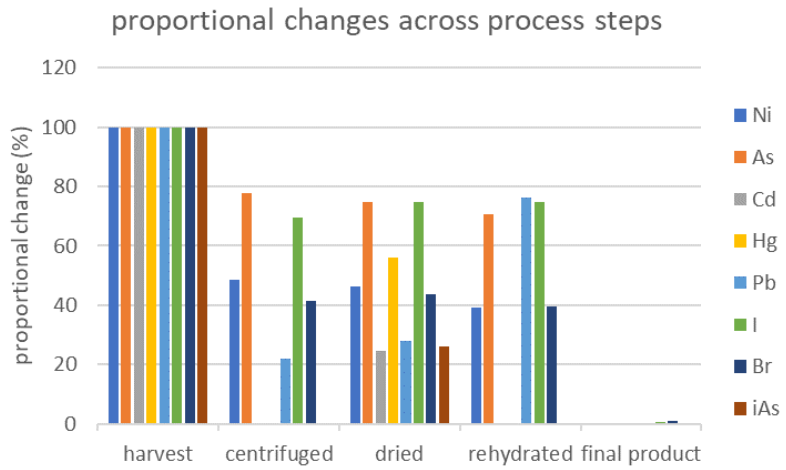

Figure 8 *Proportional changes in elemental concentrations across process steps normalised to the starting material (here "harvest", set to 100%).*

Dilution in the cheese curd reduced all measured elements except I and Br below LOQ (Fig 8). Based on the addition of 0,4% dried *Ulva* to the cheese, the ripened cheese shows higher I and Br concentrations than expected (Fig 9). Note here, that the expected concentrations are based on dried *Ulva* as the weight of rehydrated *Ulva* added to the cheese curd was not available, and that I and Br could be compared only as the other elements in the cheese curd fell below LOQ.

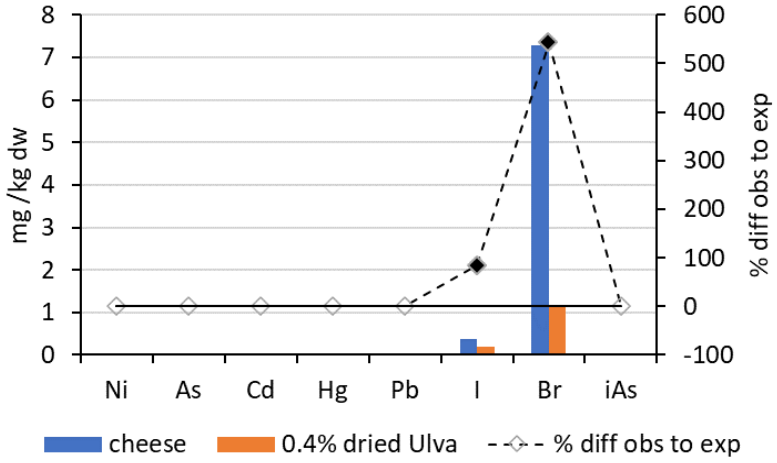

Figure 9 *Comparison of elemental concentrations expected by the addition of seaweed and the measured concentrations in the final product. The bar plot shows the elemental concentrations (mg kg^{-1} dw) for the seaweed addition (orange) and the final product (blue). The line plot shows the proportional difference between the observed vs the expected values, whereby values > 0 indicate that the final product contains a higher concentration of that element than expected by the seaweed addition alone. Full diamonds indicate that the element was quantifiable, empty diamonds indicate that the element was < LOQ in one or both samples.*

3.1.1.2 Plant-based sausages

Overall, dried *Palmaria* showed higher concentrations of the tested elements, in particular As and I, compared to dried *Ulva*. In both starting products, Hg remained below detection limit. Unsurprisingly, dilution of the rehydrated seaweed into the raw sausage dough reduces all elements by 90% (Ni) or more (Pb, I, total As) compared to the dried starting material (dried *Ulva* and *Palmaria*). Pan frying the product did not introduce much changes in the elemental concentrations, except for a Cd spike, the origin of which remains unclear but is likely not associated with the product itself (Fig 10).

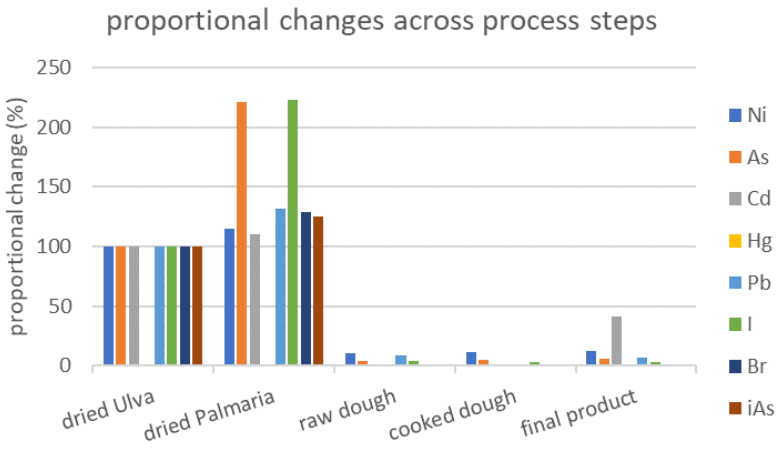

Figure 8 *Proportional changes in elemental concentrations across process steps normalised to the starting material (here "dried" Ulva, set to 100%).*

As no direct sample of the rehydrated *Ulva* and *Palmaria* was available, we compared the elemental concentrations in 9% of equal parts (hence 4.5% each) dried *Ulva* and *Palmaria* with those measured in the raw sausage dough. The dough contained less I, total As and Pb than expected from the contribution of the dried seaweed (likely an effect of the rehydration step that was not measured). The other element could not be compared as their concentrations in one or both samples was <LOQ (Fig 11). However, given that Br in the dough decreased to <LOQ suggests that the dough also contained less Br than expected from the added seaweed.

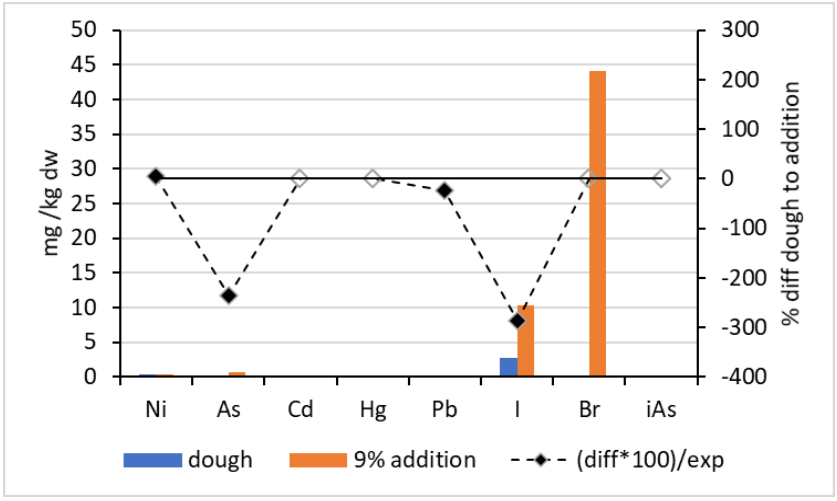

Figure 9 *Comparison of elemental concentrations expected by the seaweed addition and the measured concentrations in the final product (raw sausage dough). The bar plot shows the elemental concentrations (mg kg^{-1} dw) for the seaweed addition (orange) and the raw dough (blue). The line plot shows the proportional difference between the observed vs the expected values, whereby values < 0 indicate that the final product contains a lower concentration of that element than expected by the seaweed addition. Full diamonds indicate that the element was quantifiable in both samples, empty diamonds indicate that the element was < LOQ in one or both samples.*

3.1.1.3 Plant-based protein for animal feed

Breaking up the starting material with a carbohydrase step reduced all measured elements to 60-67% in the pellet compared to the starting material with the exception of Ni (99%) and Hg (107%). However, Hg concentrations overall were close to the LOQ (see Annex 1 for the measured concentrations) which means that the actual difference in concentration between presence and absence is very small. Only relatively low

amounts of all elements were dissolved into the carbohydrase supernatant and subsequently discarded (again, Hg is close to the LOQ). The carbohydrase pellet was treated by a protease step, in which the largest part of the elements stayed in the protease pellet. Only low amounts were transferred to the protease supernatant (the product of interest) with remaining 8% Ni, 4% total As and Br, 3% I and 2% Pb as compared to the starting material (Fig 12).

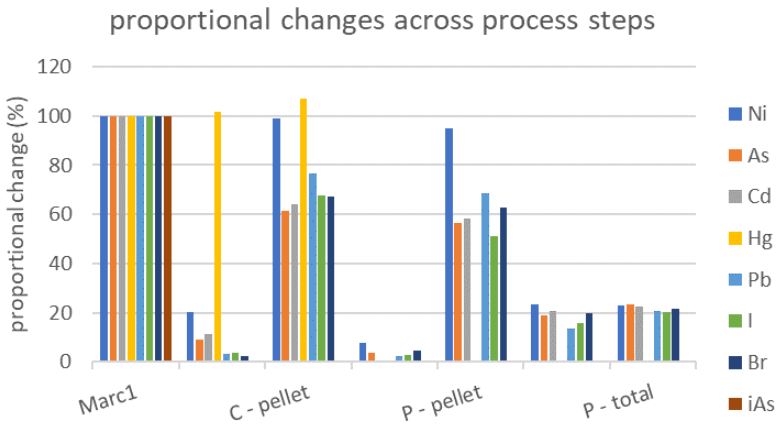

Figure 10 *Proportional changes in elemental concentrations across process steps normalised to the starting material (here "Exp 1 Marc1", set to 100%).*

3.1.2 Saccharina latissima

3.1.2.1 Plant-based burgers

As in the plant-based sausages, the dilution of the seaweed in the raw burger dough reduced most of the elemental concentrations to 4-7% (I, Br, Pb, total As) of the starting material with the exception of Ni (62%) and Cd (49%). Further processing steps did not cause any further reduction in the elemental concentrations. Pan frying the product also didn't change the elemental concentrations (Fig 13).

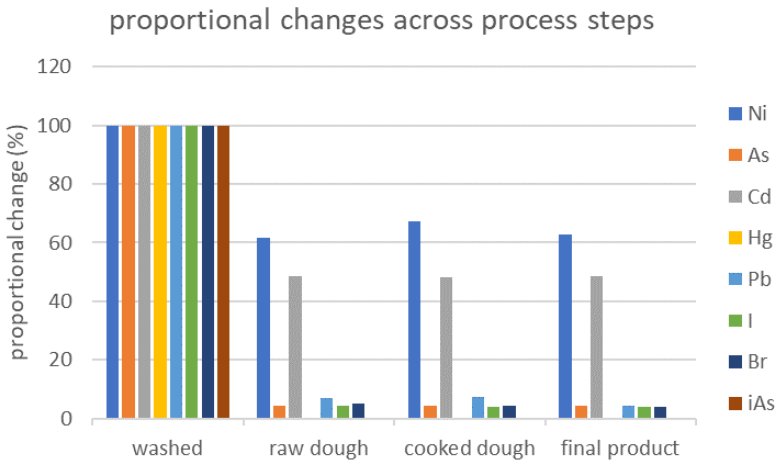

Figure 11 *Proportional changes in elemental concentrations across process steps normalised to the starting material (here "washed" Saccharina, set to 100%).*

When comparing the elemental concentrations measured in the raw burger dough with the amounts expected through the addition of 9% fresh seaweed, some elements occurred in higher concentrations than expected (Ni and Cd) whereas other occurred in lower than expected concentrations (I, Br, Pb, and total As)

(Fig 14). Note though that no sample of the frozen *Saccharina* added to the dough was available, hence we used the concentrations in the fresh ("washed") *Saccharina* for this calculation.

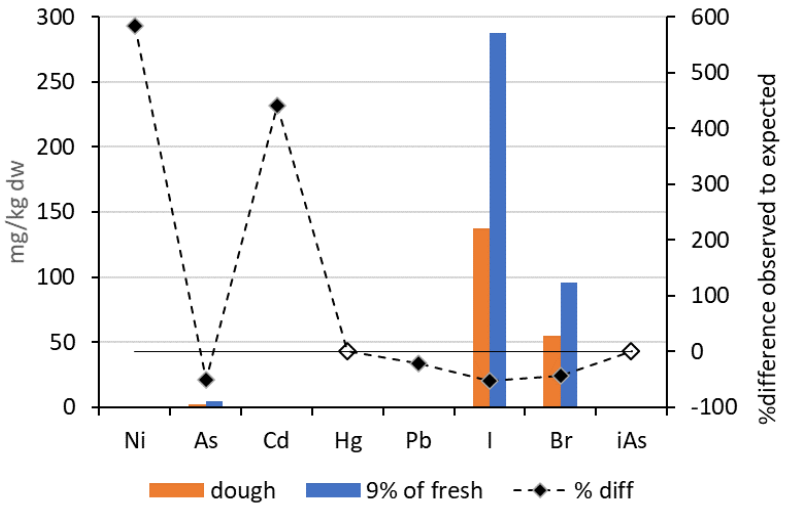

Figure 12 *Comparison of elemental concentrations expected by the seaweed addition and the measured concentrations in the final product (raw burger dough). The bar plot shows the elemental concentrations (mg kg^{-1} dw) for the seaweed addition (orange) and the raw dough (blue). The line plot shows the proportional difference between the observed vs the expected values, whereby values < 0 indicate that the final product contains a lower concentration of that element than expected by the seaweed addition, while values > 0 indicate higher concentrations than expected. Full diamonds indicate that the element was quantifiable in both samples, empty diamonds indicate that the element was < LOQ in one or both samples.*

3.1.2.2 Recreated seaweed cheese process

Recreating the cheese making process using fresh *Saccharina* showed mixed results. Washing, centrifuging and drying of the *Saccharina* resulted in reduction of some elements, notably in total As (47%,), I (50%), and Br (58%). However, other elements showed (strongly) increased concentrations, such as Ni (204%), Pb (142%), and Cd (110%). Rehydration in little water decreased some elemental concentrations a little bit further, such as I (30%), total As (32%), and Br (40%). But the same step also rather notably increased Cd to 176% and Pb to 404% compared to the starting material (Fig 15).

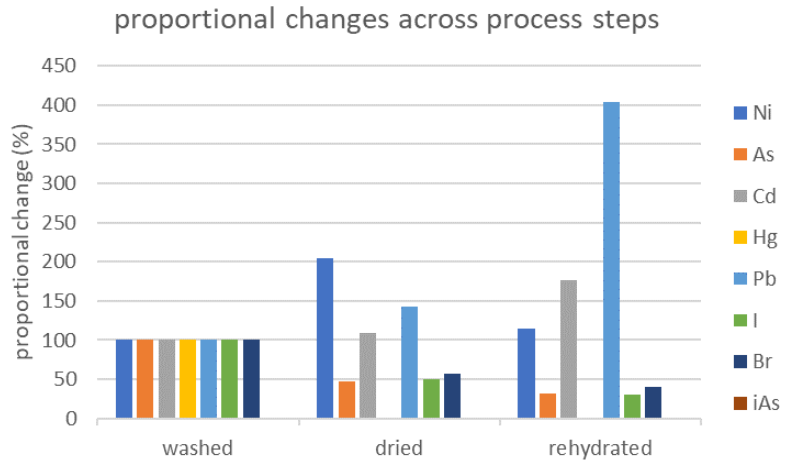

Figure 13 *Proportional changes in elemental concentrations across process steps normalised to the starting material (here "washed" Saccharina, set to 100%).*

3.1.2.3 Fermented and dried *Saccharina*

Based on the same starting material as 3.1.2.2, fermenting and subsequent drying of *Saccharina* showed mixed results. Fermenting lead to slight increases in all elements in the range of 110%, except for a reduction of Cd to 85%, and of Hg to < LOQ. The minimal reduction of As may well be in the range of measurement uncertainty. Drying resulted in even slightly higher concentrations and made it possible to quantify Hg again. However, rehydration of the dried seaweed did reduce all elemental concentrations to 32-51% (except Pb at 82%) compared to the starting material (Fig 16).

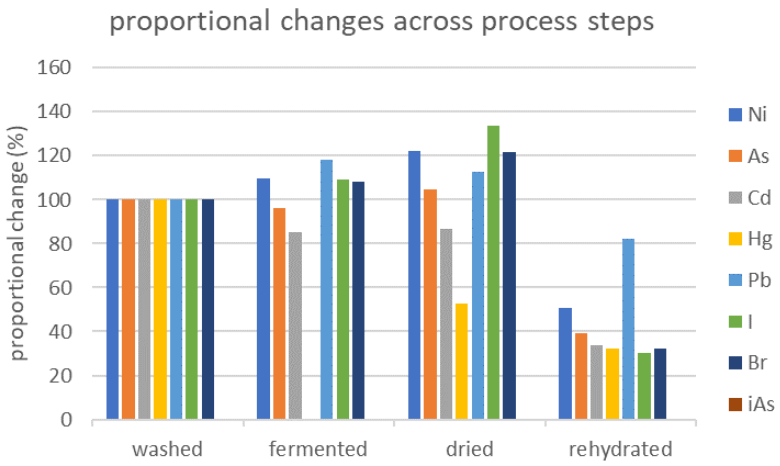

Figure 14 *Proportional changes in elemental concentrations across process steps normalised to the starting material (here "washed" Saccharina, set to 100%).*

3.1.2.4 Dried and rehydrated *Saccharina*

Also based on the same fresh material as 3.1.2.2, drying again shows mixed results with increases in some elements, such as Ni (153%), Pb (150%), I (148%), and Br (142%), and decreases in others, such as Cd (84%) and Hg (51%) relative to the starting material. Subsequent rehydration of the dried seaweed reduced all elemental concentrations to more or less the starting concentrations in Ni, Pb, I and Br, as well as to slightly further reduced levels for Cd and Hg (Fig 17).

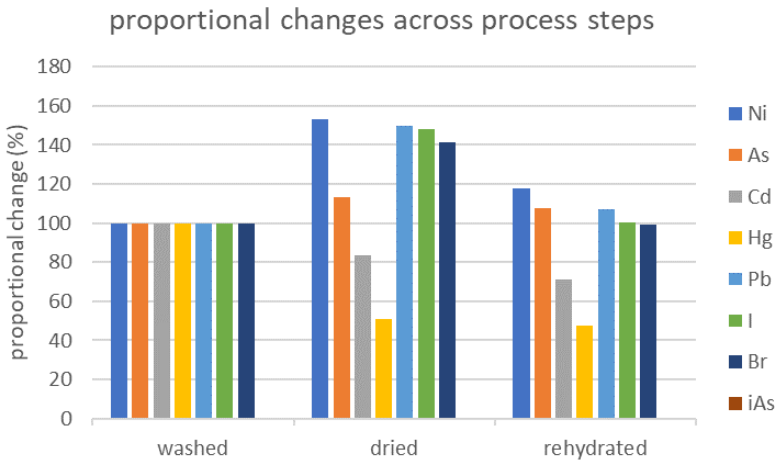

Figure 15 *Proportional changes in elemental concentrations across process steps normalised to the starting material (here "washed" Saccharina, set to 100%).*

3.1.3 Highest concentrations measured per chain

Ulva, *Saccharina* and *Palmaria* (Dulse) based production chains differ in the observed maximum elemental concentrations. Notably, the highest concentrations of I, Br and total As are observed in *Saccharina* based chains, with the highest values in dried *Saccharina* samples (Table 4). Generally, the highest elemental concentrations are observed in the fresh or in the dried seaweeds, and not all elemental concentrations decrease along the processing chain (see Annex 1 for data).

Table 4 *Measured maximum elemental concentrations per chain and element (mg kg^{-1} dry weight).*

mg kg^{-1} dw	*Ulva* Seaweed cheese	*Ulva* Plant based sausage	*Ulva* Animal feed protein	*Dulse* Plant based sausage	*Saccharina* Plant based burger	*Saccharina* Recreated cheese process	*Saccharina* Fermented - dried	*Saccharina* Dried - rehydrated
Ni	6,2	3,8	1,1	4,3	0,8	2,5	1,3	2,6
As	6,9	4,8	3,3	10,9	53,6	35,8	77,2	87,0
Cd	0,1	0,1	0,1	0,1	0,1	0,3	0,2	0,2
Hg	0,06	-	0,005	-	-	-	0,1	0,1
Pb	8,1	0,5	0,4	0,7	2,5	4,2	1,2	2,0
I	95	72	147	161	3207	2252	5532	6357
Br	891	431	212	558	1071	1076	2040	2630
iAs	1,5	0,3	0,2	0,5	-	-	0,5	0,7

3.2 Proportional changes in contaminant concentrations in subsequent processing steps

Due to the differences in sequential steps between production chains, as well as due to the issue that not all steps were sampled by the producers, the dataset does not allow testing the effect of specific steps in a structural manner (Table 5). The most often assessed subsequential steps are from dried seaweed to rehydrated seaweed (4 chains), from centrifuged to dried (2 chains), and from raw dough containing a fraction of seaweed to a cooked dough (2 chains, heating and chilling) to the pan-fried final product (2 chains, heating).

Table 5 *Summary of often a pair of subsequent processing steps was measured in this study*

Subsequent steps measured	Number of production chains
Centrifuged-dried	2
Fermented-dried	1
Dried-rehydrated	4
Raw dough-cooked dough	2
Cooked dough – final product	2
Marc1-carbohydrase	1
Carbohydrase-protease	1

3.2.1 Dried – rehydrated seaweed: elemental concentration changes

In four chains, the step from dried to rehydrated seaweed was measured with no missing intermittent steps: namely in one *Ulva*-based chain (seaweed cheese) and three *Saccharina*-based chains (recreated seaweed cheese, fermented-dried, and dried-rehydrated). Rehydrating occurred in low water to seaweed ratio using just enough water so that all water was absorbed. Most measured elements show a reduction relative to the dried material concentrations, except Pb and Cd in the seaweed cheese, and the recreated seaweed cheese processes (Fig 18). The strongest reductions were observed in the fermented seaweed process chain. Prior

fermentation may increase mobility of elements, however, the fermentation step in our dataset (n=1) seemed to increase elemental concentrations, so that the net effect of fermenting and drying still resulted in higher elemental concentrations compared to the fresh or washed starting material.

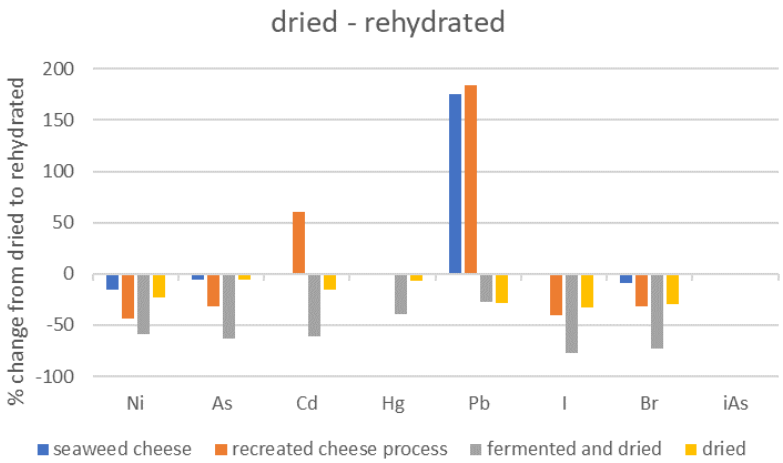

Figure 16 *Proportional change in elemental concentrations (%) due to rehydration of dried seaweed in four processing chains.*

4 Discussion

4.1 Most promising processing steps for reduction of elemental concentrations

Literature sources show that steps that allow washing out of the water soluble elements and molecules of seaweeds (e.g., washing, blanching, rehydrating in copious water) are generally effective in reducing the elemental concentrations in seaweeds. Increased temperature (blanching, cooking) or prior steps that increase the water-solubility of specific elements or break down the cell walls (e.g., fermentation, cutting, carbohydrase) likely increases the effect of the washing steps. However, most literature focusses on the reduction in iodine and (total) arsenic as the two most worrisome elements, thereby neglecting the changes in other elements, undesired as well as desired ones.

Our survey results suggest that washing, particularly in a high water to seaweed ratio and in fresh water with high osmotic potential seems to lower the concentration of most of the measured elements (e.g. *Ulva* cheese chain). Equally, rehydrating dried seaweed using warm to boiling temperature water also reduced the concentrations of most measured elements (e.g. *Saccharina* fermented-dried, *Saccharina* dried and both cheese processes). Breaking up the seaweed with a carbohydrase and protease also shows a strong reduction in elemental concentrations to 8% or less than compared to the starting material in the final fraction of interest (which is the supernatant after the protease step in the animal feed protein process).

4.2 Processing steps increasing elemental concentrations

Somewhat unexpectedly, our dataset shows a fair number of examples in which the elemental concentrations increased during the seaweed processing even though all measurements were uniformly recalculated as dry weight concentrations. One possible explanation can be that, in some cases, the amount of analysed material differed depending on the initial moisture content of the sample, which influences the LOQ cut-off concentration. Hence, in some cases an element would be recorded below LOQ for a moist sample whereas it would be quantifiable above LOQ after, e.g., drying. This probably applies for many of the cases in which Hg seemingly randomly appeared and disappeared, rendering those changes an artefact of the method employed. However, our dataset also included a fair number of cases in which marked increases in elemental concentrations were far above LOQ, and therefore need to be explained by another mechanism. We observed increases in some elemental concentrations notably upon drying (e.g. *Saccharina* and *Ulva* cheese processes), with fermentation (*Saccharina* fermented-dried), with rehydration (e.g. *Saccharina* and *Ulva* cheese processes), and with pan-frying. In some cases an inadvertent contamination by another ingredient can play a role such as other food ingredients added to a dough, but also from the water used for rehydration or from residues from a frying pan, other cases are not easily explained (e.g. effects after drying when no other substances were added). In such cases, the perceived increase in elemental concentrations could also reflect that carbon was lost (e.g. through heating) while other elements were retained. Lastly, increasing elemental concentrations could also suggest that the processing enhanced extractability from a modified food matrix (Ho and Rendang 2022) although this should not play a role in our dataset as the employed ICP method measures all atoms of an element independent from their extractability.

4.3 Caveats

We observed a number of limitations in this dataset that hamper detailed advice. These limitations partially come from the exploratory nature of this study with samples taken by producers. First of all, subsequent samples often represent several intermediate steps in one go rather than true step-wise samples along a processing chain. This hinders allocation of changes to a specific step in the procedure and only allows assessment of net effects over all intermediate processing steps. Secondly, as samples were offered in duplicates, identification of outliers is difficult. Nevertheless, if two samples of the same processing step show large divergence, that indicates that the source material was not particularly homogeneous. These limitations can be remedied by a more targeted and controlled experimental approach (PROCESS II).

4.4 Advice for follow-up study PROCESS II

Our take along advice for the follow-up study includes:

- Making sure to include a benchmark or baseline against which the samples in the process chain can be compared;
- Including industry relevant steps that have not been captured in the food production processes above, such as freezing, blanching or shredding / cutting;
- Sampling each step separately, without merging two or more production steps and without the addition of extra ingredients (e.g. dough). In particular, following steps should be consistently sampled:
  - Fresh harvest - washed (or blanched);
  - Washed - dried;
  - Dried - rehydrated;
- Sampling of all external additions (e.g. water used for washing, dough before addition of seaweed);
- Sampling of all waste streams (e.g. discarded water after washing or rehydration);
- Measuring weights of all samples and ingredients to allow mass balance calculation;
- Measuring dry weight concentrations for all samples;
- Working in triplicates to allow assessment of outliers;
- Ensuring that sample volume and sampling technique result in a representative sample of the process step.

4.5 Opportunities for the reduction of contaminants

Choice of appropriate seaweed for production: Literature and to a limited degree also our survey results show that seaweed species vary widely in their iodine and other elemental concentrations. Choosing the right seaweed species can hence have a large impact on the elemental composition of the end product, in particular if the end product consists largely of (unrefined or little refined) seaweed biomass. But even in seaweed species that are known for their high iodine contents, choices in harvesting method, location and season can make a large difference. For example, the elemental concentrations of *Saccharina* are known to vary with season, iodine contents are lower when the seaweed is grown in low salinity, and exposure to stress (e.g. exposure to air) can further trigger an efflux of iodine from the seaweed.

Washing: Washing of seaweed not only removes sand and attached organisms, but it also allows for osmotic or direct efflux of elements into the washing water. This effect is likely stronger in water with high osmotic potential and if the washing step is repeated with fresh batches of water. Combining washing with elevated temperatures seems to promote the loss of elements further, based on literature sources.

Drying: Literature suggests that drying at high temperatures may reduce iodine concentrations. In our case studies, we didn't observe this effect, however, our sample size of observations is too low for a conclusive statement.

5 Conclusion

Overall, our survey of seven processing chains shows that elemental concentrations vary strongly across the production processes. While our survey lacks a good coverage of sampling subsequent processing steps, we can, conditionally, conclude the following:

Seaweed species vary in their elemental concentrations. Here, *Ulva* shows generally much lower iodine, bromine and total arsenic concentrations than *Saccharina*. Some processing steps showed the reduction of elemental concentrations as expected from the literature, as for example, washing in the *Ulva* seaweed cheese process. In particular, rehydration of dried seaweed reduced the measured elemental concentrations (in dry weight) in several different processing chains. Other processing steps showed an unexpected increase in some elemental concentrations, as for example in the *Saccharina* dried and rehydrated process. This unexpected increase in elemental concentrations could have methodological but also processing-relevant causes, and hence warrants further research. Extraction of proteins from seaweed through carbohydrase followed by a protease step seemed to separate the proteins successfully from the elements of concern, and may present a promising way forward for the production of safe for consumption seaweed-based proteins.

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Annex 1 Elemental concentrations dataset

seaweed	process	step	moisture	wet weight measured								dry weight calculated: conc moist sample / (1- proportional moisture)							
				Ni	As	Cd	Hg	Pb	I	Br	iAs	Ni	As	Cd	Hg	Pb	I	Br	iAs
			(m/m) %	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Ulva	Seaweed cheese	harvest	76,3	0,88	1,40	<0.02	<0.015	0,61	20	211	0,2106	3,71	5,91			2,57	84	890	0,89
Ulva	Seaweed cheese	harvest	76,7	0,66	1,20	<0.02	<0.015	0,49	17	183	0,1790	2,83	5,15			2,10	73	785	0,77
Ulva	Seaweed cheese	harvest	72,6	1,70	1,90	0,03	0,02	2,10	21	177	0,4043	6,20	6,93	0,11	0,05	7,66	77	646	1,48
Ulva	Seaweed cheese	harvest	70,6	1,40	1,70	0,03	<0.015	1,80	10	81	0,2830	4,76	5,78	0,11		6,12	34	276	0,96
Ulva	Seaweed cheese	centrifuged	85,8	0,26	0,65	<0.02	<0.015	0,08	6	42	<0.11	1,83	4,58			0,54	44	296	
Ulva	Seaweed cheese	centrifuged	86,3	0,21	0,61	<0.02	<0.015	0,08	6	36	<0.11	1,53	4,45			0,60	41	263	
Ulva	Seaweed cheese	centrifuged	83,2	0,42	0,80	<0.02	<0.015	0,24	10	48	<0.11	2,50	4,76			1,43	60	286	
Ulva	Seaweed cheese	centrifuged	84,3	0,41	0,74	<0.02	<0.015	0,23	7	36	<0.11	2,61	4,71			1,46	41	229	
Ulva	Seaweed cheese	dried	10,5	1,40	3,90	0,02	0,02	0,62	37	279	0,1523	1,56	4,36	0,03	0,03	0,69	41	312	0,17
Ulva	Seaweed cheese	dried	10,7	1,40	3,90	0,02	0,02	0,52	36	231	0,1474	1,57	4,37	0,03	0,03	0,58	40	259	0,17
Ulva	Seaweed cheese	dried	9,4	2,30	4,20	0,02	0,03	1,70	55	258	0,3343	2,54	4,64	0,02	0,04	1,88	61	285	0,37
Ulva	Seaweed cheese	dried	8,7	2,20	4,00	0,02	0,03	1,80	53	253	0,3375	2,41	4,38	0,03	0,04	1,97	58	277	0,37
Ulva	Seaweed cheese	rehydrated	93,2	0,12	0,37	<0.005	<0.0035	0,22	2	15	<0.11	1,76	5,44			3,24	35	221	
Ulva	Seaweed cheese	rehydrated	93,5	0,09	0,29	<0.005	<0.0035	0,11	3	18	<0.11	1,34	4,46			1,69	40	277	
Ulva	Seaweed cheese	rehydrated	91,4	0,19	0,35	<0.005	<0.0035	0,70	8	25	<0.11	2,21	4,07			8,14	95	291	
Ulva	Seaweed cheese	rehydrated	91,6	0,13	0,24	<0.005	<0.0035	0,09	3	20	<0.11	1,55	2,86			1,01	30	238	
Ulva	Seaweed cheese	final product	33,4	<0.1	<0.05	<0.02	<0.015	<0.015	0	5	<0.11						0	8	
Ulva	Seaweed cheese	final product	33,3	<0.1	<0.05	<0.02	<0.015	<0.015	0	5	<0.11						0	7	
Ulva	plant-based sausages	dried	17,9	3,10	3,90	0,10	<0.015	0,43	59	354	0,2735	3,78	4,75	0,12		0,52	72	431	0,33
Ulva	plant-based sausages	dried	17,7	2,90	3,80	0,10	<0.015	0,42	57	351	0,2550	3,52	4,62	0,12		0,51	70	427	0,31
Dulse	plant-based sausages	dried	10,8	3,80	8,70	0,12	<0.015	0,62	143	485	0,4087	4,26	9,75	0,13		0,70	161	544	0,46
Dulse	plant-based sausages	dried	10,5	3,70	9,80	0,12	<0.015	0,60	138	499	0,3097	4,13	10,95	0,13		0,67	155	558	0,35
Ulva	plant-based sausages	raw dough	66,0	0,14	0,07	<0.02	<0.015	<0.015	1	<7.8	<0.11	0,41	0,22				2		
Ulva	plant-based sausages	raw dough	61,0	0,13	0,07	<0.02	<0.015	0,02	1	<7.8	<0.11	0,33	0,18			0,04	3		
Ulva	plant-based sausages	cooked dough	61,3	0,17	0,08	<0.02	<0.015	<0.015	1	<7.8	<0.11	0,44	0,20				2		
Ulva	plant-based sausages	cooked dough	60,2	0,16	0,10	<0.02	<0.015	<0.015	1	<7.8	<0.11	0,40	0,25				3		
Ulva	plant-based sausages	final product	53,8	0,21	0,16	0,02	<0.015	0,02	1	<7.8	<0.11	0,45	0,35	0,05		0,03	2		
Ulva	plant-based sausages	final product	53,8	0,19	0,08	<0.02	<0.015	<0.015	1	<7.8	<0.11	0,41	0,18				2		
Ulva	animal feed protein	Marc1	18,0	0,88	2,40	0,05	0,00	0,29	121	174	0,1318	1,07	2,93	0,06	0,00	0,35	147	212	0,16
Ulva	animal feed protein	Marc1	18,0	0,88	2,70	0,06	<0.0035	0,30	116	169	0,1280	1,07	3,29	0,07		0,37	141	206	0,16
Ulva	animal feed protein	C - supernatant	3,1	0,21	0,28	0,01	0,00	0,01	5	5	<0.11	0,22	0,29	0,01	0,00	0,01	5	5	
Ulva	animal feed protein	C - supernatant	3,1	0,21	0,27	0,01	0,00	0,01	5	5	<0.11	0,22	0,28	0,01	0,00	0,01	5	5	
Ulva	animal feed protein	C - pellet	11,0	0,95	1,80	0,04	0,00	0,26	77	121	<0.11	1,07	2,02	0,04	0,00	0,29	87	136	
Ulva	animal feed protein	C - pellet	11,0	0,94	1,60	0,04	<0.0035	0,23	96	129	<0.11	1,06	1,80	0,04		0,26	107	145	
Ulva	animal feed protein	P - supernatant	3,0	0,08	0,12	<0.005	<0.0035	0,01	4	9	<0.11	0,08	0,12			0,01	4	9	
Ulva	animal feed protein	P - supernatant	3,0	0,08	0,11	<0.005	<0.0035	0,01	4	9	<0.11	0,08	0,11			0,01	4	9	
Ulva	animal feed protein	P - pellet	8,5	1,00	1,60	0,04	<0.0035	0,23	61	113	<0.11	1,09	1,75	0,04		0,25	67	124	
Ulva	animal feed protein	P - pellet	8,5	0,86	1,60	0,04	<0.0035	0,22	73	126	<0.11	0,94	1,75	0,04		0,24	80	138	
Ulva	animal feed protein	C - total	5,5	0,24	0,59	0,01	<0.0035	0,06	23	39	<0.11	0,25	0,62	0,01		0,06	25	42	
Ulva	animal feed protein	C - total	5,5	0,23	0,52	0,01	<0.0035	0,04	20	39	<0.11	0,24	0,55	0,01		0,04	21	42	
Ulva	animal feed protein	P - total	5,5	0,24	0,70	0,02	<0.0035	0,07	23	41	<0.11	0,25	0,74	0,02		0,07	24	44	
Ulva	animal feed protein	P - total	5,5	0,22	0,67	0,01	<0.0035	0,07	32	45	<0.11	0,23	0,71	0,01		0,08	34	47	

seaweed	process	step	moisture	wet weight measured								dry weight calculated: conc moist sample / (1- proportional moisture)							
				Ni	As	Cd	Hg	Pb	I	Br	iAs	Ni	As	Cd	Hg	Pb	I	Br	iAs
			(m/m) %	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Saccharina	plant-based burger	washed	86,0	0,11	7,50	0,02	<0.015	0,35	449	150	<0.11	0,79	53,57	0,14		2,50	3207	1071	
Saccharina	plant-based burger	washed	85,6	0,11	7,70	0,02	<0.015	0,33	459	152	0,1563	0,76	53,47	0,15		2,29	3188	1056	1,09
Saccharina	plant-based burger	raw dough	67,4	0,16	0,82	0,02	<0.015	0,07	48	19	<0.11	0,49	2,52	0,07		0,21	147	58	
Saccharina	plant-based burger	raw dough	65,5	0,16	0,78	0,02	<0.015	0,05	44	18	<0.11	0,46	2,26	0,07		0,14	128	52	
Saccharina	plant-based burger	cooked dough	62,7	0,20	0,91	0,03	<0.015	0,09	45	17	<0.11	0,54	2,44	0,07		0,24	121	46	
Saccharina	plant-based burger	cooked dough	62,5	0,19	0,78	0,03	<0.015	0,04	47	16	<0.11	0,51	2,08	0,07		0,11	125	43	
Saccharina	plant-based burger	final product	60,8	0,19	0,88	0,03	<0.015	0,04	50	16	<0.11	0,48	2,24	0,07		0,11	128	41	
Saccharina	plant-based burger	final product	59,2	0,20	0,93	0,03	<0.015	0,04	50	18	<0.11	0,49	2,28	0,07		0,11	123	44	
Saccharina	recreated cheese process	washed	84,1	<0.1	10,40	0,03	<0.015	0,09	463	217	<0.11		65,41	0,19		0,58	2912	1365	
Saccharina	recreated cheese process	washed	83,5	0,13	11,90	0,03	<0.015	0,11	745	253	<0.11	0,79	72,12	0,18		0,67	4515	1533	
Saccharina	recreated cheese process	washed	85,3	0,11	10,60	0,03	0,02	0,12	498	234	<0.11	0,75	72,11	0,22	0,12	0,82	3388	1592	
Saccharina	recreated cheese process	washed	85,0	0,19	11,30	0,02	<0.015	0,17	648	281	<0.11	1,27	75,33	0,15		1,13	4320	1873	
Saccharina	recreated cheese process	dried	87,7	0,31	4,40	0,03	<0.015	0,12	193	93	<0.11	2,52	35,77	0,20		0,98	1569	756	
Saccharina	recreated cheese process	dried	86,9	0,17	4,10	<0.02	<0.015	0,17	295	141	<0.11	1,30	31,30			1,30	2252	1076	
Saccharina	recreated cheese process	rehydrated	86,6	0,11	2,80	0,04	<0.015	0,30	126	71	<0.11	0,82	20,90	0,33		2,24	940	530	
Saccharina	recreated cheese process	rehydrated	88,6	0,15	2,80	0,04	<0.015	0,48	153	83	<0.11	1,32	24,56	0,32		4,21	1342	728	
Saccharina	fermented	fermented	84,0	0,17	12,00	0,03	<0.015	0,18	603	282	<0.11	1,06	75,00	0,16		1,13	3769	1763	
Saccharina	fermented	fermented	81,0	0,22	12,20	0,02	<0.015	0,17	763	305	<0.11	1,16	64,21	0,12		0,89	4016	1605	
Saccharina	fermented	fermented	84,9	0,16	10,40	0,03	<0.015	0,18	680	285	<0.11	1,06	68,87	0,17		1,19	4503	1887	
Saccharina	fermented	fermented	84,1	0,13	10,60	0,03	<0.015	0,09	673	257	<0.11	0,82	66,67	0,18		0,57	4233	1616	
Saccharina	fermented	dried	3,4	1,10	71,00	0,17	0,07	1,10	4433	1750	0,3593	1,14	73,50	0,18	0,07	1,14	4589	1811	0,37
Saccharina	fermented	dried	3,4	1,10	69,00	0,17	0,07	1,10	4501	1846	<0.11	1,14	71,43	0,18	0,07	1,14	4659	1911	
Saccharina	fermented	dried	4,2	1,10	74,00	0,14	0,06	0,62	5299	1954	0,4120	1,15	77,24	0,15	0,06	0,65	5532	2040	0,43
Saccharina	fermented	dried	4,1	1,10	73,00	0,14	0,06	0,64	5257	1909	0,4572	1,15	76,12	0,15	0,06	0,67	5482	1991	0,48
Saccharina	fermented	rehydrated	91,5	<0.1	2,60	<0.02	<0.015	0,09	67	37	<0.11		30,59			1,01	790	431	
Saccharina	fermented	rehydrated	79,9	0,11	6,00	0,01	0,01	0,10	311	130	<0.11	0,55	29,85	0,06	0,04	0,50	1548	648	
Saccharina	fermented	rehydrated	64,8	0,14	8,10	0,02	<0.015	0,16	376	165	<0.11	0,40	23,01	0,06		0,45	1068	469	
Saccharina	dried	dried	5,3	0,73	82,40	0,15	0,06	0,84	4375	1899	0,3340	0,77	87,01	0,16	0,06	0,89	4620	2005	0,35
Saccharina	dried	dried	11,6	2,30	64,30	0,14	0,06	1,80	5620	2325	0,6159	2,60	72,74	0,16	0,07	2,04	6357	2630	0,70
Saccharina	dried	dried	10,8	1,40	69,50	0,13	0,05	1,10	5131	1978	0,3328	1,57	77,91	0,15	0,06	1,23	5752	2217	0,37
Saccharina	dried	dried	5,4	0,73	81,20	0,15	0,06	0,59	5381	2038	0,2915	0,77	85,84	0,16	0,06	0,62	5688	2154	0,31
Saccharina	dried	rehydrated	88,6	0,13	9,00	0,02	0,01	0,10	232	82	<0.11	1,14	78,95	0,14	0,06	0,86	2031	722	
Saccharina	dried	rehydrated	88,7	0,12	8,40	0,01	0,01	0,10	628	276	<0.11	1,06	74,34	0,12	0,06	0,85	5557	2439	

Table A1 *Compilation of all data used in the present report, sorted by seaweed species, processing chain and processing steps. We report the moisture of the sample ("moisture") and the results of the elemental analysis of the "moist" sample ("wet weight measured") as well as the calculated dry weight based concentrations ("dry weight calculated"). Results below LOQ are highlighted in red, the shade of red indicates different LOQ levels depending on the initial moisture of the sample. Note that results below LOQ are not used in the calculations of the dry weight based concentrations. Note also two samples highlighted in yellow where the moisture results are much higher than expected.*

Annex 2 Averages and proportional changes dataset

			averages dry weight								proportional changes in relation to the starting material							
seaweed	**process**	**step**	**Ni**	**As**	**Cd**	**Hg**	**Pb**	**I**	**Br**	**iAs**	**Ni**	**As**	**Cd**	**Hg**	**Pb**	**I**	**Br**	**iAs**
			mg/kg	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
Ulva	Seaweed cheese	harvest	4,38	5,94	0,11	0,05	4,62	67	649	1,02	100	100	100	100	100	100	100	100
Ulva	Seaweed cheese	centrifuged	2,12	4,63			1,01	47	268		48	78			22	69	41	
Ulva	Seaweed cheese	dried	2,02	4,44	0,03	0,03	1,28	50	283	0,27	46	75	24	56	28	75	44	26
Ulva	Seaweed cheese	rehydrated	1,72	4,21			3,52	50	257		39	71			76	75	40	
Ulva	Seaweed cheese	final product						0	7							1	1	
Ulva	plant-based sausages	dried	3,65	4,68	0,12		0,52	71	429	0,32	100	100	100		100	100	100	100
Dulse	plant-based sausages	dried	4,20	10,35	0,13		0,68	158	551	0,40	115	221	110		132	223	128	125
Ulva	plant-based sausages	raw dough	0,37	0,20			0,04	3			10	4			8	4		
Ulva	plant-based sausages	cooked dough	0,42	0,22				2			12	5				3		
Ulva	plant-based sausages	final product	0,43	0,26	0,05		0,03	2			12	6	41		7	3		
Ulva	animal feed protein	Marc1	1,07	3,11	0,07	0,005	0,36	144	209	0,16	100	100	100	100	100	100	100	100
Ulva	animal feed protein	C - supernatant	0,22	0,28	0,01	0,005	0,01	5	5		20	9	11	102	3	3	2	
Ulva	animal feed protein	C - pellet	1,06	1,91	0,04	0,005	0,28	97	141		99	61	64	107	77	67	67	
Ulva	animal feed protein	P - supernatant	0,08	0,12			0,01	4	9		8	4			2	3	4	
Ulva	animal feed protein	P - pellet	1,02	1,75	0,04		0,25	73	131		95	56	58		68	51	63	
Ulva	animal feed protein	C - total	0,25	0,59	0,01		0,05	23	42		23	19	21		14	16	20	
Ulva	animal feed protein	P - total	0,24	0,73	0,01		0,07	29	45		23	23	22		21	20	22	
Saccharina	plant-based burger	washed	0,77	53,52	0,14		2,40	3197	1063	1,09	100	100	100		100	100	100	100
Saccharina	plant-based burger	raw dough	0,48	2,39	0,07		0,17	137	55		62	4	49		7	4	5	
Saccharina	plant-based burger	cooked dough	0,52	2,26	0,07		0,18	123	44		67	4	48		7	4	4	
Saccharina	plant-based burger	final product	0,49	2,26	0,07		0,11	125	42		63	4	48		4	4	4	
Saccharina	recreated cheese process	washed	0,93	71,24	0,19	0,12	0,80	3784	1591		100	100	100	100	100	100	100	
Saccharina	recreated cheese process	dried	1,91	33,54	0,20		1,14	1911	916		204	47	110		142	50	58	
Saccharina	recreated cheese process	rehydrated	1,07	22,73	0,33		3,22	1141	629		114	32	176		404	30	40	
Saccharina	recreated cheese process	washed	0,93	71,24	0,19	0,12	0,80	3784	1591		100	100	100	100	100	100	100	
Saccharina	fermented	fermented	1,02	68,69	0,16		0,94	4130	1718		110	96	85		118	109	108	
Saccharina	fermented	dried	1,14	74,57	0,16	0,06	0,90	5065	1938	0,43	122	105	87	53	112	134	122	
Saccharina	fermented	rehydrated	0,47	27,82	0,06	0,04	0,65	1135	516		51	39	34	32	82	30	32	
Saccharina	recreated cheese process	washed	0,93	71,24	0,19	0,12	0,80	3784	1591		100	100	100	100	100	100	100	
Saccharina	dried	dried	1,43	80,87	0,16	0,06	1,20	5604	2252	0,43	153	114	84	51	150	148	142	
Saccharina	dried	rehydrated	1,10	76,64	0,13	0,06	0,85	3794	1581		118	108	71	47	107	100	99	

Table A2 *Compilation of all averaged data used in the present report, sorted by seaweed species, processing chain and processing steps. We report averages of replicates per processing step and processing chain of the dry-weight based elemental concentrations ("averages dry weight). By setting the starting material to 100%, changes in elemental concentrations along the processing chain were expressed as % remaining in the intermediate or end product in relation to the starting material.*

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