Paper I

Research Article

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Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed

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Abstract

BACKGROUND: The objectives of this study were to examine protein and amino acid composition, lipid and fatty acid composition, along with a range of essential minerals in common Norwegian seaweed species representing the red (*Palmaria palmata* and *Vertebrata lanosa*), green (*Cladophora rupestris*, *Enteromorpha intestinalis* and *Ulva lactuca*) and brown (*Alaria esculenta*, *Laminaria digitata*, *Laminaria hyperborea*, *Fucus vesiculosus* and *Pelvetia canaliculata*) classes and assess their potential as alternatives to cereals in food and feed. As macroalgae accumulate heavy metals, arsenic, cadmium and mercury were also analyzed.

RESULTS: Proteins ranged from 34 to 123 g kg^{-1} dry weight (DW) and the essential amino acid levels may cover both human and salmonid requirements. Lipids were low (6–58 g kg^{-1} DW), but the red algae had high relative content of long-chained omega-3 fatty acids (32–34 % of the fatty acids). Iodine contents were particularly high in the *Laminaria* species. Of the heavy metals only arsenic levels may be of concern.

CONCLUSION: In total, the red alga *P. palmata* was regarded as the best alternative to cereals in food and feed. For several of the other species, single-component extraction for the ingredients market may be better than using the whole product.

Keywords: macroalgae; characterization; protein; lipids; iodine; selenium

INTRODUCTION

The world's population is expected to increase up to 9.1 billion people within the next 40 years. Thus a considerable growth in food and feed production worldwide is demanded.[1] Cereals are the single most important food group worldwide and the three species corn, rice and wheat make up approximately 60% of the world's total food energy intake.[2] It is anticipated that the largest increase in food and feed production will take place within this food group. However, increased cultivation of cereals demands larger arable land areas and increased freshwater supply – both scarcity factors in many countries. An additional drawback considering cereals as the main food energy source is their lack of certain important nutrients. The protein content is generally low and they are also deficient in several essential amino acids, in particular lysine. Long-chain omega-3 fatty acids (LC n-3 PUFAs) and some minerals, iodine in particular, are other nutrients in which cereals are deficient.

As a consequence of the increase in aquaculture production in the last decades, cereals have also become increasingly important in feed production. The lack of important nutrients may give adverse effects, such as reduced growth[3,4] and altered biochemical composition of fish.[5] All of these aspects indicate that the search for new, highly nutritive food and feed sources, preferably applying less strain to the environment, is necessary.

Macroalgae, or seaweeds, are a very diverse group of plants occurring in marine environments worldwide. Botanically they are classified after phylum, class, order, family and genus (species). Colloquially they are usually divided into three main groups corresponding to the phylum; rhodophyta (red algae), chlorophyta (green algae) and phaeophyta (brown algae). Common growth features shared by macroalgae are low nutrient demands, high growth rate and no need for freshwater supply. In Asia, seaweeds are a part of the traditional diet and are frequently used in both food and feed. According to FAO statistics, South Korea, China and Japan have the highest intake of seaweed, with a daily consumption of 46, 22 and 4 g per capita, respectively.[6] The most frequently consumed species are the brown algae *Undaria pinnatifida* (wakame) and *Saccharina japonica* (kombu or konbu), along with the red algae *Porphyra* sp. (nori). The nutritional properties

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of these species are well characterized and they all contain high levels of protein, covering the requirements of all of the essential amino acids.[7,8] Wakame and kombu also are rich sources of important minerals, especially iodine,[7] while nori contains some LC n-3 PUFAs.[8] During the last decades, even more algae species have been considered as potential sources of nutrition and a number of papers focusing on nutritional characteristics, including fatty acid composition,[9] amino acid profiles[10] and dietary fiber,[11] of these have been published. Potential health benefits from direct consumption of seaweeds have also been reviewed.[12]

Utilization of seaweed as food, feed and fertilizers was common in northern Europe from around the year 900 up until approximately 1750, especially in periods of food shortage. This is well documented in, for instance, Icelandic genealogies and Norwegian clerical notes. Following industrialization and improved living conditions for people in general, the utilization of these resources became less frequent. Nowadays, in Norway, the utilization of seaweeds for food purposes is limited to industrial production of thickeners, such as alginate, agar and carrageenan, all extracted from brown algae harvested from wild resources. However, Norway has a long coastline and the potential for cultivation of algae is large. Utilizing this potential could form the basis of a new biomarine industry in Norway. As the climatic conditions are important for growth rate and nutritional composition of algae, selecting species having their natural habitat along the Norwegian coastline would probably be optimal. Nutritional composition would again determine their potential ranges of use, such as their ability to become substitutions or supplements to cereals in food and feed. So far, a complete nutritional characterization of key algae species common in Norwegian waters has not been reported.

Thus the objectives of this study were to examine the nutritional composition, limited to proteins, lipids and minerals, of common Norwegian seaweed species representing the red (*Palmaria palmata* and *Vertebrata lanosa*), green (*Cladophora rupestris*, *Enteromorpha intestinalis* and *Ulva lactuca*) and brown (*Alaria esculenta*, *Laminaria digitata*, *Laminaria hyperborea*, *Fucus vesiculosus* and *Pelvetia canaliculata*) classes and, based on nutritional requirements, assess their potential as alternatives to cereals in food and feed. As macroalgae accumulate heavy metals, arsenic, cadmium and mercury levels were also analyzed, for discovery of potential health risks associated with increased intake.

EXPERIMENTAL

Algae samples

Samples of ten common marine macroalgae species, from all of the three main groups (two red, three green and five brown, of which two were wracks and three kelps), were harvested off the coast of Norway. In order to minimize the natural seasonal variations in biochemical composition, all samples were harvested in May and June (2010 and 2012). This is the optimal time of harvesting as it is at the end of the growth season and before epiphytic fouling becomes a problem.[13] All species, except *P. palmata* and *U. lactuca*, were collected in the latitudinal range of 67–69° N. The two aforementioned species, however, could not be retrieved in this area during the sampling period and were therefore collected further south (62–63° N) (Table 1). Samples of entire algae were flushed with freshwater and subsequently frozen before transport to the laboratory. Meals of corn, rice, soy and wheat were purchased in a local supermarket.

The water content of algae is generally high, but there is a large variability between species. This could complicate comparisons between species and hence it was decided to freeze-dry all materials prior to analyses and do all comparisons on a dry weight basis. All raw material samples were freeze-dried using a Vir-Tis Genesis 35EL freeze dryer (SP Industries, Gardiner, NY, USA). Pending chemical analyses, freeze-dried samples were stored in darkness at room temperature. Within 1 month after freeze-drying, all samples were subjected to proximate analysis (water, lipid and ash), fatty acid composition, free and total amino acid composition/protein and mineral analysis.

All reagents used in this study were of analytical grade and purchased from Sigma Chemical Co (St Louis, MO, USA), unless otherwise stated.

Analytical methods

Water

Water content was determined using a modified version of the AOAC method 950.46B,[14] whereby approximately 2.5 g freeze-dried material was dried at 105 °C until constant weight and water content was determined gravimetrically. Analyses were performed in triplicate.

Lipids

Total lipids were extracted from the water-free sample using petroleum ether according to AOAC method 945.16.[14] Lipid content was determined gravimetrically. Analyses were performed in triplicate.

Ash

Ash content was determined using a modified version of AOAC method 938.08.[14] The water- and lipid-free sample was combusted at 500 °C for 12 h and ash content was determined gravimetrically. Analyses were performed in triplicate.

Fatty acid composition

Lipids were extracted using Folch's method,[15] substituting chloroform with dichloromethane for health and safety reasons. Extracted lipid samples were redissolved to a concentration of approximately 10 g L^{-1} in dichloromethane: methanol (2:1, v/v). Trans-methylation of the samples was performed according to Stoffel *et al.*,[16] with the same modifications as described by Maehre *et al.*[17] Chromatographic analysis and identification of fatty acids was performed as described previously.[17]

Protein and amino acid analysis

Free amino acids were extracted according to Mierke-Klemeyer *et al.*,[18] dissolving approximately 0.2 g freeze-dried material in a mixture of 9 mL distilled H_2O and 1 mL 20 mmol L^{-1} norleucine (internal standard). One milliliter of 35% sulfosalicylic acid was added for removal of proteins and large peptides, followed by centrifugation at 4000 × *g* for 10 min. Prior to analysis, aliquots of 200 µL of the supernatants were diluted to a suitable concentration in lithium citrate buffer at pH 2.2.

For analysis of total amino acids, approximately 40 mg freeze-dried material was dissolved in a mixture of 0.7 mL distilled H_2O and 0.5 mL of 20 mmol L^{-1} norleucine (internal standard). Concentrated hydrochloric acid (12 mol L^{-1}) was added to obtain a final concentration of 6 mol L^{-1}. In order to minimize oxidation, samples were flushed with nitrogen gas for 15 s before hydrolysis at 110 °C for 24 h according to Moore and Stein.[19] Following hydrolysis, 100 µL aliquots of the hydrolysates were evaporated

Table 1. Summary of macroalgae species included in the study

Species	Common name	Class	Harvesting area	Harvesting time
Alaria esculenta	Winged kelp	Brown (kelp)	Sommarøy, Troms county (69° N, 18° E)	2010
Laminaria digitata	Oarweed	Brown (kelp)	Sommarøy, Troms county (69° N, 18° E)	2010
Laminaria hyperborea	Tangle	Brown (kelp)	Sommarøy, Troms county (69° N, 18° E)	2010
Fucus vesiculosus	Bladderwrack	Brown (wrack)	Sommarøy, Troms county (69° N, 18° E)	2010
Pelvetia canaliculata	Channeled wrack	Brown (wrack)	Brensholmen, Troms county (69° N, 18° E)	2010
Cladophora rupestris	Mekong weed	Green	Skjerstadfjorden, Nordland county (67° N, 14° E)	2010
Enteromorpha intestinalis	Gut weed	Green	Skjerstadfjorden, Nordland county (67° N, 14° E)	2010
Ulva lactuca	Sea lettuce	Green	Trondheimsfjorden, Sør-Trøndelag county (63° N, 9° E)	2012
Palmaria palmata	Dulse	Red	Voldsfjorden, Møre og Romsdal county (62° N, 5° E)	2012
Vertebrata lanosa	Wrack siphon weed	Red	Oldervik, Troms county (69° N, 19° E)	2012

under nitrogen gas until complete dryness. Prior to analysis the samples were redissolved to a suitable concentration in lithium citrate buffer at pH 2.2.

All amino acids were analyzed chromatographically and identified as described previously,[17] using a Biochrom B30 amino acid analyzer (Biochrom Co, Cambridge, UK). Protein content was calculated from the sums of individual amino acid residues (molecular weight of each amino acid after deduction of the molecular weight of water) as recommended by FAO.[20]

Minerals

All multi-element determination was carried out on inductively coupled plasma mass spectrometry (ICPMS, Agilent 7500c) after microwave-assisted wet digestion (Ethos Pro microwave system, Milestone, Holger Teknologi, Oslo, Norway) in duplicates of 0.20–0.25 g freeze-dried material.[21] The method has been adopted as an NMKL (Nordic Committee on Food Analysis) method[22] as well as a CEN (European Normalization Organization) method EN 15763:2009.[23] Iodine concentrations were determined according to Julshamn *et al.*[24] Trueness and precision in the analyses were ensured by analyzing certified reference materials from the National Institute of Standards and Technology (NIST) and National Research Council (Ottawa, Canada). The methods are accredited according to NS-EN-ISO 17025, and the laboratories at NIFES are frequently participating in proficiency tests.

Statistics

Statistical analysis was performed using SPSS 19 (SPSS Inc., Chicago, IL, USA). Tests of normality (Shapiro–Wilk's test) and homogeneity of variance (Levene's test) returned normal distribution with unequal variance for all species and chemical variables. Hence one-way analysis of variance (ANOVA) was performed, followed by the Dunnet's T3 post hoc test for evaluation of statistics. Means were considered significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Selection of species

There were two inclusion criteria for the algae in this study: one was that species should represent all of the three main classes of macroalgae (red, green and brown); the other was that they should be easily accessible. The biochemical variation between species is considered to be due partly to phylogenetic differences and partly to seasonal and geographical conditions, such as water temperature and light amount and intensity.[25–28] In order to minimize the latter, it was decided to harvest all samples at the same time of year and from the narrowest geographical area possible. As mentioned in the Experimental section, *P. palmata* and *U. lactuca* could not be retrieved in the original sampling area and had to be collected further south along the Norwegian coastline. Macroalgae grow all over the world and species diversity is greater in temperate and tropical regions than in the polar regions.[29] Some of the species included in this study are widely distributed across climatic zones; others exist in polar and cold temperate regions exclusively. However, all of the included species have relatives of the same phylum, class, order and family in other climatic zones.[30] In broad outline, biochemical characteristics of the included species may therefore be valid also for other species than those included.

Protein and amino acid composition

Protein is a major factor when assessing potential health benefits of a food product as it is the essential nutrient for growth. Not only the amount of protein but also the protein quality is important. Protein quality of a food product is often evaluated by its contents and composition of essential amino acids (EAA) or, in some cases, by its chemical score. Of the 20 amino acids, nine are considered essential for humans: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. For most fish, arginine is also essential, owing to lack of a functional urea cycle. The chemical score equals the lowest value returned when calculating the ratio between each EAA in the food protein and the corresponding EAA in a reference protein proposed by FAO/WHO.[31] Proteins of animal source normally have a chemical score of 1.0, while cereal proteins normally range from 0.4 to 0.6. Legumes, beans and nuts normally range in between these. The chemical scores of the algae in this study ranged from 0.75 to 1.0 (Table 2), which indicate that the protein quality of algae is superior to most terrestrial plants.

Variability between species was evident both in the amino acid composition (Table 2) and protein contents (Table 3) of the samples. The lowest protein content was found in the green alga *C. rupestris*, at 34 g kg^{-1} DW. Four of the five brown algae analyzed contained 50–60 g kg^{-1} protein on a dry weight basis, which is within the same range as the cereals corn, rice and wheat (Table 4). The two red algae, *P. palmata* and *V. lanosa*, along with the green algae *E. intestinalis* and *U. lactuca* and the brown alga *A. esculenta*, were richer in protein, ranging from 90 to 120 g kg^{-1} DW. The

Table 2. Total amino acids in ten macroalgae species ($n = 5$)

	A. esculenta	*L. digitata*	*L. hyperborea*	*F. vesiculosus*	*P. canaliculata*	*C. rupestris*	*E. intestinalis*	*U. lactuca*	*P. palmata*	*V. lanosa*
Essential amino acids (EAA)										
Threonine	5.1 ± 0.4c	3.8 ± 0.3b	3.5 ± 0.3b	3.4 ± 0.4b	3.5 ± 0.1b	2.2 ± 0.3a	8.0 ± 0.8def	6.2 ± 0.3d	7.1 ± 0.2e	7.8 ± 0.3f
Valine	5.5 ± 0.3c	3.6 ± 0.3b	3.5 ± 0.2b	3.7 ± 0.6ab	3.9 ± 0.2b	2.4 ± 0.3a	8.4 ± 1.3cde	7.1 ± 0.1d	9.6 ± 0.4e	7.6 ± 0.3d
Methionine	2.4 ± 0.3cde	1.8 ± 0.1bc	1.6 ± 0.1b	1.5 ± 0.2b	1.4 ± 0.1b	0.9 ± 0.1a	2.3 ± 0.4bcde	2.2 ± 0.1d	3.1 ± 0.2e	1.8 ± 0.2bcd
Isoleucine	3.8 ± 0.5bc	2.7 ± 0.1b	2.2 ± 0.2a	2.7 ± 0.3ab	3.0 ± 0.0b	1.6 ± 0.4a	5.9 ± 1.0cde	4.4 ± 0.2c	6.5 ± 0.1d	7.2 ± 0.2e
Leucine	7.5 ± 0.9c	5.2 ± 0.1b	4.5 ± 0.5b	5.0 ± 0.7b	5.2 ± 0.3b	2.7 ± 0.5a	9.5 ± 1.2cde	8.5 ± 0.3c	11.3 ± 0.3e	9.9 ± 0.2d
Phenylalanine	4.8 ± 0.5c	3.4 ± 0.3b	3.1 ± 0.1b	3.3 ± 0.4b	3.4 ± 0.1b	2.1 ± 0.3a	7.4 ± 1.1 cdef	6.0 ± 0.2d	7.1 ± 0.2e	8.2 ± 0.2f
Lysine	5.3 ± 0.5c	3.7 ± 0.2b	3.4 ± 0.3b	4.3 ± 0.6bcd	3.7 ± 0.2b	2.1 ± 0.4a	6.4 ± 0.9c	5.1 ± 0.2c	8.9 ± 0.4d	12.6 ± 0.3e
Histidine	1.6 ± 0.2bcde	1.2 ± 0.1b	1.2 ± 0.1bc	1.1 ± 0.1b	1.0 ± 0.1ab	0.7 ± 0.2a	2.1 ± 0.4bcde	1.6 ± 0.1 cd	1.8 ± 0.1de	2.0 ± 0.0e
Tryptophan	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Non-essential amino acids (NEAA)										
Aspartic acid[a]	8.4 ± 0.7c	6.2 ± 0.2b	5.9 ± 0.4b	8.3 ± 1.1bc	5.9 ± 0.3b	3.5 ± 0.4a	14.6 ± 1.8de	9.0 ± 0.3c	13.1 ± 0.3e	12.3 ± 0.2d
Serine	5.2 ± 0.4c	3.6 ± 0.1b	3.5 ± 0.3b	3.5 ± 0.5ab	3.6 ± 0.1b	2.2 ± 0.3a	7.8 ± 1.1cde	5.9 ± 0.3c	8.4 ± 0.2e	7.7 ± 0.3d
Glutamic acid[a]	20.1 ± 1.1e	8.5 ± 0.6b	8.6 ± 0.6b	17.9 ± 2.2 cde	15.0 ± 1.2 cd	5.7 ± 0.8a	18.2 ± 2.0de	12.2 ± 0.5c	21.3 ± 0.5e	16.3 ± 0.4d
Proline	5.1 ± 0.8abc	3.9 ± 0.2b	3.5 ± 0.5ab	3.1 ± 0.5ab	3.2 ± 0.2a	2.9 ± 0.3a	6.6 ± 1.0c	5.8 ± 0.5c	9.7 ± 0.3d	10.8 ± 0.5d
Glycine	5.7 ± 0.6b	4.1 ± 0.2a	3.8 ± 0.2a	3.8 ± 0.5a	4.1 ± 0.2a	3.3 ± 0.4a	8.5 ± 0.9 cd	7.3 ± 0.2c	9.6 ± 0.4d	8.9 ± 0.2d
Alanine	18.9 ± 1.1 g	5.2 ± 3.0abcef	6.2 ± 0.5b	5.0 ± 0.7b	5.5 ± 0.3b	3.1 ± 0.5a	14.7 ± 1.8dg	10.1 ± 0.3de	12.2 ± 0.5df	7.6 ± 0.5c
Cysteine	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	0.5 ± 0.2bd	1.4 ± 0.4cef	1.0 ± 0.2de	0.5 ± 0.1bc	2.1 ± 0.1f
Tyrosine	2.9 ± 0.4c	1.8 ± 0.2b	1.6 ± 0.1ab	1.5 ± 0.2ab	1.4 ± 0.1a	1.5 ± 0.3ab	3.8 ± 0.5 cd	3.4 ± 0.4c	4.7 ± 0.2de	5.4 ± 0.4e
Arginine	4.8 ± 0.6 cd	3.4 ± 0.2bc	3.0 ± 0.1ab	3.2 ± 0.4ab	3.2 ± 0.2ab	2.5 ± 0.3a	7.4 ± 1.2def	6.0 ± 0.3d	8.6 ± 0.3f	7.0 ± 0.4e
Sum TAA	107.2 ± 6.6c	62.2 ± 4.2b	58.9 ± 3.3b	71.2 ± 8.4b	66.8 ± 3.2b	40.1 ± 5.0a	132.9 ± 17.1 cd	101.5 ± 3.9c	143.6 ± 3.7d	135.1 ± 2.7d
Relative amount EAA (%)	33.5 ± 1.4a	40.9 ± 2.2cdefg	38.9 ± 0.3 cd	35.0 ± 1.1ab	37.7 ± 0.6bc	36.7 ± 0.9abcd	37.5 ± 0.4be	40.3 ± 0.4 g	38.6 ± 0.2c	42.3 ± 0.1f
Chemical score	0.84	1.00	0.89	0.80	0.82	0.96	0.91	0.92	0.75	0.87

Values are expressed as mean ± SD and in g kg^{-1} DW. Different letters in each row indicate significant differences ($P < 0.05$) between species.
[a] Values for aspartic acid and glutamic acid represent the sums of aspartic acid + asparagine and glutamic acid + glutamine respectively, as asparagine and glutamine are present in their acid forms after acidic hydrolysis.
n.d., not detected; n.a., not analyzed.

protein contents found in the algae in this study seem to be generally lower than reported in other studies.[26–28] This may partly be explained by geographical and seasonal variation,[28] but also by methodological differences. The most common method of protein determination in foods is the Kjeldahl method for determination of total nitrogen, where total nitrogen is converted to protein using a nitrogen-to-protein conversion factor usually set to 6.25. This factor is, however, not universal, as the amino acid composition varies substantially between different food proteins. In addition, not all nitrogen found in foods is protein bound, and nitrogen-containing molecules, such as urea, ammonia and nitrates, may be present in variable amounts in various foods. For most materials a conversion factor of 6.25 is too high and hence the protein content will be overestimated using this factor.[32] Alternative conversion factors have therefore been presented for many food items,[32] and for seaweed an average conversion factor of 4.92 has been suggested.[33] In this study, however, the protein contents are reported as the sums of individual amino acid residues following acidic hydrolysis, according to the recommendations from FAO.[20] A drawback using the present method is that tryptophan is completely destroyed during acidic hydrolysis and hence could not be analyzed.

Our results show that proteins from all three main classes of macroalgae are able to cover the human requirements for EAA.[34] The three cereals most frequently used for food, corn, rice and wheat are low in both protein and in some EAA. The limiting amino acid is usually lysine. One gram of algae meal of the three species with the highest protein levels, *E. intestinalis*, *P. palmata* and *V. lanosa*, contain equal or higher amounts of all of the EAA compared to meals of the three cereals and, in particular, the lysine contents were three to nine times higher. Combining these facts, algae with high protein contents are better protein sources than corn, rice and wheat. The relative amount of free amino acids (FAA) ranged from approximately 2% to 14.5% – generally lowest in the green algae and highest in the red (Table 5). For most humans, the digestion of proteins is adequate and a specific need for FAAs has not been established. There is, however, one exception: taurine. This amino acid is exclusively free, i.e. not bound to proteins and considered as conditionally essential, as the biosynthesis may be inadequate in some life stages, in particular during infancy.[35] Taurine is involved in many physiological processes[36] and there is evidence that it mitigates several of the risk factors of CVD, such as blood lipid composition,[37] endogenous thrombolytic activity[38] and blood pressure.[39] Plant materials are normally devoid of taurine. Although at quite low levels (0.4 ± 0.1 g kg^{-1} DW), taurine was found in *C. rupestris*, *P. palmata* and *V. lanosa*, which further strengthens the algae's potential as an addition or replacement of other plant materials in the diet.

The EAA requirements for fish (salmonids) are somewhat higher than for humans.[40] Except for the sulfur-containing AA (methionine + cysteine), several of the algae in this study contained sufficient amounts of all EAA. In fish feed the most common plant ingredients are corn, wheat and soy. As described earlier, the combination of protein content and quality make algae, especially red algae, a superior ingredient to corn and wheat. The protein content of soy meal is, however, much higher (approximately 240 g kg^{-1}) than in algae and substitution of this ingredient will therefore not be feasible. In soy, methionine is the limiting amino acid and the content of this amino acid in the algae protein was approximately three times higher than in soy. Using algae protein as a supplement to soy could therefore be a possibility. Due to an immature digestive tract and low proteolytic capacity in the early life stages of marine fish larvae, a high proportion of FAA in general

Table 3. Proximate composition of ten macroalgae ($n = 3$ for water, lipid and ash; $n = 5$ for protein)

	A. esculenta	*L. digitata*	*L. hyperborea*	*F. vesiculosus*	*P. canaliculata*	*C. rupestris*	*E. intestinalis*	*U. lactuca*	*P. palmata*	*V. lanosa*
Water content fresh algae (g kg^{-1} WW)[a]	826.4 ± 4.8b	839.8 ± 0.9b	833.1 ± 2.0b	656.6 ± 20.8a	615.6 ± 22.7a	852.4 ± 2.2c	923.8 ± 3.5d	854.3 ± 8.2bc	819.5 ± 7.7bc	823.8 ± 6.3bc
Water content freeze-dried algae (g kg^{-1} DW)[b]	53.9 ± 0.5de	68.1 ± 0.6 g	52.7 ± 0.3d	61.0 ± 0.1f	49.0 ± 1.2 cd	26.3 ± 1.2a	60.9 ± 2.7cdefg	47.5 ± 0.6c	37.8 ± 1.1b	57.0 ± 0.8ef
Lipids* (g kg^{-1} DW)[c]	15.0 ± 4.3abc	8.5 ± 2.2a	11.4 ± 2.6ab	26.5 ± 0.4b	37.4 ± 0.8c	8.8 ± 1.4a	22.0 ± 6.5abc	13.3 ± 1.7a	13.9 ± 2.4ab	18.0 ± 5.4abc
Lipids** (g kg^{-1} DW)[c]	13.0 ± 0.5bc	11.3 ± 0.5ab	14.2 ± 2.6acd	35.1 ± 0.6e	58.1 ± 1.7f	6.3 ± 1.3a	10.3 ± 0.9ab	26.2 ± 1.4d	13.3 ± 0.5bc	13.0 ± 0.4ab
Proteins*** (g kg^{-1} DW)[c]	91.1 ± 5.7c	53.1 ± 3.4b	50.2 ± 2.8b	61.1 ± 7.2b	57.2 ± 2.8b	34.2 ± 4.3a	113.3 ± 14.6 cd	86.5 ± 3.3c	122.6 ± 3.1d	115.6 ± 2.3d
Ash (g kg^{-1} DW)[c]	245.6 ± 5.6b	244.3 ± 0.3bc	287.5 ± 2.2d	209.2 ± 1.0a	212.4 ± 2.2a	778.0 ± 8.7 g	552.9 ± 21.8f	293.1 ± 8.4 cd	422.3 ± 5.6e	287.8 ± 1.6d

Values are expressed as mean ± SD. Different letters in each row indicate significant differences ($P < 0.05$) between the different macroalgae species.
[a] Weight expressed as g kg^{-1} of the original wet weight (WW) samples.
[b] Weight expressed as g kg^{-1} of the freeze-dried material (DW).
[c] Adjusted DW expressed as g kg^{-1}, with respect to the residual water in the sample after freeze-drying.
* Lipids – ether extraction.
** Lipids – dichloromethane/methanol extraction.
*** Protein calculated from total amino acid residues.

Table 4. Protein content and total amino acid composition in corn, rice, soy and wheat meals ($n = 3$)

	Corn meal	Rice meal	Soy meal	Wheat meal
Protein	41.1 ± 0.6	36.9 ± 1.3	237.7 ± 4.2	76.3 ± 1.7
Essential amino acids (EAA)				
Threonine	1.7 ± 0.1	1.7 ± 0.1	11.5 ± 0.4	2.5 ± 0.1
Valine	2.5 ± 0.1	3.0 ± 0.1	14.5 ± 0.1	4.1 ± 0.2
Methionine	0.9 ± 0.0	1.0 ± 0.1	2.6 ± 0.1	1.2 ± 0.1
Isoleucine	1.9 ± 0.0	2.0 ± 0.1	14.0 ± 0.1	3.3 ± 0.1
Leucine	7.6 ± 0.1	4.3 ± 0.2	24.6 ± 0.5	6.9 ± 0.2
Phenylalanine	2.8 ± 0.1	2.7 ± 0.1	16.5 ± 0.6	4.9 ± 0.2
Lysine	1.2 ± 0.0	1.9 ± 0.1	20.0 ± 1.0	2.1 ± 0.0
Histidine	1.3 ± 0.0	1.0 ± 0.0	6.7 ± 0.2	1.8 ± 0.0
Tryptophan	n.a.	n.a.	n.a.	n.a.
Non-essential amino acids (NEAA)				
Aspartic acid[a]	2.2 ± 0.0	3.1 ± 0.1	22.9 ± 0.4	2.5 ± 0.1
Serine	2.4 ± 0.0	2.4 ± 0.1	15.2 ± 0.3	4.5 ± 0.2
Glutamic acid[a]	9.9 ± 0.2	8.2 ± 0.4	51.8 ± 1.3	31.7 ± 0.7
Proline	5.4 ± 0.2	2.4 ± 0.0	15.3 ± 0.5	10.8 ± 0.4
Glycine	1.6 ± 0.0	2.1 ± 0.1	11.2 ± 0.2	3.1 ± 0.1
Alanine	4.0 ± 0.1	2.7 ± 0.1	12.2 ± 0.2	2.8 ± 0.1
Cysteine	0.4 ± 0.0	0.3 ± 0.1	3.1 ± 0.2	1.1 ± 0.0
Tyrosine	0.5 ± 0.0	0.5 ± 0.0	10.0 ± 0.4	1.5 ± 0.1
Arginine	1.9 ± 0.0	3.8 ± 0.1	23.7 ± 0.7	3.7 ± 0.2
Sum TAA	48.0 ± 0.7	43.0 ± 1.5	275.8 ± 4.9	88.6 ± 2.0
Relative amount EAA (%)	41.3 ± 0.3	40.9 ± 0.3	40.0 ± 0.2	30.3 ± 0.2
Chemical score	0.47	0.86	0.79	0.47

Values are expressed as mean ± SD and in g kg^{-1} DW.
[a] Values for aspartic acid and glutamic acid represent the sums of aspartic acid + asparagine and glutamic acid + glutamine respectively, as asparagine and glutamine are present in their acid forms after acidic hydrolysis.
n.a., not analyzed.

is considered to increase the growth and amino acid utilization potential.[41,42] The green algae in this study had a relative FAA content below 5%, while the other species ranged from 8% to 14.5% (Table 5), which is comparable to rotifers used in start feeding of marine fish larvae.[17]

Lipid and fatty acid composition

The lipid contents were relatively low in all of the species analyzed, ranging from 6 to 58 g kg^{-1} DW using Folch's extraction, the lowest being *C. rupestris* and the highest being *P. canaliculata* (Table 3). Both lipid levels and variability between species are in accordance with other studies on marine algae.[43,44]

The main fatty acids of the algae are shown in Table 6 and substantial species variability was also evident in the fatty acid composition. The fatty acid profiles differing most from the others were those of the green algae. They contained very low concentrations of fatty acids longer than 18 C atoms, resembling fatty acid profiles of terrestrial plants more than those of marine organisms. Among the brown and red algae, the two brown algae *F. vesiculosus* and *P. canaliculata* had a lower relative content of saturated fatty acids, higher monounsaturated fatty acids and n-6 content, along with lower n-3 content than the other species. Also the ratio between n-6 and n-3 fatty acids was higher in these species.

In addition to the generally low lipid content, one of the concerns regarding a diet mainly consisting of cereals is their lack of LC n-3 PUFAs, in particular eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3). These fatty acids have been shown to attenuate risk factors associated with development and progression of cardiovascular diseases (CVDs), in particular by reduction of inflammation. None of the algae contained DHA, while the content of EPA varied considerably between species. While the relative content of this fatty acid in the red algae *P. palmata* and *V. lanosa* was 32–34% of the fatty acids, which is in accordance with other studies,[44] the green algae were almost devoid of the same fatty acid. Despite the high relative EPA content in red algae, they still cannot be considered a very good dietary source of LC n-3 PUFAs owing to their low total lipid content. The European Food Safety Authority (EFSA) state that a daily intake of 250 mg EPA + DHA may prevent development of CVDs.[45] Achieving this amount of EPA by consumption of red algae alone is highly unlikely as the daily intake would have to be 130–160 g, which is three to four times the average daily consumption in South Korea.

Oils from terrestrial plants are often rich in n-6 fatty acids. Imbalance between n-6 and n-3 fatty acids in tissues and cell membranes has been suggested to be among the initiators of inflammation processes in the body. As inflammation processes are involved in several lifestyle-related diseases, a balanced intake of the different types of fatty acids could therefore reduce the occurrence of some of these diseases. A ratio between n-6 and n-3 of 2–5:1 has been suggested as optimal, but in the Western world today the actual ratio is 15–17:1.[46] Most of the algae in this study were quite low in n-6 fatty acids, the relative content being 1–14% of the fatty acids in red and green algae, and 13–23% of the fatty acids in the brown algae. The ratio between n-6 and n-3 was within or lower than the recommended range for all of the algae.

The fatty acid composition of the diet is also important for fish, in particular for cold-water marine species. For instance, LC n-3 PUFAs are crucial for a number of physiological processes, such as temperature regulation and membrane viscosity.[47,48] The requirements of EPA + DHA for marine species listed by the US National Research Council (NRC)[49] range from 5 to 20 g kg^{-1} diet. The natural fatty acid composition of most wild marine species is characterized by high content of LC n-3 PUFAs and very low content of n-6 fatty acids, and hence a low ratio between n-6 and n-3. Substitution of fish meal and oil with cereals in aquaculture feeds will alter the biochemical composition of the produced fish, as recently shown in a comparison study of fatty acid composition of wild and farmed Atlantic salmon.[5] Neither for use in fish feed, the algae content of EPA was sufficient in order to cover the daily requirements set by the NRC. However, compared to terrestrial plants, the fatty acid composition of algae is more similar to that of wild marine fish. Hence substituting cereals with algae in fish feed could contribute to an improved fatty acid composition of the farmed fish.

Ash and mineral content

Also the ash contents, giving a rough estimate of the total mineral contents of the algae, varied between species and were especially high in *C. rupestris* (approximately 780 g kg^{-1} DW), *E. intestinalis* (approximately 550 g kg^{-1} DW) and *P. palmata* (approximately 420 g kg^{-1} DW) (Table 3). A more detailed overview of the mineral composition of the algae is presented in Table 7.

Minerals are metallic elements present in various forms and amounts in almost all tissues. Their functions in the human body

Table 5. Free amino acids in ten macroalgae species ($n = 5$)

	A. esculenta	*L. digitata*	*L. hyperborea*	*F. vesiculosus*	*P. canaliculata*	*C. rupestris*	*E. intestinalis*	*U. lactuca*	*P. palmata*	*V. lanosa*
Essential amino acids (EAA)										
Threonine	0.3 ± 0.1bc	0.3 ± 0.1c	0.3 ± 0.0c	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	0.2 ± 0.0b	0.2 ± 0.0b
Valine	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	0.1 ± 0.0b	0.2 ± 0.0c
Methionine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isoleucine	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	0.1 ± 0.0b	0.1 ± 0.0b
Leucine	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	0.1 ± 0.0b	0.1 ± 0.0b
Phenylalanine	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	0.1 ± 0.0b	1.5 ± 0.1c
Lysine	0.2 ± 0.0bcd	n.d.a	n.d.a	0.3 ± 0.1d	n.d.a	n.d.a	n.d.a	n.d.a	0.1 ± 0.0b	0.2 ± 0.0c
Histidine	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	0.1 ± 0.0b
Tryptophan	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Non-essential amino acids (NEAA)										
Taurine	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	0.4 ± 0.1b	n.d.a	n.d.a	0.4 ± 0.1b	0.4 ± 0.1b
Aspartic acid	1.3 ± 0.1f	1.0 ± 0.1e	1.2 ± 0.1ef	0.7 ± 0.1 cd	0.5 ± 0.1c	n.d.a	n.d.a	0.2 ± 0.0b	3.0 ± 0.0 g	0.7 ± 0.0d
Serine	0.4 ± 0.1c	0.2 ± 0.0c	0.2 ± 0.0c	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	0.3 ± 0.0c	0.1 ± 0.0b
Asparagine	n.d.a	n.d.a	n.d.a	1.7 ± 0.3b	n.d.a	n.d.a	3.1 ± 0.3c	n.d.a	n.d.a	n.d.a
Glutamic acid	2.9 ± 0.0e	0.7 ± 0.1ab	1.0 ± 0.1bc	1.6 ± 0.2 cd	1.4 ± 0.2bcd	0.4 ± 0.0a	1.4 ± 0.1d	0.9 ± 0.0b	6.1 ± 0.1f	1.1 ± 0.1c
Glutamine	1.0 ± 0.1f	0.4 ± 0.1bc	0.5 ± 0.1be	1.7 ± 0.3 g	0.7 ± 0.1 cd	n.d.a	0.7 ± 0.1de	n.d.a	0.7 ± 0.1d	0.4 ± 0.0b
Proline	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	4.0 ± 0.1b	5.0 ± 0.3c
Glycine	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	0.1 ± 0.0b	n.d.a	0.3 ± 0.0c	0.1 ± 0.0b	0.2 ± 0.0b
Alanine	9.4 ± 0.3e	2.2 ± 0.1d	2.2 ± 0.1d	0.8 ± 0.2abc	0.9 ± 0.2bc	0.3 ± 0.1a	1.0 ± 0.1de	0.5 ± 0.0b	1.0 ± 0.0c	0.4 ± 0.0a
Cysteine	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a
Tyrosine	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	0.2 ± 0.1b
Arginine	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	0.1 ± 0.0b	0.1 ± 0.0c
Sum FAA	15.5 ± 0.3 g	4.9 ± 0.2d	5.3 ± 0.3de	6.8 ± 1.1de	3.5 ± 0.5c	1.3 ± 0.1a	6.2 ± 0.5e	1.9 ± 0.1b	16.2 ± 0.3g	10.8 ± 0.4f

Values are expressed as mean ± SD and in g kg^{-1} DW. Different letters indicate significant differences ($P < 0.05$) between species.
n.d., not detected; n.a., not analyzed.

are widespread, from bone mineralization via blood pressure regulation to protection from oxidative stress. The requirements for humans vary from a few micrograms per day up to above 1 g per day. No single food source is optimal for all minerals, but a mixed diet should provide sufficient amounts of most minerals. A few trace minerals, in particular selenium and iodine, are nevertheless associated with deficiency disorders.

Iodine is essential for the synthesis of thyroid hormones, which are important metabolism regulators both in humans and in fish. An inadequate intake of this mineral may lead to development of iodine deficiency disorders, such as goiter and cretinism. Insufficient daily intake of iodine, with subsequent deficiency, is common worldwide. An estimate from 2003 states that 35.2% of the world's general population has insufficient intake of iodine and that the prevalence of goiter is 15.8%.[50] Iodization of salt has so far been the main instrument in countering this phenomenon and, although the global situation has improved over the last decade, iodine deficiency still remains a global health challenge.[51] Fish and seaweed, which are very good food sources of iodine, obtain most of their iodine from the water.[49] Seaweed is known to be especially rich in this mineral. This was confirmed in this study, where iodine contents ranged from 21 to 3500 mg kg^{-1} DW, which is 10–1400 times the mean value of marine fish.[52] However, some toxic reactions of excess iodine intake have been reported and a level of upper tolerable intake of 1100 µg d^{-1} for adults has therefore been set.[53] Seaweed has been identified as one of the risk factors for exceeding this level and the results from this study showed that iodine levels in both of the *Laminaria* species, along with *V. lanosa*, were very high and that a daily intake of only 0.3–1.0 g would exceed the tolerable limit for humans. Little is known on possible iodine toxicity in fish, but a recent publication states that high-dose enrichment of this mineral in rotifers alters the morphology of the thyroid follicles in cod larvae.[54]

Selenium is regarded as one of the most important endogenous antioxidants, as it is a constituent of glutathione peroxidases (GPx), which is a class of antioxidative enzymes. It is also central in the thyroid hormone regulation, and a diet deficient in selenium may therefore lead to, and even intensify, some of the same conditions as seen in iodine deficiencies. According to the new Nordic nutrition recommendations, the recommended daily intake of selenium is 50–60 µg d^{-1}.[55] The selenium levels in the algae in this study ranged from 0.02 to 0.53 mg kg^{-1} DW, where the two red algae *P. palmata* and *V. lanosa* ranged highest. In order to cover the daily recommendation of selenium, the intake would have to be 357 and 94 g of these two species, respectively.

Heavy metals

Apart from the many positive traits associated with intake of algae, there are also concerns to take into account. Algae are known for their ability to take up, store and accumulate heavy metals which may be detrimental to human health. In this study the amounts of arsenic, cadmium and mercury were analyzed (Table 7). Several adverse health effects have been associated with intake of all of these heavy metals. Examples may be peripheral vascular disease and various cancers for arsenic, renal tubular dysfunction for cadmium and impaired mental development for mercury.[56] Limits for assumed safe intakes of contaminants, so-called provisional tolerable weekly intake (PTWI) values, have been set by the FAO/WHO Joint Expert Committee of on Food Additives (JECFA)

Table 6. Fatty acid composition of ten macroalgae species ($n = 3$)

	Alaria esculenta	*Laminaria digitata*	*Laminaria hyperborea*	*Fucus vesiculosus*	*Pelvetia canaliculata*	*Cladophora rupestris*	*Enteromorpha intestinalis*	*Ulva lactuca*	*Palmaria palmata*	*Vertebrata lanosa*
C14:0	8.9 ± 0.2b	9.1 ± 0.1b	9.0 ± 0.8bcd	12.4 ± 0.0d	9.5 ± 0.2bc	9.0 ± 3.2abcd	1.8 ± 0.5a	0.9 ± 0.1a	10.2 ± 0.2c	2.2 ± 0.2a
C16:0	26.9 ± 0.4d	21.7 ± 0.3c	23.3 ± 0.4c	11.4 ± 0.2b	9.8 ± 0.1a	31.8 ± 2.8cde	23.2 ± 1.2 cd	26.0 ± 0.4d	31.9 ± 0.5e	25.6 ± 0.1d
C18:0	1.7 ± 0.0c	0.8 ± 0.1ab	1.3 ± 0.5abcd	0.6 ± 0.0b	1.9 ± 0.0d	n.d.a	n.d.a	n.d.a	1.6 ± 0.1 cd	0.9 ± 0.1b
Sum SAT	37.4 ± 0.6e	31.5 ± 0.3d	33.7 ± 0.7d	24.3 ± 0.2b	21.2 ± 0.3a	40.8 ± 0.5f	25.0 ± 0.7bc	26.9 ± 0.4c	43.7 ± 0.6 g	28.6 ± 0.2c
C16:1, n7	1.5 ± 0.0 cd	0.9 ± 0.0b	n.d.a	1.1 ± 0.1bc	1.4 ± 0.0 cd	8.3 ± 1.1ef	1.8 ± 0.1de	0.9 ± 0.0b	1.4 ± 0.1bcd	8.0 ± 0.0f
C18:1, n9	23.9 ± 0.5bd	17.8 ± 0.5ce	26.5 ± 4.2acdfg	46.0 ± 0.4 g	40.8 ± 0.3f	10.5 ± 2.1abc	5.4 ± 3.3be	0.8 ± 0.0ab	5.1 ± 1.1ab	5.6 ± 1.0ab
C18:1, n7	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	8.6 ± 2.5abcd	15.2 ± 0.9d	14.6 ± 0.0d	2.2 ± 0.0b	9.0 ± 0.2c
Sum MUFA	25.4 ± 0.5d	18.7 ± 0.5bc	26.5 ± 4.2abcdef	47.1 ± 0.3f	42.2 ± 0.3e	27.3 ± 1.0d	22.4 ± 2.3bd	16.3 ± 0.0b	8.7 ± 1.1a	22.6 ± 0.8 cd
C16:3, n3	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	1.2 ± 0.1b	2.6 ± 0.0c	n.d.a	n.d.a
C16:4, n3	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	2.1 ± 0.8ab	7.6 ± 0.4c	7.1 ± 0.0bc	n.d.a	n.d.a
C18:2, n6	8.2 ± 0.2e	9.5 ± 0.0d	5.0 ± 0.2c	8.0 ± 0.0e	13.4 ± 0.1 g	7.4 ± 0.8bcdef	5.8 ± 0.6bcde	10.5 ± 0.0f	0.6 ± 0.0a	4.1 ± 0.1b
C18:3, n3	5.1 ± 0.0e	7.5 ± 0.1f	3.8 ± 0.4cde	2.7 ± 0.0c	3.4 ± 0.0d	5.2 ± 1.7abcdefg	14.4 ± 0.8 h	15.2 ± 0.1gh	n.d.a	0.8 ± 0.1b
C18:4, n3	8.1 ± 0.2e	10.2 ± 0.1f	8.1 ± 0.8def	2.2 ± 0.1c	1.9 ± 0.1c	4.3 ± 1.4abcdef	6.1 ± 0.2d	6.3 ± 0.1d	n.d.a	1.0 ± 0.1b
C20:2, n6	n.d.a	n.d.a	n.d.a	0.9 ± 0.0b	1.0 ± 0.0c	n.d.a	n.d.a	n.d.a	n.d.a	0.7 ± 0.0b
C20:3, n6	n.d.a	n.d.a	n.d.a	1.0 ± 0.0b	2.1 ± 0.0d	n.d.a	n.d.a	n.d.a	n.d.a	1.5 ± 0.0c
C20:4, n6	4.6 ± 0.0c	7.0 ± 0.1e	7.6 ± 0.8cde	7.4 ± 0.1e	8.2 ± 0.3e	n.d.a	n.d.a	n.d.a	0.7 ± 0.0b	5.6 ± 0.2d
C20:5, n3	7.1 ± 0.1d	11.4 ± 0.1e	9.5 ± 1.0cde	3.7 ± 0.0bc	4.0 ± 0.1bc	2.5 ± 0.6ab	0.8 ± 0.1a	0.9 ± 0.2a	34.3 ± 0.1f	32.1 ± 1.0f
C22:5, n3	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	n.d.a	1.2 ± 0.0b	1.2 ± 0.0b	n.d.a	n.d.a
Sum PUFA	33.2 ± 0.2b	45.6 ± 0.2d	34.2 ± 3.2abcd	25.8 ± 0.1a	34.0 ± 0.3b	21.5 ± 3.1b	37.1 ± 0.8ab	42.6 ± 0.1c	35.4 ± 0.5b	45.8 ± 0.9 cd
n3	20.4 ± 0.3b	29.1 ± 0.2cde	21.5 ± 2.2abcdfh	8.6 ± 0.1a	9.3 ± 0.2a	14.1 ± 2.3ab	31.3 ± 1.4defgh	32.1 ± 0.1 fg	34.3 ± 0.1hi	33.8 ± 0.9egi
n6	12.8 ± 0.1 g	16.5 ± 0.1 h	12.7 ± 1.0efghi	17.2 ± 0.1i	24.6 ± 0.3j	7.4 ± 0.8bcd	5.8 ± 0.6b	10.5 ± 0.0cf	1.1 ± 0.4a	11.9 ± 0.0de
n6/n3	0.6 ± 0.0d	0.6 ± 0.0d	0.6 ± 0.0d	2.0 ± 0.0e	2.6 ± 0.1f	0.5 ± 0.0 cd	0.2 ± 0.0ab	0.3 ± 0.0b	0.0 ± 0.0a	0.4 ± 0.0bc
n.i.	4.0 ± 0.9ab	4.2 ± 0.5ab	5.6 ± 0.3b	2.8 ± 0.1a	2.6 ± 0.0a	10.3 ± 1.9abc	15.5 ± 1.0c	14.2 ± 0.4c	12.2 ± 0.5c	3.0 ± 0.2a

Values are given as mean ± SD and in percent of total fatty acid content. Different letters indicate significant differences ($P < 0.05$) between species.
n.d., not detected; n.i., not identified.

Table 7. Macrominerals, microminerals and heavy metals in ten macroalgae species ($n = 2$)

	A. esculenta	*L. digitata*	*L. hyperborea*	*F. vesiculosus*	*P. canaliculata*	*C. rupestris*	*E. intestinalis*	*U. lactuca*	*P. palmata*	*V. lanosa*
Macrominerals										
Calcium	8000	10 000	8000	12 000	8300	29 000	5 500	3 500	3600	6400
Magnesium	8700	8 400	6400	7 400	9600	12 000	15 000	26 000	5300	6000
Phosphorus	2300	1 200	1600	840	730	870	1 200	500	2700	1100
Microminerals										
Copper	2.4	1.6	1.7	1.8	2.6	17	4.9	6	4.9	8
Iron	87	58	120	92	130	10 000	6 000	210	100	480
Iodine	220	3 100	3500	130	210	63	130	21	260	1300
Manganese	5.6	3.1	6.5	34	8.6	240	130	11	11	20
Selenium	0.041	0.021	0.033	0.03	0.035	0.066	0.028	0.049	0.14	0.53
Zinc	49	24	22	26	31	30	25	8	29	81
Heavy metals										
Arsenic	48	64	55	41	28	9.4	4.9	7.9	10	9.3
Cadmium	3.4	0.1	0.48	1.2	0.48	0.091	0.12	0.092	0.48	3.8
Mercury	<0.005	0.006	0.007	0.011	0.047	0.006	0.014	0.005	0.005	0.01

Values are expressed as mean of two parallels and in mg kg^{-1} DW.

for all of these heavy metals. The unit for PTWI is generally μg kg^{-1} body weight per week and the respective values are 15 for arsenic, 7 for cadmium and 3.3 for mercury.[57] The toxic potential of the heavy metals is also dependent on their physical state; while arsenic is most toxic in its inorganic form, mercury is most toxic in its organic form (methylated mercury, MeHg). The arsenic content in the algae analyzed in this study was quite high, ranging from 4.9 to 64 mg kg^{-1} DW, while levels of cadmium (ranging from 0.092 to 3.8 mg kg^{-1} DW) and mercury (ranging from 0.005 to 0.047 mg kg^{-1} DW) were lower. In order to reach the PTWI levels for a person weighing 70 kg, the weekly consumption would have to be 16–214 g for arsenic, 0.13–5 kg for cadmium and 4.8–46 kg for mercury. These results show that of the analyzed heavy metals only arsenic levels may be of concern in these species. However, as a speciation of the chemical forms of arsenic was not performed in this study, only the total arsenic content is presented. As

previously mentioned, the inorganic form of arsenic is the more toxic and speciation studies have shown that the predominant form of arsenic in algae is the organic.[58] In addition, many algae are able to metabolize inorganic arsenic into organic forms.[59]

In conclusion, the total composition of the red algae species *P. palmata* makes it the best candidate for utilization in food and feed. At the other end, the species least suitable for utilization seems to be the green alga *C. rupestris*. The combination of beneficial compounds and compounds possessing potential health risks are evident in several of the analyzed species. For instance, both the red alga *V. lanosa* and the brown alga *A. esculenta* could serve as a good protein sources, but the level of iodine (*V. lanosa*) and arsenic (*A. esculenta*) complicate the utilization of the whole plant as algae meal. This implies that extraction of single compounds, such as proteins, minerals and/or fatty acids, for use as ingredients in food and feed production may be a better strategy.

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