SLUTTRAPPORT

På sporet av ny mat – innhold og biotilgjengelighet av jod, uorganisk arsen, kadmium og kobber fra tare

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I Sammendrag

Det er stor etterspørsel etter mer kunnskap om innhold og biotilgjengelighet av jod, uorganisk arsen og metaller i forhold til bruk av tare som fôringrediens og som mat. En optimalisert analysemetode for måling av jod og uorganisk arsen i tare har blitt utarbeidet. Videre har vi i prosjektet undersøkt hvordan prosessering og lagring påvirker innholdet av jod i tare, i tillegg er det også undersøkt hvordan sesongvariasjonen påvirker nivåene av jod og kadmium i tare langs kysten. Biotilgjengeligheten av jod, uorganisk arsen, arsen, kadmium og kobber, samt effekten av et høyt jodinntak fra sukkertare har blitt vurdert i en gnagermodell. En SWOT analyse har blitt utarbeidet for å undersøke bruken av tare som ingrediens i fôr til laks.

Summary

There is a great demand for more knowledge regarding content and bioavailability of iodine, inorganic arsenic and metals in relation to the use of kelp as a feed ingredient and as food. An optimized method for measuring iodine and inorganic arsenic in kelp has been developed. Furthermore, in the project we evaluated how processing and storage affect the iodine content, and how seasonal variation affects the levels of iodine and cadmium in kelp along the coast. The bioavailability of iodine, inorganic arsenic, total arsenic, cadmium and copper, in addition to the health effect of a high iodine intake from sugar kelp has been assessed in a rodent model. A SWOT analysis has been prepared to investigate the use of kelp as an ingredient in salmon feed.

2 Innledning

Tang og tare omfatter alle større havalger, men ofte defineres tang som arter knyttet til fjæresonen, mens tare vokser dypere. I Asia har tang og tare historisk blitt brukt som tilsetning i mat, men i Norge og Europa har det hovedsakelig blitt brukt som i dyrefôr spesielt i kystnære strøk. Tang og tare blir tilsatt i flere produkter nå sammenlignet med tidligere og har blitt en vanligere ingrediens i vårt kosthold. Innholdet av jod i makroalger varierer veldig avhengig av familie og art, men generelt er innholdet av jod høyest i brunalger, noe lavere i rødalger og lavest i grønnalger. I tillegg til å ha et høyt innhold av jod har også flere havalger et betydelig innhold av mineraler, metaller og fremmedstoffer, hvor spesielt innhold av uorganisk arsen, kadmium og delvis kobber er ansett som problematisk. Første delmål i prosjektet er å optimalisere og validere analysemetodene for å kvantifisere uorganisk arsen og jod i alger. En validering av jodinnholdet i alger er helt nødvendig for å kunne beregne anbefalt inntak av jod og jodprodukter. På grunn av mange ulike arsenforbindelser i alger, er det nødvendig med en optimalisering analysemetodene for å bestemme mengden uorganisk arsen så korrekt som mulig. Andre delmål er å kvantifisere jod og metallinnhold i alger fra ulik lokasjon og sesong. Prøvene som er analysert er tatt fra ulike tidspunkt i sesongen, i tillegg er prøvene også fra ulike steder rundt langs norskekysten. Prosjektet kartlegger systematisk betydningen av lokalisasjon og sesong i forhold til innhold av jod og metaller. Det ble også undersøkt hvordan oppvarming, prosessering/bearbeiding og lagring påvirker innholdet av jod i sukkertare. Tredje delmål av prosjektet er å øke kunnskap om helseeffekten av sukkertare i dietten, med hovedfokus på biotilgjengeligheten av jod. Biotilgjengeligheten av jod, arsen, kadmium og kobber ble undersøkt ved at dietter tilsatt sukkertare med høyt jodinnhold eller jod i form av KI ble gitt rotter og utskillelsen ble kvantifisert i urin og avføring. Forsøket ble gjennomført etter OECD sine retningslinjer, og ulike parametere og helseeffekter ble undersøkt etter 90 dager med sukkertare i dietten. Morfologiske endringer av skjoldbruskkjertelen og hormonene TSH, T3 og T4 ble kvantifisert. Tolv metabolitter knyttet til skjoldbruskkjertelen ble målt i plasma, i tillegg til metabolomics fra levervev. Siste delen av sluttrapporten inneholder en SWOT analyse hvor potensiale for bruk av tare som fôringrediens i lakseoppdrett blir utredet.

3 Problemstilling, formål og arbeidspakker

Hovedmål for prosjektet

Å øke kunnskapen om innhold og biotilgjengelighet av jod og metaller fra norsk sukkertare for potensielt bruk som mat og fôringrediens.

Delmål for prosjektet

Delmål 1: Å optimalisere analysemetode for riktig bestemmelse av jod og uorganisk arsen i tare

Delmål 2: Å øke kunnskap om betydning av lokalitet (geografisk spredning), sesong (høsta januar – mai) og prosessering/ bearbeiding/ lagring for mengde jod og metaller i sukkertare.

Delmål 3: Å øke kunnskapen om (og hvordan) tare i maten påvirker helsen til gnagere

Arbeidspakkebeskrivelse/ gjennomføring

Arbeidspakke 1. Innhold av jod, uorganisk arsen, kadmium og kobber i norsk sukkertare. Arbeidspakke-leder: Arne Duinker, NIFES. Samarbeidspartnere: Jens Jørgen Sloth (Dansk Teknisk Universitet, DTU), Veronika Sele (NIFES), Harald Sveier (Ocean Forest).

Arbeidspakke 2. Biotilgjengelighet og helseeffekter og av jod, uorganisk arsen, kadmium og kobber fra tare i dietten. Arbeidspakke-leder: Bjørn Liaset, NIFES. Samarbeidspartnere: Marc Berntsen, Heidi Amlund, Lisbeth Dahl, Arne Duinker (alle NIFES), Jens Jørgen Sloth (DTU).

Arbeidspakke 3. Potensiale for bruk av tare som fôringrediens i lakseoppdrett – en SWOT analyse. Arbeidspakke-leder: Erik-Jan Lock.

4-6 Sammendrag/konklusjoner fra hver arbeidspakke

(Hver arbeidspakke har et vedlegg som inneholder prosjektgjennomføring, resultater, diskusjon og konklusjon i form av en rapport eller et manuskript)

Arbeidspakke 1: Innhold av jod, uorganisk arsen, kadmium og kobber i norsk sukkertare

Sammendrag Arbeidspakke 1.1 og 1.2: Vedlegg 1: Innhold av jod, uorganisk arsen, kadmium og kobber i norsk sukkertare

Konklusjon

- En metode til bestemmelse av jod i tang og tare er identifisert (EN17050:2017). Metoden har vist at gi troverdige resultater for analyse av jod i tare. Metoden er utviklet på DTU og implementert på både DTU og HI.

- Et sertifisert referansemateriale av en brunalge (SRM3232, Laminaria) med sertifisert verdi for jod er identifisert og anskaffet. Analyser av dette materiale med EN17050 viser at metoden gir korrekte resultater for denne prøvetype.

- Systematiske isokroniske forsøk over 8 uker ble gjennomført for at undersøke stabiliteten av jod i algebiomasse og algeekstrakter. Resultatene viste at jod i biomasse er ganske stabilt og tørt tarepulver kan oppbevares i lengre tid uten tap av jod. Jodinnholdet i tareekstrakt er stabilt så lenge beholderen er lukket, mens i åpne beholdere vil det skje et tap av jod for syreekstrakter.

Sammendrag av forsøk knyttet til stabilitet: Vedlegg 2: Report on investigation of stability of iodine in seaweed biomass and seaweed extracts

Conclusion

- According to the study, there is no need for storing the dry samples in which iodine is to be determined in the fridge or freezer, since temperature up to 60 °C does not affect iodine content in dry seaweed samples for at least 8 weeks. At room temperature (around 20 °C) samples can be stored for at least 6 months without changes in iodine content.
- Extracts can be prepared at least 8 weeks before measurement and stored at room temperature regardless of the solution pH (between 4 and 10) without questioning the trueness of the total iodine results, since iodine content does not change under these conditions. However, if extracts are stored at higher temperatures (up to 60 °C), water loss should be evaluated by weight and considered in calculations.
- If iodine is present in the acidic solution in inorganic form (I^- or IO_3^-) there is a risk to be lost due to volatilization. Use of alkaline extraction is therefore preferred over acidic digestion as a sample preparation procedure.

Arbeidspakke 1.3:

Rapport med oppdaterte data knyttet til denne arbeidspakken: Vedlegg 3: Knowledge update on macroalgae food and feed safety

Sammendrag

Geografisk variasjon

Tare ble dyrket etter standardisert oppsett på 9 ulike lokaliteter fra sør til nord i Norge. Både kadmium, uorganisk arsen og jod viste signifikant variasjon mellom lokalitetene. For kadmium ble det funnet en sammen med breddegrad ved at kadmium økte 4 ganger fra sør til nord (0,3-1,5). De to nordligste lokalitetene hadde konsentrasjoner som var høyere enn det som er tillatt for ingredienser i fiskefôr.

Sesongvariasjon

For jod var det en generell økning gjennom sesongen, noe maskert av stor variasjon. For en lokalitet i Trøndelag var det en økning fra 2500 mg/kg tørrvekt i uke 16 til nærmere 4000 uke 22 og 24. For en lokalitet i Troms var økningen fra 2000mg/kg i uke 18 til 5000 i uke 27. Uke 24 var det imidlertid 3000 på 1 m dyp mot 4600 på 8 m dyp. Taren vokste i hele denne perioden, og avveininger må evt. tas mellom biomasse og lavere jodinnhold. Kadmium viste ingen klar sesongvariasjon, men en del variasjon med dybde. Kadmium var generelt lavere i mer saktevoksende tare på 8 m enn på 1 m. På tross av stor variasjon var det til tide klare forskjeller. For eksempel var konsentrasjonen av Cd i Troms dobbelt så høy på 8 m (2 mg/kg tørrvekt) i forhold til 1 m i uke 24. 8 m er vel ikke så realistisk dyrkningsdybde, men eksempelet viser likevel at dybde er av betydning. Dette kan være viktig i nord der konsentrasjonene er høyest.

Tilberedning/prosessering

Tørking av tare bidrar ikke til vesentlig reduksjon av jod. Her er det en del variasjon, fra 25 % reduksjon til ingen endring selv etter oppbevaring av tørket tare i fuktig miljø. Ved koking i 15 minutter: Vi finner 10-50 % av jod igjen i kokt tare og 20-40 % igjen i kokevannet. Tap til luft blir ca 50 %. Ved videre koking av kokevannet reduseres jod innholdet ytterligere, og etter innkoking var 15-20 % av opprinnelig jod fra taren igjen. I ettertid er disse forsøkene gjentatt, men uten den samme reduksjon i jodinnhold. Vi jobber fortsatt med å finne ut hvordan prosessen med tap av jod til luft kan anvendes for jodreduksjon i tareprodukter.

Steking av både fersk og tørket tare viser også en vesentlig nedgang i jod innholdet, og lite finnes igjen i oljen. Estimert tap til luft ved avdamping av jod er fra ca. 25 til over 80 %.

Arbeidspakke 2. Biotilgjengelighet og helseeffekter og av jod, uorganisk arsen, kadmium og kobber fra tare i dietten

Manuskript knyttet til arbeidspakke 2: Vedlegg 4: Iodine bioavailability and accumulation of arsenic and cadmium in rats fed sugar kelp (Saccharina Latissima)

Summary:

Mild iodine deficiency and suboptimal iodine status are prominent issues in several European countries, due to generally lower intake of our main sources of iodine, including dairy products and seafood. Brown algae have a high content of iodine, but the iodine bioavailability have been reported to be lower than potassium iodide, and highly dependent of species. Further, potential negative effects of other compounds in algae, such as cadmium (Cd) and arsenic (As) have also been addressed. In the present study, we compared the bioavailability of iodine from farmed sugar kelp (Saccharina latissimi) with potassium iodide (KI) in female Wistar IGS rats. The experimental diets contained 14-17 mg/kg or 160-200 mg/kg iodine, and fecal and urinary iodine excretion were monitored. 94-95 % of the total iodine intake was excreted in urine from rats fed both the low and high dose of potassium iodide. Urinary iodine excretion was lower in rats given iodine from sugar kelp, but still relatively high (73-81%) and accompanied by increased fecal iodine excretion. Overall, a reduction in iodine bioavailability was observed in rats fed farmed sugar kelp compared to KI. No significant adverse effects related to body weight development, feed efficiency and plasma markers for liver or kidney damage were observed in rats fed very high levels of iodine from either KI or sugar kelp. Reduction in plasma free T4 (fT4) and T4 were observed in rats fed the highest dose of iodine, but no significant effects on circulating levels of TSH and free T3 were detected. Total As levels in feces and urine indicate that 60-80% of total As and more than 93% of Cd ingested was excreted in rats fed 0.5 and 5% kelp. Liver metabolomic profiling demonstrates that a high inclusion of sugar kelp (5%) in the diet for 13-weeks of feeding has pronounced effects on liver metabolites involved in cholesterol syntheses and the redox potential, and potentially reduces the hepatoprotective effects.

Conclusion:

- More than 73 % of iodine ingested from sugar kelp (Saccharina Latissima) was excreted in urine within 24-h, compared to 94-95 % in rats fed potassium iodide (KI). Iodine excretion in feces was four times higher in rats fed iodine from sugar kelp than KI.
- More than 60% of total arsenic (As) and 93% of cadmium (Cd) ingested from sugar kelp was excreted in feces and/or urine within 24 hours. Inorganic As (iAs) was below the limit of quantification (LOQ) in liver tissue of rats fed sugar kelp.
- Body weight, feed efficiency and plasma markers for liver damage or kidney failure were not altered after a 13-week period with a daily intake of iodine of 220-237 or 2000-2300 ug.
- Excessive iodine intake did not cause significant changes in circulating thyroid stimulating hormone (TSH) or the thyroid regulated hormone triiodothyronine (T3) in rats fed either iodine from kelp or KI. Reduced levels of plasma thyroxine (T4) were observed in rats fed the highest dose of iodine from both sugar kelp and KI.

- Rats fed the highest dose of sugar kelp (5%) had a distinct metabolite profile in liver, separating from the other dietary groups. Several metabolites involved in cholesterol syntheses and affecting the redox potential were altered.

Arbeidspakke 3. Potensiale for bruk av tare som fôringrediens i lakseoppdrett – en SWOT analyse

Rapport knyttet til arbeidspakke 3: Vedlegg 5: Rapport: SWOT analysis seaweed in aquafeed

Summary:
Finfish aquaculture has been a quickly developing industry during the past three decades and is expected to continue this growth in the foreseeable future. The aquafeed industry has to keep up with this growth and maybe even faster, since many of the traditional forms of finfish culture (e.g. in Asia) are being intensified which often results in the use of formulated feeds. The main ingredients in aquafeed nowadays come from terrestrial sources, with the exception of fishmeal and –oil. Even though it is a well-known fact that a kg of fish fillet needs considerable less resources than a kg of beef or even pork, aquaculture will inevitably put more pressure on land resources. The blue economy aims to produce more food from the sea to satisfy the global need for nutrients. Finfish aquaculture is often used as an example of how to achieve this. Indeed, the potential is large, however this is only possible if we manage to harvest the primary producers from the sea as well. Seaweed is one of the primary producers in the marine food chain, similar to plants in the terrestrial food chain. Although seaweed are taxonomically not plants, many parallels between seaweed and plants exists. Both can be a valuable source of nutrients that can be used by animals higher up the food chain, but both also contain anti-nutritional factors, preventing them from being preyed on by these animals. The effect of a plain soybean meal on the development of enteritis in Atlantic salmon is well known and similar effects are seen of peas and other vegetable products. In commercial diets it is highly processed protein concentrates of these plant products that are used. This removes or reduces many of these anti-nutritional factors and simultaneously concentrates the protein content of the product. Salmon is a carnivorous fish that requires protein, lipid and micronutrients for healthy growth, the requirement for carbohydrates is very low. Seaweed is mainly made-up of carbohydrates that cannot be used by the fish. This raises the obvious questions about post-harvest processing of seaweed to make nutrients more accessible and remove anti-nutritional factors, which currently are underdeveloped or non-existing. A fractionation of the seaweed biomass is needed where high-end products (e.g. alginates) can offset a large part of the production and processing costs. The lack of seaweed processing and diversification of the processing is the major hurdle for the use of seaweed in aquafeed. This analysis will elaborate on the strengths and weaknesses of using seaweeds in feed for fish and pinpoints future changes that could stimulate (opportunities) or raise barriers (threats) in the application of marine macroalgae in aquafeed.

7 Hovedfunn

- En metode til bestemmelse av jod i tang og tare er utviklet (EN17050:2017), og analyser av sertifisert referansemateriale av en brunalge (SRM3232, Laminaria) gir god nøyaktighet for denne prøvetypen. Metoden er utviklet på DTU og implementert på både DTU og HI. Videre har vi også vist at jod i biomasse er ganske stabilt og tørt tarepulver kan oppbevares i lengre tid uten tap av jod.

- Mengden jod i tare viser generell økning gjennom sesongen, men stor variasjon ble observert mellom ulike lokalisasjoner. Kadmiumnivået i tare viste tydelig sesongvariasjon, men også betydelig variasjon med havdybde. Steking, tørking og koking av tare medførte betydelig reduksjon i jod-innholdet, men reduksjonen varierte avhengig av prosessering.

- Jod fra sukkertare hadde en signifikant lavere biotilgjengelighet (73%) sammenlignet mot kaliumjodid (95%) når dette ble gitt i dietten til gnagere. Fire ganger høyere nivå av jod ble målt i avføringen til gangere gitt sukkertare sammenlignet med gnagere gitt kaliumjodid. Overdrevent inntak av jod fra sukkertare eller kaliumjodid medførte ingen endring i thyreoideastimulerende hormon (TSH) og få endringer ble observert i de jod regulerte hormonene knyttet til skjoldbruskkjertelen. Redusert nivåer av tyroksin (T4) ble observert ved inntak av de høyeste dosene jod fra både tare og kaliumjodid. Det ble ikke observert noen negative fysiologiske effekter ved inntak av sukkertare over en 13 ukers-periode, men rotter gitt høyeste dose sukkertare hadde en noe endret metabolsk profil i lever.

8. Leveranser

- Oppstartsmøte med referansegruppe,
- Potensiale for bruk av tare som fôringrediens i lakseoppdrett – en SWOT analyse (AP 3)
- Resultater fra prosessering og tilberedning av tare (AP 1.3)
- Forberedelser gnagerforsøk (framstilling taremel, fôr, fôranalyser, søknad godkjenning dyreforsøk etc) (AP 2.1 og 2.2)
- Optimalisering av analyse metode for måling av jod uorganisk arsen (AP 1.1)
- Optimalisering av analyse metode for måling av jod (AP 1.2)
- Ferdigstille dyreforsøk 1 med analyser (AP 2.1)
- Ferdigstille metabolomics data fra levervev og thyroidea metabolitter i plasma fra gnagerforsøket (AP 2.2)
- Analysedata fra geografisk og sesongmessig variasjon (1.3)
- Delta på to seminar/konferanser i prosjektperioden.
- Ferdigstille vitenskapelig manuskript
- Sluttrapport

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WP 1: Innhold av jod, uorganisk arsen, kadmium og kobber i norsk sukkertare

1. Problemstilling og formål

Alger er havets grønnsaker, og interessen for alger som mat er økende i hele Europa. Mye av denne interessen skyldes høyt innhold av en rekke næringsstoffer og virkestoffer, samt fokus på ny mat med lavt «karbon fingerprint». Alger kan også ha enkelte utfordringer i forhold til mattrygghet, da de kan inneholde høye konsentrasjoner av jod, uorganisk arsen og kadmium. Et annet metall som er aktuelt i forbindelse med taredyrking i nærheten av lakseoppdrett er kobber, ettersom kobber brukes som antibegroingsmiddel i merder.

Delarbeidspakke 1.1. Validering av arsen-spesieringsmetode til spesifikk bestemmelse av uorganisk arsen i alger

Arsen er et element som finnes naturlig i høyere konsentrasjoner i marine prøver sammenlignet med terrestriske prøver. Arsen finnes i mengde forbindelser, både vannløselige og fettløselige, og organiske og uorganiske forbindelser. Uorganisk arsen er karsinogent og kan føre til ulike typer kreft, blant annet i lunger, hud og nyre (EFSA 2009). I 2009 publiserte EFSA en vitenskapelig opinion på arsen i mat i 2009, der det ble estimert at inntaket av uorganisk arsen i en voksen populasjon er høy og i området av $BMDL_{01}$ (0.3 til 8 µg/kg kroppsvekt per dag) for kreft i lunger, hud og nyre, samt hudlesjoner. I 2014 oppdaterte EFSA eksponeringsestimatet, og de oppdaterte estimatene var lavere, men fremdeles i området av BMDLs (EFSA 2014). Matvarer som f.eks. korn, ris og drikkevann er kilder til uorganisk arsen. Fisk og sjømat er også ansett som kilder som bidrar til eksponering av uorganisk arsen (EFSA 2009).

For uorganisk arsen, er det maksimumsgrenser for ris og risprodukter (EU 1881/2006 and amendments). Det er etablert øvre grense for totalmengden arsen i fôr og fôrmidler. Det er også en fotnote i regelverket som sier at operatøren skal kunne vise at innholdet av uorganisk arsen er under 2 mg/kg. Det er ikke etablert øvre grenser for uorganisk arsen i sjømat eller produkter av sjømat.

Alger har en kompleks kjemi når det kommer til arsenforbindelser. Alger inneholder ofte mer av de organiske arsenforbindelsene kallet arsenoribosider, eller arsensukker (Feldmann and Krupp 2011). Noen alger kan også inneholde høye konsentrasjoner av uorganisk arsen, som for eksempel den brune algen hijiki (*Sargassm fusiforme) (Taylor, Goodale et al. 2017)* . Noen arter av andre brunalger har det også blitt vist å kunne inneholde relativt høy andel av uorganisk arsen (Taylor, Goodale et al. 2017).

Den Europeiske kommittee for standardisering (CEN) har publisert en metode for bestemmelse av uorganisk arsen i mat og matvarer ved å benytte HPLC-ICPMS (EN 16802:2016). Metodens prinsipp er å bestemme uorganisk arsen som summen av arsenitt (As(III)) og arsenat (As(V)) ved å benytte HPLC koblet til ICPMS. Innen den instrumentell analysen blir prøven ekstrahert ved sur oppslutning, og As(III) blir oksidert til As(V) ved å benytte hydrogenperoksid i ekstraksjonsløsningen. Uorganisk arsen kan på denne måten bli bestemt som As(V) ved å benytte anionbytter HPLC-ICPMS[1]. Ved analyser av alger, har det vært mistanke om interferenser med andre arsenforbindelser i analysemetoden. Formålet med denne delarbeidspakken var å sikre at den anvendte analysemetode optimeres slik at bestemmelsen av uorganisk arsen blir korrekt og det ikke er interferens fra andre arsenforbindelser ved analyse av makroalger og tare.

[1] Væskekromatografi koblet til et induktivt koblet plasma massespektrometer.

2. Prosjektgjennomføring

Den analytiske metoden for bestemmelse av uorganisk arsen (EN 16802:2016) baserer seg på en separasjon av arsenforbindelser ved bruk av ionebytterkromatografi. Det benyttes en anionbytter HPLC kolonne, som binder til seg anioner, som arsenat ($As(O^-)_3$). I metoden benyttes er en isokratisk mobilfase som gir en separasjon av uorganisk arsen fra andre organiske arsenforbindelser, med en analysetid på totalt 8 minutter. Utfordringen med korte og effektive metoder vil være å få separert uorganisk arsen fra organisk arsenforbindelser i prøvetyper som inneholder en høy andel organiske arsenforbindelser, og særlig når prøvetypen inneholder andre former for organiske arsenforbindelser enn arsenobetaine. Alger og tare kan inneholde en større andel arsensukker, og noen av disse forbindelsene kan potensielt elueres i samme område som retensjonstiden til uorganisk arsen.

For å optimere analysemetoden slik at bestemmelsen av uorganisk arsen blir korrekt, og for å forsikre at det ikke er interferens fra andre arsenforbindelser, ble det vurdert og testet ut ulike fremgangsmåter;

1. *Bruk av andre kromatografiske prinsipper for separasjon av uorganisk arsen*

Det ble undersøkt hvordan arsenforbindelsene i tareprøver ble separert på HPLC kolonner basert med andre separasjonsprinsipper, både kationbytter kromatografi og omvendt-fase kromatografi. Formålet var å undersøke om ekstrakter av tareprøver som hadde høye konsentrasjoner av uorganisk arsen (9 – 11 mg/kg tørrvekt[2]) kunne inneholde organisk arsenforbindelser, som muligens ble holdt igjen på en HPLC kolonne med annen type stasjonærfase.

2. *Fraksjonering av uorganisk arsen topp, og analyse med HR-MS*

Det ble fraksjonert ut topper av uorganisk arsen fra tareprøver ved å samle opp eluated i retensjosntidsvinduet til uorganisk arsen når analyseres for uorganisk arsen[2]. Formålet var å analysere disse fraksjonene for arsenforbindelser med høyoppløselig massespektrometri (HR-MS).

3. *Bruk av gradient-eluering og anionbytter kromatografi*

Den isokratiske metoden for uorganisk arsen ble videreutviklet fra å være en isokratisk metode til en gradient-elureringsmetode der separasjonen baserer seg på to ulike mobilfaser. Formålet var å få en mer gradvis separasjon av arsenforbindelsene slik at separasjon av uorganisk arsen ble forbedret.

3. Oppnådde resultater og diskusjon

1. *Bruk av andre kromatografiske prinsipper for separasjon av uorganisk arsen*

Utvalgte tareprøver som ved tidligere analyser hadde vist høye nivå av uorganisk arsen, samt prøver som hadde vist at den kromatografiske toppen for uorganisk arsen var asymmetrisk, ble analysert ved å benytte andre kromatografiske prinsipper for separasjonen. Resultatene fra disse analyser viste at arsenforbindelsene i ekstraktene ikke ble holdt tilbake på en kationbytter-kolonne. Det var dermed ikke mulig å verifisere om det var andre organiske arsenforbindelser i toppen for uorganisk arsen ved å benytte denne fremgangsmåten. Lignende resultater ble oppnådd når ekstraktene ble analysert med omvendt-fase kromatografi, der det var dårlig separasjon av uorganisk arsen og arsenobetaine. Det ble fra forsøkene konkludert med at disse kromatografiske prinsippene var lite egnet for å separere uorganisk arsen fra andre organiske arsenforbindelser.

2. *Fraksjonering av uorganisk arsen topp, og analyse med HR-MS*

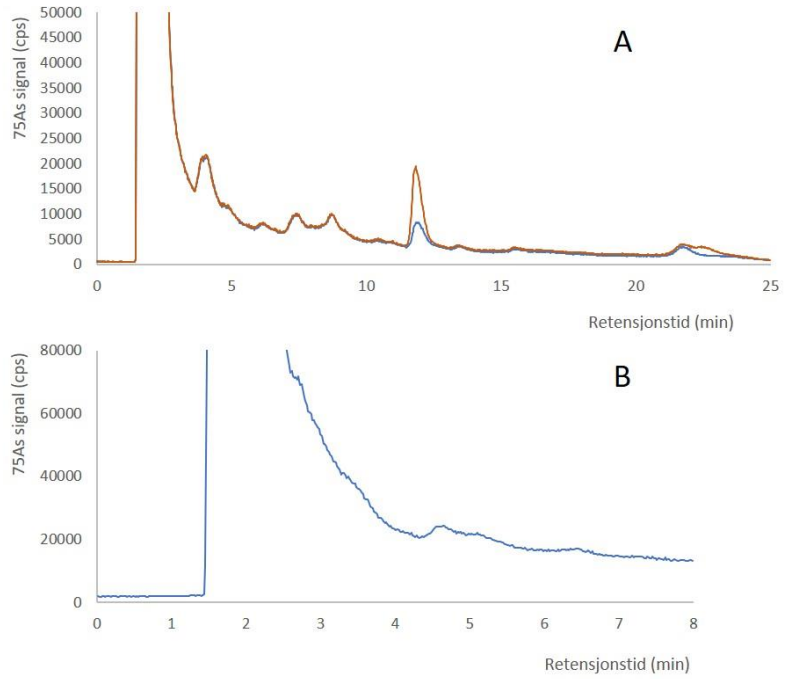
Fraksjoneringen av uorganisk arsen topp ble utført for utvalgte tareprøver med høye konsentrasjoner av uorganisk arsen. Men HR-MS analysene ble ikke utført som planlagt. Det var både analytiske og praktiske utfordringer som var grunnen til dette. Det var utfordringer med å få tilgang på

[2] Bestemt ved å benytte CEN metode (EN 16802:2016).

instrumentet, samt at fraksjoner inneholder salter fra den ioniske kromatografiske separasjonen som kan forårsaker utfordringer ved analyse med elektrospray MS. Det ble ut fra dette bestemt å heller fokusere på å optimalisere den nåværende analysemetoden for bestemmelse av uorganisk arsen i tare. Dette var mer hensiktsmessig med tanke på å kunne implementere en rutinemetode for uorganisk arsen bestemmelse i tare.

3. Bruk av gradient-eluering og anionbytter kromatografi

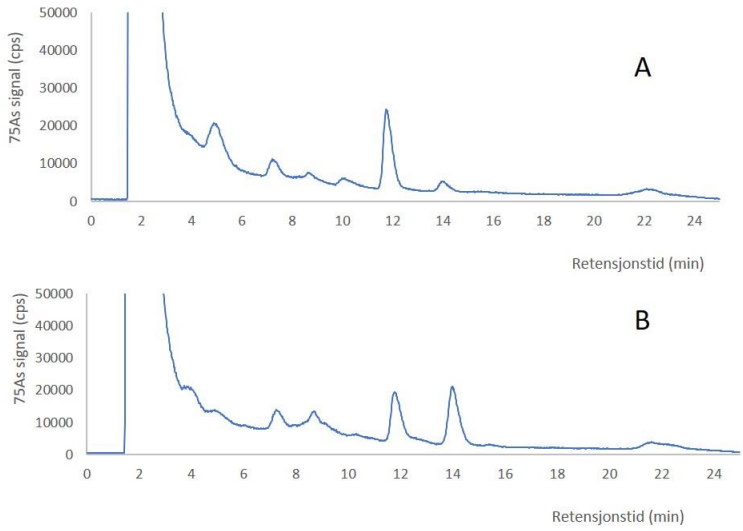
Med utgangspunkt i CEN metoden for uorganisk arsen, ble denne videreutviklet fra å være en isokratisk metode til å benytte en gradient eluering. Ved å benytte to mobilfaser; 3% MeOH (A) og 60 mM ammonium karbonat, pH 9.3 (B), ble separasjonen av uorganisk arsen forbedret. Gradient-elueringen startet på 20% innblanding av mobilfase B, som ble gradvis økt til 100% innblanding av B ved 18 min. Etter 20 min ble igjen mengde mobilfase B redusert til 20% innblanding. Analysetiden ble totalt forlenget til 25 minutter. Metoden gav en tydelig forbedret separasjon av uorganisk arsen fra de andre organisk arsenforbindelsene i tare (Figur 1A og 1B).



Figur 1. Et HPLC-ICPMS kromatogram av grisetang når analysert med gradient-elueringsmetoden (A); topp for uorganisk arsen er ved r.t. 11 min, her vist ved å tilsette uorganisk arsen standard (rød linje) til prøveekstraktet (blå linje). Til sammenligning er samme prøve analysert med den isokratiske standard metoden for bestemmelse av uorganisk arsen (B); topp for uorganisk arsen er ved r.t. 4.5 min.

Metoden ble videre benyttet for å bestemme uorganisk arsen i ulike tareprøver der det hadde vært erfaringsmessig utfordrende å definere den kromatografiske toppen til uorganisk arsen ved bruk av CEN metoden. Resultatene viste at flere av disse tareprøvene inneholdt en rekke arsenforbindelser som eluerte nær toppen for uorganisk arsen, som for eksempel sukkertare og grisetang (Figur 2A og B). Ettersom ICP-MS ikke gir noen informasjon om den kjemiske strukturen til forbindelsene, så har ikke disse arsenforbindelser blitt identifisert. Det arbeides nå med å validere den utviklede gradientmetoden for uorganisk arsen. Resultatene viser så langt god presisjon for bestemmelse av

uorganisk arsen med RSD(%) under 10% for sertifiserte referansemateriale, in-house referansemateriale og ulike algeprøver (n = 5) ved analyse på ulike tidspunkt (n = 6). Resultatene for uorganisk arsen for det sertifiserte referansematerialet B211 (ris, NIST) er på 129 ± 8 µg/kg (n=6) som er nær den sertifiserte verdien på 124 ± 11 µg/kg. Det er dessverre ikke mulig på nåværende tidspunkt å få tak i sertifiserte referansematerialer av tare som er sertifisert for uorganisk arsen. Videre arbeid vil fokusere på å sammenligne metoden og resultatene ved utførelse av analysene ved både HI og DTU i Danmark.



Figur 2. *Sukkertarer (A) og Blæretang (B) analysert for uorganisk arsen med gradient-elueringsmetoden. Uorganisk arsen eluerer ved retensjonstid 12 min.*

Konklusjon

Det har blitt utviklet en analysemetode som baserer seg på gradient-eluering anionbytter HPLC-ICPMS. Metoden viser en forbedret separasjon av uorganisk arsen i komplekse prøver som tang og tare. Det er startet en valideringsprosess av metoden, og videre arbeid vil bl.a. fokusere på å sammenligne metoden og resultatene ved utførelse av analysene ved både HI og DTU i Danmark.

Delarbeidspakke 1.2. Optimalisering og validering av analysemetode til bestemmelse av jod i alger

Problemstilling og formål

Makroalger inneholder generelt mye jod, og noen tarearter har svært høye konsentrasjoner, rundt 4 000 mg/kg tørrvekt eller ca 700 mg/kg våtvekt. Med en anbefalt øvre grense for daglig inntak av jod på 600 µg bør man i teorien ikke innta mer enn under ett gram tørket tare per dag. Et høyt inntak av jod påvirker skjoldbruskkjertelens funksjon og kan forårsake hypotyreose eller hypertyreose. I enkelte tilfeller er det også vist økt forekomst av jod-indusert struma. Personer med en normalt fungerende skjoldbruskkjertel kan i utgangspunktet håndtere et jodinntak på omkring 1 mg/dag over flere måneder, mens andre igjen vil få hypotyreose eller hypertyreose bare ved et inntak på 0.3 mg/dag; altså et jodinntak som er på 2 ganger lavere enn daglig anbefalt inntak.

De fleste analyser av sporelementer i biologiske prøver kan gjøres ved bruk av ICPMS etter syreoppslutning av prøven. Dette gjelder ikke for jod som i sur oppløsning vil danne en flyktig forbindelse hyrogeniodid (HI). Derfor er de fleste metoder til bestemmelse av jod basert på en alkalisk ekstraksjon. Jod kan være potensielt ustabilt, og denne ustabiliteten av jod i både prøvemasse og i løsning har vært grunnlag for diskusjon av jod-data.

For å kunne vurdere inntaket og dermed eksponering til jod er det viktig å ha kvalitetssikrede analytiske data. Det primære formål med denne delarbeidspakke var å sikre at analysemetoden for bestemmelse av jod i alger er etablert og validert, slik at kvalitetssikrede og troverdige data kan oppnås i prosjektet. I tillegg var formålet å undersøke stabiliteten av jod i biomasse og i ekstrakter bli nærmere undersøkt.

Gjennomføring

Jod ble bestemt i alger ved å anvende en metode som er utviklet og valideret ved DTU. Metoden baseres på basisk ekstraksjon ved bruk av TMAH (tetramethylammonium hydroxide) og deretter bestemmelse av jod i ekstraktet ved bruk av ICP-MS, ved bruk av ekstern kalibrering og med intern standard. Metoden blev i 2017 godkjent som europæisk standard for analyse av jod i animalske fôrprodukter (EN17050:2017) (CEN 2017). I den sammenheng blev metoden testet for analyse av et taremel (som fôringrediens) med gode resultater. For å kvalitetsikre analyseresultatene er det vanligt at inkludere et sertifiseret referansemateriale i analysen. I 2018 kom det første algebaserte referansemateriale med sertifisert verdi for jod på markedet fra National Institute of Standards and Technology (NIST). Dette materiale har navnet SRM 3232 kelp powder og er basert på et pulver av laminaria. Både DTU og HI har anskaffet dette materiale og anvendt det i analysene av jod i tang og tare. Disse analyser har bekreftet at den anvendte metodeprinsipp i EN17050:2017 gir korrekt resultat. Tilgjengeligheten av en standardmetode og et sertifisert referansemateriale for jod som kunne verifisere korrektheten av jod-analysene gjorde at det planlagte eksperimenter med NAA (Neutron Activation Analysis) blev nedprioritert. I stedet for blev det besluttet å anvende ressurserne på et studie av stabiliteten av jod i tare og i tare-ekstrakt. Disse forsøk har til formål å bidra med vigtig og ny informasjon om stabiliteten av jod i tare og tare-ekstrakt og gi informasjon relatert til oppbevaring av tare-prøver og -ekstrakter.

Resultater

Stabilitetsforsøkene er beskrevet i delrapport (finnes i appendix, på engelsk). Forsøkene er utført etter de standardiserte prinsippene i (Lamberty, Schimmel et al. 1998) som anvendes til å dokumentere

stabiliteten av referansematerialer. Forsøket ble satt opp som angitt i nedenstående tabell med variasjon av temperatur, tid og prøvetype (se appendix for ytterligere detaljer).

Temperatur	Time	Type of sample
• F → på frys (–20 °C) • R → rom T (approx. 20 °C) • H → høy T (60 °C)	• 3W → 3 uker • 5W → 5 uker • 8W → 8 uker • LP → lengre periode (6 months)	• SB → biomasse • EW → ekstraksjon i vann • EAc → ekstraksjon i syre • EAl → ekstraksjon i base

I løpet av forsøket ble prøvene etter endt behandling satt på frys (referansepunkt) og samlet sammen til analysen, som ble utført isokronisk for å minske eventuelle usikkerheter fra selve analysen.

Alle jod analyser er utført på DTU ved bruk av EN17050:2017 metoden.

Resultanene viste:

- Jodinnholdet i biomasse som ble eksponert for forskjellige temperaturer og tid var stabilt i de 8 uker som forsøket varte. Resultatene tyder dermed på at tørr biomasse kan oppbevares ved romtemperatur over lengre tid uten at jodinnholdet minker, eller blir påvirket.
- Jodinnholdet i forskjellige ekstrakter (vann, syre og base) oppbevart ved forskjellige temperaturer og tid i lukkende beholdere var stabilt over de 8 uker i forsøket, men også helt opp til 6 måneder. Dette betyr at ekstrakter kan oppbevares i lukkede beholdere uten tap av jod over lengre tid.
- Ved oppbevaring av syreekstrakter med åpne beholdere skjer et tap av jod (opp til 30% tap over 2 uker). Det skjer et større tap av jodid (I-) enn jodat (IO3-) i syreekstrakter.

Konklusjoner

- En metode til bestemmelse av jod i tang og tare er identifisert (EN17050:2017). Metoden har vist at gi troverdige resultater for analyse av jod i tare. Metoden er utviklet på DTU og implementert på både DTU og HI.
- Et sertifisert referansemateriale av en brunalge (SRM3232, Laminaria) med sertifisert verdi for jod er identifiset og anskaffet. Analyser av dette materiale med EN17050 viser at metoden gir korrekte resultater for denne prøvetype.
- Systematiske isokroniske forsøg over 8 uker blev gjennomført for at undersøke stabiliteten av jod i alge biomasse og alge ekstrakter. Resultatene viste at jod i biomasse er ganske stabilt og tørt tarepulver kan oppbevares i lengre tid uten tap. Jodinnholdet i tareekstrakt er stabilt, så lenge beholdere er lukket, mens i åpne beholdere vil det skje et tap av jod for syre ekstrakter.

Referanser:

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EFSA (2009). "Scientific Opinion on Arsenic in Food." EFSA Journal **7**(10).
EFSA (2014). "Dietary exposure to inorganic arsenic in the European population." EFSA Journal **12**(3).
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Appendix:

- Ana Jerse, Report on investigation of stability of iodine in seaweed biomass and seaweed extracts, DTU Food, 2019.

Report on investigation of stability of iodine in seaweed biomass and seaweed extracts

Project: På sporet av ny mat – innhold og biotilgjenglighet av jod, uorganisk arsen, kadmium og kobber fra tare.

1. Background

Volatile nature of iodine is often considered a problem in iodine analysis, especially when working with acidic solutions. There is, however, not much evidence available on this topic and no systematic test on the stability of iodine in seaweed or seaweed extracts was found in the literature.

The purpose of this study was therefore to find if total amount of iodine is changed in 8-week period at different temperatures.

2. Plan of the experiment

Samples of *Saccharina latissima* (dry biomass sample or extract in solutions with different pH) were exposed to two different temperatures (20 °C and 60 °C) in addition to the "reference temperature" which was –20 °C, for 3, 5 or 8 weeks. In addition, two biomass subsamples were kept at room temperature for 0.5 year. All the samples were kept in the dark for the entire period of the experiment.

Labelling system: temperature-time-type of sample-duplicate

Temperature	Time	Type of sample	Duplicate
• F → freezer (–20 °C) • R → room T (approx. 20 °C) • H → high T (60 °C)	• 3W → 3 weeks • 5W → 5 weeks • 8W → 8 weeks • 6M → 6 months	• SB → solid biomass (approx. 1 g of dry sample) • EW → extraction in water • EAc → extraction in acid • EAl → extraction in alkaline	• a • b • B1/B2 (blanks)

Scheme:

bottle lable	sample\week	1	2	3	4	5	6	7	8
SB_1	F-SB_a								
SB_2	F-SB_b								
SB_3	R-3W-SB_a								
SB_4	R-3W-SB_b								
SB_5	H-3W-SB_a								
SB_6	H-3W-SB_b								
SB_7	R-5W-SB_a								
SB_8	R-5W-SB_b								
SB_9	H-5W-SB_a								
SB_10	H-5W-SB_b								
SB_11	R-8W-SB_a								
SB_12	R-8W-SB_b								
SB_13	H-8W-SB_a								
SB_14	H-8W-SB_b								
SB_15	R-6M-SB_a								→
SB_16	R-6M-SB_b								→

EW_B1	EW_blank_a
EW_B2	EW_blank_b
EW_1	F-EW_a
EW_2	F-EW_b
EW_3	R-3W-EW_a
EW_4	R-3W-EW_b
EW_5	H-3W-EW_a
EW_6	H-3W-EW_b
EW_7	R-5W-EW_a
EW_8	R-5W-EW_b
EW_9	H-5W-EW_a
EW_10	H-5W-EW_b
EW_11	R-8W-EW_a
EW_12	R-8W-EW_b
EW_13	H-8W-EW_a
EW_14	H-8W-EW_b

EAc_B1	EAc_blank_a
EAc_B2	EAc_blank_b
EAc_1	F-EAc_a
EAc_2	F-EAc_b
EAc_3	R-3W-EAc_a
EAc_4	R-3W-EAc_b
EAc_5	H-3W-EAc_a
EAc_6	H-3W-EAc_b
EAc_7	R-5W-EAc_a
EAc_8	R-5W-EAc_b
EAc_9	H-5W-EAc_a
EAc_10	H-5W-EAc_b
EAc_11	R-8W-EAc_a
EAc_12	R-8W-EAc_b
EAc_13	H-8W-EAc_a
EAc_14	H-8W-EAc_b

EAl_B1	EAl_blank_a
EAl_B2	EAl_blank_b
EAl_1	F-EAl_a
EAl_2	F-EAl_b
EAl_3	R-3W-EAl_a
EAl_4	R-3W-EAl_b
EAl_5	H-3W-EAl_a
EAl_6	H-3W-EAl_b
EAl_7	R-5W-EAl_a
EAl_8	R-5W-EAl_b
EAl_9	H-5W-EAl_a
EAl_10	H-5W-EAl_b
EAl_11	R-8W-EAl_a
EAl_12	R-8W-EAl_b
EAl_13	H-8W-EAl_a
EAl_14	H-8W-EAl_b

Start of the experiment: 14. 11. 2018
3 weeks: 5. 12. 2018
5 weeks: 19. 12. 2018
8 weeks: 9. 1. 2019
Longer period: 15. 5. 2019

3. Additional experiments

Due to the results of the main experiment (see Results section), two additional experiment was performed.

3.1 Water loss

Iodine content in extracts at 60 °C seemed to increase over time, so we assumed that some water might be lost during the experiment, despite the fact that tubes were closed. Since this was not expected, tubes were not weighed before and after the experiments and therefore another experiment was performed with water, but at the same temperature and time.

3.2 Stability of iodide and iodate in 2% HNO_3

Unexpected results were observed also for iodine content in acidic extracts. Therefore, stability of two iodine species – iodide (I^-) and iodate (IO_3^-) was further tested in 2% HNO_3.

4. Procedures

4.1 Stability of iodine in seaweed biomass and extracts

a) Biomass samples

Iodine in biomass samples was determined following the standard method for determination of iodine in animal feed by ICP-MS, DS/EN 17050:2017. To 0,3 mL of sample, 5 mL of Milli-Q water and 1 mL of 25% TMAH were added and tubes were placed to the oven for 3 h at 90 °C. After cooling, extracts were diluted with Milli-Q water to 50 mL. Before measurement, samples were further diluted 10000-fold with 0.5% TMAH. Te was added as an internal standard in concentration 1 ng Te/mL of measured solution. Iodine was measured by ICP-MS (Thermo iCapQ) and quantification was done based on external calibration curve, prepared in 0,5% TMAH in the range between 0.1 and 20 ng I/mL. Iodine was extracted from samples on January 16, 2019 and measured on January 18, 2019. In the samples that were stored at room temperature for 6 months, iodine was measured by Annette Landin on August 6, 2019.

b) Extracts

For stability of extract, first extraction solutions were prepared. Besides Milli-Q water also Milli-Q water of which pH was adjusted to 4 with HNO_3 for acidic extraction or to 10 with NH_3 for alkaline extraction. Then 6 mL of corresponding solution was added to 0.3 g of sample. Tubes were placed in the oven for 3 h at 90 °C. After cooling, samples were centrifuged (5 min, 7000 g). Supernatant was transferred into the 15 mL tubes, but since it was not possible to transfer clear supernatant only, 15 mL tubes were centrifuged again (5 min, 7000 g). After second centrifugation, clear supernatant was transferred into another 15 mL tube which were used for the experiment. Extractions were performed on November 14, 2018.

After the experiment, extracts were diluted in the same rate as they were for the biomass samples after extraction. To water extracts 25% TMAH was added in the amount that resulted in 0.5% TMAH in the solution and samples were further diluted with 0,5% TMAH. For acidic and alkaline extracts, pH was adjusted with 25% TMAH to 12.7–12.8, which is a pH of 0.5% TMAH. Samples were further diluted with 0.5% TMAH. Calibration curves were prepared in the range between 0.1 and 20 ng I/mL for each type of extracts individualy in the same media. Therefore, a solution with corresponding pH was prepared first, then pH was adjusted with TMAH, these solutions were further diluted in the same rate as samples and finally used to prepare calibration curves. Iodine was measured by ICP-MS (Thermo iCapQ) on January 24, 2019.

4.2 Test of water loss

Tubes were filled with 2 mL of Milli-Q water (approx. the amount of extract in the main experiment), weighed and left at –20 °C (reference), 20 °C and 60 °C. After each period, corresponding tubes were cooled to room temperature, weighed and stored in the freezer until the end of the experiment. Tubes were stored at the same place as the samples in previous experiment. Water loss obtained in the experiment was used to correct determined iodine content from the main experiment.

4.3 Stability of iodide and iodate in acid

Three replicates of blank samples (2% HNO_3) and two replicates of solution of each iodine species (10 ng I/mL in 2% HNO_3) were prepared for each tested period: 0 days (reference), 1, 2, 3, 7, 10 and 14 days. To each tube, 5 mL of corresponding solution was added. Solutions were stored in open tubes in fume hood for the stated period. After that period, tubes were closed and stored in the freezer until measurement. Tubes were weighed before the experiment and the relevant tubes after each period to evaluate water loss during the experiment, which was later considered in calculation of the results.

After the experiment, 0.9 mL of 25% TMAH was added, since it was previously tested that 5 mL of 2% HNO_3 + 0.9 mL of 25% TMAH gives pH around 12.8, which is the pH of 0.5% TMAH that is usually used for iodine determination by ICP-MS. Te (1000 ng/mL, 0.1 mL) was added to each tube as an internal standard. Samples were diluted to 10 mL with 0.5% TMAH.

Calibration curve was prepared in the range between 0.1 and 5 ng I/mL in the same matrix as samples (25 mL 2% HNO_3 + 4.5 mL 25% TMAH, diluted to 50 mL with 0.5% TMAH).

5. Results

5.1 Stability of iodine seaweed biomass and extracts

a) Biomass samples

Results of iodine content in seaweed dry biomass samples exposed to different temperatures for different time periods are shown in Figure 1. Iodine content remained at the same level regardless of time and temperature of storage. Statistics analysis were not performed, the error bars in Figure 1 represent standard deviation of 2-3 determinations.

a) Extracts

Results of iodine content in extracts are shown in Figure 2. The trend in all extracts was the same, regardless of pH. Calculated to content in the sample, we can say that iodine in water, acidic and alkaline media was extracted in the same rate as when using standard method for iodine determination. Iodine content in extracts, stored at room temperature did not change over 8 weeks. Statistics analysis were not performed, the error bars in Figure 2 represent standard deviation of 2-3 determinations. On the other hand, iodine content in extracts, stored at higher temperature (60 °C) increased over time. Since statistical analysis was not performed, we cannot say if there are statistically significant differences or not, but there certainly is a trend of increasing iodine content in these extracts over time. Although the tubes with extracts were closed, probably some of the liquid evaporated and thus iodine was concentrated in the solution. Since we did not expect evaporation from closed tubes, we did not weighed the tubes before and after experiment. Therefore, another experiment was performed to investigate this further (see section 5.2).

There is a common believe that iodine is unstable in acidic solutions. Analyte can be lost by formation of volatile iodine species in acid. However, the results from this study does not show any loss of iodine from acidic extracts even after 8 weeks at 60 °C. Iodine content as well as the overall trend in acidic extracts was the same as in water and alkaline extracts. We cannot argue that we did not lose iodine because the tubes were closed which would prevent evaporation/volatilization, since some liquid most probably evaporated and so could volatile iodine species.

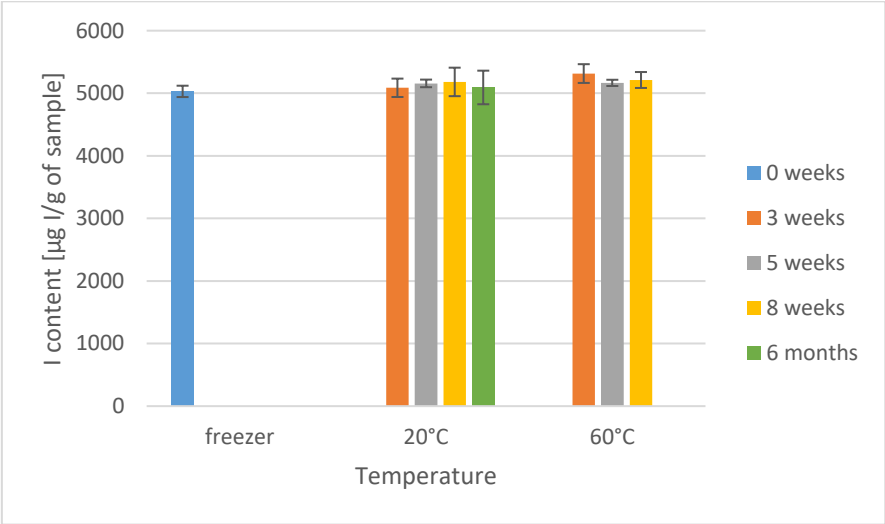

Figure 1: Iodine content in seaweed biomass samples exposed to different temperature for different periods. Reference sample was stored in freezer for the entire period of experiment (blue column).

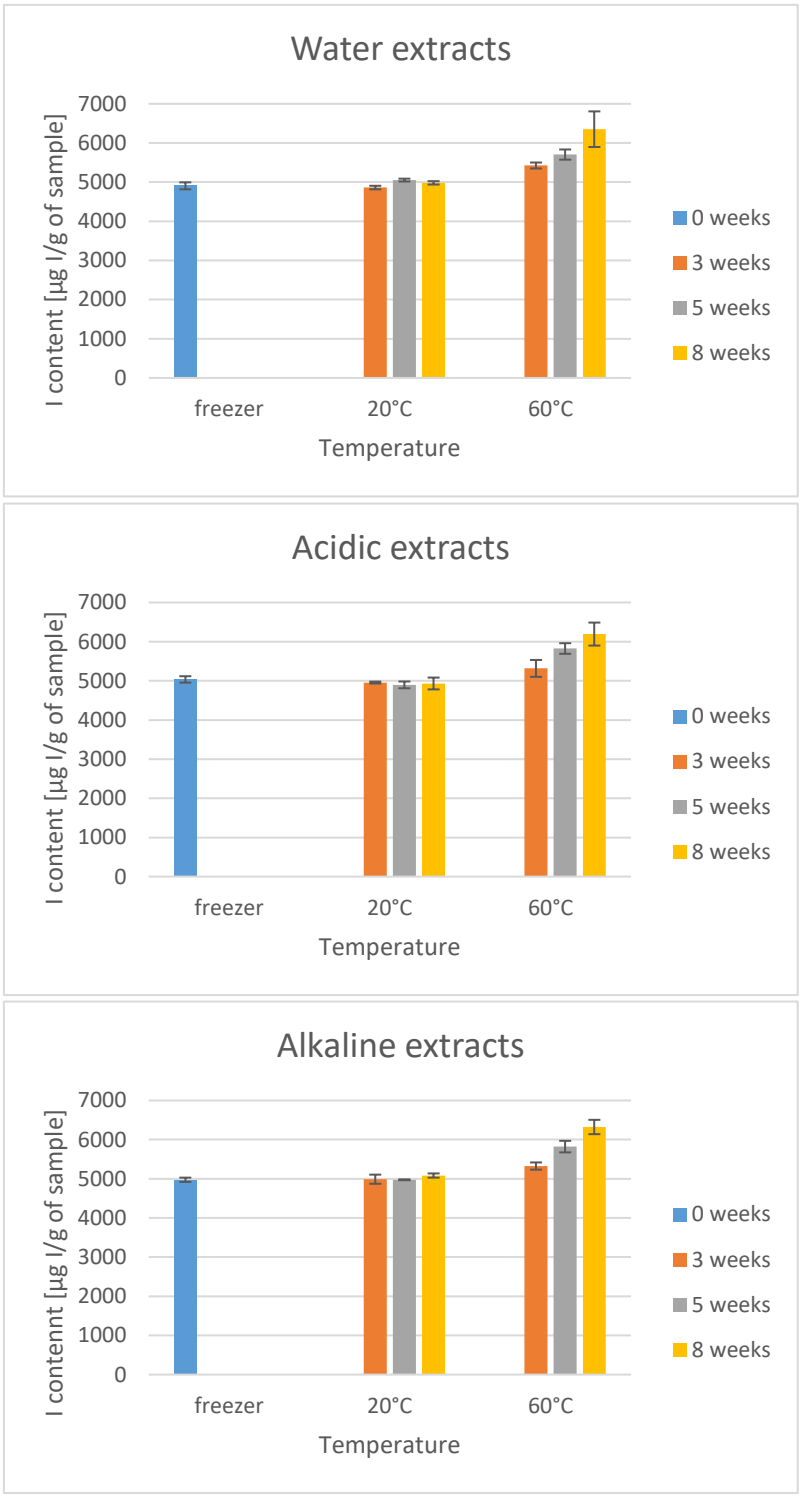

Figure 2: Iodine content in extracts of seaweed samples exposed to different temperature for different periods. Reference sample was stored in freezer for the entire period of experiment (blue column).

5.2 Water loss

As show on Figure 2, iodine content in extracts at 60 °C seemed to increased over time. However, In additional experiment we found that some water was actually lost even though the tubes were closed. When the results were recalculated considering the loss, iodine content remained stable also at 60 °C after 8 weeks (Figure 3).

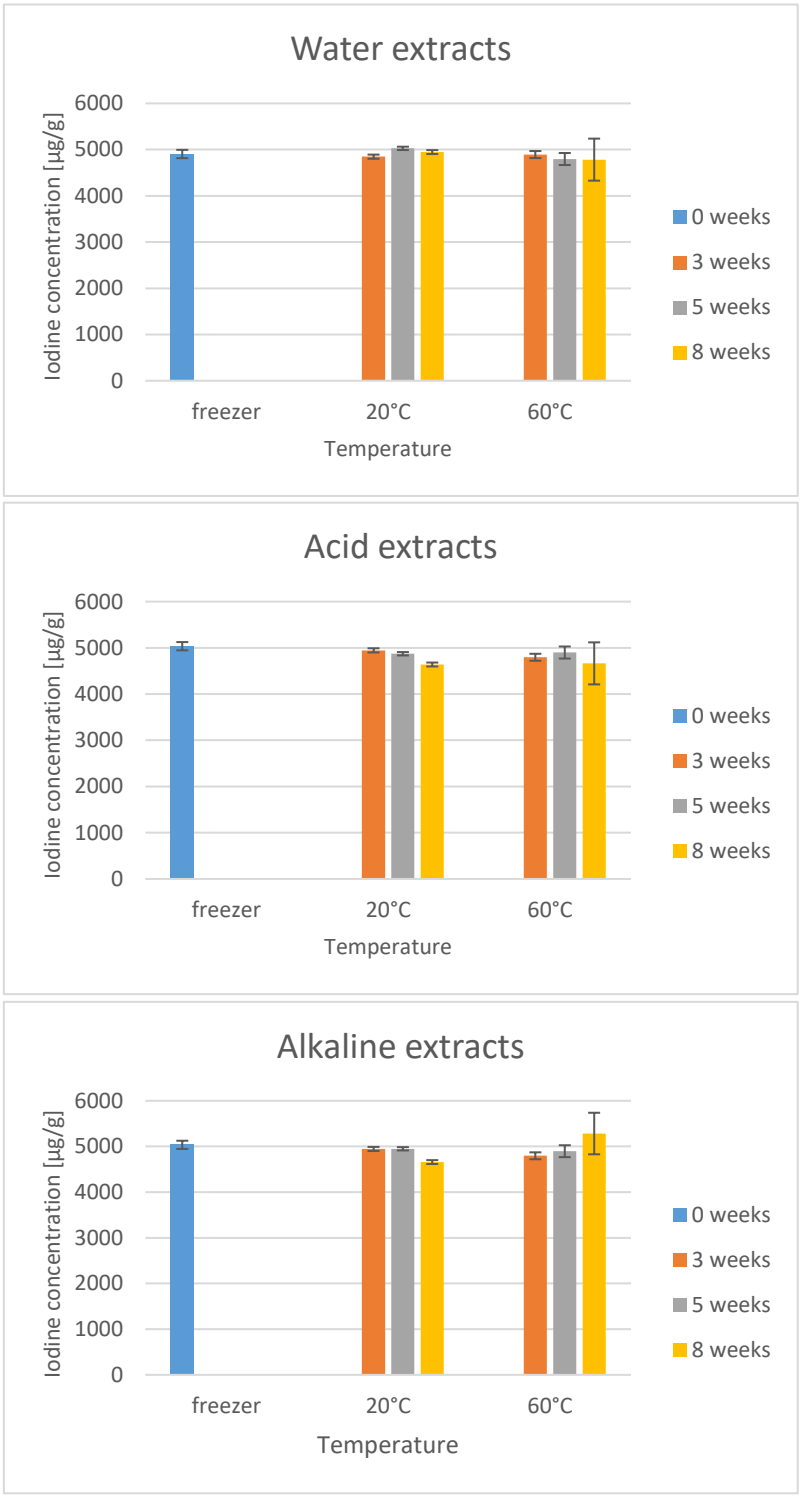

Figure 3: Iodine content in extracts of seaweed samples exposed to different temperature for different periods – recalculated results considering water loss. Reference sample was stored in freezer for the entire period of experiment (blue column).

5.1 Stability of iodide and iodate in acid

Iodine is usually considered volatile in acid solutions and therefore it was unexpected that iodine content in acidic extracts remained stable even at 60 °C for 8 weeks. Although the tubes were closed, water partly evaporated during 8 weeks, and so could iodine. Additional experiment was performed with iodide and iodate solution to evaluate loss due to volatilization. The results are presented in Figure --- as percent of the reference value, which was the one that was stored directly in the freezer at the beginning of the experiment.

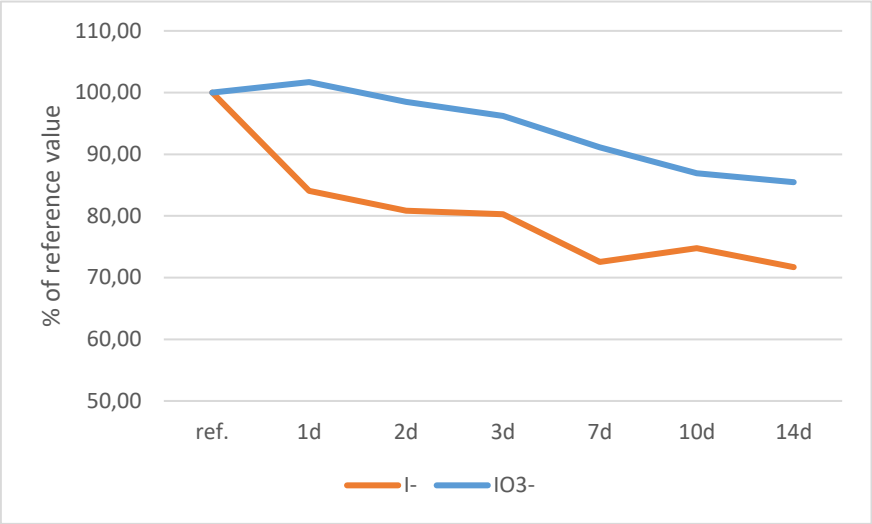

Figure 4: Loss of iodide and iodate in 2% HNO3 during 14 days.

Very small differences were observed for iodate in the first three days, while later the concentration in the tube decreased more. After 14 days, iodine concentration was approx. 15% lower than at the beginning when iodate was used. On the other hand, more than 15% of iodide was lost in the first day, but the concentration was then quite stable for the next two days (2 and 3). Another drop in iodine concentration was observed after 7 days (approx. 28% less than at the beginning), but it was similar after 10 and 14 days.

These results suggest that iodine is indeed volatile in acidic solution, more in the form of iodide than in the form of iodate, and acid digestion might not be a good option for sample preparation. However, no loss was observed from acidic seaweed extracts. It is possible that closed tubes prevented the loss. Another explanation would be that the majority of iodine in the seaweed extract was not in inorganic form.

6. Conclusions

According to the study, there is no need for storing the dry samples in which iodine is to be determined in fridge or even freezer, since temperature up to 60 °C does not affect iodine content in dry seaweed samples for at least 8 weeks. At room temperature (around 20 °C) samples can be stored for at least 6 months without changes in iodine content.

Extracts can be prepared at least 8 weeks before measurement and stored at room temperature regardless of the solution pH (between 4 and 10) without questioning the trueness of the total iodine results, since iodine content does not change under these conditions. However, if extracts are stored at higher temperatures (up to 60 °C), water loss should be evaluated by weight and considered in calculations.

If iodine is present in the acidic solution in inorganic form (I^- or IO_3^-) there is a risk to be lost due to volatilization. Use of alkaline extraction is therefore preferred over acidic digestion as sample preparation procedure.

KNOWLEDGE UPDATE ON MACROALGAE FOOD AND FEED SAFETY

based on data generated in the period 2014-2019 by the Institute of Marine Research, Norway

Arne Duinker, Malin Kleppe, Even Fjære, Irene Biancarosa, Hilde Elise Heldal, Lisbeth Dahl og Bjørn Tore Lunestad (HI)

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Sammendrag (norsk):

It has previously been addressed that some species of macroalgae may contain elevated levels of iodine, cadmium and inorganic arsenic. With the increased amount of data in the present report it is now possible to discriminate between individual species that have high levels of these components and others that are within the normal range. Among the updates are also new data on microbiology and iodine reduction that may contribute to a better understanding of this food group that is new to most Europeans.

This report follows up a previous report from 2016 pointing out knowledge-gaps in the area of food and feed safety regarding macroalgae. The levels of iodine, cadmium and inorganic arsenic were pointed out as the main challenges for macroalgae as food. Since 2016 a substantial number of samples of Norwegian macroalgae have been analysed, with data from 27 species and 14 of these with five or more samples. The report is based on about 400 analyses of cadmium, mercury, lead and iodine and 332 analyses of inorganic arsenic. This allows for a more detailed evaluation of many individual species compared to the previous report. The present report answers to a request from the Norwegian Food Safety Authority for updates on filling of the knowledge gaps from the previous report.

The main updates are:

- New data on iodine and metals with increased resolution at the species level.
- New data on inorganic arsenic identifies a group of macroalgae that hold substantially higher inorganic arsenic concentration than the normal range.
- Bioavailability of 73-78 % of iodine from sugar kelp was found in a rat model study
- Imported species with Asian origin had similar levels of iodine and heavy metals to closely related species from Norwegian waters.
- New results demonstrate iodine reduction in kelp through drying, boiling and frying.
- New data on kainic acid in dulse shows relatively low levels also in Norwegian dulse.
- New knowledge on microbiology shows that for products that have been heat treated some attention as to cold storage due to the possible presence of spore forming bacteria should be given, similar to what applies for other heat treated products as for example dairy products. Spore formers pose a low challenge for fresh or dried seaweed used directly.
- Data on macro- and microminerals are presented.
- Experimental use of macroalgae for fish feed via insects shows that macroalgae provides marine nutrients into the feed chain, but also that there is a risk that some batches of seaweed are exceeding the maximum levels for cadmium and arsenic in animal feed

The variation of inorganic arsenic concentrations is particularly large, both between and within species. Four species showed clearly higher levels than the rest. Oar weed (*Laminaria digitata*) showed a range from very low to very high concentrations, with more than 50 % of the samples showing high levels. The highest concentrations are at the level of the Asian produced hijiki (*Sargassum fusiforme*), that was also analysed. Several countries around the world have issued warnings for hijiki. Two local relatives of hijiki also show high concentrations, but more samples are needed to confirm this. A few samples of the close relative to oar weed, tangle (*Laminaria hyperborea*), show low concentrations of inorganic arsenic. More samples are needed to confirm this, but tangle may provide an alternative to oar weed for wild harvest.

In accordance with the previous report, cadmium concentrations are highest in the brown and red algae, but without a distinct group of macroalgal species with higher level than the rest as for inorganic arsenic.

Iodine levels are highest among the brown algae, in accordance with the previous report. The clearly highest levels are found in the kelp species in the *Saccharina* and *Laminaria* genera, with typical values between 2000 and 6000 mg/kg dry weight within both genera. Perennial brown algae, like wracks and others that do not shed the blade during winter, are lower, while green and red algae, including the popular sushi seaweed Nori, are relatively low in iodine.

The levels of lead in the various macroalgae are generally low. The levels of mercury are also low, and the proportion of the toxic form, methyl mercury, also seems to be low.

The higher number of data in this update also allows more precise consideration of the main commercial farmed species in Norway, sugar kelp (*Saccharina latissima*) and winged kelp (*Alaria esculenta*). Neither species are among the high-level species regarding inorganic arsenic and both are intermediate in cadmium concentrations. Regarding iodine, sugar kelp is among the high-level species and winged kelp intermediate.

For some of the species, some information is available on effects of biotic and abiotic factors on the content of

cadmium, iodine and inorganic arsenic. However, large variations are found in metal concentrations, and more knowledge of the factors causing such variation is needed to allow more predicable product quality for the seaweed industry.

Recent data on radioactivity in macroalgae is reviewed and the Norwegian monitoring programme, which includes macroalgae, is described. In general, and in according with the previous report, no radioactivity levels of concern with respect to food safety is found in seafood, including macroalgae.

Regarding microbiology, data on winged kelp and sugar kelp are described. Low microbial numbers for total aerobic count were found in all samples as well as low incidence of cold adapted bacteria and spore-forming bacteria. Furthermore, there were no detection of indicators of faecal contamination as enterococci and coliforms, nor pathogenic vibrios or *Listeria monocytogenes*. However, in several of the examined samples, spore forming *Bacillus* spp. were isolated and seems able to pose a challenge if not processing and storage conditions take their possible presence into account. To avoid revival and growth of *Bacillus* spores in heat treated products, continuous chilling is necessary. *Bacillus* spores possess a low risk for dried products and other products that are not heated.

Results are presented from a project on the iodine and metals in sugar kelp. Health effects of high iodine intake as well as bioavailability of iodine from sugar kelp was studied in a 13 weeks rodent trial. No harmful effects of high iodine or other content in the kelp could be found in any of the study groups. Healthy rats have high tolerance for iodine even at the high level in the present study, and the rat model was hence not suited for evaluation of harmful effects of high iodine intake. On the other hand, the high tolerance of the iodine intake allowed to conclude that none of the other components of the kelp had a negative impact on the health of the rats. The high tolerance of iodine in rats also made it possible to study the bioavailability of iodine from kelp at very high concentrations. Iodine availability was lower, but still high, from kelp (80 %) compared to iodine added as potassium iodide (95 %), and the kelp fed rats had more iodine in feces. The project also studied the geographical variation in iodine and metals in sugar kelp along the Norwegian coast in a standardised growth trial. The results showed no geographical trend in iodine or inorganic arsenic, while a clear increase in cadmium was seen from south to north. Another study examined the effect of dehydration and cooking on iodine in sugar kelp. Iodine is reduced through drying, boiling and frying processes, with prolonged simmering showing a substantial reduction in total in kelp and stock.

Results on nutrients with trace metals (iron, zinc and selenium) and macro minerals (calcium, potassium, magnesium, sodium and phosphorus) are described.

A SWOT analysis showed the potential of seaweed as a source of protein and other nutrients for salmon feed but emphasises the same challenges with anti-nutrients and accessibility of nutrients as for other non-animal protein sources. An alternative method for using macroalgae biomass in fish feed is via insect larvae, which overcomes the problem of high carbohydrate content in the macroalgae. Results from an international project showed that a seaweed-enriched media resulted in a more “marine” nutritional profile of the insect larvae. However, there is a risk that some batches of seaweed are exceeding the maximum levels for cadmium and arsenic in animal feed.

The process of submitting data on macroalgae to the EFSA database is described, and codes for the various species and products are given.

More knowledge is still needed in the field of food and feed safety regarding macroalgae. In particular, more data is needed for the species with low number of analyses in the present report, and in particular the species with high levels of inorganic arsenic and cadmium relative to the normal range. More data on inorganic arsenic in tangle should be acquired to explore the indication that the levels are drastically lower than the closely related oar weed. More data is also needed in general on seasonal and geographical variasion as well as on the bioavailability of cadmium and inorganic arsenic relative to other food and feed sources.

Sammendrag (engelsk):

Det har tidligere blitt påpekt at noen arter av makroalger kan ha høye nivåer av jod, kadmium og uorganisk arsen. Med et langt høyere antall data i denne rapporten er det nå mulig å skille mellom arter som har høye nivåer av disse elementene og andre som er innenfor normalområdet. Oppdateringen inneholder også data på mikrobiologi og jodreduksjon som kan bidra til bedre forståelse av denne gruppen av matvarer som er ny for de fleste europeere.

Denne rapporten følger opp en tidligere rapport fra 2016 der det ble pekt på kunnskapshull i forhold til makroalger og mattrygghet og som trygt fôr. Innhold av jod, kadmium og uorganisk arsen ble utpekt som hovedutfordringer for bruk av

makroalger som mat. Siden 2016 har det blitt analysert et betydelig antall prøver av norske makroalger, med data fra 27, arter hvorav 14 av disse har fem eller flere prøver. Denne rapporten er basert på rundt 400 analyser av kadmium og jod og 332 analyser av uorganisk arsen. Dette gjør det mulig med en mer detaljert vurdering av mange enkelt-arter sammenliknet med forrige rapport. Rapporten svarer opp forespørsel fra Mattilsynet der det bes om ny kunnskap som kan fylle kunnskapshullene fra den forrige rapporten.

De viktigste oppdateringene er:

- Nye data på jod og metaller med økt oppløsning på artsnivå.
- Nye data på uorganisk arsen viser en liten gruppe makroalger som har betydelig høyere konsentrasjoner enn normalnivå hos de andre artene.
- Biotilgjengelighet av jod på 80 % ble funnet i en studie med rottemodell.
- Importerte arter med asiatisk opprinnelse hadde nivåer av jod og tungmetaller tilsvarende nært beslektede arter fra Norge.
- Nye data viser reduksjon av jodinnhold i tare ved tørking, koking og steking.
- Nye data på kainsyre i søl viser relativt lave nivåer også i norsk søl.
- Ny kunnskap i forhold til mikrobiologi viser at det bør tas forholdsregler som god kjøling av varmebehandlede produkter av makroalger, tilsvarende det som er gjeldende for andre varmebehandlede produkter som for eksempel melkeprodukter, som følge av mulig forekomst av sporedannende bakterier. Sporedannende bakterier utgjør liten utfordring for ferske eller tørkede produkter brukt direkte.
- Data på makro- og mikromineraler er presentert.
- Eksperimentell bruk av makroalger som fôr til fisk via insekter viser at makroalger bidrar med viktige marine næringsstoffer til fôrkjeden, men også at det kan være risiko for at noen partier av makroalger kan overstige grenseverdiene for kadmium og arsen i dyrefôr.

Variasjonen i konsentrasjoner av uorganisk arsen var spesielt høy, både innen og mellom arter. Fire arter hadde klart høyere konsentrasjoner enn de resterende artene. Fingertare (*Laminaria digitata*) hadde et spenn i konsentrasjoner fra svært lave til svært høye verdier, der over 50 % av prøvene hadde høye konsentrasjoner. De høyeste konsentrasjonene i fingertare var på nivå med den asiatisk produserte hijiki (*Sargassum fusiforme*) som også ble analysert. Flere land rundt om i verden har gitt ut advarsler mot å spise hijiki. To lokale slektninger av hijiki hadde også høye konsentrasjoner, men det bør tas flere prøver for å bekrefte dette. Noen få prøver av stortare (*Laminaria hyperborea*), som er nært beslektet til fingertare, hadde svært lave konsentrasjoner av uorganisk arsen. Det bør tas flere prøver for å bekrefte dette, men stortare kan være et alternativ til fingertare for villhøsterne.

I samsvar med den forrige rapporten var konsentrasjonene av kadmium høyest i brunalger og rødalger, men uten en adskilt gruppe arter med høyere nivåer enn resten, slik det ble funnet for uorganisk arsen.

Innhold av jod var høyest blant brunalgene, i samsvar med forrige rapport. De klart høyeste konsentrasjonene ble funnet innenfor slektene *Saccharina* og *Laminaria*, med typiske konsentrasjoner mellom 2 000 og 6 000 mg/kg tørrvekt innenfor begge slektene. Flerårige brunalger, som tangsorter med flere som ikke mister bladene om vinteren, har lavere konsentrasjoner, mens grønnalger og rødalger, inkludert den populære sushi-arten Nori, har relativt lavt innhold av jod.

Innholdet av bly i makroalger er generelt lavt. Nivåer av kvikksølv er også lave, og andelen av den toksiske formen metylkvikksølv ser også ut til å være lav.

Basert på den økte mengden data i denne oppdateringen er det nå mulig med en grundigere vurdering av sukkertare (*Saccharina latissima*) og butare (*Alaria esculenta*), som er de viktigste artene som dyrkes i Norge. Ingen av disse er blant artene med høyt innhold av uorganisk arsen og begge har middels høye konsentrasjoner av kadmium. Når det gjelder jodkonsentrasjoner er disse høye hos sukkertare og middels høye hos butare.

For noen av artene har vi nå informasjon om effekt av biotiske og abiotiske faktorer på innholdet ad kadmium, jod og uorganisk arsen. Det er imidlertid stor variasjon i disse dataene, og det er behov for mer kunnskap om faktorer som er opphavet til denne variasjonen, slik at produktkvaliteten kan bli mer forutsigbar for næringen.

Nyere data på radioaktivitet i makroalger er presentert og det norske overvåkningsprogrammet, som inkluderer makroalger, er beskrevet. Generelt, og i samsvar med den forrige rapporten, er det ikke funnet nivåer av radioaktivitet som er problematisk i forhold til mattrygghet, i sjømat, inkludert makroalger.

Data på mikrobiologi i sukkertare og butare er gjennomgått. Lave verdier for totalt aerobt kimtall samt lav forekomst av kuldeadapterte og sporedannende bakterier ble funnet i alle prøvene. Videre ble det ikke detektert indikatorer på fekal kontaminering som enterokokker og coliforme bakterier, eller patogene vibrio eller *Listeria monocytogenes*. Det ble

imidlertid isolert sporedannende *Bacillus* spp. fra flere av de undersøkte prøvene, noe som kan gi en utfordring om ikke forhold under prosessering og lagring tar hensyn til disse sporedannerne. For å unngå aktivering og vekst av *Bacillus* sporer i varmebehandlede produkter er det nødvendig med kontinuerlig kjøling. *Bacillus* sporer er et lite problem for tørkede produkter og produkter som ikke er varmebehandlet.

Rapporten presenterer resultater fra et prosjekt på jod og metaller i sukkertare. Helseeffekter av høyt jodinntak samt biotilgjengelighet av jod fra sukkertare ble studert i et 13-ukers rotteforsøk. Ingen negative effekter av høyt jodinnhold eller andre komponenter i taren ble funnet i noen av forsøksgruppene. Friske rotter har høy toleranse for jod, selv ved det høye inntaket i dette forsøket, og rottemodellen var dermed ikke egnet til å vurdere effekter av høyt jodinntak. På den annen side gjorde denne toleransen det mulig å konkludere at heller ingen av de andre komponentene i tare hadde negative helseeffekter hos rottene. Den høye toleransen for jod gjorde det også mulig å vurdere biotilgjengeligheten av jod fra tare i høye konsentrasjoner. Tilgjengeligheten var lavere, men fortsatt høy, fra tare (80 %) sammenliknet med jod tilsatt som kaliumjodid (95 %), og rottene som fikk tare i fôret hadde mer jod i feces. Prosjektet undersøkte også geografiske variasjoner i innhold av jod og metaller i sukkertare langs norskekysten i et standardisert dyrkingsforsøk. Resultatene viste ingen geografiske trender i jod eller uorganisk arsen, mens en klar økning i konsentrasjoner av kadmium fra sør til nord ble funnet. Det ble også sett på effekt av tørking tilberedning av sukkertare på innhold av jod. Innholdet av jod ble redusert både ved tørking, steking og småkoking, og vedvarende småkoking viste en vesentlig nedgang i tare og kraft til sammen.

Rapporten presenterer også data på næringsstoff med sporminerаler (jern, sink og selen) og makromineraler (kalsium, kalium, magnesium, natrium og fosfor).

En SWOT analyse viste potensialet til makroalger som en kilde til protein og andre næringsstoff til laksefôr, men fremhever de samme utfordringene i forhold til antinæringsstoff og tilgjengelighet av næringsstoff som for andre ikke-animalske proteinkilder. En alternativ metode for å bruke makroalger til fiskefôr er via insektlarver, noe som omgår utfordringen med høyt innhold av karbohydrater i makroalgene. Resultater fra et internasjonalt prosjekt viste at medium tilsatt makroalger ga en mer «marin» ernæringsprofil til insektlarvene. Det er imidlertid en risiko for at grenseverdien for kadmium og arsen i dyrefôr overstiges i noen partier med makroalger.

Prosessen med innsending av data til EFSA databasen er beskrevet, og koder for de ulike artene og produktene er oppgitt.

Det er behov for mer kunnskap innen feltene mattrygghet og trygt fôr når det gjelder makroalger. Det er behov for mer data for arter med færrest antall analyser i denne rapporten, og spesielt for arter med høye verdier av uorganisk arsen og kadmium i forhold til normalnivåene. Mer data på uorganisk arsen i stortare er nødvendig for å undersøke videre indikasjonene på at nivåene i denne arten er så mye lavere enn den nært beslektede fingertaren. Det er også behov for mer data generelt på sesongmessig og geografisk variasjon og i tillegg mer kunnskap om tilgjengelighet av kadmium og uorganisk arsen fra makroalger i forhold til andre mat- og fôrressurser.

Innhold

1 - Introduction

In 2016, a technical report on potential risks posed by macroalgae for application as feed and food, from a Norwegian perspective was published by the Institute of Marine Research (Duinker et al., 2016) . The Norwegian Food Safety Authority (NFSA) has now requested the Institute of Marine Research (IMR) for an updated knowledge status on macroalgae, with special emphasis on data on iodine and metals and new data that could change the conclusions from the 2016 report. Other data on macroalgal food and feed safety generated by the IMR since 2016, that might fill some of the knowledge gaps pointed out in the 2016 report, are also requested.

One of the main conclusions from the 2016 report was that macroalgae, in particular brown algae, may contain elevated levels of inorganic arsenic, total arsenic and cadmium. Further, the amount of data on Norwegian seaweed was too low, allowing only general conclusions. In 2018, EFSA made a call for data in the commission recommendation (EU) 2018/464 on the monitoring of metals and iodine in seaweed, halophytes and products based on seaweed, including both local and imported products.

Since 2016 the amount of chemical data generated by the IMR on Norwegian as well as imported macroalgae has increased substantially, and a monitoring programme was started according to EFSAs requirements. The main updated conclusion is that it now can be discriminated between individual species that have high levels of these components and others that are within the normal range. The 2016 report also revealed a lack of information on seasonal and geographical variation, which is still lacking except for a few species as discussed below. Some work has also been done on the presence of bacteria on kelp and will be summarised here. Studies on bioavailability of metals from kelp have been performed together with studies on the effect of cooking on iodine content, and ongoing student work on iodine is also described. Work on the use of macroalgae in fish feed is summarised as well as analyses of minerals. Finally, the process of submitting occurrence data to EFSA is described and discussed.

2 - Material and methods

Samples were retrieved by field work by IMR staff, kelp growers and wild harvesters during the period 2014-2019. Samples were freeze dried and homogenised before further analysis. The analyses are ISO accredited for most elements and otherwise analysed in our accredited labs, hence fulfilling the request in EFSA's call for data that the analyses should be carried out in accordance with Annex III to Regulation (EC) No 882/2004. Samples of arame, kombu, hijiki, wakame and nori are imported from production in Asia.

An overview of the species collected is given in Table 1.

Table 1 . List of species collected in the period 2014-2019 with Latin, English and Norwegian names.

	Latin name	English name	Norwegian name
Brown algae	***Alaria esculenta***	Winged kelp	Butare
	Ascophyllum nodosum	Rockweed	Grisetang
	Chorda filum	Dead man's rope	Martaum
	Eisenia bicyclis	Arame	Arame
	Fucus serratus	Toothed wrack	Sagtang
	Fucus spiralis	Spiral wrack	Kaurtang
	Fucus vesiculosus	Bladderwrack	Blæretang
	Halidrys siliquosa	Halidrys siliquosa	Skolmetang
	Himanthalia elongata	Thongweed	Remtang
	Laminaria digitata	Oar weed	Fingertare
	Laminaria hyperborea	Tangle	Stortare
	Pelvetia canaliculata	Channelled wrack	Sauetang
	Saccharina latissima	Sugar kelp	Sukkertare
	Saccharina spp	Kombu	Kombu
	Sargassum fusiforme	Hijiki	Hijiki
	Sargassum muticum	Wireweed	Japansk drivtang
	Undaria pinnatifida	Wakame	Wakame
Green algae	***Codium fragile***	Green sea fingers	Pollpryd
	Ulva intestinalis	Gutweed	Tarmgrønske
	Ulva lactuca	Sea lettuce	Havsalat
	Ulva spp	Green nori	Green nori
Red algae	***Chondrus crispus***	Irish moss	Krusflik
	Palmaria palmata	Dulse	Søl
	Porphyra purpurea	Purple laver	Purpurfjærehinne
	Porphyra spp	Nori	Nori
	Porphyra umbilicalis	Pink laver	Vanlig fjærehinne
	Vertebrata lanosa	Wrack siphon weed	Grisetangdokke

Determination of metals (including Cd, Hg, Pb, Fe, Zn and Se) by ICPMS (IMR method 197)

Two parallels were weighed from each sample. The metals were determined by inductively coupled plasma-mass spectrometry (ICP-MS) after decomposing in microwave oven as described by Julshamn et al. (2007). The method is

accredited for cadmium (Cd), mercury (Hg), lead (Pb), zinc (Zn) and selenium (Se).

Determination of macro minerals (Na, Mg, K, Ca and P) by ICPMS (IMR method 382)

Two parallels were weighed from each sample. The concentrations of the macro minerals (Na, Mg, K, Ca and P) were determined by inductively coupled plasma-mass spectrometry (ICP-MS), after acid wet digestion in a microwave oven. The concentrations were determined using an external calibration (standard curve) and the method is accredited according to ISO 17025.

Determination of inorganic arsenic by HPLC-ICPMS (IMR method 261)

The sample was added 10 ml 0.07 mol/l HNO_3 in 3 % H_2O_2 and extracted in microwave oven for 20 minutes at 90 °C. The sample was then cooled, centrifuged and filtrated prior to analysis. Inorganic arsenic was selectively separated from other arsenic compounds by anion exchange HPLC and determined as As5+ by ICP-MS. The method is accredited according to iso-17025.

Determination of iodine

Samples were added 1 ml tetrametylammonium hydroxyide (TMAH) and 5 ml deionized water before extraction at 90 ° C ± 3 ° C for 3h. The samples were then diluted and centrifuged. Prior to quantification, the samples were filtered through a 0.45 µ m single use syringe and disposal filter. Tellurium which was used as an internal standard in order to correct for instrument drift. Iodine concentration in the samples was determined by inductively coupled plasma-mass spectrometry (ICP-MS).

3 - Results and discussion

3.1 – Occurrence of inorganic arsenic, iodine, cadmium, lead and mercury in macroalgae

Both fresh and dried macroalgae have been sampled and analysed. Dried products show higher concentrations than fresh, since dry matter percentage typically range from 10 to 30 % (see appendix) resulting in 3-10 times higher concentrations of metals after drying. As an example, median iodine concentration in 141 samples of fresh sugar kelp (*Saccharina latissima*) was 410 mg/kg wet weight while 16 samples of dried products had median concentration of 3650 mg/kg dry weight. For comparison purposes, all concentrations are converted to dry weight basis in the following review. 27 species have been sampled and analysed, and 14 of these have five or more samples.

The tables include both mean and median values, since in many instances the mean values are affected by a few atypically high values, and median values are more representable for the typical values. The 25 % quartile range is presented in addition to the minimum and maximum values for the same reason, and the upper 75 % quartile value is more suited for comparison of typically high values, not affected by a few extreme values.

At present there are no maximum levels (MLs) for minerals in seaweed as food in the EU, except for mercury, and a call for data for the period 2018-2021 has been made to generate a basis for evaluating establishment of MLs. As stated in the Commission Recommendation (EU) 2018/464 on the monitoring of metals and iodine in seaweed, halophytes and products based on seaweed: “For arsenic, cadmium and lead, maximum levels (MLs) for various foodstuffs are established under Commission Regulation (EC) No 1881/2006. However, currently no MLs are established for these substances in seaweed and halophytes, except for the MLs established under this Regulation for food supplements consisting exclusively or mainly of seaweed or products derived from seaweed”. Concentrations in fresh seaweed have been converted to dry weight concentrations for comparisons with dry seaweed products. Dry weight percentages are presented in the appendix.

3.1.1 - Inorganic arsenic

Content of inorganic arsenic show a particularly high variation (Table 1). Among the types of seaweed that have been sourced in Norwegian waters from mainly wild harvest, but also from cultivation, the oar weed (*Laminaria digitata*) shows the highest concentrations. The variation spans from the lowest concentrations to the highest concentrations in Table 1, and the highest concentrations are at the level of the Asian produced hijiki (*Sargassum fusiforme*), which several European countries (including Norway), USA and Canada have issued warnings for due to the high level of inorganic arsenic and increased risk of cancer. 50 % of the samples of oar weed have concentrations of inorganic arsenic above 24 mg/kg dry weight, and the three highest samples have concentrations between 63 and 79 mg/kg dry weight. Also cultivated oar weed showed very high concentrations. In a small study, inorganic arsenic was seen to increase substantially in oar weed between March and June (Duinker, 2014) , but samples submitted by the industry in 2018 showed an opposite pattern. The concentration in one sample of Hijiki was 59 mg/kg dry weight, and levels from literature typically vary between 50 and 90 mg/kg dry weight (Rose et al., 2007; Yamashita, 2014) . A related species to hijiki, the invasive wireweed (*Sargassum muticum*) also showed high concentration of inorganic arsenic in four samples with concentrations between 48 and 68 mg/kg dry weight. A native species, *Halidrys siliquosa* (sea oak, family *Sargassaceae* also including the *Sargassum* species), is also related to Hijiki and had concentrations between 2.4 and 42 mg/kg dry weight. These two latter species are not commonly used at the moment. On the other side, a species that is closely related to oar weed with quite similar morphology, *Laminaria hyperborea* (tangle), showed concentrations in the lower end of all seaweed species between 0.03 and 0.04 mg/kg dry weight in four samples analysed. Several companies report a shift from *L. digitata* to *L. hyperborea* in their products. More samples are needed of both the *Sargassum* related species and *L. hyperborea* to confirm these findings.

The species with high concentrations of iAs range in median concentrations from 22 to 59 mg/kg dry weight, and there is then a distinct gap down to the other species where median concentrations are 100 times lower from 0.23 and below.

Table 2. Inorganic arsenic in macroalgae, mg/kg dry weight. Species presented in order by decreasing median concentrations. Cell colouring corresponds to brown, green and red algae.

Latin name	Common name	N	Mean	Median	Min-max	25 % Quartiles
Sargassum fusiforme	Hijiki	1	59	59		
Sargassum muticum	Wireweed	4	54	51	48-68	49-60
Halidrys siliquosa	Sea oak	8	12	7.6	2.4-42	4.7-13
Laminaria digitata	Oar weed	40	24	21	0.06-79	6.9-39
Chondrus crispus	Irish moss	2	0.23	0.23	0.21-0.25	0.21-0.25
Ulva spp	Green nori	1	0.21	0.21		
Saccharina latissima	Sugar kelp	77	0.17	0.16	0.03-0.67	0.11-0.22
Chorda filum	Dead man's rope	2	0.15	0.15	0.03-0.27	0.03-0.27
Vertebrata lanosa	Wrack siphon weed	19	0.27	0.15	0.04-1.04	0.11-0.24
Codium fragile	Green sea fingers	2	0.14	0.14	0.07-0.21	0.07-0.21
Ulva intestinalis	Gutweed	6	0.18	0.13	0.02-0.44	0.05-0.33
Alaria esculenta	Winged kelp	33	0.77	0.11	0.03-2.7	0.08-0.22
Fucus serratus	toothed wrack	18	0.14	0.1	0.01-0.56	0.06-0.18
Fucus vesiculosus	Bladderwrack	23	0.2	0.1	0.02-1.64	0.07-0.19
Pelvetia canaliculata	Channelled wrack	2	0.1	0.1	0.09-0.12	0.09-0.12
Ulva lactuca	Sea lettuce	10	0.14	0.09	0.03-0.45	0.06-0.12
Palmaria palmata	Dulse	23	0.22	0.09	0.02-1.03	0.04-0.28
Ascophyllum nodosum	Rockweed	22	0.11	0.04	<0.01-1.21	0.02-0.12
Fucus spiralis	Spiral wrack	2	0.04	0.04	0.03-0.05	0.03-0.05
Himanthalia elongata	Thongweed	4	0.04	0.04	0.01-0.05	0.02-0.05
Porphyra purpurea	Purple laver	3	0.09	0.04	0.03-0.19	0.03-0.19
Laminaria hyperborea	Tangle	4	0.036	0.037	0.03-0.041	0.032-0.04
Undaria pinnatifida	Wakame	5	0.03	0.03	<0.01-0.06	0.03-0.04
Porphyra spp	Nori	11	0.08	0.03	0.01-0.3	0.02-0.13
Eisenia bicyclis	Arame	1	0.02	0.02	0.02-0.02	0.02-0.02
Saccharina spp	Kombu	4	0.03	0.02	0.02-0.05	0.02-0.04
Porphyra umbilicalis	Pink laver	5	0.03	0.02	0.01-0.06	0.02-0.05

3.1.2 - Cadmium

Regarding cadmium, the levels are in accordance with the 2016-report (Duinker et al., 2016), but the available information of specific species is improved. There is no group of species that show drastically elevated concentrations like for inorganic arsenic, and the concentration ranges overlap (Table 2). Red and brown algae have the highest concentrations, while green algae are very low in cadmium. Sugar kelp has the highest concentrations for the northern localities as discussed below ("Geographic variation of cadmium in farmed sugar kelp").

Table 3. Cadmium concentrations in macroalgae, mg/kg dry weight. Species presented in order by decreasing median concentrations. Cell colouring corresponds to brown, green and red algae.

Latin name	Common name	N	Mean	Median	Min-max	25 % Quartiles
Vertebrata lanosa	Wrack siphon weed	18	3.4	3.3	2.1-5	2.7-3.9
Undaria pinnatifida	Wakame	5	2.7	3.1	0.72-4	2.6-3.2
Sargassum fusiforme	Hijiki	1	2.3	2.3	2.3-2.3	2.3-2.3
Fucus serratus	Toothed wrack	19	1.9	1.8	0.88-3.3	1.5-2.4
Porphyra spp	Nori	11	1.7	1.5	0.41-3.4	0.87-2.3
Alaria esculenta	Winged kelp	40	1.5	1.3	0.3-4.8	1-1.7
Fucus vesiculosus	Bladderwrack	27	1.4	1.2	0.41-3.1	0.79-2
Saccharina latissima	Sugar kelp	148	0.94	0.65	0.16-3.1	0.41-1.4
Eisenia bicyclis	Arame	1	0.6	0.6	0.6-0.6	0.6-0.6
Himanthalia elongata	Thongweed	5	0.78	0.56	0.39-1.8	0.45-0.66
Porphyra umbilicalis	Pink laver	6	0.57	0.49	0.19-1.3	0.39-0.56
Fucus spiralis	Spiral wrack	3	0.67	0.47	0.45-1.1	0.45-1.1
Saccharina spp	Kombu	4	0.46	0.47	0.15-0.75	0.29-0.63
Laminaria hyperborea	Tangle	1	0.82	0.82	0.82	0.82
Porphyra purpurea	Purple laver	3	0.67	0.39	0.17-1.5	0.17-1.5
Sargassum muticum	Wireweed	2	0.37	0.37	0.09-0.64	0.09-0.64
Pelvetia canaliculata	Channelled wrack	3	0.3	0.3	0.24-0.36	0.24-0.36
Ascophyllum nodosum	Rockweed	24	0.29	0.28	0.16-0.47	0.23-0.32
Chorda filum	Dead man's rope	2	0.27	0.27	0.07-0.47	0.07-0.47
Palmaria palmata	Dulse	26	0.37	0.25	0.05-1.6	0.15-0.37
Laminaria digitata	Oar weed	33	0.38	0.22	0.033-1.9	0.17-0.53
Chondrus crispus	Irish moss	2	0.21	0.21	0.14-0.28	0.14-0.28
Halidrys siliquosa	Sea oak	2	0.19	0.19	0.09-0.28	0.09-0.28
Ulva intestinalis	Gutweed	7	0.24	0.18	0.08-0.55	0.14-0.28
Ulva lactuca	Sea lettuce	12	0.17	0.15	0.08-0.34	0.12-0.22
Ulva spp	Green nori	1	0.08	0.08	0.08-0.08	0.08-0.08
Codium fragile	Green sea fingers	2	<0.06	0.06	<0.06-0.06	<0.06-0.06

3.1.3 - Iodine

The concentrations of iodine are in accordance with the 2016 report (Duinker et al., 2016), but with more detailed information at the species level. The kelp species in the *Saccharina* and *Laminaria* families, both in Norway and Asia, have the highest levels with typical concentrations between 3 000 and 4 000 mg/kg dry weight and maximum concentrations around 10 000 mg/kg dry weight (Table 3), which is higher than any other food group. Winged kelp and the related Wakame from Asia, however, have a concentration range one tenth or less of the *Laminaria* and *Saccharina* species, which is part of the reason that winged kelp is getting more popular for cultivation in Norway these days. In general, the brown perennial species are intermediate, and the green and red algae are relatively low in iodine concentrations. One exception here is *Vertebrata lanosa* (wrack siphon weed) which grows as a symbiont on *Ascophyllum* and has ten times higher iodine concentrations compared to the other red algae.

Table 4. Iodine concentrations in macroalgae, mg/kg dry weight. Species presented in order by decreasing median concentrations. Cell colouring corresponds to brown, green and red algae.

Latin name	Common name	N	Mean	Median	Min-max	25 % Quartiles
Laminaria digitata	Oar weed	33	5 100	5 000	1400-10000	3600-6400
Laminaria hyperborea	Tangle	1	4 200	4 200	4200-4200	4200-4200
Saccharina latissima	Sugar kelp	150	3 700	3 500	670-10000	2600-4600
Saccharina spp	Kombu	4	2 800	2 600	2100-4000	2100-3500
Vertebrata lanosa	Wrack siphon weed	18	2 500	2 200	710-6200	1900-3000
Chorda filum	Dead man's rope	2	850	850	120-1600	120-1600
Alaria esculenta	Winged kelp	30	840	740	70-2400	450-1100
Halidrys siliquosa	Sea oak	2	690	690	670-710	670-710
Ascophyllum nodosum	Rockweed	24	710	670	320-1500	510-800
Fucus serratus	Toothed wrack	20	650	620	280-1000	530-760
Sargassum fusiforme	Hijiki	1	490	490	490-490	490-490
Eisenia bicyclis	Arame	1	450	450	450-450	450-450
Fucus vesiculosus	Bladderwrack	27	380	310	140-830	210-520
Sargassum muticum	Wireweed	2	300	300	120-480	120-480
Chondrus crispus	Irish moss	2	260	260	200-330	200-330
Palmaria palmata	Dulse	26	300	260	15-790	130-430
Pelvetia canaliculata	Channelled wrack	3	210	200	200-220	200-220
Undaria pinnatifida	Wakame	5	150	160	39-280	110-170
Fucus spiralis	Spiral wrack	3	150	150	140-150	140-150
Ulva intestinalis	Gutweed	7	130	130	29-240	41-220
Ulva lactuca	Sea lettuce	12	110	100	37-290	53-120
Ulva spp	Green nori	1	92	92	92-92	92-92
Porphyra purpurea	Purple laver	3	67	79	22-100	22-100
Porphyra umbilicalis	Pink laver	6	68	69	14-140	15-100
Himanthalia elongata	Thongweed	5	90	59	41-230	58-61
Porphyra spp	Nori	11	51	37	8-100	32-85
Codium fragile	Green sea fingers	2	23	23	17-29	17-29

For adults, recommended daily intake of iodine is 150 µg and maximum daily intake is 600 µg. Nori sheets contained

about 60 µg per sheet, and between 2 and 10 sheets should cover the range 150-600 µg iodine. 1 ml (1/5 teaspoon) of dried kelp flakes weighing approximately 100 mg correspond to approximately 400 µg of iodine, given a typical concentration of 4 000 mg/kg dry weight. Similar calculations could make producers able to advice intake to the consumers.

3.1.4 - Lead

There are generally low levels of lead in seaweed (Table 5), although large variation is seen within each species with a few relatively high values 5-10 times higher than the 75% quartile for some of the species. Such high values should be followed up in future studies.

Table 5. Lead concentrations in macroalgae, mg/kg dry weight. Species presented in order by decreasing median concentrations. Cell colouring corresponds to brown, green and red algae.

Latin name	Common name	N	Mean	Median	Min-max	25 % Quartiles
Sargassum fusiforme	Hijiki	1	1.6	1.6		
Codium fragile	Green sea fingers	2	1.4	1.4	0.4-2.3	0.4-2.3
Undaria pinnatifida	Wakame	5	0.76	0.93	<0.22-1.1	0.63-0.99
Ulva spp	Green nori	1	0.85	0.85		
Ulva intestinalis	Gutweed	7	0.89	0.67	0.21-3	0.36-0.82
Vertebrata lanosa	Wrack siphon weed	18	0.96	0.59	0.24-3.3	0.36-1.3
Chorda filum	Dead man's rope	2	0.37	0.37	<0.21-0.52	0.21-0.52
Chondrus crispus	Irish moss	2	0.35	0.35	0.3-0.41	0.3-0.41
Fucus spiralis	Spiral wrack	3	0.33	0.32	0.27-0.4	0.27-0.4
Fucus serratus	Toothed wrack	19	0.44	0.29	<0.13-1.7	0.2-0.5
Halidrys siliquosa	Sea oak	2	0.28	0.28	0.024-0.54	0.024-0.54
Sargassum muticum	Wireweed	1	0.28	0.28		
Ulva lactuca	Sea lettuce	12	0.62	0.27	<0.2-2.8	0.22-0.73
Porphyra umbilicalis	Pink laver	6	0.38	0.26	<0.22-0.84	<0.22-0.67
Laminaria hyperborea	Tangle	1	<0.25	<0.25		
Alaria esculenta	Winged kelp	38	0.66	0.24	<0.055-4.4	0.21-0.86
Pelvetia canaliculata	Channelled wrack	3	0.4	0.24	<0.21-0.75	<0.21-0.75
Saccharina latissima	Sugar kelp	148	0.33	0.24	<0.22-5.7	<0.22-0.27
Fucus vesiculosus	Bladderwrack	26	0.49	0.23	<0.077-3.3	0.19-0.34
Palmaria palmata	Dulse	25	0.33	0.23	<0.039-1.1	<0.21-0.41
Porphyra purpurea	Purple laver	3	0.24	0.23	0.055-0.44	0.055-0.44
Ascophyllum nodosum	Rockweed	24	0.34	0.22	<0.052-1.9	0.099-0.29
Porphyra spp	Nori	11	0.28	0.22	<0.21-0.8	<0.21-0.24
Eisenia bicyclis	Arame	1	<0.21	<0.21		
Saccharina spp	Kombu	4	<0.21	<0.21		
Himanthalia elongata	Thongweed	5	<0.2	<0.2		
Laminaria digitata	Oar weed	33	0.15	0.19	<0.021-0.64	0.065-0.21

3.1.5 - Mercury

The levels of mercury are generally low. Most mercury concentrations were below the limit of quantification (loq), and only 100 of 406 samples were above loq. Only five of the species had more than 50% of mercury concentrations above loq so that mean concentrations could be presented (Table 6). However, this does not reflect higher concentrations of mercury as loq was quite variable. Samples with high total mineral content were diluted more than other samples, resulting in higher loq, and some species had hence more concentrations above loq but lower concentrations than other species.

The highest max concentrations were found in sugar kelp, oar weed and wrack siphon weed with 0.08, 0.07 and 0.07 mg/kg dry weight, respectively.

Table 6. Mercury concentrations, mg/kg dry weight. Number of samples above loq and total number of samples are presented. Upper-bound mean concentration is given when more than 50% of samples are above loq. For species with concentrations above loq, max concentration is given as the highest concentration above loq, although the highest loq is above this concentration for most species. Cell coloration is according to brown, green and red algae.

Latin name	Common name	N>LOQ / total N	Mean	Median	Min-max
Alaria esculenta	Winged kelp	6/38		<0.044	<0.0043-0.054
Ascophyllum nodosum	Rockweed	12/24		<0.031	<0.0078-0.033
Chorda filum	Dead man's rope	0/2		<0.048	<0.045-<0.051
Eisenia bicyclis	Arame	0/1		<0.052	
Fucus serratus	toothed wrack	9/19		<0.015	<0.0045-0.015
Fucus spiralis	Spiral wrack	2/3	0.02	<0.008	<0.046-0.005
Fucus vesiculosus	Bladderwrack	14/27	0.028	0.022	<0.0065-0.022
Halidrys siliquosa	Halidrys siliquosa	1/2			<0.042-0.006
Himanthalia elongata	Thongweed	0/5		<0.043	<0.0049-<0.051
Laminaria digitata	Oar weed	17/33	0.03	0.024	<0.0059-0.067
Laminaria hyperborea	Tangle	0/1		<0.051	
Pelvetia canaliculata	Channelled wrack	1/3		<0.035	<0.033-0.042
Saccharina latissima	Sugar kelp	17/148		<0.047	<0.0098-0.081
Saccharina spp	Kombu	0/4		<0.052	<0.051-<0.053
Sargassum fusiforme	Hijiki	0/1		<0.056	
Sargassum muticum	Wireweed	0/2		<0.044	<0.04-<0.048
Undaria pinnatifida	Wakame	0/5		<0.053	<0.053-<0.054
Codium fragile	Green sea fingers	0/2		<0.05	<0.042-<0.058
Ulva intestinalis	Gutweed	3/7		<0.045	<0.005-0.011
Ulva lactuca	Sea lettuce	4/12		<0.045	<0.0035-0.009
Ulva spp	Green nori	0/1		<0.053	
Chondrus crispus	Irish moss	2/2	0.006	0.0059	0.005-0.007
Palmaria palmata	Dulse	4/26		<0.043	<0.0042-0.005
Porphyra purpurea	Purple laver	2/3	0.006	0.005	<0.005-0.007
Porphyra spp	Nori	0/11		<0.052	<0.048-<0.055
Porphyra umbilicalis	Pink laver	1/6		<0.043	<0.005-0.007
Vertebrata lanosa	Wrack siphon weed	5/17		<0.047	<0.017-0.068

3.1.6 - Commercially cultivated species: Sugar kelp and winged kelp

The higher number of data in this update also allows more precise consideration of sugar kelp and winged kelp, which are the most common species for both cultivation and wild harvest in Norway at the moment. Neither of these species are among the high concentration species regarding inorganic arsenic. Sugar kelp is number seven and winged kelp number 12 in the list based on decreasing median concentrations of inorganic arsenic (Table 2). For the winged kelp one extreme value of inorganic arsenic was seen with more than ten times higher concentration than the 75 % percentile. This could be associated with epiphyte algae as suggested below (3.2), and this should be followed up with further studies. Winged kelp and sugar kelp have intermediate cadmium concentrations present as number six and eight on the list with decreasing median values (Table 3). Regarding iodine, sugar kelp is among the species with the highest concentrations and winged kelp intermediate (Table 4). The higher concentrations of cadmium in sugar kelp are found in more northern areas as discussed below ("Geographic variation of cadmium in farmed sugar kelp").

3.2 - Factors affecting the levels of metals in kelp

As discussed below ("Geographic variation of cadmium in farmed sugar kelp"), cadmium concentrations in farmed sugar kelp showed a clear increase from south to north in Norway. The same section also discusses how iodine is reduced during cooking and lack of seasonal variation for farmed kelp.

During a master thesis study of wild and cultivated sugar kelp and winged kelp (Kleppe, 2016), cadmium decreased with increasing size of the plants, which suggests that fast growing individuals have lower concentrations of cadmium than slow growing individuals. Cadmium concentrations also decreased from the stipes and growth zone towards the tip. Iodine showed a similar trend within the plants, but no clear correlation with size. Inorganic arsenic showed no consistent variation but was usually highest in the mid-section of the blades of both species. Fouling was found to increase metal concentrations with higher cadmium concentrations in areas of sugar kelp covered with the bryozoan *Membranipora membranacea* , while an unknown filamentous alga increased concentrations of inorganic arsenic substantially in wild winged kelp. Differences were seen between wild and cultivated plants, but the differences were inconsistent between the two species and between the different minerals.

The amount of data on season, geography, exposure etc. are still scarce and more studies are needed. The variation even within the same locality (data not shown) for cultivated kelp is surprisingly high, and more knowledge of the factors causing such variation is needed to allow more predicable product quality for the seaweed industry.

3.3 - Radioactivity

Macroalgae are known to effectively concentrate radionuclides from seawater and are therefore widely used as a bio-indicator for radioactive pollution in the marine environment (e.g. Keogh, 2006; Kershaw et al., 2005) . For example, *Ascophyllum nodosum* are known to have a high uptake of the radionuclide ^{99}Tc (Sjøtun et al., 2011) , with increasing concentrations from young to older growth segments (Heldal and Sjøtun, 2010) . Nevertheless, few publications describe food safety aspects of radionuclides in macroalgae. Tuo et al. (2016) found no higher levels of either natural (^{238}U, ^{226}Ra, ^{228}Ra, ^{40}K) or anthropogenic (^{137}Cs) radionuclides in different seaweed species compared to other seafood, vegetables or meat products. Similarly, Moreda-Piñeiro et al. (2011) concluded that radiation levels in typical Japanese and Korean foodstuff, which include seaweed, are safe and at the same level as other countries.

The Norwegian marine monitoring programme Ra dioactivity in the M arine E nvironment (RAME) (www.dsa.no) charts trends of radionuclides in the marine environment. The results show that levels of the beta emitter ^{99}Tc in *F. vesiculosus* collected along the Norwegian coast in the period 2012-2014 did not exceed 70 Bq/kg dry weight (d.w.) (Skjerdal et al., 2017). Further, the levels of the gamma emitter ^{137}Cs, in *F. vesiculosus* collected in the same area and period ranged from 0.17 Bq/kg (d.w.) to 3.2 Bq/kg (d.w.). Levels of the alpha emitter ^{239,240}Pu were below the detection limit in samples collected in the same area and period (Skjerdal et al., 2017) . There are no maximum permitted levels for ^{99}Tc and ^{239,240}Pu in foodstuffs in Norway. The maximum permitted level for ^{137}Cs in foodstuffs set by Norwegian authorities after the Chernobyl accident is 600 Bq/kg. The ^{137}Cs-levels in macroalgae along the Norwegian coast are

far below this and should be of little or no concern to seafood consumers. In general, no radioactivity levels of concern with respect to food safety is found in seafood. Levels of natural radionuclides are generally higher than levels of anthropogenic radionuclides. For example, Tuo et al. (2016) found that the natural radionuclide ^{40}K accounted for around 87% of the total dose from the radionuclides ^{238}U, ^{226}Ra, ^{228}Ra, ^{40}K and ^{137}Cs in foodstuff from coastal areas of China.

3.4 - Other components

A few samples of kelp have been analysed for **dioxins and PCB's** and show low concentrations, in accordance with the 2016 report. 13 samples of dulse were analysed for **kainic acid** at the Danish Technical University and showed a range from 5 to 180 µg/g dry weight and hence confirm relatively low levels of this toxin. The toxicity of kainic acid has not yet been determined. However, in order to reach the hazardous levels of kainic acid similar to what was dosed in mice and rat experiments, a total amount of about 30 kg dry dulse of the 130 µg/g dulse would have to be eaten (Mouritsen et al., 2013) . Recently, higher values up to 500 µg/g dry weight has been found in Danish dulse, but still almost 10 kg would be needed for toxic responses.

3.5 - Microbiology

Since 2016 IMR worked with microbiology in an industry project. Blikra et al. (2019) describe the food quality and microbial safety of the two brown macroalgae winged kelp (*Alaria esculenta)* and sugar kelp (*Saccharina latissima*) harvested and processed in Norway. Samples included raw and frozen kelp. The authors found for all samples low microbial numbers (1–3 log colony forming units/g) for total aerobic count, cold adapted bacteria and spore-forming bacteria. Furthermore, there were no detection of indicators of faecal contamination as enterococci and coliforms, nor pathogenic vibrios or *Listeria monocytogenes* .

However, in several of the examined samples, *Bacillus* spp. were isolated and seems able to pose a challenge if not processing and storage conditions take their possible presence into account.

Bacteria in the genus *Bacillus* may grow under aerobic and anaerobic conditions. The bacteria and their spores are widely distributed in the environment and has been isolated from a wide variety of foods, especially of plant origin, but also from meat, fish, and dairy products. The most important species in food microbiology is *B. cereus* , and bacteria in this group have been involved in several food borne infections and intoxications.

Most strains of *B. cereus* are able to grow in low-acid foods at temperatures down to 10 ^{o} C and up to 55 ^{o} C (optimum 30 to 40 ^{o} C). During the last decades, some strains of *B. cereus* have been found able to grow at temperatures down to 4 ^{o} C. *Bacillus* species has been isolated from *sous vide* cod fillets at 5 ^{o} C and in many other *sous vide* products. Food containing more than 10^4 *B. cereus* cells per g may not be safe for consumption. This number is far above what was seen by Blikra et al., 2019.

Several foods like milk and rice are also known to contain *Bacillus* spp., and it is common routine that heat-treated food products containing milk or rice are stored cold to prevent *Bacillus* spp. from growing. Heat treatment usually gives a temperature high enough to kill vegetative bacterial cells and any competitive microbiota, but not *Bacillus* spores. After such heat treatment, spores may be reactivated and give multiplication of *Bacillus* without competing bacteria present. Control of *Bacillus cereus* is efficiently obtained by chilling, except for some few cold-adapted strains that mainly pose a challenge for dairy products. The same precautions should hence be taken for heat treated products containing macroalgae due to possible presence of *Bacillus* spp. spores.

3.6 – Bioavailability, geographical variation and effect of cooking on sugar kelp composition

This project has not yet been finalised and reported, but summary of the findings this far, is given below. The project as financed by the Norwegian Seafood Research Fund (FHF) and carried out in collaboration with the Technical University of Denmark (DTU) and the kelp producer Ocean Forest.

Original project title: «På sporet av ny mat»

Experimental diet for rats added sugar kelp (Saccharina latissima)

We have performed a 13-week feeding trial with female Wistar IGS rats to compare the health effects and bioavailability of iodine from farmed sugar kelp with potassium iodine. No signs of toxic responses were found in animals with 0.5 and 5% sugar kelp or comparable amounts of potassium iodine in the diet. No difference in body weight, feed intake, heart-, kidney- or live weight were observed in the animals fed dried kelp in the diet at 0.5 and 5%. Biomarkers for liver and kidney damage measured in plasma were not increased in rats given potassium iodide or iodine from sugar kelp. No significant differences in serum concentrations of the thyroid stimulating hormone (TSH) and thyroid hormone T3 concentrations were observed. However, a small but significant reduction in serum T4 was observed in the rats fed the highest concentration of sugar kelp and potassium iodide.

Collectively, the observed high tolerance of iodine in rats agree with previous studies (Calil-Silveira et al., 2016; Yoshida et al., 2014). Yoshida et. al (2014) reported no significant effects on serum T3 and T4 concentration in rats despite an iodine intake up to 3500 µg iodine per day from kelp.

Based on available literature and the results from our experiment, it seems clear that rats have a high tolerance for iodine, even at the high intake of sugar kelp (5% of the diet) in the present study. The rat model used was hence not suited for evaluation of negative effects of iodine. On the other hand, the high tolerance of the iodine allowed to conclude that none of the other components of the kelp had a negative impact on the health of the rats. The high tolerance of iodine in rats also made it possible to study the bioavailability of iodine from kelp at very high concentrations.

Bioavailability of iodine from sugar kelp in rats

From previous studies both in animals (Yoshida et al., 2014) and humans (Aquaron et al., 2002; Combet et al., 2014), based on excretion of iodine in urine, there are indications that the bioavailability of iodine is lower from kelp compared to the salt form of iodine, potassium iodide. Most of absorbed iodine is excreted in the urine, and only a small fraction is retained in the thyroid gland or found in circulation. The amount of iodine excreted in urine is hence used as a measure for the amount of absorbed iodine over a time period. By monitoring urinary iodine excretion in rats from the 13-week feeding trial, the bioavailability of iodine from sugar kelp was evaluated. Urine excretion of iodine reflects the dietary intake in all experimental groups. 94-95 % of total iodine intake was excreted in urine in both the low and high dose of potassium iodide. Urine excretion in rats given iodine from sugar kelp, show a significantly lower excretion level in urine with 73-78% bioavailability of iodine. This is still relatively high, but significantly lower compared to potassium iodide. These results are in agreement with data reported on bioavailability of iodine in humans using both potassium iodide (96.4%) and seaweed (93 and 75%) (Aquaron et al., 2002). A lower bioavailability of iodine from seaweed were also observed when comparing three iodine-rich foods in a human intervention pilot study. Iodine excretion in urine were 86%, 87% and 60% with 36 hours collection after consumption of fish, milk or seaweed, respectively (Redway et al., 2018). The rat model used in our study was probably well suited for evaluation of iodine availability, although it was not suited for evaluation of toxic effects as discussed above.

In contrast to earlier reports we quantified iodine in feces. A lower urine excretion of iodine in rats given sugar kelp was accompanied by a significant increase in iodine found in feces. Diets supplemented with potassium iodide resulted in 0.5-1 % of the iodine in feces on average. In contrast, rats given kelp in the diet had on average 8% of total iodine in feces, representing iodine not absorbed in the intestine from the kelp diet.

Bioavailability of total and inorganic arsenic, cadmium and copper from sugar kelp in rats

More than 70 % of ingested total arsenic in rats fed the a diet with 5 % sugar kelp was found in the 24 hours feces collection. This is in accordance with a high proportion of arsenic being organic, hence having a high availability. For cadmium, the amounts in the 24 hours feces collection were almost identical to the ingested amount, indicating very low availability. This is in accordance with a generally low availability of inorganic metals forms. Some cadmium was assimilated, though, as seen from the significant increase in cadmium in liver and kidney in the 5% diet group. Regarding copper, the concentrations in the kelp were too low to give differences in the feed between the groups, and there were no differences in the liver or kidney samples.

Regarding inorganic arsenic, rats and other rodents are not suitable models since these animals metabolise and excrete inorganic arsenic at high rates. Inorganic arsenic was only detected in the 5 % kelp group feed, and no concentrations above limit of quantification (LOQ) could be found in the liver or kidneys samples from any of the groups.

The effect of cooking on iodine levels in sugar kelp

Iodine is water soluble, and iodide (I^-), probably the main form in kelp, reacts with water forming the volatile hydrogen iodide. Several forms of cooking were performed to assess the effect of iodine content of the sugar kelp. Boiling in water for 15 minutes released 50-90 % of iodine to the water, and of this iodine 50% was released to air. Continued simmering of the stock reduced further 50 % over 15 minutes. Frying released in average 50 % (25-80) of the iodine. Drying of sugar kelp increases concentrations of all ellements ten times just by removing the water, which makes 90 % of the wet weight. However, when comparing iodine concentrations on dry weight, it was seen that about 25 % of the iodine evaporated during drying.

Geographic variation of cadmium in farmed sugar kelp

In cooperation with SINTEF (Macrosea project), a standardised growth trial was performed on localities all along the coast of Norway, and samples were analysed for metals at the IMR. The results showed a clear trend with increasing concentrations of cadmium from south to north. The levels in the north (68-70N) were above 1.0 mg/kg dry weight which is the limit for inclusion in fish feed, and more than four times higher than the southern samples (58-60N). Inorganic arsenic and iodine did not show a similar trend.

3.7 - Student projects on iodine

There are three relevant master theses at the IMR with main focus on iodine. Two of these are based on purchased seaweed products from Norwegian web shops. The products are analysed for iodine, total arsenic, mercury, lead and cadmium. This work also includes blood and urine samples from some seaweed eaters and are analysed for iodine status and thyroid markers. The third study has included four of the purchased seaweed products in a vegan week menu. These daily menus are analysed with and without seaweed for iodine and metals. All three master theses were defended in June 2020 and the main results will be published in peer reviewed journals in 2020-21.

3.8 - Nutrient analyses

The trace minerals, iron (Fe), zinc (Zn) and selenium (Se), were analysed together with the heavy metals and metalloids and hence with the same number of analyses. The macro minerals, calcium (Ca), potassium (K), magnesium (Mg), sodium (Na) and phosphorus (P), were analysed separately with in total 104 analyses. Seven species had three or more samples. The results are presented in Table 7 and Table 8.

Table 7 . Selenium (Se), iron (Fe) and zinc (Zn) medians (± 25% percentiles) concentrations in macroalgae, mg/kg dry weight. Species are presented in order by decreasing median concentrations. Cell colouring corresponds to brown, green and red algae.

		N	Se			N	Fe			N	Zn	
Vertebrata lanosa	Wrack siphon weed	18	0.83	(0.69-1.2)	Hijiki	1	820		Wrack siphon weed	18	95	(18-100)
Sargassum fusiforme	Hijiki	1	0.22		Green nori	1	480		Tangle	1	76	
Porphyra spp	Nori	11	0.21	(0.16-0.25)	Wrack siphon weed	18	340	(260-540)	Toothed wrack	18	65	(19-86)
Ulva spp	Green nori	1	0.2		Irish moss	2	260	(190-330)	Pink laver	6	59	(6-70)
Chondrus crispus	Irish moss	2	0.19	(0.14-0.23)	Gutweed	5	230	(160-580)	Hijiki	1	39	
Undaria pinnatifida	Wakame	5	0.17	(0.16-0.23)	Nori	11	160	(90-180)	Wakame	5	37	(5-41)
Palmaria palmata	Dulse	23	0.16	(0.14-0.36)	Wireweed	2	160	(32-280)	Bladderwrack	27	36	(27-60)
Porphyra purpurea	Purple laver	3	0.16	(0.046-0.39)	Wakame	5	150	(120-160)	Oar weed	33	36	(33-41)
Sargassum muticum	Wireweed	2	0.14	(0.08-0.2)	Spiral wrack	3	130	(120-240)	Winged kelp	37	35	(38-49)
Ulva intestinalis	Gutweed	5	0.14	(0.11-0.38)	Sea lettuce	10	120	(90-300)	Thongweed	5	35	(5-39)
Laminaria hyperborea	Tangle	1	0.13		Channelled wrack	3	120	(25-300)	Dead man's rope	2	30	(2-47)
Fucus serratus	Toothed wrack	18	0.12	(0.089-0.12)	Purple laver	3	110	(89-330)	Rockweed	24	29	(24-47)
Codium fragile	Green sea fingers	2	0.12	(0.1-0.13)	Pink laver	6	110	(58-110)	Dulse	23	28	(26-33)
Ulva lactuca	Sea lettuce	10	0.12	(0.068-0.22)	Dulse	23	100	(84-160)	Channelled wrack	3	25	(3-36)
Alaria esculenta	Winged kelp	37	0.11	(0.09-0.15)	Toothed wrack	18	78	(46-110)	Sugar kelp	146	24	(148-32)
Chorda filum	Dead man's rope	2	0.11	(0.09-0.13)	Bladderwrack	27	68	(41-130)	Nori	11	22	(11-35)
Eisenia bicyclis	Arame	1	0.1		Rockweed	24	62	(33-95)	Wireweed	2	19	(2-29)
Saccharina spp	Kombu	4	0.1	(0.098-0.1)	Winged kelp	37	56	(44-68)	Arame	1	16	
Fucus spiralis	Spiral wrack	3	0.099	(0.095-0.11)	Halidrys siliquosa	2	56	(16-96)	Green nori	1	13	
Pelvetia canaliculata	Channelled wrack	3	0.095	(0.051-0.098)	Green sea fingers	2	51	(36-65)	Gutweed	5	12	(7-18)
Saccharina latissima	Sugar kelp	146	0.095	(0.074-0.12)	Sugar kelp	146	44	(31-65)	Sea lettuce	10	11	(12-14)
Himanthalia elongata	Thongweed	5	0.091	(0.049-0.094)	Oar weed	33	39	(18-78)	Spiral wrack	3	7	(3-8)
Fucus vesiculosus	Bladderwrack	27	0.089	(0.071-0.11)	Kombu	4	33	(20-41)	Green sea fingers	2	5.9	(2-5.9)
Ascophyllum nodosum	Rockweed	24	0.084	(0.044-0.096)	Arame	1	32		Irish moss	2	5.6	(2-49)
Halidrys siliquosa	Halidrys siliquosa	2	0.08	(0.034-0.13)	Dead man's rope	2	23	(9.2-37)	Kombu	4	5.6	(4-7)
Porphyra umbilicalis	Pink laver	6	0.077	(0.067-0.098)	Tangle	1	20		Purple laver	3	2	(3-18)
Laminaria digitata	Oar weed	33	0.065	(0.042-0.091)	Thongweed	5	20	(14-22)	Halidrys siliquosa	2	2	(2-17)

Table 8 . Calcium (Ca), potassium (K), magnesium (Mg), Sodium (Na) and phosphorus (P) concentrations medians (± 25% percentiles) in macroalgae, mg/kg dry weight. Sorting order by decreasing median concentrations. Cell colouring: Brown, green and red algae.

		N	Ca			N	K			N	Mg			N	Na			N	P	
Sargassum muticum	Wireweed	2	22 000	(22000-22000)	Dulse	6	99 000	(43000-140000)	Green sea fingers	1	31 000		Green sea fingers	1	170 000		Winged kelp	12	3 700	(2700-4100)
Ulva intestinalis	Gutweed	2	22 000	(14000-29000)	Sugar kelp	51	91 000	(73000-130000)	Sea lettuce	2	26 000	(25000-28000)	Sugar kelp	51	48 000	(43000-56000)	Dulse	6	3 200	(2500-3700)
Alaria esculenta	Winged kelp	12	19 000	(17000-22000)	Oar weed	5	80 000	(40000-82000)	Wireweed	2	11 000	(7200-14000)	Winged kelp	12	46 000	(40000-65000)	Purple laver	3	3 200	(2900-3300)
Fucus vesiculosus	Bladderwrack	4	18 000	(15000-24000)	Winged kelp	12	62 000	(57000-66000)	Gutweed	2	9 900	(8700-11000)	Oar weed	5	44 000	(39000-56000)	Green sea fingers	1	2 700	
Fucus spiralis	Spiral wrack	2	18 000	(17000-18000)	Wireweed	2	61 000	(48000-75000)	Winged kelp	12	9 800	(8800-12000)	Thongweed	1	39 000		Irish moss	1	2 400	
Himanthalia elongata	Thongweed	1	18 000		Thongweed	1	47 000		Thongweed	1	9 400		Wrack siphon weed	1	36 000		Oar weed	5	2 300	(1600-3600)
Codium fragile	green sea fingers	1	18 000		Wrack siphon weed	1	47 000		Irish moss	1	9 000		Toothed wrack	1	32 000		Sea lettuce	2	2 300	(2200-2400)
Ascophyllum nodosum	Rockweed	6	16 000	(15000-17000)	Halidrys siliquosa	1	36 000		Rockweed	6	8 500	(8000-9500)	Rockweed	6	31 000	(27000-35000)	Gutweed	2	2 100	(1700-2500)
Fucus serratus	toothed wrack	1	16 000		Toothed wrack	1	30 000		Sugar kelp	51	8 100	(7300-8900)	Bladderwrack	4	25 000	(20000-31000)	Sugar kelp	51	2 000	(1200-2600)
Halidrys siliquosa	Halidrys siliquosa	1	16 000		Sea lettuce	2	30 000	(27000-34000)	Spiral wrack	2	7 900	(7500-8200)	Wireweed	2	25 000	(20000-29000)	Pink laver	2	2 000	(1500-2600)
Saccharina latissima	Sugar kelp	51	15 000	(12000-17000)	Irish moss	1	30 000		Channelled wrack	1	7 900		Spiral wrack	2	24 000	(20000-27000)	Thongweed	1	1 500	
Laminaria digitata	Oar weed	5	15 000	(13000-15000)	Bladderwrack	4	27 000	(21000-30000)	Toothed wrack	1	7 400		Channelled wrack	1	23 000		Wireweed	2	1 500	(1200-1700)
Pelvetia canaliculata	channelled wrack	1	14 000		Purple laver	3	26 000	(15000-31000)	Oar weed	5	7 200	(6500-9600)	Sea lettuce	2	19 000	(8300-30000)	Wrack siphon weed	1	1 400	
Chondrus crispus	Irish moss	1	13 000		Spiral wrack	2	25 000	(21000-28000)	Bladderwrack	4	7 100	(6200-8100)	Irish moss	1	18 000		Bladderwrack	4	1 300	(740-1700)
Vertebrata lanosa	Wrack siphon weed	1	7 200		Pink laver	2	22 000	(13000-31000)	Wrack siphon weed	1	6 700		Pink laver	2	18 000	(5300-30000)	Halidrys siliquosa	1	1 100	
Ulva lactuca	Sea lettuce	2	6 000	(5100-6900)	Rockweed	6	17 000	(16000-25000)	Halidrys siliquosa	1	6 200		Dulse	6	15 000	(4100-20000)	Spiral wrack	2	960	(820-1100)
Porphyra purpurea	Purple laver	3	4 800	(2300-5400)	Channelled wrack	1	17 000		Pink laver	2	5 000	(4400-5700)	Halidrys siliquosa	1	13 000		Toothed wrack	1	760	
Palmaria palmata	Dulse	6	4 300	(2500-18000)	Gutweed	2	16 000	(12000-20000)	Purple laver	3	4 700	(3600-17000)	Purple laver	3	7 200	(6200-100000)	Rockweed	6	720	(440-830)
Porphyra umbilicalis	Pink laver	2	2 900	(2200-3500)	Green sea fingers	1	14 000		Dulse	6	2 800	(1200-3300)	Gutweed	2	7 000	(5500-8500)	Channelled wrack	1	700	

3.9 – Seaweed as a salmon feed resource

IMR conducted a SWOT analysis of the use of macroalgae in fish feed (Lock and Belghit, 2018) . Although seaweed are taxonomically not plants, many parallels between seaweed and plants exists. Both can be a valuable source of nutrients that can be used by animals higher up the food chain, but both can also contain anti-nutritional factors, preventing them from being preyed on. The effect of a plain soybean meal on the development of enteritis in Atlantic salmon is well known and similar effects are seen of peas and other vegetable products. In commercial diets, it is highly processed protein concentrates of these plant products that are used today in Norway. Processing removes or reduces some of the anti-nutritional factors and simultaneously concentrates the protein content of the product. Salmon is a carnivorous fish that requires protein, lipid and micronutrients for healthy growth, while the requirement for carbohydrates is very low. Seaweed is mainly made-up of carbohydrates that cannot be used by the fish. This raises the obvious questions about post-harvest processing of seaweed to make nutrients more accessible and remove anti-nutritional factors, which currently are underdeveloped or non-existing. A fractionation of the seaweed biomass is needed where high-end products (e.g. alginates) can offset a large part of the production and processing costs. The lack of seaweed processing and diversification of the processing is the major hurdle for the use of seaweed in aquafeed. The SWOT analysis elaborates on the strengths and weaknesses of using seaweeds in feed for fish and pinpoints future changes that could stimulate (opportunities) or raise barriers (threats) in the application of marine macroalgae in aquafeed.

The high content of carbohydrates in seaweed limits the use of seaweed as a major component in fish feed. The use of insect larvae for processing seaweed is a way to overcome this problem by increasing the protein and lipid concentrations and decreasing the carbohydrate content. The insect larvae are then a promising feed source for fish aquaculture that was examined in the project Aquafly.

Larvae of the black soldier fly (*Hermetia illucens* ; BSF) were reared on plant-based media enriched with the brown alga *Aschophyllym nodosum* (rockweed), in increasing percentages (from 0 to 100 % seaweed inclusion) (Liland et al., 2017) . All the tested substrates allowed for BSF larval growth and development, although the best growth performance, nutrient utilization and retention were observed in larvae fed up to 50 % seaweed inclusion in the medium (Liland et al., 2017) . The larvae fed seaweed-enriched media had a more “marine” profile than the control larvae, with the eicosapentaenoic acid (20:5n-3) (EPA), iodine and vitamin E introduced in the larvae from the seaweed in the media. The nutritional profile of the larvae fed seaweed better suited the dietary requirements of Atlantic salmon for these nutrients (NRC, 2011) , compared to larvae fed media without seaweed.

The legislation in the European Union (EU) has set maximum levels (MLs) for undesirable substances in feed and food stuff (EC Directive 2002/32; Commission Regulation (EC) No 1881/2006). When seaweed is used as feeding substrate for insect larvae, levels of heavy metals and arsenic in the medium should be within MLs set by the legislation, as insects are considered full-fledged “farmed animals” by the EU (Regulation (EC) No 1069/2009; Commission Regulation (EU) 2017/893). Mixing seaweed biomass (rockweed) with the plant-based medium for BSF larvae, increased the concentrations of heavy metals (cadmium, lead and mercury) and arsenic in the media for the larvae, when more seaweed was included (Biancarosa et al., 2018) .

Accumulation of cadmium and total arsenic in the insect larvae fed with different batches of rockweed was sometimes higher than the MLs. In a feeding trial, insect meal had concentrations above ML for arsenic, while whole feed concentrations were below ML (Biancarosa et al., 2019) . Salmon were fed increasing levels of insect meal replacing fish meal, and levels of cadmium, mercury and lead in the feed were reflected in the salmon fillets. Interestingly, arsenic levels were similar in all feed groups, but total arsenic decreased with increasing insect meal in the diet, suggesting low availability of the forms of arsenic in the insect meal.

4 - EFSA data submission

Initial work was done during the autumn of 2019 attempting to submit data on macroalgae to the EFSA database, using the new SSD2 format. However, for most of the species the necessary codes needed for the submission was not established and added to the database until after the deadline for submission in October 2019. Upon request a list of seaweed species were added to the FOODEX database catalogue, but not in the reporting hierarchy directly. These species can then be specified as sources within the different types of seaweed, like Sea belt as "Brown algae, SOURCE = Sea belt (as organism)" as seen in the table below.

Table 9. Codes for macroalgae species for reporting to the EFSA FOODEX database.

Common name	Latin name	Code	Text
Winged kelp/dabberlocks	*Alaria esculenta*	A00VK#F01.A18BQ	Brown algae, SOURCE = Dabberlocks (as organism)
Rockweed	*Ascophyllum nodosum*	A170Z	Rockweed
Irish moss	*Chondrus crispus*	A00VG	Carrageen mosses
Dead man's rope	*Chorda filum*	A00VK#F01.A18BK	Brown algae, SOURCE = Dead man's rope (as organism)
Green sea fingers	*Codium fragile*	A00VB#F01.A18BJ	Green algae, SOURCE = Green sea fingers (as organism)
Toothed wrack	*Fucus serratus*	A00VK#F01.A0B6S	Brown algae, SOURCE = Toothed wrack (as organism)
Spiral wrack	*Fucus spiralis*	A00VK#F01.A18BL	Brown algae, SOURCE = Spiral wrack (as organism)
Bladderwrack	*Fucus vesiculosus*	A00VK#F01.A0CRR	Brown algae, SOURCE = Bladder wrack (as organism)
Halidrys siliquosa	*Halidrys siliquosa*	A00VK#F01.A18BM	Brown algae, SOURCE = Sea oak (as organism)
Thongweed	*Himanthalia elongata*	A00VN	Sea spaghetti
Oar weed	*Laminaria digitata*	A00VK#F01.A0B6M	Brown algae, SOURCE = Tangle (as organism)
Tangle	*Laminaria hyperborea*	A00VK#F01.A0B6N	Brown algae, SOURCE = North European kelp (as organism)
Dulse	*Palmaria palmata*	A00VJ	Dulse
Channelled wrack	*Pelvetia canaliculata*	A00VK#F01.A18BH	Brown algae, SOURCE = Channelled wrack (as organism)
Purple laver	*Porphyra purpurea*	A00VH	Laver (species-specific code is missing)
Pink laver	*Porphyra umbilicalis*	A00VH	Laver (species-specific code is missing)
Sugar kelp	*Saccharina latissima*	A00VK#F01.A0B6Q	Brown algae, SOURCE = Sea belt (as organism)
Gutweed	*Ulva intestinalis*	A00VB#F01.A0B6F	Green algae, SOURCE = Hollow green nori (as organism)
Sea lettuce	*Ulva lactuca*	A00VD	Sea lettuce
Wrack siphon weed	*Vertebrata lanosa*	A00VE#F01.A18BG	Red algae, SOURCE = Wrack siphon weed (as organism)

Codes for dried seaweed should be reported in the following format as informed by EFSA secretary October 2019:

A00ZQ#F01.A0B6Q$F27.A00VK	Dried vegetables, SOURCE = Sea belt (as organism), SOURCE-COMMODITIES = Brown algae
A00ZQ#F01.A05MJ$F27.A00VB	Dried vegetables, SOURCE = Sea lettuce (as organism), SOURCE-COMMODITIES = Green algae
A00ZQ#F01.A05MP$F27.A00VE	Dried vegetables, SOURCE = Dulse (as organism), SOURCE-COMMODITIES = Red algae

In addition, codes for wild and cultivated seaweed should be found to address the call for data. Data for 353 samples of macroalgae were submitted by the IMR in time for the dealine of October 1, 2020.

5 - References

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6 - Appendix

Table 10. Dry weight percentages in samples of fresh and dried macroalgae (imported products sold dry). Species are presented with decreasing median values. Cell colouring according to brown, red and green algae.

Latin name	Common name	N	Mean	Median	Min-max	25 % Quartiles
Saccharina spp	Kombu, dried	4	97	97	95-99	95-98
Eisenia bicyclis	Arame, dried	1	96	96		
Porphyra spp	Nori, dried	10	95	95	83-99	94-97
Undaria pinnatifida	Wakame, dried	5	94	94	93-95	94-94
Ulva spp	Green nori, dried	1	94	94		
Sargassum fusiforme	Hijiki, dried	1	90	90		
Ascophyllum nodosum	Rockweed	17	30	31	18-37	28-34
Pelvetia canaliculata	channelled wrack	1	31	31		
Fucus vesiculosus	Bladderwrack	20	31	28	19-94	25-30
Halidrys siliquosa	Halidrys siliquosa	8	25	24	22-35	22-24
Fucus serratus	toothed wrack	16	23	23	17-28	21-24
Fucus spiralis	Spiral wrack	1	20	20		
Laminaria digitata	Oar weed	28	19	18	14-25	15-21
Vertebrata lanosa	Wrack siphon weed	15	16	17	8.6-22	13-18
Alaria esculenta	Winged kelp	28	15	16	7.9-22	12-18
Laminaria hyperborea	Tangle	4	16	16	14-17	15-16
Ulva lactuca	Sea lettuce	11	16	16	9.4-28	11-18
Palmaria palmata	Dulse	20	17	16	12-34	13-20
Himanthalia elongata	Thongweed	2	15	15	14-16	14-16
Sargassum muticum	Wireweed	4	13	13	11-15	12-15
Chorda filum	Dead man's rope	1	13	13		
Saccharina latissima	Sugar kelp	156	13	12	6-26	10-15
Porphyra purpurea	Purple laver	1	11	11		
Ulva intestinalis	Gutweed	5	10	8	7.3-13	7.9-13
Codium fragile	Green sea fingers	2	5	5	4.8-5.2	4.8-5.2

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Seaweed as feed ingredient in aquafeed

– a SWOT analysis

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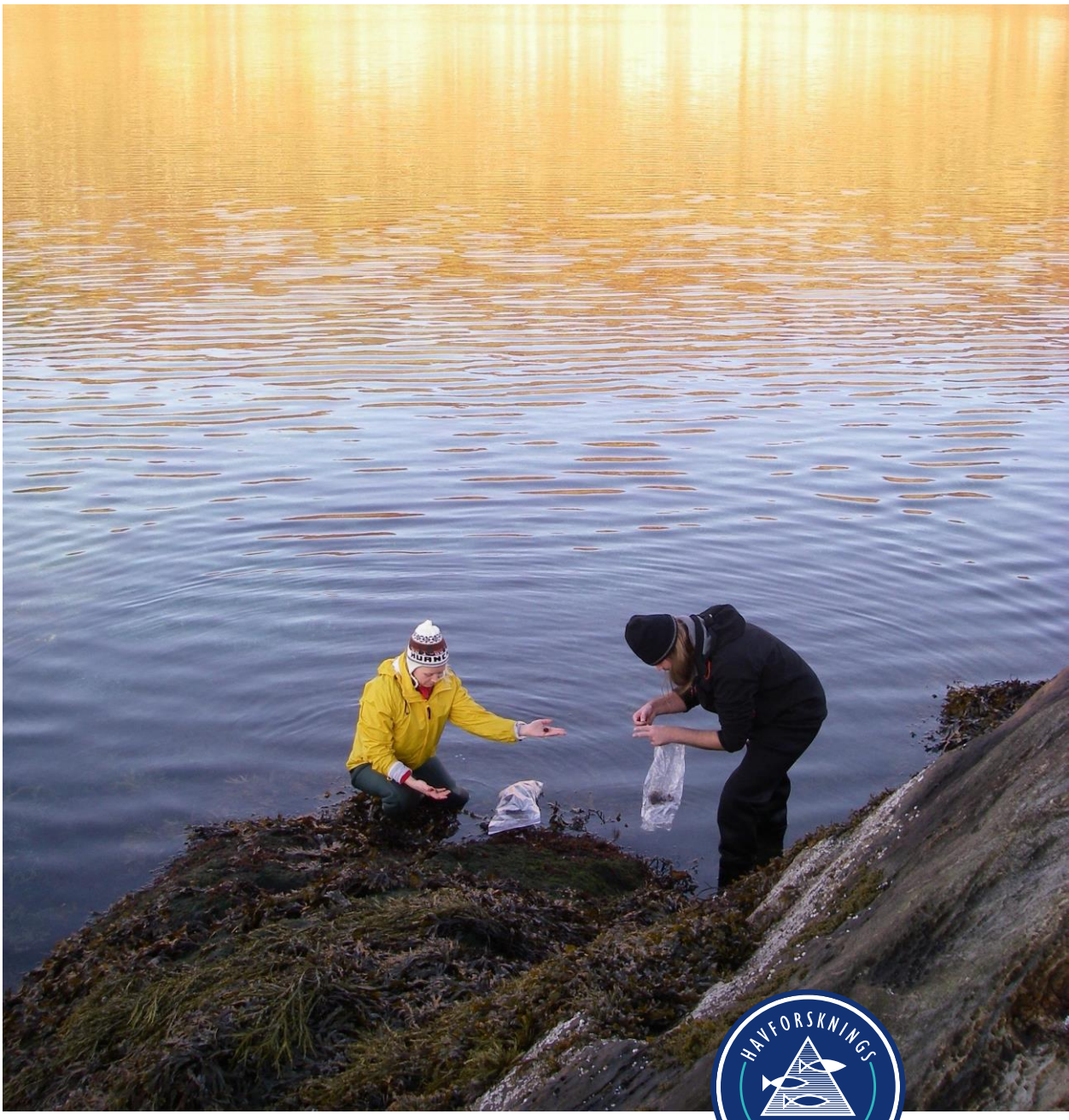

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Summary (Norwegian):
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Summary (English):
Finfish aquaculture has been a quickly developing industry during the past three decades and is expected to continue this growth in the foreseeable future. The aquafeed industry has to keep up with this growth and maybe even faster, since many of the traditional forms of finfish culture (e.g. in Asia) are being intensified which often results in the use of formulated feeds. The main ingredients in aquafeed nowadays come from terrestrial sources, with the exception of fishmeal and –oil. Even though it is a well-known fact that a kg of fish fillet needs considerable less resources than a kg of beef or even pork, aquaculture will inevitably put more pressure on land resources. The blue economy aims to produce more food from the sea to satisfy the global need for nutrients. Finfish aquaculture is often used as an example of how to achieve this. Indeed, the potential is large, however this is only possible if we manage to harvest the primary producers from the sea as well. Seaweed is one of the primary producers in the marine food chain, similar to plants in the terrestrial food chain. Although seaweed are taxonomically not plants, many parallels between seaweed and plants exists. Both can be a valuable source of nutrients that can be used by animals higher up the food chain, but both also contain anti-nutritional factors, preventing them from being preyed on by these animals. The effect of a plain soybean meal on the development of enteritis in Atlantic salmon is well known and similar effects are seen of peas and other vegetable products. In commercial diets it is highly processed protein concentrates of these plant products that are used. This removes or reduces many of these anti-nutritional factors and simultaneously concentrates the protein content of the product. Salmon is a carnivorous fish that requires protein, lipid and micronutrients for healthy growth, the requirement for carbohydrates is very low. Seaweed is mainly made-up of carbohydrates that cannot be used by the fish. This raises the obvious questions about post-harvest processing of seaweed to make nutrients more accessible and remove anti-nutritional factors, which currently are underdeveloped or non-existing. A fractionation of the seaweed biomass is needed where high-end products (e.g. alginates) can

offset a large part of the production and processing costs. The lack of seaweed processing and diversification of the processing is the major hurdle for the use of seaweed in aquafeed. This analysis will elaborate on the strengths and weaknesses of using seaweeds in feed for fish and pinpoints future changes that could stimulate (opportunities) or raise barriers (threats) in the application of marine macroalgae in aquafeed.

Emneord (norsk):
1. [Emneord]

Subject heading (English):
1. [Subject heading]

Project Manager

Research Group Manager

Content

1 Strengths

The inherent strength of seaweed for this analysis (aquafeed) will be from a nutritional perspective. There is a decent amount of documentation of nutritional composition of seaweed both from Norway and abroad [1-6]. The most relevant macronutrient from seaweed is protein. Depending on the species and the season, seaweeds can contain varying protein levels (50-300 g/kg dry weight (DW) [1, 2, 6]. The green and red types of seaweed contain the highest protein levels (150-300 g/kg) and the brown seaweed on average the lowest [1, 6]. Of the brown species it is however the kelp species (like Saccharina latissima and Alaria esculenta) that contain the highest protein levels, around 100-200 g/kg [1, 4, 6]. These species are most likely to be cultured on a large scale for food and feed. Generally, seaweed species contain all the essential amino acids required for animal growth and health, including fish. In addition to the higher content in acidic amino acids, some red seaweed (Palmaria palmata) possess a high concentration of methionine which is higher than reported for leguminous pulses [7]. These seaweed species also contain a high concentration of taurine [7], which can be beneficial in fish nutrition and is not detected in plant meals. Many peptides have been identified in marine macroalgae with therapeutic properties [4]. Among them, the antioxidant peptide glutathione has been found in the Norwegian marine macroalgae [8]. In terms of other antioxidant systems, some cysteine-oxoforms metabolites (hypotaurine and cysteine-sulfinic acid) were also detected in the green and the brown seaweeds [8, 9].Seaweed are usually low in lipids, but in some species the typical marine omega n-3 long chain fatty acid (eicosapentaenic acid, 20:5n-3) can contain up to 0.5% (of dry weight) [10]. The ratio between n-6 and n-3 fatty acids is considered an index for evaluating the nutritional value of a dietary lipid source with respect to human and animal development and health. The n-6:n-3 ratio varied between the phyla but also between different species belonging to the same phylum, for example the three seaweeds phyla collected along the Norwegian coast had an n-6:n-3 ratio around 1:1 [1, 2], which does not exceed the ratio of 5:1, as recommended by the world Health Organization (WHO). Furthermore, the red and the brown seaweed are rich source of bioactive lipid metabolites, the oxylipins [8], which act as signaling molecules and provide innate immunity against different stress factors [11]. There are companies pressing oil out of dried seaweed targeting specific markets, but for aquafeed it is an unlikely future ingredient due to pricing [12].Seaweed is rich in iodine. For human consumption this would have its benefits however for fish nutrition it is

less of an importance. Certain species like, Laminaria sp. (stortare), currently one of the main species harvested along the Norwegian coast can contain extremely high levels of iodine[1, 13]. At these concentrations it would pose a health risk for consumers, it is unclear whether these high iodine levels would pose as risk for the fish. Julshamn and colleagues (2006) fed Atlantic salmon diets containing up to 80 times their requirement for iodine. After 150 days of feeding, this did not affect their health. Fillet iodine levels were moderately increased [14], which could be beneficial to the nutritional value of these fish for human consumption. Seaweed also contains other essential elements, like calcium, iron, manganese, zinc, selenium, magnesium, and phosphorus. All of these are required by the fish, however there is little known on the availability of these minerals for fish. In their natural environments, seaweeds are exposed to various biotic and abiotic stress factors. As a result, seaweeds contain many forms of antioxidants including vitamins and pigments. Seaweeds are a good source of some water-(B(1), B(2), B(12), C) and fat-soluble (β-carotene with vitamin A activity, vitamin E) vitamins. The water-soluble vitamin C is present in large amounts in brown, green and red seaweeds, such as Gracilaria spp, which contain 25 mg/100 g wet weight [15, 16]. Green seaweed, like Ulva lactuca, can provide as well a high level of vitamin B12 [17]. Moreover, the level of β-carotene found in the seaweeds is high and can exceed those measured in carrots, e.g. in Gracilaria chilensis [18]. Furthermore, brown algae contain high level of the eight forms of vitamin E (tocopherols and tocotrienols) [1, 8], which are known as a strong antioxidant compounds with many beneficial health effects and required by salmon. The exploitation of seaweed has been mainly focused on the industrial production of thickeners, stabilizers or gelling agents in the food industry, such as carrageenan, agar and alginates [19]. These compounds could be used as binders in the feed pellets since they are naturally present in the seaweed meal. Finally, seaweed is rich in many other and unknown bioactive compounds, for an overview please see Holdt and Kraan (2011) [4]. A lot of these bioactive compounds are found in the carbohydrate fraction, such as the polyols, which are the common storage compound of brown algae. This phylum is also a rich source of sulfated polysaccharides [8]. There is not much known about the effect of these compounds in aquafeed, however these bioactive compounds, which are considered as functional food ingredients, have beneficial health effects in humans and mammals [20, 21]. Beyond the nutritional composition, using seaweed as a functional feed ingredient can provide specific benefits to the fish, e.g. preventive health care through nutritional means. However, in a seaweed meal there is a whole collection

of compounds and some compounds could be detrimental to the fish's health. The few studies that exist show that a small inclusion of algae (between 2.5 and 10% of the diet composition) in aquafeed resulted in positive effects such as; increase in growth performance, carcass quality, intestinal microbiota, improve stress and immune response and disease resistance [22-27]. For example, the dietary inclusion of Gracilaria or Fucus spp (2.5-7%) into the diets of European seabass (Dicentrarchus labrax) improved the immune and antioxidant response without affecting the growth performances [26]. Moreover, feeding rainbow trout (Oncorhynchus mykiss) with diets supplemented with 5% Gracilaria sp, improved flesh quality traits (higher color intensity and juiciness) and enriched the content of flesh iodine than fish fed the control diet [28]. However, high inclusion of algae (18%, Porphyra sp.) in aquafeed showed impaired growth in thick-lipped grey mullet (Chelon labrosus) when compared with non-supplemented diets [29].

2 Weaknesses

The nutritional composition of seaweed varies highly between different species, but also within species belonging to the same phylum. Nutrient content varies between locations, time of the year, wild vs cultured, etc. For example, the brown algae, *A. nodosum* contains 45 g protein/Kg DW, while the red algae, *P. dioica* contains 310 g protein/kg DW when sampled at the same location at the same day [1, 6]. Moreover, seaweeds are usually low in lipids (0.9±3.7% of dry weight) and do not contain docosahexaenoic acid (22:6n-3 DHA).

Seaweeds are rich in minerals, giving a seaweed meal a (too) high ash content. Certain seaweeds species contain a very high concentration of iodine (*L. digitata*, 10 000 mg/kg DW) [1], manganese and zinc [30]. There is no data available on the availability of these minerals for fish.

Seaweeds accumulate undesirable elements, such as arsenic and cadmium. These concentrations can be so high that the level exceeds the maximum level allowed for the use as a feed ingredients [1, 2, 31, 32].

If a whole seaweed meal is used a large portion will be indigestible complex carbohydrates that will not be used by the fish species. Furthermore, seaweeds contain substances with anti-nutritional activity such as lectins, protease inhibitors, goitrogens, allergens, anti-vitamins, and toxins (e.g. kainic acid), which can reduce the digestibility and bioavailability of other ingredients.

3 Opportunities

Norway has an extensive coastline (100,000 km characterized by fjords and islands), which is among the world's longest and most productive but also has a well-established aquaculture sector offering suitable preconditions for developing large-scale cultivation of seaweed biomass, both wild-harvested and cultivated. There are currently also many projects focused on seaweed cultivation, which can optimize production and reduce costs. Cultivation of seaweed has the advantage of better control over the material and harvesting time can be optimized according to certain nutritional parameters.

Seaweed from long lines can be used not only in feed production but also for production of food, nutraceuticals, fertiliser, soil amendment, fine chemicals, cosmeceuticals and pharmaceuticals. Seaweeds contain many commercially interesting compounds (e.g. alginates). If the industry can develop into a fractionation of the seaweed biomass, where the high-end products (for e.g. cosmetics, food) can offset most of the costs of production and processing, than the remainder can be turned into a protein concentrate. Furthermore, seaweeds in proximity to fish farms, can function as extractive components within a cultivation food web. Reducing the environmental impact of intensive fish aquaculture, integrated multitrophic aquaculture systems add value to the investment in finfish aquaculture by increasing the yield of total biomass produced on a single site.

4 Threats

The major threat is the lack of processing facilities for seaweed at this moment. When looking at the terrestrial production chain it is very well developed compared to the marine production chain (besides fish). A dried whole seaweed meal is not the way forward, it has to be processed into e.g. a seaweed protein concentrate.

Pricing of commodities: this is a threat to any new feed ingredient. Based on the amino acid composition, seaweed is comparable to vegetable proteins. A seaweed protein concentrate therefore has to compete with a soybean protein concentrate for price.

Harvesting seaweeds, specifically the brown algae, using designed trawling equipment, remains controversial as the removal of and interference with natural habitats has the potential to affect local biodiversity and ecosystem integrity, and may contribute to coastal erosion.

5 SWOT analysis

The attributes are ordered in our perceived importance. Every column starts with the most important Strength, Weakness, Opportunity or Threat.

STRENGTHS	**WEAKNESSES**
Vegetable type protein High levels of bioactive amino acids, metabolites and peptides *taurine* *cysteine-oxoforms metabolites* *glutathione, γ-aminobutyric acid (GABA)* *mycosporine-like amino acids* Bio-active compounds with antioxidant properties *sugar alcohol metabolites (mannitol, sorbitol)* *sulfated polysaccharides* *Pigments (carotenoids, fucoxanthin)* *oxylipins* Rich in valuable micronutrients, like I, Zn and Mn High abundance of fucosterol and α-tocopherol Binding properties (complex poly-saccharides)	High concentration of complex carbohydrates High concentrations of cadmium, arsenic (inorganic and organic) and mercury High variation in nutrient content (species, geography, season) Anti-nutritional compounds *lectins* *protease inhibitors* *goitrogens* *allergens* *anti-vitamins* High ash content *iodine* *zinc* *manganese* *calcium* Low in lipid content
OPPORTUNITIES	**THREATS**
Possibility to build the aquaculture production on marine primary producers – Blue Economy Seaweed abundantly present along the Norwegian coast Local sourcing of cultivated seaweed Synergies with existing aquaculture (IMTA)	Lack of processing/refinement Low pricing of commodities Environmental impact of harvesting wild seaweed

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