CHAPTER 24

Seaweed Proteins and Amino Acids as Nutraceuticals

Monika Černá[1]

Contents

Abstract

Seaweeds demonstrate original and interesting nutritional characteristics. Protein concentration ranges from 5% to 47% of dry basic. Its value depends particularly on species and the environmental conditions. Seaweed protein is a source of all amino acids, especially glycine, alanine, arginine, proline, glutamic, and aspartic acids. In algae, essential amino acids (EAAs) represent almost a half of total amino acids and their protein profile is close to the profile of egg protein. In case of non-EAAs, all three groups (green, brown, and red seaweeds) contain the similar amount. Red seaweed seems to be a good source of protein because its value reaches 47%.

Department of Food Technology and Microbiology, Faculty of Technology, Tomas Bata University in Zlín, Zlín, Czech Republic
[1] Corresponding author: Monika Černá, *E-mail address:* cerna@ft.utb.cz

Advances in Food and Nutrition Research, Volume 64
ISSN 1043-4526, DOI: 10.1016/B978-0-12-387669-0.00024-7

The issue of protein malnutrition supports the trend to find a new and cheap alternative source of protein. Algae could play an important role in the above-mentioned challenge because of relatively high content of nitrogen compounds. Algae may be used in the industry as a source of ingredients with high nutritional quality.

I. CRITERIA AND SIGNIFICANCE OF DIETARY PROTEIN IN HUMAN

A. Role of amino acids and protein in the human body

Proteins are an essential component of the diet needed for the survival of animals and human (Friedman, 1996) and a major source of carbon, which is contained in about 16%. Proteins may be divided into fibrous and globular proteins according to the shape of their tertiary structures (Zimmermann, 2003). Proteins could also be grouped into seven categories with reference to their functions: enzyme catalysis, defense, transport, support, motion, regulation, and storage proteins (Losos *et al.*, 2008). Proteins play a very important role in all processes in the human body. These processes are facilitated by enzymes, which are actually specialized proteins increasing the reaction rate without its changes. Deficiency of enzymes causes a slower reaction. Enzymes are a globular type of proteins and they are absolutely different in the composition (Marshall, 2005). Variety of globular proteins has a unique ability to transport substances across cell membranes; others use their shape to recognize foreign microbes and cancer cells. Proteins also have regulatory roles within the cell—turning on and shutting off genes during genesis. Moreover, proteins also receive information and act as cell-surface receptors. However, fibrous proteins play a structural role. These fibers include collagen in skin, keratin in hair, fibrin in blood clots, ligaments, tendons, and bonds. They are the most abundant type of proteins in a vertebrate body (Losos *et al.*, 2008). Proteins are needed to initiate every biochemical process in the body, and they provide invaluable source of energy (Marshall, 2005). In cells, proteins are continually being made and resolved in a process known as a protein turnover. The supply of amino acids (amino acid pool) is derived from either food or body proteins collected in the cell and circulating blood and stands ready to be incorporated in proteins and other compounds or use for energy.

B. Human amino acid and protein requirements

Recommended dietary allowance of good-quality protein is 0.83 g/kg of body weight per day with the protein digestibility-corrected amino acid score value of 1.0 (WHO, 2002). Acceptable macronutrient distribution

range for protein is 10–35% of energy for adults (Food and Nutrition Board, 2005). If the body synthesizes more proteins than it degrades and intakes, nitrogen status becomes positive, but protein gained over the need is degraded and stored as the body fat. If the body degrades more proteins than it synthesizes and loses, nitrogen status becomes negative. This nitrogen status is found in the body of people who are starving or suffering from other stresses such as burns, injuries, infections, etc. (Whitney and Rolfes, 2008). There is a need to considerate not only the total intake of protein but also the quality of protein. The protein quality is discussed in terms of biological value (or net protein utilization used in animal growth studies) (FAO/WHO, 1991). Amino acid composition and protein digestibility are the main factors influencing protein quality. Proteins are linear polymers of 20 different amino acids (essential and nonessential). To prevent protein degradation, dietary protein has to provide at least nine essential amino acids (EAAs), other amino groups, and energy for the synthesis of the rest. Generally, food of animal origin provides high-quality proteins (except from gelatin). Plant proteins have more amino acids patterns and tend to be limiting in one or more EAAs. Protein digestibility/bioavailability (comprises digestibility, chemical integrity, etc.) depends on many factors (Kies, 1981). The first is the source of proteins. Digestibility of most animal and plant proteins ranges between 90–99% and 70–90%, respectively (Gropper *et al.*, 2008; Whitney and Rolfes, 2008). The influence of other food components should be considered. For example, dietary fiber might reduce the available energy by 2–3% at a moderate level and by the additional 2–3% in vegetable diets (FAO/WHO, 1985). It also depends on an accepter of proteins. Different animal species have different digestive enzyme systems and other physiological/biochemical differences in their gastrointestinal systems. Physiological and psychological stress may cause profound changes in the activity of gastrointestinal tract as well (Kies, 1981).

According to WHO (2002), protein requirement could be defined as ''the lowest level of dietary protein intake that will balance the losses of nitrogen from the body and thus maintain the body protein mass, in persons at energy balance with modest levels of physical activity, plus, in children or in pregnant or lactating women, the needs associated with the deposition of tissues or the secretion of milk at rates consistent with good health.'' Protein and amino acid requirements are differentiated into the safe individual intake and safe population intake (this value is greater than the safe individual intake in most cases). The requirements are suggested for health and disease condition of all age groups (eventually sexes) and women during pregnancy and lactation as well (Tables 24.1 and 24.2). The recommendations for developing countries are also stated. They were based as a sum of the total protein content of the diet (total

TABLE 24.1 Proteins and amino acid requirements (WHO, 2002)

Age (years)/average weight (kg)	Protein intake (g/day)	Amino acids intake (mg/kg/day)			
		Lysine	Sulfur amino acids	Threonine	Tryptophan
1/9.8	11.6	45.0	22.0	23.0	6.4
5/19.7	17.1	35.0	18.0	18.0	4.8
12/45.6	40.5	35.0	17.0	18.0	4.8
16 (boy)/66.5	57.9	33.0	16.0	17.0	4.5
16 (girl)/56.4	47.4				
>18/70.0	58.0	30.0	15.0	15.0	4.0

TABLE 24.2 Proteins requirements (WHO, 2002)

Prenatancy	Extra protein intake (g/kg)
1 trimester	1
2 trimester	10
3 trimester	31
Lactation	**Extra protein intake (g/kg)**
<6 months	19
>6 months	13

nitrogen × 6.25) and the available protein in the diet, which is calculated as total protein × protein digestibility-corrected amino acid score value (digestibility factor × amino acid score) (WHO, 2002). Nutritional value of proteins has been evaluated by comparing their amino acid composition with the reference protein according to WHO (2002) or with egg or soya protein used as a pattern.

There are several additional methods available for determining protein quality of food and evaluating protein adequacy of the diet. Nitrogen balance studies assess intake of dietary nitrogen and also measurement and summation of nitrogen losses from the body. Chemical score (amino acid score) involves determination of amino acids composition (with using amino acid analyzer or high-performance liquid chromatography techniques) of a test protein (reference or egg pattern). Biological value of protein shows how much nitrogen is retained in the body for the maintenance and growth rather than it is absorbed. Other methods possible to use to assess the protein quality are protein efficiency ratio or net protein utilization (Gropper *et al.*, 2008).

C. The role of protein in human health

Proteins, unlike fatty acids and carbohydrates, are not stored in the body, but they are deaminated followed by the oxidation of carbon skeleton through the pathways of glucose or fat metabolism, or they are stored in the form of glycogen or fat. It depends on the specific amino acid and the energy balance at the time. Nitrogen waste is excreted in urine as either urea or ammonia. Considering the body need of proteins for sustaining the essential physiological functions, diet low in protein could be deficient in many important vitamins and minerals in comparison to protein-rich diet. During fasting and starvation, muscle provides a source of amino acids. The balance between protein synthesis and resolution is closely regulated (Becker and Smith, 2006). FAO/WHO (De Onís *et al.*, 1993) defines malnutrition as ''the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions.'' In the world, especially in developing countries, protein-energy malnutrition is one of the most common health problems in both children and adults (Stephenson *et al.*, 2000). Protein-energy malnutrition plays a fundamental role in developing countries due to poverty, minimal medical care, endemic infections, and poor sanitary conditions. This manifests particularly in children, who are offered little food or food of inadequate quality for their needs. Studies in Nigeria found that protein-energy malnutrition has been the second most frequent cause of death of children less than 5 years (Nnakwe, 1996). More than 70% of children with protein-energy malnutrition live in Asia, 26% in Africa, and 4% in Latin America and the Caribbean (WHO, 2000). However, Grover and Ee (2009) mentioned that protein-energy malnutrition also occurred in developed countries.

Protein deficiency has negative effects on all organs. It has been proved that it has adverse effects on the brain, immune system, and kidney function (Food and Nutrition Board, 2005). Feoli *et al.* (2006) reported that protein malnutrition increased oxidative damage of lipids and proteins gained from rat brain areas. This malnutrition may be the indication of important mechanism of changes in the brain development. Low protein diet could cause genetic damage as well. Frequency of chromosomal aberrations in peripheral blood lymphocytes was nearly seven times higher in malnourished infants than in eutrophic children (Padula *et al.*, 2009). However, results of recent study show that the effect of protein and energy intake on plasma insulin-like growth factor I (IGF-I) is not temporary, and that long-term protein (0.73 g/kg body weight/day, 9% of energy) and calorie (1989 kcal/day) restriction may cause chronic decrease of plasma IGF-I concentrations independent on the body fat mass. These data suggest that a lower protein and particularly calorie intake may have some additional protective effects against some types of cancer (Fontana *et al.*, 2006).

Epidemiologic studies point out that the modern lifestyle of developed countries with high calorie and protein consumption, low physical activity, and obesity increases the risk of cancer (Calle and Kaaks, 2004). It has been reported that general diet in Western Europe and the United States contains 1.5–2 times higher amounts of protein. High protein intakes may increase urinary calcium excretion, although the effect on calcium balance is controversial, as amino acids increase the efficiency of intestinal absorption. High protein intake is connected with the increasing urinary nitrogen excretion, vasopressin plasma levels, creatinine clearance, glomerular filtration rate, kidney hypertrophy, renal hemodynamics, and eicosanoid production in renal tubules (Bankir and Kriz, 1995). Moreover, the relationship between high protein intake and risk of renal cell cancer (Chow *et al*., 1994) or prostate cancer (Vlajinac *et al*., 1997) has been discussed. Other health effects of high protein intake, such as diabetic nephropathy, have not been clarified yet.

D. Bioavailability, digestion, and absorption of amino acids

Bioavailability of proteins (or amino acids) is a popularized term for an important factor of determining protein quality. It is usually considered as a level, up to which amino acids or small peptides from a sample protein consumed by a living organism are finally transported into the body. Therefore, it includes digestibility and absorption mechanisms. Some showings of bioavailability of food proteins should be given by the results of valid *in vitro* digestibility methods (Kies, 1981). Normally, protein digestion starts in the stomach by the activity of HCl, which denatures quaternary, tertiary, and secondary structures of proteins and causes the activation of pepsinogen to pepsin. Final products of gastric protein digestion include especially large polypeptides, oligopeptides, and free amino acids. Protein digestion is completed by intestinal enzymes in the lumen of the small intestine (Gropper *et al*., 2008). Amino acids are absorbed along the entire small intestine, most of them particularly in the proximal small intestine. Amino acids are transported by specific carriers.

Transport of amino acids across the apical membrane is not only via sodium-dependent symporters but also due to the proton-motive force and the gradient of other amino acids providing to absorb amino acids from the lumen efficiently. In the basolateral membrane, antiporters work together with facilitators to release amino acids without depleting cells of valuable nutrients. Individual amino acids are mostly transported by more than one transporter, affording a backup capacity for the absorption during the mutational inactivation of a transport system (Bröer, 2008).

Briefly, neutral and anionic amino acids are transferred by Na^+ symporters during the secondary active transport from the lumen into

mucosal cells and then they are transported into the blood with carriers or by diffuse. If Na^+ was absent, no accumulation was observed. There is recent evidence that methionine transport is made separately of other neutral amino acids transport. Cationic amino acids (arginine, lysine, and ornithine) are partly taken up into the enterocytes by Na^+-independent mechanisms, as the membrane potential is a driving force for their uptake. Anionic amino acids are already resolved in the mucosal cells. They also have their own (Na^+ and K^+ dependent) carrier systems, and finally neutral amino acids apply several different transporters (Despopoulos and Silbernagl, 2009; Preston *et al.*, 1974). In addition, diacidic amino acids (glutamic and aspartic acids) are largely transaminated to alanine during the absorption. Dipeptides and tripeptides are usually absorbed more rapidly than free amino acids. Inside enterocytes, peptides are hydrolyzed, and amino acids are released together with those absorbed by amino acid transporters. A number of specific amino acid absorption disorders are congenital and combined with similar defects of renal tubular reabsorption. A lot of inborn disorders influence amino acid transport in epithelial cells, such as cystinuria, lysinuric protein intolerance, Hartnup disorder, iminoglycinuria, dicarboxylic aminoaciduria, and some other less well-described disturbances of amino acid transport (Bröer, 2008).

II. PROTEIN AND AMINO ACIDS IN SEAWEEDS

Seaweeds have been used as human food, particularly in China, Japan, and the Republic of Korea for several centuries. Recently, seaweeds have appeared in the cuisine of North America, South America, and Europe as well (FAO, 2003). Because of their low content of energy but high concentration of dietary fibers, minerals, and vitamins, they seem to be a good source of healthy food (Ito and Hori, 1989). Algae provide a significant amount of nitrogen compounds, namely, amino acids and proteins as well (Darcy-Vrillon, 1993; Fleurence, 1999a; Oohusa, 1993).

A. Factors influencing the amino acid and protein content

Very common seaweeds, which are used as human food, are species of red algae *Porphyra* (nori), brown algae *Laminaria* (kombu), and *Undaria* (wakame) (FAO, 2002). USDA (2010) shows that the raw seaweed *Undaria* spp. contains approximately 3.0% of proteins and, with regard to 80% moisture content, it has about 15.2% of protein of dry matter. Admittedly, protein values range from 5% to 47% according to the species, environmental conditions, habitats, maturity, and applied methods used for protein and amino acid determination (Ito and Hori, 1989).

Seaweed proteins contain all amino acids, which significantly depend on the seasonal period (Fleurence, 1999a; Galland-Irmouli *et al*., 1999). Generally, the highest protein value has been found during the period of winter–early spring and the lowest during summer–early autumn (Galland-Irmouli *et al*., 1999). According to Denis *et al.* (2010), in red algae *Grateloupia turuturu*, the maximal concentration of protein was observed from January to April and the lowest amount of protein from July to August. The minimum of protein value in summer could be connected with the destruction of phycobiliproteins (Galland-Irmouli *et al*., 1999). In contrary to the other compounds (ash or dietary fiber), proteins were put through large changes within the year. Moreover, it was reported that different protein levels depend on the specific areas, too. For example, Yaich *et al.* (2011) mentions that *Ultrica lactuca*, which come from the littoral area of Tunisia, contains almost by 50% higher amount of protein than the same species from Philippines. From the data gained by Renaud and Luong-Van (2006), it is evident that the highest concentration of protein was found in red algae collected in summer (4.8–12.8%), while it was significantly lower during winter.

Protein concentration varied especially within the same species among populations (McDermid and Stuercke, 2003). Generally, the protein amount of brown seaweeds is low. It has been reported that it is lower than 15% (Fleurence, 1999a). On the contrary, red seaweeds contain a high quantity of protein, and this value is often comparable with the amount of other foods such as soybean or eggs. There were established some differences in the protein value between red and green seaweeds. In agreement with Qasim (1991), in brown seaweeds, there was determined the interval of 21–28%, in green 14–26%, and in red seaweeds 11–24%. Roslin (2003) found a minimum of protein at the level of 1.5% in green algae. It was observed that red alga *Gracilaria changii* contains a relatively high amount of protein, approximately 34% of dry weight (Norziah and Ching, 2000), which is comparable with the value of protein in green peas (USDA, 2010). Nevertheless, protein quantity is very changeable in dependence on different species, for example, red alga *Corallina officinalis* provided a very low protein content, approximately 7% (Marsham *et al*., 2007). Generally, the protein content decreases in the order of the seaweed group: red $>$ green $>$ brown. In Tables 24.3 and 24.4, there are presented protein content of some seaweed demonstrating their variability.

Many researchers have assessed protein content by measuring nitrogen content and multiplying it by different conversion factors. In the case of seaweed, the nitrogen-to-protein factor ranges from 3.75 to 5.72 (Lorenço *et al*., 2002); therefore, the traditional value might overestimate the protein content. However, nitrogen in seaweeds is a component of many types of molecules in addition to protein, such as DNA, ATP, etc. High amount of polysaccharides could limit the accessibility of protein

TABLE 24.3 Protein content (%, dry matter) of red seaweed

Red seaweed	Protein	Methods	References
Ahnfeltiopsis concinna	5.7	Lowry	McDermid and Stuercke (2003)
Amansia multifida	25.6	Kjeldahl	Ramos *et al.* (2000)
Asparagopsis taxiformis	6.1	Lowry	McDermid and Stuercke (2003)
Bryothamnion seaforthii	17.3	Kjeldahl	Ramos *et al.* (2000)
Bryothamnion triquertrum	11.8	Kjeldahl	Ramos *et al.* (2000)
Corallina officinalis	6.9	Kjeldahl	Marsham *et al.* (2007)
Corallina officinalis	2.3	Kjeldahl	Ramos *et al.* (2000)
Digenea simplex	15.6	Kjeldahl	Ramos *et al.* (2000)
Enantiocladia duperreyi	19.5	Kjeldahl	Ramos *et al.* (2000)
Eucheuma cottonii	9.8	Kjeldahl	Matanjun *et al.* (2009)
Eucheuma denticulatum	4.9	Lowry	McDermid and Stuercke (2003)
Gelidiella acerosa	31.1	Biuret	Manivannan *et al.* (2009)
Gracilaria birdiae	7.1	Bradford	Gressler *et al.* (2010)
Gracilaria domingensis	6.2	Bradford	Gressler *et al.* (2010)
Gracilaria folifera	7.0	Biuret	Manivannan *et al.* (2008)
Gracilaria changgi	34.5	Kjeldahl	Norziah and Ching (2000)
Gracilaria lemaneiformis	7.7	Kjeldahl	Ramos *et al.* (2000)
Gracilaria salicornia	5.6	Lowry	McDermid and Stuercke (2003)
Grateloupia turuturu	22.9	Kjeldahl	Denis *et al.* (2010)
Halymenia formousa	21.2	Lowry	McDermid and Stuercke (2003)
Hypnea charoides	18.4	Kjeldahl	Wong and Cheung (2000)
Hypnea japonica	19.1	Kjeldahl	Wong and Cheung (2000)
Hypnea valentiae	8.3	Biuret	Manivannan *et al.* (2008)
Chondrus ocellatus	8.3	Lowry	McDermid and Stuercke (2003)
Laurencia filiformis	18.3	Bradford	Gressler *et al.* (2010)
Laurencia intricata	4.6	Bradford	Gressler *et al.* (2010)
Laurencia nidifica	3.2	Lowry	McDermid and Stuercke, 2003
Ochtodes secundiramea	10.1	Bradford	Gressler *et al.* (2011)
Palmaria palmata	18.3	Kjeldahl	Galland-Irmouli *et al.* (1999)
Plocamium brasiliense	15.7	Bradford	Gressler *et al.* (2011)
Porphyra sp.	31.3	Kjeldahl	Dawczynski *et al.* (2007)
Porphyra sp.	44.0	Kjeldahl	Marsham *et al.* (2007)
Porphyra vietnamensis	16.5	Lowry	McDermid and Stuercke (2003)
Solieria filiformis	21.3	Kjeldahl	Ramos *et al.* (2000)
Vidalia obtusiloba	18.1	Kjeldahl	Ramos *et al.* (2000)

Kjeldahl method based on the nitrogen-to-protein factor 6.25.

TABLE 24.4 Protein content (%, dry matter) of green and brown seaweed

	Protein	Methods	References
Green seaweed			
Caulerpa lentillifera	9.7	Lowry	McDermid and Stuercke (2003)
Caulerpa lentillifera	10.4	Kjeldahl	Matanjun *et al.* (2009)
Caulerpa sertularioides	20.0	Kjeldahl	Ramos *et al.* (2000)
Cladophora glomerata	20.4	Biuret	Manivannan *et al.* (2009)
Cladophora glomerata	14.1	Kjeldahl	Akköz *et al.* (2011)
Codium reediae	7.0	Lowry	McDermid and Stuercke (2003)
Codium tomentosum	6.1	Biuret	Manivannan *et al.* (2008)
Enteromorpha compressa	12.3	Biuret	Manivannan *et al.* (2009)
Enteromorpha flexuosa	7.9	Lowry	McDermid and Stuercke (2003)
Enteromorpha intestinalis	15.2	Kjeldahl	Akköz *et al.* (2011)
Enteromorpha intestinalis	16.4	Biuret	Manivannan *et al.* (2008)
Halimeda macroloba	28.9	Biuret	Manivannan *et al.* (2009)
Halimeda tuna	23.1	Biuret	Manivannan *et al.* (2009)
Monostroma oxyspermum	9.6	Lowry	McDermid and Stuercke (2003)
Ulva fasciata	8.8	Lowry	McDermid and Stuercke (2003)
Ulva fasciata	6.3	Kjeldahl	Ramos *et al.* (2000)
Ulva lactuca	7.1	Kjeldahl	Wong and Cheung (2000)
Ulva lactuca	27.2	Kjeldahl	Ortiz *et al.* (2006)
Ulva lactuca	3.3	Biuret	Manivannan *et al.* (2008)
Ulva lactuca	8.5	Kjeldahl	Yaich *et al.* (2011)
Ulva reticulata	13.5	Biuret	Manivannan *et al.* (2009)
Ulva reticulata	20.0	Biuret	Shanmugam and Palpandi (2008)
Brown seaweed			
Dictyota acutiloba	12.0	Lowry	McDermid and Stuercke (2003)
Dictyota sadvicensis	6.4	Lowry	McDermid and Stuercke (2003)
Durvillaea antartica (leaves)	10.4	Kjeldahl	Ortiz *et al.* (2006)
Durvillaea antartica (stem)	11.6	Kjeldahl	Ortiz *et al.* (2006)
Hizikia fusiforme	11.6	Kjeldahl	Dawczynski *et al.* (2007)
Laminaria sp.	7.5	Kjeldahl	Dawczynski *et al.* (2007)
Padina gymnospora	17.1	Biuret	Manivannan *et al.* (2008)
Padina gymnospora	11.2	Kjeldahl	Ramos *et al.* (2000)
Padina pavonica	13.6	Biuret	Manivannan *et al.* (2009)

TABLE 24.4 (*continued*)

	Protein	Methods	References
Sargassum echinocarpum	10.3	Lowry	McDermid and Stuercke (2003)
Sargassum fluitans	12.8	Kjeldahl	Ramos *et al.* (2000)
Sargassum obtusifolium	13.0	Lowry	McDermid and Stuercke (2003)
Sargassum polycystum	5.4	Kjeldahl	Matanjun *et al.* (2009)
Sargassum tenerimum	12.4	Biuret	Manivannan *et al.* (2008)
Sargassum vulgare	16.3	Kjeldahl	Ramos *et al.* (2000)
Undaria pinnatifida	19.8	Kjeldahl	Dawczynski *et al.* (2007)

Kjeldahl method based on the nitrogen-to-protein factor 6.25.

(Fleurence, 1999b). For example, McDermid and Stuercke (2003) used Lowry's method (Lowry *et al.*, 1951), which is specific for protein. Another method used for the estimation of protein is Biuret method (Manivannan *et al.*, 2008). Gressler *et al.* (2011) reported that the value of soluble protein obtained by Bradford's method (with bovine serum albumin as a standard) was quite similar to that determined by the method based on the nitrogen-to-protein factor 4.43.

B. Amino acid composition of seaweeds

All amino acids are presented in seaweeds (Matanjun *et al.*, 2009). With respect to total EAAs in the FAO/WHO (1991) pattern, seaweeds (especially red and green) seem to be able to contribute to adequate levels of total EAA (Wong and Cheung, 2000). On the contrary, Matanjun *et al.* (2009) reported higher amounts of amino acid (AA) in green seaweeds than in red and brown. Despite this, many papers indicated that EAAs in red algae represented almost half of total AAs and it meant the ratio of EAA to AA about 0.4–0.5. The ratio of EAA to nonessential amino acids (NEAAs) was about 0.7–0.9 (Gressler *et al.*, 2010; Norziah and Ching, 2000). These data agreed with the results of Galland-Irmouli *et al.* (1999) who reported that proteins of *Palmaria palmata* contained 26–50% of EAAs of AA, and its protein profile was close to the profile of egg protein. Wong and Cheung (2000) found EAA on the level of 42–48% in red and green seaweeds. Methionine and cysteine were detected in a high amount in red seaweeds than in green and brown (Qasim, 1991), but the value showed low amounts in red algae, less than 0.3% and 0.1%, respectively (Gressler *et al.*, 2011). The highest EAA was phenylalanine in species belonging to three groups: red, green, and brown algae (Matanjun *et al.*, 2009). Glycine,

alanine, arginine, proline, glutamic, and aspartic acids composed together a large part of the AAs fraction, whereas AAs tyrosine, methionine, and cysteine occurred in a lower amount (Gressler *et al*., 2010; Norziah and Ching, 2000) on contrary to the results of Qasim (1991). However, Ortiz *et al.* (2006) concluded that brown alga *Durvillaea antartica* stood out of a high level of histidine and valine. Seaweeds have the amounts of glutamic and aspartic acids in a range from 15% to 44%.

In the case of NEAAs, glutamic and aspartic acid constituted a predominant quantity of AA. In brown seaweeds, it represented 20–44% (Fleurence, 1999a; Munda, 1977; Wong and Cheung, 2000), in green seaweeds 26–32% (Fleurence *et al*., 1995), and in red seaweeds 14–19% (Fujiwara-Arasaki *et al*., 1984), respectively. Glutamic acid (or a salt form) is intricately involved in sustaining proper function of the brain and its mental activity. Aspartic acid, in a form of energy, helps initialize two of the body's most important pathways (Krebs and urea cycles) (Braverman *et al*., 2003). But it is needed to consider that no author analyzed all AAs, namely, tryptophan and cysteine, consequential in differences in a sum of AAs (Gressler *et al*., 2011). In general, all three groups, green, brown, and red seaweeds, contain the similar amount of NEAA (Matanjun *et al*., 2009). Seaweeds contain also nonprotein nitrogenous materials, such as free AAs, chlorophyll, nitrate and nitrite nitrogen, ammonium ions, and nucleic acids. Only free AAs are probably in a connection with typical flavors and taste, especially glutamic and aspartic acid (Yaich *et al*., 2011), and also glycine and alanine (Norziah and Ching, 2000). Therefore, it could be assumed that this amount is not significant because of a very comparable value of the crude protein and AA content (Qasim, 1991).

Many methods with different analysis condition are used for the determination of AAs. These methods conclude two steps: hydrolysis of substrate, and chromatographic separation and detection of residues. Hydrolysis is the most critical part which is affected by several factors such as temperature, time, hydrolysis agent, and additives (Fountoulakis and Lahm, 1998). Weiss *et al*. (1998) focused on the effects of the hydrolysis method on the AA content and composition of protein with a conclusion that the conventional hydrolysis delivered more accurate data in comparison with the microwave radiation-induced hydrolysis.

C. Nutritional evaluation

Protein quality is evaluated by different methods with various patterns of standard; hence it is very difficult to compare the published data. Chosen standard, which is used for the computation of these factors, strongly affects the limiting AA. Rama *et al.* (1964) identified the highly significant correlation with the biological value of 11 protein samples from 15.

In spite of this fact, in most studies, amino acid score (AAS), index of essential amino acid, and egg protein have been applied. Seaweeds have usually low AAS (about 20–67%); nevertheless, it is common similarly to other plants (Matanjun *et al.*, 2009). Matanjun *et al.* (2009) showed that *Sargassum polycystum* had AAS higher (67%) than soybeans (47%) or casein (58%) and then this value could be compared with beef (69%).

As limiting acids depending on the species and standard protein, lysine was indicated (Matanjun *et al.*, 2009, Mišurcová *et al.*, 2010; Norziah and Ching, 2000; Ortiz *et al.*, 2006; Ramos *et al.*, 2000), also leucine and isoleucine (Ortiz *et al.*, 2006), and further as the second limiting amino acid, it was methionine (Ramos *et al.*, 2000).

In comparison to the standard protein, seaweed proteins (as well as other plant proteins) are not full-valued proteins because of low amounts of some amino acids. Nevertheless, the presence of all EAAs in the considerable quantities indicates that seaweed proteins are nutritionally superior to the terrestrial plant proteins (Qasim, 1991). In Tables 24.3 and 24.4, there are presented protein contents of some seaweed demonstrating their variability.

In vitro protein digestibility of seaweed proteins is influenced by the species, seasonal period, and content of antinutritional factors such as phenolic and polysaccharides (Fleurence, 1999a; Mabeau and Fleurence, 1993). Activity of proteolytic enzymes may be reduced due to the reaction of amino acids with oxidized phenolic compound (Wong and Cheung, 2001). Differences between the prediction of protein quality (based on amino acid content/amino acid requirement ratios) and the actual protein quality (based on the performance in living organisms) seem to be a reason of variations in the utilization of the amino acids comprised in different proteins.

REFERENCES

Akköz, C., Arslan, D., Ünver, A., Özcan, M. M., and Yimaz, B. (2011). Chemical composition, total phenolic and mineral contents of *Enteromorpha intestinalis* (L.) Kűtz. and *Cladophora glomerata* (L.) Kűtz. seaweeds. *J. Food Biochem.* **35**, 513–523.

Bankir, L. and Kriz, W. (1995). Adaptation of the kidney to protein intake and to urine concentrating activity: Similar consequences in health and CRF. *Kidney Int.* **47**, 7–24.

Becker, G. W. and Smith, K. (2006). Basic metabolism III: Protein. *Surgery* **24**, 115–120.

Braverman, E. R., Pfeiffer, C. C., Blum, K., and Smayda, R. (2003). Glutamate amino acids. *In* ''The Healing Nutrients Within: Facts, Findings, and New Research on Amino Acids'', (Ch. Hirsch, Ed.), pp. 165–200. Basic Health Publications, Inc., Laguna Beach.

Bröer, S. (2008). Amino acid transport across mammalian intestinal and renal epithelia. *Physiol. Rev.* **88**, 49–286.

Calle, E. E. and Kaaks, R. (2004). Overweight, obesity and cancer: Epidemiological evidence and proposed mechanisms. *Nat. Rev. Cancer* **4**, 579–591.

Chow, W. H., Gridley, G., McLaughlin, J. K., Mandel, J. S., Wacholder, S., Blot, W. J., Niwa, S., and Fraumeni, J. F. (1994). Protein intake and risk of renal cell cancer. *J. Natl. Cancer Inst.* **86**, 1131–1139.

Darcy-Vrillon, B. (1993). Nutritional aspects of the developing use of marine macroalgae for the human food industry. *Int. J. Food Sci. Nutr.* **44**, 23–35.

Dawczynski, Ch., Schubert, R., and Jahreis, G. (2007). Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chem.* **103**, 891–899.

De Onís, M., Monteiro, C., Akré, J., and Clugston, G. (1993). The worldwide magnitude of protein-energy malnutrition: An overview from the WHO Global Database on Child Growth. *Bull. World Health Org.* **71**, 703–712.

Denis, C., Morançais, M., Li, M., Deniaud, E., Gaudin, P., Wielgosz-Collin, G., Barnathan, G., Jaouen, P., and Fleurence, J. (2010). Study of the chemical composition of edible red macroalgae *Grateloupia turuturu* from Brittany (France). *Food Chem.* **119**, 913–917.

Despopoulos, A. and Silbernagl, S. (2009). Nutrition and digestion. *In* ''Color Atlas of Physiology''. pp. 228–267. Thieme, New York.

FAO (2002). Prospects for seaweed production in developing countries. FAO Fisheries Circular, 968. FAO, Rome.

FAO (2003). A guide to the seaweed industry. FAO Fisheries Technical Paper 441. FAO, Rome.

FAO/WHO (1985). Report of a Joint FAO/WHO/UNU Expert Consultation. WHO Technical Report Series 724. Energy and protein requirements. WHO Press, Geneva.

FAO/WHO (1991). Report of a Joint FAO/WHO/UNU Expert Consultation. FAO Food and Nutritional Paper 51. Protein Quality Evaluation. WHO Press, Rome.

Feoli, A. M., Siqueira, I. R., Almeida, L., Tramontina, A. C., Vanzella, C., Sbaraini, S., Schweigert, I. D., Netto, C. A., Perry, M. L. S., and Gonçalves, C. A. (2006). Effects of protein malnutrition on oxidative status in rat brain. *Nutrition* **22**, 160–165.

Fleurence, J. (1999a). Seaweed proteins: Biochemical, nutritional aspects and potential uses. *Trends Food Sci. Technol.* **10**, 25–28.

Fleurence, J. (1999b). The enzyme degradation of algal cell walls: A useful approach for improving protein accessibility? *J. Appl. Phycol.* **11**, 313–314.

Fleurence, J., Le Coeur, C., Mabeau, S., Maurice, M., and Landrein, A. (1995). Comparison of different extractive procedures for proteins from the edible seaweeds *Ulva rigida* and *Ulva rotundata*. *J. Appl. Phycol.* **7**, 577–582.

Fontana, L., Klein, S., and Holloszy, J. O. (2006). Long-term low-protein, low-calorie diet and endurance exercise modulate metabolic factors associated with cancer risk. *Am. J. Clin. Nutr.* **84**, 1456–1462.

Food and Nutrition Board (2005). Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). The National Academies Press, Washington, DC.

Fountoulakis, M. and Lahm, H. W. (1998). Hydrolysis amino acid composition analysis of proteins. *J. Chromatogr. A* **826**, 109–134.

Friedman, M. (1996). Nutritional value of proteins from different food sources. A review. *J. Agric. Food Chem.* **44**, 6–29.

Fujiwara-Arasaki, T., Mino, N., and Kuroda, M. (1984). The protein value in human nutrition of edible marine algae in Japan. *Hydrobiologia* **116–117**, 513–516.

Galland-Irmouli, A. V., Fleurence, J., Lamghari, R., Luçon, M., Rouxel, C., Barbaroux, O., Bronowicki, J. P., Villaume, Ch., and Guéant, J. L. (1999). Nutritional value of proteins from edible seaweed *Palmaria palmata* (dulse). *J. Nutr. Biochem.* **10**, 353–359.

Gressler, V., Yokoya, N. S., Fujii, M. T., Colepicolo, P., Mancini-Filho, J., Torres, R. P., and Pinto, E. (2010). Lipid, fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. *Food Chem.* **120**, 585–590.

Gressler, V., Fujii, M. T., Martins, A. P., Colepicolo, P., Mancini-Filho, J., and Pinto, E. (2011). Biochemical composition of two red seaweed species grown on the Brazilian coast. *J. Sci. Food Agric.* **91**, 1687–1692.

Gropper, S. S., Smith, J. L., and Groff, J. L. (2008). Protein. *In* ''Advanced Nutrition and Human Metabolism'', (P. Adams, Ed.), pp. 179–250. Wadsworth, Belmont.

Grover, Z. and Ee, L. C. (2009). Protein energy malnutrition. *Pediatr. Clin. North Am.* **56**, 1055–1068.

Ito, K. and Hori, K. (1989). Seaweed: Chemical composition and potential food uses. *Food Rev. Int.* **5**, 101–144.

Kies, C. (1981). Bioavailability: A factor in protein quality. *J. Agric. Food Chem.* **29**, 435–440.

Lorenço, S. O., Barbarino, E., De-Paula, J. C., Otávio da Pereira, L. S., and Marquez Lanfer, U. M. (2002). Amino acid composition, protein, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. *Phycol. Res.* **50**, 233–241.

Losos, J. B., Mason, K. A., and Singer, S. R. (2008). Proteins. *In* ''Biology'', (P. E. Reidy and A. L. Winch, Eds), pp. 43–53. McGraw Hill Companies, Inc., New York.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

Mabeau, S. and Fleurence, J. (1993). Seaweed in food products: Biochemical and nutritional aspects. *Trends Food Sci. Technol.* **4**, 103–107.

Manivannan, K., Thirumaran, G., Karthikai Devi, G., Hemalatha, A., and Anantharaman, P. (2008). Biochemical composition of seaweeds from Mandapam coastal regions along southeast cost of India. *Am-Euras. J. Bot.* **1**, 32–37.

Manivannan, K., Thirumaran, G., Karthikai Devi, G., Anantharaman, P., and Balasubramanian, T. (2009). Proximate composition of different group of seaweeds from Vedalai Coastal waters (Gulf of Mannar): Southeast coast of India. *Middle East J. Sci. Res.* **4**, 72–77.

Marshall, K. (2005). Role of proteins in the body. *In* ''Basic Health Publications User's Guide to Protein and Amino Acids'', (J. Challem and T. Dukrin, Eds), pp. 15–20. Basic Health Publications, Inc., Laguna Beach.

Marsham, S., Scot, G. W., and Tobin, M. L. (2007). Comparison of nutritive chemistry of a range of temperate seaweeds. *Food Chem.* **100**, 1331–1336.

Matanjun, P., Mohamed, S., Mustapha, N. M., and Muhammad, K. (2009). Nutrient content of tropical edible seaweeds, *Eucheuma cottoni*, *Caulerpa lentillifera* and *Sargassum polycysum*. *J. Appl. Phycol.* **21**, 75–80.

McDermid, K. J. and Stuercke, B. (2003). Nutritional composition of edible Hawaiian seaweeds. *J. Appl. Phycol.* **15**, 513–524.

Mišurcová, L., Kráčmar, S., Klejdus, B., and Vacek, J. (2010). Nitrogen content, dietary fiber, and digestibility in algal food products. *Czech. J. Food Sci.* **28**, 27–35.

Munda, I. M. (1977). Differences in amino acid composition of estuarine and marine fucoids. *Aquat. Bot.* **3**, 273–280.

Nnakwe, N. (1996). The effect and causes of protein-energy malnutrition in Nigerian children. *Nutr. Res.* **15**, 785–794.

Norziah, M. H. and Ching, C. Y. (2000). Nutritional composition of edible seaweed *Gracilaria changii*. *Food Chem.* **68**, 69–76.

Oohusa, T. (1993). Recent trends in nori products and markets in Asia. *J. Appl. Phycol.* **5**, 155–159.

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., Navarrete, E., Osorio, A., and Rios, A. (2006). Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. *Food Chem.* **99**, 98–104.

Padula, G., Salceda, S., and Seoane, A. I. (2009). Protein-energy malnutrition contributes to increased structural chromosomal alteration frequencies in Argentinean children. *Nutr. Res.* **29**, 35–40.

Preston, R. L., Schaeffer, J. F., and Curran, P. F. (1974). Structure-affinity relationships of substrates for the neutral amino acid transport system in rabbit ileum. *J. Gen. Physiol.* **64**, 443–467.

Qasim, R. (1991). Amino acids composition of some common seaweeds. *Pak. J. Pharm. Sci.* **4**, 49–54.

Rama, P. B., Norton, H. W., and Johnson, C. (1964). The amino acid composition and nutritive value of proteins. V. Amino acid requirements as a pattern for protein evaluation. *J. Nutr.* **82**, 88–92.

Ramos, M. V., Monteiro, C., Moreira, R. A., and Carvalho, A. F. F. U. (2000). Amino acid composition of some Brazilian seaweed species. *J. Food Biochem.* **24**, 33–39.

Renaud, S. M. and Luong-Van, J. T. (2006). Seasonal variation in the chemical composition of tropical Australian marine macroalgae. *J. Appl. Phycol.* **18**, 381–387.

Roslin, S. (2003). Seasonal variations in the protein content of marine algae in relation to environmental parameters in Arockiapuram coast. *Seaweed Res. Utilin.* **16**, 13–16.

Shanmugam, A. and Palpandi, C. H. (2008). Biochemical composition and fatty acid profile of the green alga *Ulva reticulata*. *Asian J. Biochem.* **3**, 26–31.

Stephenson, L. S., Lathan, M. C., and Ottesen, E. A. (2000). Global malnutrition. *Parasitology* **121**, 5–22.

USDA (2010). Composition of Foods Raw, Processed, Prepared. USDA National Nutrient Database for Standard Reference. Release 23. U.S. Department of Agriculture, Beltsville.

Vlajinac, H. D., Marinkovic, J. M., Ilic, M. D., and Kocev, N. I. (1997). Diet and prostate cancer: A case-control study. *Eur. J. Cancer* **33**, 101–107.

Weiss, M., Manneberg, M., Juranville, J.-F., Lahm, H.-W., and Fountoulakis, M. (1998). Effect of the hydrolysis method on the determination of the amino acid composition of proteins. *J. of Chromatogr. A.* **795**, 263–275.

Whitney, E. and Rolfes, S. R. (2008). Protein: Amino acids. *In* ''Understanding Nutrition'', (P. Adams, Ed.), pp. 180–211. Thomson Higher Education, Belmont.

WHO (2000). Turning the Tide of Malnutrition: Responding to the Challenge of the 21st Century. WHO, Geneva.

WHO (2002). Report of Joint WHO/FAO/UNU Expert Consultation, WHO Technical Report Series 935. Protein and Amino Acid Requirements in Human Nutrition. WHO Press, Geneva.

Wong, K. H. and Cheung, P. C. K. (2000). Nutritional evaluation of some subtropical red and green seaweeds. Part I—Proximate composition, amino acid profiles and some physico-chemical properties. *Food Chem.* **71**, 475–482.

Wong, K. H. and Cheung, P. C. K. (2001). Nutritional evaluation of some subtropical red and green seaweeds. Part II. In vitro protein digestibility and amino acid profiles of protein concentrates. *Food Chem.* **72**, 11–17.

Yaich, H., Garna, H., Besbes, S., Paquot, M., Blecker, Ch., and Attia, H. (2011). Chemical composition and functional properties of *Ulva lactuca* seaweed collected in Tunisia. *Food Chem.* **128**, 895–901.

Zimmermann, K. H. (2003). Proteins. *In* ''An Introduction to Protein Informatics'', (M. Ismail, Ed.), pp. 5–22. Springer-Verlag, New York, LLC.