Nitrogen Content, Dietary Fiber, and Digestibility in Algal Food Products

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Abstract

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The basic nutritional aspects and parameters of freshwater and marine algal food products are described. Blue-green algae (*Spirulina pacifica*, *S. platensis*), green algae (*Chlorella pyrenoidosa*), red algae (*Palmaria palmata*, *Porphyra tenera*), and brown algae (*Eisenia bicyclis*, *Hizikia fusiformis*, *Laminaria japonica*, *Undaria pinnatifida*) were used for this purpose. The ash content, total nitrogen, dietary fibers, and *in vitro* digestibility of the above-mentioned algal species were studied. The ash contents amounted to 8–11% (for freshwater) and 9–33% (for marine) of the weights of the algal samples. The total nitrogen contents were analysed using a modified Winkler's method; in the process, higher nitrogen contents were observed in freshwater algae than in marine ones. For the analysis of dietary fiber contents, the instrument Ankom^{220} Fibre Analyser was used. The marine brown algae species were generally assigned higher contents of dietary fiber than the freshwater algal products. The results of the dietary fiber analysis differed with the methodologies used. Pepsin, pancreatin, and a combination of both were applied for the study of *in vitro* digestibility. Generally, brown algae showed the worst digestibility in comparison with other algal food products.

Keywords: algae; cyanobacteria; nitrogen; digestibility; dietary fiber

From the perspective of the food industry, positive nutritional values, such as high contents of dietary fibers and proteins, and low contents of lipids were found in algae. In addition, algae contain different secondary metabolites (e.g. vitamins, saccharides, volatile compounds, and phenols) that could be used as antioxidants, antibiotics, and/or virostatic agents. Food products prepared from algae could involve not only positive but also negative or disputable effects in mammalian organism. For example, higher contents of toxic elements (e.g. cadmium) or fucotoxins (algal protective compounds against herbivore attack and pathogens) in algal food products are undesirable. Bearing these considerations in mind, it is important to study the nutritional aspects and chemical compositions of algae in detail. Primarily, macroscopic marine algae (multicellular algae, species

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of genus *Laminaria* and *Undaria*) were used in the food industry as food and as a source of fucocoloids. However, the discovery of new flocculation, filtration, and extraction procedures saw the beginning of the use of microscopic algal species (unicellular algae, species of genus *Chlorella* and *Spirulina*) as food for humans and feed for animals (Grima *et al*. 2003). Since recently, these new untraditional sources of protein and nitrogen nutrients from water organisms and also from waste by-products (e.g. potato tuber skin Lachman *et al*. 2005; Bárta & Bártová 2008) have been explored for their utilisation in feed- and food-stuffs.

The application of algae in the food industry, pharmaceutical development, and biotechnology processes have been described in different review papers and book series. Food algal chemistry and technology were recently discussed (Skulberg 2000; Mundt *et al*. 2001; Pulz & Gross 2004; Thajuddin & Subramanian 2005; Cardozo *et al*. 2007; Leflaive & Ten-Hage 2007; Eriksen 2008; Gantar & Svircev 2008). It is evident that digestibility as well as the contents of dietary fiber and bioactive compounds in algae can play an important role in the evaluation of algal food quality. Digestibility is studied on the basis of nitrogen consumption before and after the process of digestion, which is usually studied under *in vitro* conditions using enzymes such as pepsin (Mabeau & Fleurence 1993). However, several analytical methodologies for the study of bioactive compounds in algal material are known. Usually, different extraction and purification techniques in combination with liquid or gas chromatography with on-line diode-array, electrochemical or mass spectrometry detectors are used (Herrero *et al*. 2006; El Hattab *et al*. 2007; Klejdus *et al*. 2009). Finally, antioxidant, biological, and toxicological activities of single compounds or algal extracts have been recently studied.

The article presented here provides a detailed study of the nutritional parameters of different algae used as food. For our purpose were used: freshwater blue-green algae (*Spirulina pacifica* and *S. platensis*) and green alga (*Chlorella pyrenoidosa*); marine red algae (*Palmaria palmata and Porphyra tenera*), and brown algae (*Eisenia bicyclis*, *Hizikia fusiformis, Laminaria japonica*, and *Undaria pinnatifida*); for other details see Table 1. The examination of dietary fibers, digestibility, and total nitrogen in the above-mentioned algal species is described.

MATERIAL AND METHODS

Chemicals, sample preparation, and ash determination. Algal samples (Table 1) were homogenised with a mixer (Vorwerk Thermomix TM 31, Asbach, Germany); particle size 1 mm. All chemicals were of p.a. purity (Lachema, Chemapol, Lach-Ner, Brno, Czech Republic) if not otherwise stated. Dry weights were determined using desiccation of algal samples (5 g) in Venticell desiccators (BMT, Brno, Czech Republic) at 105°C. The ash content was determined using incineration (5 h) of the respective algal sample (1 g) in a muffle furnace 018 LP (Elektrické pece Svoboda, Světice u Říčan, Czech Republic) at 550°C.

Total nitrogen analysis. For the analysis of total nitrogen in the algae, the modification of the previously described methodology according to Winkler was used. The total nitrogen was calculated according to the equation:

$$\mathrm{TN} = \frac{a \times 10^{-3} \times c \times M_n \times f_t \times f_d \times f_c}{m} \times 100$$

where:

TN – total nitrogen content in % (w)
a – H_2SO_4 consumption in ml
c – concentration of H_2SO_4 (25mM)
M_n – atomic weight of nitrogen (M_N = 14.01 g/mol)
f_t – titration factor (f_t = 2)
f_d – dilution factor (f_d = 5)
f_c – correlation factor (f_c = 6.25)
m – algal sample weight (m = 0.5 g)

The correlation factor 6.25 was used on the presumption that proteins contain 16% of nitrogen; for other details see (Volkmann *et al*. 2008).

Dietary fiber analysis. Dietary fiber in algal samples was analysed using an Ankom^{220} Fibre Analyzer (ANKOM Technology, New York, USA). The contents of crude fiber (CF), acid-detergent fiber (ADF), neutral-detergent fiber (NDF), and acid-detergent lignin (ADL) were determined. For these analyses, Ankom^{220} Fibre Analyzer manufacturer methodologies were used (ANKOM Technology Method 2008). For other details of dietary fiber analyses see Javorský (1987) and Mišurcová (2008).

Briefly, the samples were hydrolysed in filter bags (F 57, pore internal dimension 50 µm, ANKOM Technology, New York, USA) by using 127.5mM H_2SO_4 and 313mM NaOH separately, 45 min (for CF); cetyl trimethyl ammonium bromide (FAD 20C, ANKOM

Technology), 60 min (for ADF); sodium lauryl sulphate (FND 20C, ANKOM Technology) and α-amylase (FAA, ANKOM Technology), 75 min (for NDF). ADL was determined as ADF and the samples were subsequently treated with 72% H_2SO_4. Then, the filter bags containing the samples were washed in water (three times) and in acetone (once, 3 min). After acetone evaporation, the bags were dried at 105°C (4 h) and then incinerated in a muffle furnace at 550°C (5 h). The CF, ADF, NDF, and ADL contents were calculated according to the equation:

$$V = \frac{(m_3 - m_1c_1) - (m_4 - m_1c_2)}{m_2} \times 100$$

where

V – content of CF, ADF, NDF, or ADL in % (w) of algal sample

m_1 – weight of the bag (g)

m_2 – weight of the algal sample (g): 1 g (for CF) or 0.5 g (for ADF, NDF, and ADL);

m_3 – weight of the dried bag with the hydrolysed sample (g)

m_4 – weight of the bag with the hydrolysed sample after incineration

$c_1 = m_S/m_1$, $c_2 = m_P/m_1$ (m_s – weight of the dried bag after hydrolysis, m_p – weight of the bag ash)

Digestibility. Digestibility of algal samples was determined using several enzymes. This approach is based on *in vitro* simulations of the digestion of samples similar to those in a human body. Method A is based on the determination of nitrogen before and after the digestion with pepsin. In the case of method B, digestibility was evaluated by the determination of the decrease in organic matter before and after pepsin and pancreatin digestion in a Daisy incubator (ANKOM Technology, New York, USA). For both methods, casein (Sigma Aldrich Corp., St. Louis, USA) was applied as the reference material with 100% digestibility.

Method A: Algal samples (5 g) were incubated in 225 ml of pepsin solution at 40°C (48 h) in an incubator BT 120 (BMT a.s., Brno, Czech Republic). The incubation solution was prepared by dissolution of pepsin (2 g, 0.7 U/g, Merck KGaA, Darmstadt, Germany) in HCl (1 l, concentration 75mM) at 40°C; pH of the incubation solution was below 1.7. After the completion of incubation, HCl (7.5 ml, 7M) was added to the incubation solution for enzyme inhibition. The solution was filtred (Filtrac 390, Selecta, Spain), the filtrate (100 ml) was evaporated using an evaporator RVO-200 (INGOS Prague, Czech Republic). Then the samples were dissolved in distilled water to a final volume of 10 ml each. The dissolved samples (1 ml) were used for the determination of total nitrogen content as described in the section Total nitrogen analysis. Digestibility was calculated according to the equation:

$$D = \frac{TN_1 - TN_2}{TN_3 - TN_4} \times 100$$

where:

D – digestibility of the sample in % (w) of the dried sample

TN_1 – total nitrogen content of the sample before digestion (%)

TN_2 – total nitrogen content after digestion (%)

TN_3 – content of total nitrogen in casein before digestion (%)

TN_4 – total nitrogen content of casein after its digestion (%)

Method B. The digestibility of algal samples was evaluated by a modified method (Forejtová *et al*. 2005). For this purpose, the samples were digested with pepsin (0.7 U/g, Merck KGaA, Darmstadt, Germany) and pancreatin (protease activity 350 U/g, lipase activity 6000 U/g, amylase activity 7500 U/g, Merck KGaA, Germany). We used 0.6 g of enzyme per 1 g of algal sample in all experiments. The digestibility of the samples was determined using these enzymes separately and/or in combination.

Filter bags, containing of 0.25 g of algal samples (F 57, pore internal dimension 50 μm, ANKOM Technology, New York), were inserted into incubation bottles containing 1.7 l solution of HCl (1mM) for pepsin and phosphate buffer (pH 7.45) for pancreatin. The samples were incubated for 24 h in a Daisy incubator at 40°C. After the incubation was completed, the bags were washed using distilled water and dried at 103°C (24 h).

Pepsin + pancreatin digestion was conducted separately; 24 h with pepsin, and after that 24 h with pancreatin. Other experimental steps were the same as in the case of pepsin digestion, which is described in the previous paragraph.

The values of digestibility were calculated according to the following equations:

$$D = 100 - \frac{100 \times W_{DM}}{m_2 \times DM}$$

$$W_{DM} = m_3 - m_1c; \quad DM = \frac{DM_{\%} - m_s}{100}$$

where:

D – digestibility in % (w) of the dried sample

W_{DM} – weight of algal sample after digestion and drying (g)

DM – dry weight of the sample (g)

$DM_{\%}$ – dry weight of the sample (%)

m_1 – weight of the filter bag (g)

m_2 – weight of the sample (g)

m_3 – weight of the filter bag containing the sample after digestion and drying (g)

m_s – weight of the sample for the determination of dry weight (g)

$c = m_b/m_1$ (m_b – weight of the filter bag after digestion and drying)

Finally, digestibility D in % was calculated for each sample according to the equation:

$$DM = \frac{\text{D of sample}}{\text{D of casein}} \times 100$$

RESULTS AND DISCUSSION

In the first experiments, we determined the dry weights and ash contents in algal samples. These parameters were studied in the selected food products of edible freshwater (*Spirulina pacifica*, *S. platensis*, and *Chlorella pyrenoidosa*) and marine algae (*Palmaria palmata*, *Porphyra tenera, Eisenia bicyclis, Hizikia fusiformis, Laminaria japonica*, and *Undaria pinnatifida*); Table 1. The relative height contents of dry weights were found in all algal products (the dry weights varied in the interval from 88% to 95%; data not shown). The height values of dry weights are typical for these types of food products which were previously dried by the manufacturer.

The results of analyses clearly show that freshwater algae (on average 8.8%) contain lower ash contents than marine algae (on average 22%). The ashes were quantified as the percentage representation (w) of the residues after incineration of algal samples in a muffle furnace. Only with *E. bicyclis* (9.7%) was found a lower ash content (as opposed to other marine algal products). The ash content in algae is probably connected with the concentration of inorganic compounds and salts in water environment where the algae grow; e.g. in the selected red and brown algae from Northeastern Mediterranean Sea the ash contents varied from 17% to 27% on a dry weight basis (Polat & Ozogul 2008). Furthermore, the ash contents in the final food products could be probably affected by the washing procedures after the harvesting of the algae. The percentage representation of ash per single algal sample is shown in Figure 1.

Total nitrogen

All of the freshwater algal samples showed ca. 200% higher content of total nitrogen than the marine samples (the lowest contents of total nitrogen were found in brown algae *H. fusiformis* and *E. bicyclis*). The highest values of total nitrogen contents were found in the samples of Chlorella and in both representatives of the blue-green algae (columns A–C in Figure 2). It was observed by other authors that the food based on Spirulina usually possessed a higher content of proteins and amino acids which

Table 1. Freshwater and marine algal food products used in this study

Algae	Algae strain	Food product	Sample	Country of origin
Blue-green	*Spirulina pacifica*	Spirulina	A	Hawaii
	Spirulina platensis	Spirulina	B	India
Green	*Chlorella pyrenoidosa*	Chlorella	C	Taiwan
Red	*Palmaria palmata*	Dulse	D	USA
	Porphyra tenera	Nori	E	Japan
Brown	*Eisenia bicyclis*	Arame	F	Japan
	Hizikia fusiformis	Hijiky	G	Japan
	Laminaria japonica	Kombu	H	Japan
	Undaria pinnatifida	Wakame	I	Japan
	Undaria pinnatifida	Wakame	J	Japan

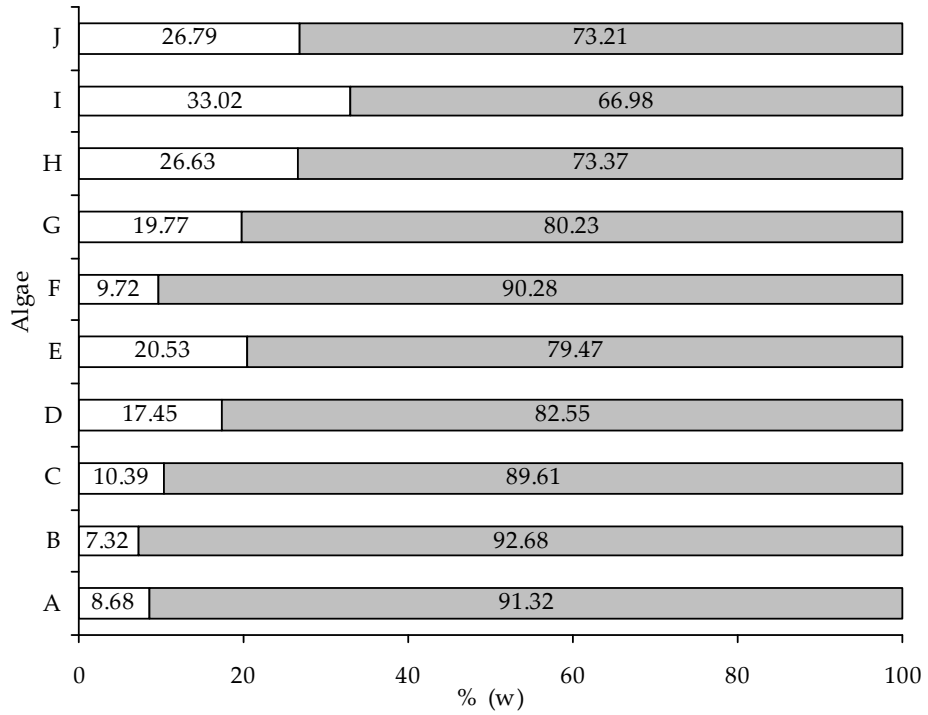

Figure 1. The percentage representations of ash contents (white columns) in freshwater and marine algae (for names of algae see Table 1). The values were calculated as ash recovered after incineration of algal samples in a muffle furnace. 100% represents total weight of the sample

are an important source of nitrogen (Campanella *et al*. 1999). These observations showed that freshwater algae could be an interesting source of nitrogen compounds (Prugar 2008).

We also observed lower contents of total nitrogen in red and brown algae. The lower contents of nitrogen in brown algae (*E. bicyclis*, *H. fusiformis*, *L. japonica*, and *U. pinnatifida*) are probably connected with the fact that brown algae generally contain lower amounts of proteins than other algal groups (Burtin 2003). For the comparison of total nitrogen contents in individual groups of algae see the inset in Figure 2. The relative standard deviations (RSDs) for the dry weight, ash, and total nitrogen analysis were lower than 1%.

Dietary fiber

Dietary fiber means the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fiber promote beneficial physiological effects including laxation and blood cholesterol or glucose attenuation (Anonymous 2001). Several authors have published their results on dietary fiber in algae; a detailed compilation of their findings is presented in the review article of Brownlee *et al*. (2005).

Here, we studied crude fiber (CF, lignocellulose complex), neutral-detergent fiber (NDF, lignocellulose complex and semisoluble hemicelulose), acid-detergent fiber (ADF, lignocellulose complex), and acid-detergent lignin (ADL) in all algae using an instrument Ankom^{220} (machine-controlled procedure). For CF, NDF, ADF, and ADL analysis was investigated the sample hydrolysis with weak acid and hydroxide, sodium lauryl sulphate, cetyl trimethyl ammonium bromide, and 72% sulphuric acid, respectively; detailed experimental procedures were received from the doctoral thesis of Mišurcová (2008). After quantification of the non-hydrolysed residues, we found different values of dietary fiber contents, depending on the method of the sample hydrolysis (Table 2). For a better comparison of the occurrence of dietary fiber in algae, the average values of all types of dietary fiber (CF, NDF, ADF, and ADL) contents in individual algae are presented in Figure 3 and

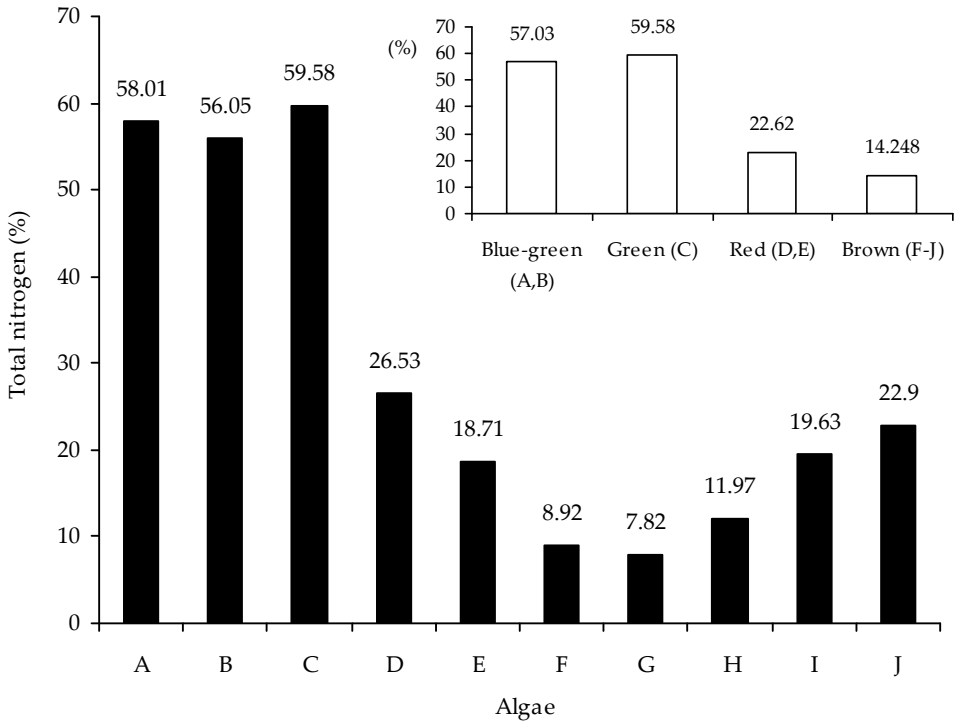

Figure 2. Total nitrogen contents in algae. In summary, the average values of total nitrogen contents in different algal groups are shown in the inset. 100% represents total weight of the sample. For the names of algae see Table 1

in its inset for different algal groups. The results showed that red and brown algae contain higher amounts of dietary fibers than freshwater algae. The RSDs for the dietary fiber analysis were in the interval of 0.06–6.1%.

The content of dietary fiber in algae is an important nutritional parameter (see above). It was found that dietary fibers can bind toxic compounds and thus eliminate their mobility in the organism of the consumer. For comparison, Burtin (2003) published a study focused on the nutritional values of seaweeds. It was concluded that fruits and vegetables (for example apple or cabbage) can contain similar amounts of dietary fibers as wakame, nori, kombu, and other algal products. However, it is difficult to compare the results acquired by dif-

Table 2. Percentage representation of dietary fibers in algae (n = 6) – x ± relative standard deviation

Algal product	CF	NDF	ADF	ADL
A	0.18 ± 0.30	4.68 ± 2.79	0.12 ± 0.12	4.56 ± 3.15
B	0.10 ± 0.11	0.22 ± 0.25	0.26 ± 0.22	3.16 ± 2.03
C	1.97 ± 0.38	2.21 ± 0.83	6.25 ± 1.52	2.71 ± 0.34
D	1.49 ± 0.23	15.13 ± 0.62	3.12 ± 0.31	0.44 ± 0.69
E	3.24 ± 0.17	28.18 ± 2.33	12.38 ± 0.45	4.36 ± 0.42
F	7.30 ± 0.29	14.55 ± 0.79	19.28 ± 0.38	3.45 ± 0.66
G	12.55 ± 0.27	20.66 ± 0.81	29.36 ± 1.20	7.51 ± 0.81
H	5.45 ± 0.46	22.08 ± 2.70	13.83 ± 1.05	0.43 ± 0.67
I	3.11 ± 0.55	13.90 ± 4.02	16.19 ± 1.87	2.93 ± 0.70
J	2.94 ± 0.06	34.88 ± 6.10	19.83 ± 0.69	4.46 ± 1.36

CF – crude fiber; NDF – neutral-detergent fiber; ADF – acid-detergent fiber; ADL – acid-detergent lignin

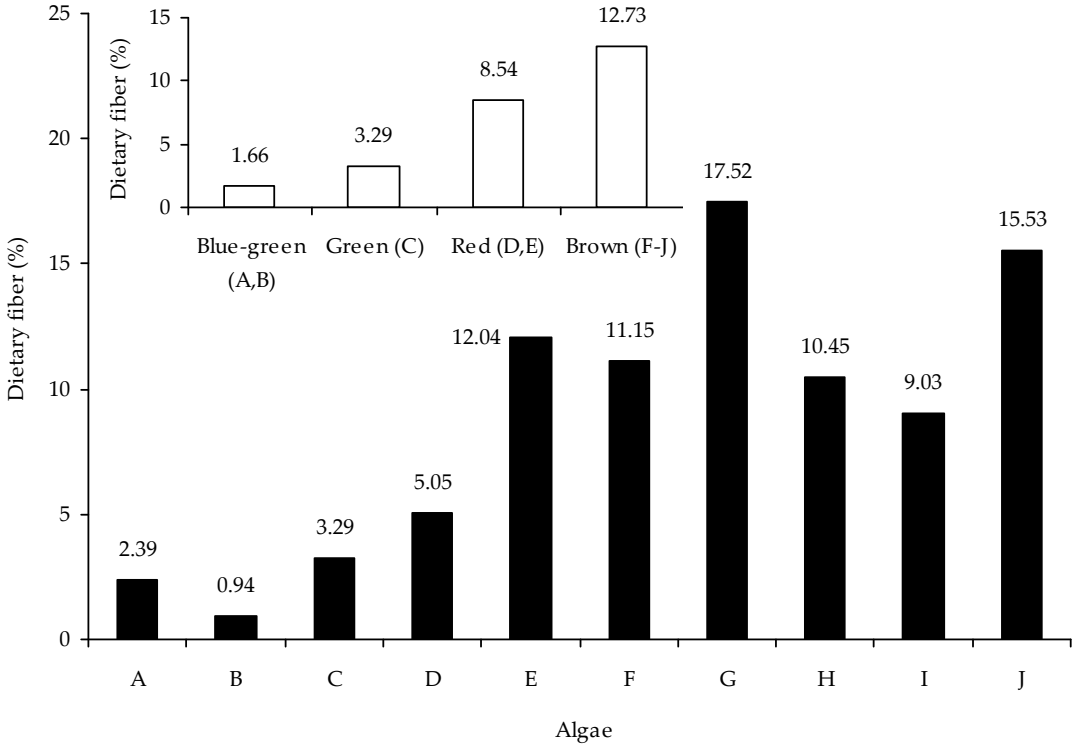

Figure 3. Contents of dietary fibers in algae. The percentage representations were calculated as the average values of crude, neutral-detergent, acid-detergent fiber, and acid-detergent lignin that are presented in Table 2. In summary, the average values of dietary fibers in different algal groups are shown in the inset. For the names of algae see Table 1

ferent methods and experimental procedures. In addition, the contents of compounds comprised by dietary fibers could be influenced by the growth conditions of algae.

Digestibility of algae

The *in vivo* digestibility of algae is not well documented, and the available studies on their assimilation by humans have not provided conclusive results. However, several authors have described a high rate of algal protein degradation *in vitro* by proteolytic enzymes such as pepsin, pancreatin, and pronase (Mabeau & Fleurence 1993).

The *in vitro* digestibility of the algal samples was determined after their incubation with pepsin. For this purpose, the experimental procedure: method A (see Material and methods) was used. For a detailed examination of digestibility, the following experimental approaches were applied based on digestion of algae with pepsin, pancreatin (mixture of protease, lipase, and amylase) and a combination of both in a special incubator (method B). In the case of method A, the highest and the lowest digestion was shown with food products H or D and G or J, respectively. The comparison of the digestibility values determined by methods A and B for different algal groups is presented in Figure 4. The impaired digestibility of brown algae may be connected with their higher content of dietary fiber, as shown in the previous

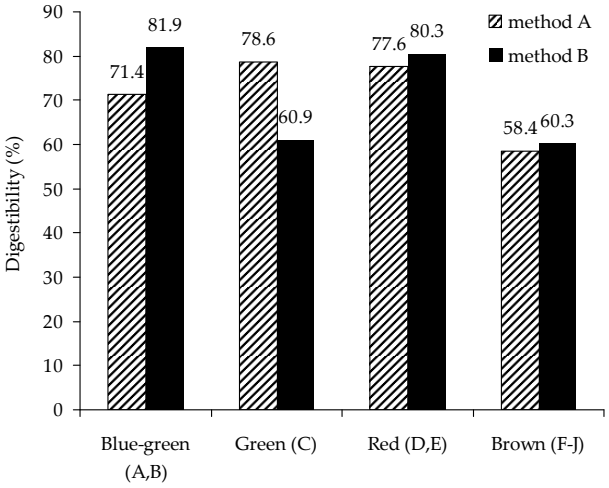

Figure 4. The digestibility of blue-green, green, red, and brown algae using pepsin. Different methodologies were used; see section Materials and methods for details. Digestibility of casein was 100%

Table 3. Percentage representation of digestibility of algal dry weights by pepsin, pancreatin, and combination of both. Digestibility of casein represents 100%. For the methodology (method B was used) and names of algae see section Materials and methods and Table 1, respectively (n = 6) – x ± relative standard deviation

Algae	Pepsin	Pancreatin	Pepsin + pancreatin
A	74.1 ± 6.8	82.9 ± 1.5	85.6 ± 1.5
B	89.6 ± 6.1	97.5 ± 0.8	94.3 ± 8.3
C	60.9 ± 3.2	79.1 ± 1.0	75.3 ± 1.1
D	87.4 ± 0.7	84.9 ± 0.3	87.3 ± 0.2
E	73.2 ± 3.8	65.9 ± 0.3	70.2 ± 0.4
F	57.6 ± 1.4	73.2 ± 1.4	57.1 ± 1.0
G	51.8 ± 0.8	65.8 ± 2.3	51.8 ± 0.8
H	70.2 ± 3.9	76.1 ± 1.2	72.1 ± 0.7
I	69.1 ± 0.4	87.5 ± 2.0	68.6 ± 0.4
J	52.8 ± 0.6	57.1 ± 1.4	52.7 ± 0.6

section (see inset in Figure 3). The RSDs for all measurements varied in the intervals 0.1–0.3% for method A and 0.2–8.3% for method B.

In the case of method B, the concrete values of digestibility of algal samples are shown in Table 3. We observed the efficiency of digestibility for most of the samples to be in the following order: pancreatin > pancreatin/pepsin > pepsin. The relationship between the digestibility efficiency and the enzyme which was used for digestion is presented in Table 3. Similarly to method A, the lowest digestibility was found for brown algal products. Finally, on the basis of the comparison of methods A and B, the following order of algae digestibility was found: B > D > A > H > I > E > C > F > G > J.

The digestibility was previously described in different papers. For example, Wong and Cheung (2000, 2001) studied *in vitro* protein digestibility of red seaweeds (*Hypnea charoides* and *H. japonica*) and green seaweed (*Ulva lactuca*). These authors showed that the protein digestibility of red seaweed (around 88%) was slightly higher than that of green seaweed.

In conclusion, we showed here that the food products from marine algae have higher contents of ash and dietary fibers than the products of freshwater algae. On the other hand, higher contents of total nitrogen were found in freshwater algal products than in marine ones. In addition, *in vitro* digestibility of brown algal products (around 60% vs. digestibility 100% for casein) was lower than of other algae analysed. We suppose that the results will be important for the evaluation of algal food product quality and the study of subsequent nutritional parameters in the future.

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