===== **PLANT PHYSIOLOGY** =====

Assessment of Fatty Acid and Amino Acid Composition of Macroalgae from the Persian Gulf to Characterize their Suitability for Nutritional Supplements

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Abstract—Macroalgae, commonly known as seaweed, can be found worldwide. Seaweeds offer a novel dietary ingredient because of their excellent biochemical composition and health protective effects of carbohydrates, proteins, amino acids (AAs), fatty acids (FAs), and bioactive compounds. This study aimed to investigate and compare the AAs and FAs profile of 10 green, red, and brown seaweeds collected from Iranian coasts. The results indicated that, red seaweeds had a much higher ash content than the other species. Furthermore, red seaweeds had the highest and lowest lipid contents (1.5−4% D.W.), with significantly ($p < 0.05$) elevated concentrations of lipids observed in *Champia globulifera*. Palmitic acid (C16:0) and oleic acid (C18:2) were the predominant saturated and unsaturated FAs found in all assayed species. The highest eicosapentaenoic acids (EPA) were found in *Cystoseira myrica* (6.02% methyl esters of FA [FAME]). All species had n-6/n-3 FA ratios (approx. ≤1) favorable for human health. The protein content was the highest in the brown species (4.3% D.W.). All samples presented a complete profile of essential AAs. In all cases, leucine and phenylalanine were predominant. The ratio of essential AAs/non-essential AAs (EAAs/NEAAs) ranged from 1.17 to 2.65, which was higher in the brown seaweeds compared with the other species. These findings highlight the potential of using seaweeds as an alternative and sustainable source of fatty acids and amino acids for nutritional supplements and industrial food processing.

Keywords: seaweed, nutritional compounds, protein, lipid, pca, persian gulf

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INTRODUCTION

Macroalgae, marine seaweed, are rich in various biologically active substances valued for their unique properties can play a vital role in the food industry. Foods, food ingredients, nutraceuticals, and feeds are the major commercial products obtained from macroalgae for both human and animal use (Śliwińska-Wilczewska et al., 2020). Global utilization of macroalgae is a multibillion dollar industry. The world production of seaweed has tripled approximately, from 10.6 million tonnes in 2000 to 32.4 million tonnes in 2018, which some species (e.g. *Caulerpa* spp., *Undaria pinnatifida*, and *Porphyra* spp.) are produced primarily as human food (FAO, 2020). Furthermore, seaweeds have several advantages that distinguish them from terrestrial crops as food sources include simple and rapid growth, ability to grow in non-arable land, highly efficient productivity, and ease of cultivation and moving (Zarei Jeliani et al., 2021, 2017).

From a nutritional point of view, seaweeds contain a high range of nutritionally beneficial compounds and they are increasingly gaining attention in many

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Abbreviations: AA—Amino acid; DHA—Docosahexaenoic; FAs—Fatty acids; EAAs—Essential amino acids; EPA—Eicosapentaenoic acids; FAMEs—Methyl esters of fatty acid; MUFA—Monounsaturated fatty acids; NEAAs—Non-essential amino acids; PUFA—Polyunsaturated fatty acids; PCA—Principal Component Analysis.

countries. They are low-calorie foods that contain high concentration of minerals (Mg, Ca, P, K, and Na), vitamins, proteins, essential amino acids (EAAs), polyunsaturated fatty acids (PUFAs) highlighting the eicosapentaenoic acid (EPA) that belong to the family of ω-3 fatty acids, and indigestible sulfated carbohydrates as thickening agents (alginate, agar, and carrageenan) (Penalver et al., 2020). These properties enable seaweed to have tremendous potential as a supplement in functional food or for the extraction of compounds. Shreadah et al. (2018) have reported that brown seaweeds are excellent sources of natural products for nutritional and pharmaceutical applications.

Both humans and most animals do not possess enzymes to form double bonds at the n-3 and n-6 positions of the FA carbon chain. Therefore, humans must obtain the essential FAs from dietary sources. Long-chain n-3 PUFAs, such as docosahexaenoic (DHA, C 22:6) and eicosapentaenoic (EPA, C 20:5) acids have favorable effects in humans in terms of nutraceutical use. Although macroalgae represented low lipid contents, their PUFA contents are equivalent or higher than those of terrestrial crops. Furthermore, seaweeds are rich in PUFAs of the n-3 and n-6 series can play a vital role in growth and are suitable for food and pharmaceutical applications (Sohrabipour et al., 2019 ; Verma et al., 2016).

Proteins are a major class of macromolecules, essential for food in the human diet. Nine of the 21 AAs most commonly used in higher organisms are classified as essential amino acids (EAAs) in humans, because they cannot be synthesized from other AAs; therefore, they must be consumed in the diet. Seaweed proteins contain significant amounts of all of the EAAs (Pirian et al., 2017, 2020). In addition, the EAA contents of some species can be compared with those of terrestrial crops. For instance, one gram of macroalgae with the high protein levels such as *Enteromorpha intestinalis* and *Palmaria palmate*, contains equal to or higher amounts of all of the EAAs compared to other protein-rich foods, such as soybean, rice, corn, and wheat (Biris-Dorhoi et al., 2020).

Although the distribution of seaweed on the Persian Gulf coastlines in the South of Iran has been studied, their nutritional properties are not completely known. Data on the nutritional value concerning FAs and AAs composition of these seaweeds are sparse (Akbary et al., 2020; Pirian et al., 2020; Pirian et al., 2017). Here, we present results for two Chlorophyceae, two Phaeophyceae, and six Rhodophyceae species of seaweeds collected from the northern coast of Persian Gulf. Our aim was to expand the currently limited database for seaweeds from Iran, examine taxonomically connected differences in their FA and AA profiles, and identify species with high levels of important PUFA and EAAs that might be potentially valuable resources for human consumption or other commercial applications.

MATERIALS AND METHODS

Sample Collection and Preparation

We gathered 10 types mature thalli of seaweed, two species (*Ulva intestinalis* Linnaeus, *Ulva clathrata* var. confervoides C. Agardh) from Chlorophyta, two species (*Sargassum angustifolium* C. Agardh, *Cystoseira myrica* S. G. Gmelin C. Agardh) from Phaeophyta, and six species (*Acanthophora muscoides* Linnaeus Bory, *Chondria papillosa* C. Agardh, *Centroceras clavulatum* C. Agardh Montagne, *Champia parvula* C. Agardh Harvey, *Champia globulifera* Børgesen and *Hypnea pannosa* J. Agardh) from Rhodophyta, from the intertidal zone of the Iranian side of the Persian Gulf in April 2019 for analysis in the present study (Table 1). Morphological identification of the analyzed algae was carried out with standard keys (Kokabi and Yousefzadi, 2015; Sohrabipour and Rabei, 2007, 2008). Immediately after collection, the seaweeds were cleaned and washed with seawater to remove sand, epiphytes and other extraneous matter before they were transported to the laboratory. Then, the samples were rinsed with distilled water and dried at room temperature. The dried samples were ground into a fine powder and kept at 4°C for further analysis.

Chemical Analysis of the Seaweeds (Total Lipid, Protein, and Ash Contents)

The total lipid content of the samples was extracted according to Bligh and Dyer (Bligh and Dyer, 1959). Briefly, the dried algal powder was placed into a glass vial and a 2 : 1 (*v/v*) mixture of chloroform: methanol was added. The mixture was heated at 60°C for 1 h and then filtered (Whatman GF/A filter) to remove the particles. The filtered crude extract was washed with 0.9% NaCl solution, mixed by vortex, and allowed to rest for 30 min until an upper and lower phase were established. The upper phase was removed and the lower one that contained the lipids was evaporated under a gentle stream of nitrogen. The lipid extract was then weighed and recorded as the percentage of dry weight of the sample.

The total protein content was estimated by the Bradford method (Bradford, 1976). The protein was measured by absorbance (595 nm) of each sample and different concentrations of bovine serum albumin (BSA) were prepared as the standard. Finally, the protein contents of the samples were estimated based on the BSA curve and expressed as percentages of dry weight.

Ash contents of the samples were determined according to the method described by the Association of Official Analytical Chemists (AOAC, 2000). Briefly, 5 g of the dried algae sample was kept at 525°C for 6 h in a blast furnace and then weighed. The ash content was expressed as the percentage of the dry weight sample.

Table 1. Seaweed species, collection details, and ash contents (% D.W.) from the Persian Gulf, Iran

	Species	Taxonomy	Latitude and longitude	Ash
Brown algae	1. *Sargassum angustifolium*	Ph, Fucophycidae, Fucales, Sargassaceae	Bandar Lengeh (Kowsar Beach) N 26°33′25″ E 54°53′29″	34.6 ± 0.0[de]
	2. *Cystoseira myrica*	Ph, Fucophycidae, Fucales, Sargassaceae	Bandar Lengeh (Kowsar Beach) N 26°33′25″ E 54°53′29″	33.1 ± 1.7[e]
Green algae	3. *Ulva intestinalis*	Chl, Ulvophyceae, Ulvales, Ulvaceae	Bandar Abbas (Ghadir Blvd) N 27°10′59″ E 56°19′09″	45.0 ± 1.0[b]
	4. *Ulva clathrata*	Chl, Ulvophyceae, Ulvales, Ulvaceae	Bandar Abbas (Ghadir Blvd) N 27°10′59″ E 56°19′09″	34.7 ± 0.4[d]
Red algae	5. *Acanthophora muscoides*	Rh, Florideophyceae, Ceramiales, Rhodomelaceae	Bandar Bushehr (Shaghab) N 28°55′07″ E 50°48′37″	39.8 ± 1.0[c]
	6. *Chondria papillosa*	Rh, Florideophyceae, Ceramiales, Rhodomelaceae	Bandar Bushehr (Shaghab) N 28°55′07″ E 50°48′37″	35.9 ± 0.7[d]
	7. *Champia parvula*	Rh, Florideophyceae, Rhodymeniales, Champiaceae	Bandar Bushehr (Shaghab) N 28°55′07″ E 50°48′37″	52.5 ± 0.5[a]
	8. *Hypnea pannosa*	Rh, Florideophyceae, Gigartinales, Cystocloniaceae	Qeshm Island (Botanical garden) N 26°58′26″ E 56°15′18″	51.0 ± 1.3[a]
	9. *Centroceras clavulatum*	Rh, Florideophyceae, Ceramiales, Ceramiaceae	Bandar Bushehr (Shaghab) N 28°55′07″ E 50°48′37″	38.2 ± 1.1[c]
	10. *Champia globulifera*	Rh, Florideophyceae, Rhodymeniales, Champiaceae	Qeshm Island (Botanical garden) N 26°58′26″ E 56°15′18″	51.4 ± 0.8[a]

Means with different letters indicate a significant difference at $p \leq 0.05$ using Duncan multiple range test.

Fatty Acids Analysis and GC-Conditions

GC with Flame Ionization Detector (GC-FID) analysis was used. The methyl esters of fatty acid (FAMEs) were prepared according to a modified method published by Miller and Berger (1985). Initially, a solution that contained 100 mg of each dried seaweeds and 2 mL NaOH (1.2 mol/L) in aqueous methanol (50%) were boiled for 30 min (saponification). For methylation, the samples were acidified with 0.6 mL of HCl (10 mol/L) followed by the addition of 1 mL methanolic BCl_3 (12%) as the catalyst, and the mixture was heated for 10 min at 85°C. Then, 1 mL of hexane/diethyl ether (1 : 1) was added for FAME extraction. Finally, 3 mL of NaOH (0.3 mol/L) was added to the extracted FAMEs. The organic phase (FAMEs) was transferred and completely evaporated by nitrogen flushing. Chloroform was added to the samples prior to injection into the gas chromatograph (Varian, 3800) that was equipped with a fused silica capillary column BPX 70 (25 m × 0.32 mm, film thickness 0.25 μm) and a flame ionization detector. The carrier gas (helium) was run through a temperature gradient of 160 to 230°C, at an increasing increment of 1.5°C min^{-1}. The initial and final times were 0 and 15 min, and the total run time was 50 min. FA identification was performed using external standards (SUPELCO F.A.M.E. Mix C4-C24). FA composition was calculated from the total identified FA area and the values were averages of at least three injections of each sample (Pirian et al. 2017; 2020).

Amino Acids Analysis

For AA analysis, the dried seaweeds were hydrolyzed with hydrochloric acid (6 N) in evacuated sealed tubes for 24 h at 110°C. After digestion, the samples were filtered and 10 μL of them were transferred to 30 mL digestion tubes and dried under vacuum at 40°C. Then samples were prepared using a mixture of sodium acetate buffer-methanol (5 : 1) and borate buffer and then derived with opaco-phthaldialdehyde. 20 mL of the prepared sample was injected into the HPLC (Column: C18, Detector: Knauer rf-530, UV Absorbance Detector), and the amino acid uptake was measured using a 570 nm UV-visible detector and a set of amino acid standards (Sigma chemicals) was analyzed with each set of experimental samples. The identification of the amino acid in the sample was carried out by comparison with retention times of the standards (Pirian et al. 2017, 2020).

Statistical Analysis

One-way ANOVA was performed to reveal statistically significant differences ($p \leq 0.05$) between the samples using SPSS software (version 26). The charts were drawn in Microsoft Excel 2016. In order to improve the clarity of the results and to study the rela-
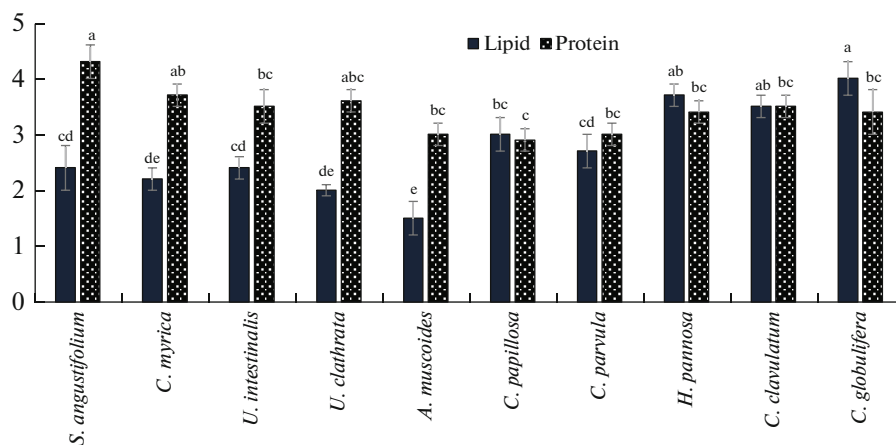
Fig. 1. Total protein and lipid contents of the analyzed seaweeds (% D.W.). Means with different letters indicate a significant difference at $p \leq 0.05$ using Duncan multiple range test.
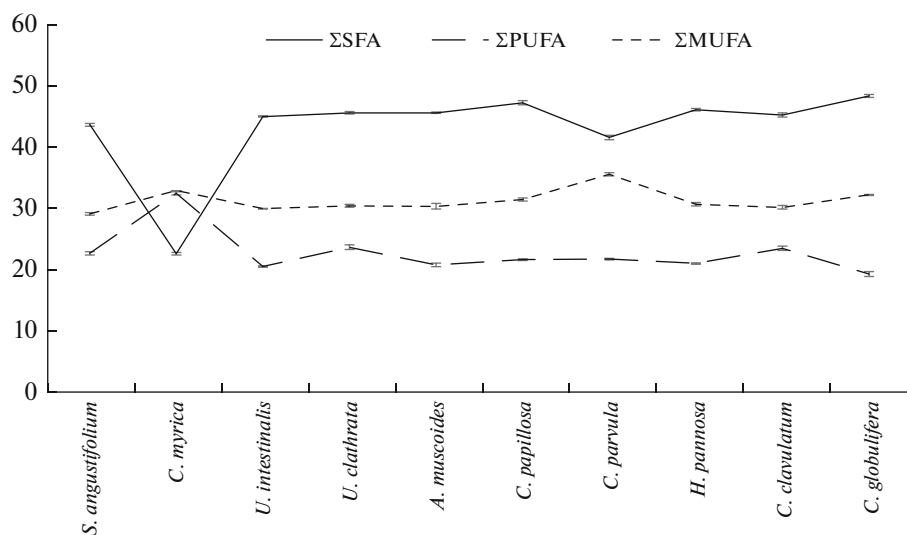

Fig. 2. Comparison of saturated fatty acids (SFAs) and Unsaturated fatty acids (USFAs) amongst analyzed seaweeds.

tionship between them a principal component analysis (PCA) was performed with publicly available Past 3.16 software.

RESULTS

The biochemical constituents (total lipid, protein, and ash contents), fatty acid and amino acid composition of 10 different seaweed species from three different genera of seaweeds collected from Iranian sea coast i.e. Bandar Lengeh, Bandar Abbas, Bandar Bushehr, and Qeshm Island are shown in Tables 1 to 3 and Figs. 1 to 5.

There were significant differences amongst seaweeds according to species and phyla for all the proximate composition variables (Table 1, Fig. 1). In this study, the ash content differed amongst the species

(33 to 52% dry weight [D.W.]). The recorded ash content ranged from 34.7−45% in green seaweeds, 35.9−52.5% in red seaweeds, and 33.1−34.6% in brown seaweeds. *C. parvula*, *C. globulifera*, and *H. pannosa* (~52% D.W.), followed by *U. intestinalis* (45.0% D.W.) had substantially greater ash content than the other species. *C. myrica* (33.1% D.W.) had the lowest ash content amongst the analyzed species.

The range of lipid content recorded was 1.5−4% D.W. Red seaweeds *C. globulifera, H. pannosa,* and *C. clavulatum* showed the highest total lipid contents (4, 3.7, and 3.5% D.W. respectively). The lipid content of *A. muscoides* were significantly lower than the other species (1.5% D.W.) (Fig. 1). Fatty acids profile of the macroalgal species expressed as percentage of FAME, are presented in Table 2. In the analyzed seaweed varieties, the sum of the saturated fatty acid (SFA) was

Table 2. Fatty acids (FAs) profile of the algal species (% methyl esters of FA [FAME])

Fatty acids (FAs)	Brown algae	Green algae			Red algae					
	S. angustifolium	C. myrica	U. intestinalis	U. clathrata	A. muscoides	C. papillosa	C. parvula	H. pannosa	C. clavulatum	C. globulifera
C12:0	0.34[a]	ND	0.35[a]	0.35[a]	0.35[a]	0.36[a]	0.37[a]	0.35[a]	0.35[a]	0.37[a]
C14:0	5.27[a]	2.21[c]	5.45[a]	5.51[a]	5.52[a]	5.72[a]	3.79[b]	5.58[a]	5.46[a]	5.85[a]
C16:0	37.13[e]	18.14[f]	38.37[d]	38.81[cd]	38.90[cd]	40.28[b]	36.52[e]	39.32[c]	38.50[cd]	41.22[a]
C18:0	0.14[b]	1.75[a]	0.14[b]	0.14[b]	0.14[b]	0.15[b]	0.15[b]	0.14[b]	0.14[b]	0.15[b]
C20:0	0.42[b]	0.61[a]	0.39[b]	0.44[b]	0.40[b]	0.41[b]	0.41[b]	0.40[b]	0.44[b]	0.42[b]
C22:0	0.43[b]	ND	0.40[b]	0.45[b]	0.40[b]	0.42[b]	0.42[b]	0.41[b]	0.45[b]	0.43[b]
C16:1 n–7	3.72[b]	9.85[a]	3.85[b]	3.89[b]	3.90[b]	4.04[b]	4.08[b]	3.94[b]	3.86[b]	4.14[b]
C18:1 n–9	24.14[f]	20.88[g]	24.95[e]	25.23[de]	25.29[de]	26.19[c]	30.25[a]	25.56[d]	25.03[de]	26.80[b]
C18:2 n–6 (LA)	3.15[b]	12.35[a]	3.25[b]	3.29[b]	3.29[b]	3.41[b]	3.44[b]	3.33[b]	3.26[b]	3.49[b]
C18:3 n–3 (ALA)	2.96[b]	3.45[a]	3.06[ab]	3.09[ab]	3.10[ab]	3.21[ab]	3.24[ab]	3.13[ab]	3.07[ab]	3.28[ab]
C18:4 n–3 (SDA)	5.53[ab]	4.59[b]	5.13[ab]	5.77[a]	5.20[ab]	5.38[ab]	5.43[ab]	5.25[ab]	5.73[a]	5.51[ab]
C20:1 n–9	1.35[b]	2.25[a]	1.25[b]	1.41[b]	1.27[b]	1.32[b]	1.33[b]	1.28[b]	1.40[b]	1.35[b]
C20:4 n–6 (ARA)	5.26[a]	6.15[a]	3.72[b]	5.49[a]	3.77[b]	3.91[b]	3.94[b]	3.81[b]	5.45[a]	4.00[b]
C20:5 n–3 (EPA)	4.98[b]	6.02[a]	4.63[b]	5.21[b]	4.69[b]	4.56[b]	4.90[b]	4.74[b]	5.17[b]	2.23[c]
C22:4 n–6	0.89	ND	0.83	0.93	0.84	0.87	0.88	0.85	0.92	0.89
ΣSFA	43.73[e]	22.71[g]	45.1[d]	45.7[cd]	45.71[cd]	47.34[b]	41.66[f]	46.2[c]	45.34[d]	48.44[a]
ΣMUFA	29.21[f]	32.98[b]	30.05[e]	30.53[de]	30.46[de]	31.55[c]	35.66[a]	30.78[d]	30.29[de]	32.29[b]
ΣPUFA	22.77[c]	32.56[a]	20.62[e]	23.78[b]	20.89[e]	21.74[d]	21.83[d]	21.11[de]	23.60[b]	19.40[f]
SFA/UFA	0.84	0.35	0.89	0.84	0.89	0.89	0.72	0.89	0.84	0.94
n-6/n-3 PUFA	0.7	1.3	0.6	0.7	0.6	0.6	0.6	0.6	0.7	0.8

ND: Not detected, ΣSFA: Sum of saturated fatty acids; ΣMUFA: Sum of monounsaturated fatty acids; ΣPUFA: Sum of polyunsaturated fatty acids; SFA/UFA: Saturated fatty acids to unsaturated fatty acids; LA: Linolenic acid; ALA: α– linolenic acid; SDA: Stearidonic acid; ARA: Arachidonic acid; EPA: Eicosapentaenoic acid. Means with different letters indicate a significant difference at $p \leq 0.05$ using Duncan multiple range test.

Table 3. Amino acids (AAs) profiles of algal species (100 g^{-1} protein)

Amino acids (AAs) g/100 g total protein	Brown algae		Green algae		Red algae					
	S. angustifolium	C. myrica	U. intestinalis	U. clathrata	A. muscoides	C. papillosa	C. parvula	H. pannosa	C. clavulatum	C. globulifera
Essential amino acids (EAA)										
Leucine	8.10[bc]	12.31[a]	8.43[b]	7.02[fg]	7.30[ef]	8.07[bcd]	7.60[cde]	7.52[def]	6.56[g]	6.48[g]
Phenyl alanine	8.01[b]	9.84[a]	6.88[cd]	6.95[cd]	8.08[b]	7.98[b]	8.42[b]	7.43[bc]	6.48[cd]	6.41[d]
Threonine	3.83[bcd]	6.62[a]	4.26[bc]	3.32[def]	4.14[bc]	3.82[bcde]	4.31[b]	3.55[cdef]	3.10[ef]	3.06[f]
Tyrosine	3.83[abc]	2.73[e]	3.06[de]	3.32[cd]	4.02[ab]	3.82[abc]	4.19[a]	3.55[bcd]	3.10[de]	3.06[de]
Methionine	3.13[b]	5.89[a]	2.95[b]	2.71[b]	3.06[b]	3.12[b]	3.18[b]	2.90[b]	2.53[b]	2.50[b]
Arginine	2.86[bcd]	8.54[a]	1.44[e]	2.48[cd]	3.25[bc]	2.85[bcd]	3.39[b]	2.66[bcd]	2.32[d]	2.29[d]
Valine	2.69[ab]	3.21[a]	3.20[b]	2.33[ab]	2.27[ab]	2.68[ab]	2.36[ab]	2.49[ab]	2.17[b]	2.15[b]
Lysine	2.47[b]	4.83[a]	2.41[b]	2.14[b]	2.11[b]	2.46[b]	2.20[b]	2.29[b]	2.00[b]	1.97[b]
Histidine	0.66[b]	5.45[a]	0.75[b]	0.57[b]	0.91[b]	0.66[b]	0.94[b]	0.81[b]	0.53[b]	0.53[b]
Non-essential amino acids (NEAA)										
Aspartate	7.81[ab]	8.11[a]	7.10[cd]	6.78[de]	7.30[bcd]	7.79[abc]	7.60[abc]	7.25[bcd]	6.32[e]	6.25[e]
Glutamate	7.52[bc]	6.12[e]	8.25[ab]	6.52[de]	8.38[a]	7.49[bc]	8.73[a]	6.98[cd]	6.08[e]	6.01[e]
Proline	4.18[bc]	3.38[cd]	4.62[ab]	3.63[cd]	5.13[a]	4.17[bc]	5.34[a]	3.88[bcd]	3.38[cd]	3.34[d]
Alanine	4.16[ab]	1.94[d]	4.68[a]	3.61[bc]	3.35[c]	4.15[ab]	3.49[bc]	3.86[bc]	3.37[c]	3.33[c]
Serine	1.70[a]	0.73[b]	1.99[a]	1.48[ab]	1.80[a]	1.70[a]	1.88[a]	1.58[ab]	1.38[ab]	1.36[ab]
Glycine	1.19[a]	0.95[a]	1.11[a]	1.03[a]	1.28[a]	1.19[a]	1.33[a]	1.10[a]	0.96[a]	0.95[a]
Cysteine	0.85[a]	1.22[a]	0.83[a]	0.73[a]	0.12[b]	0.84[a]	0.12[b]	0.78[a]	0.68[a]	0.68[a]
EAA/NEAA	1.30	2.65	1.17	1.30	1.28	1.30	1.28	1.31	1.30	1.30

Means with different letters indicate a significant difference at $p \leq 0.05$ using Duncan multiple range test.
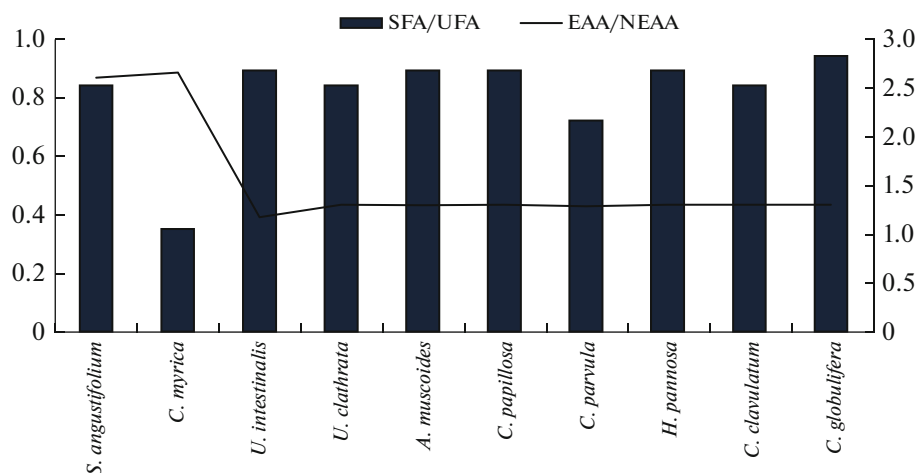
Fig. 3. Comparison between the ratios of saturated fatty acids/unsaturated fatty acids (SFAs/UFAs) and essential amino acids/non-essential amino acids (EAAs/NEAAs).
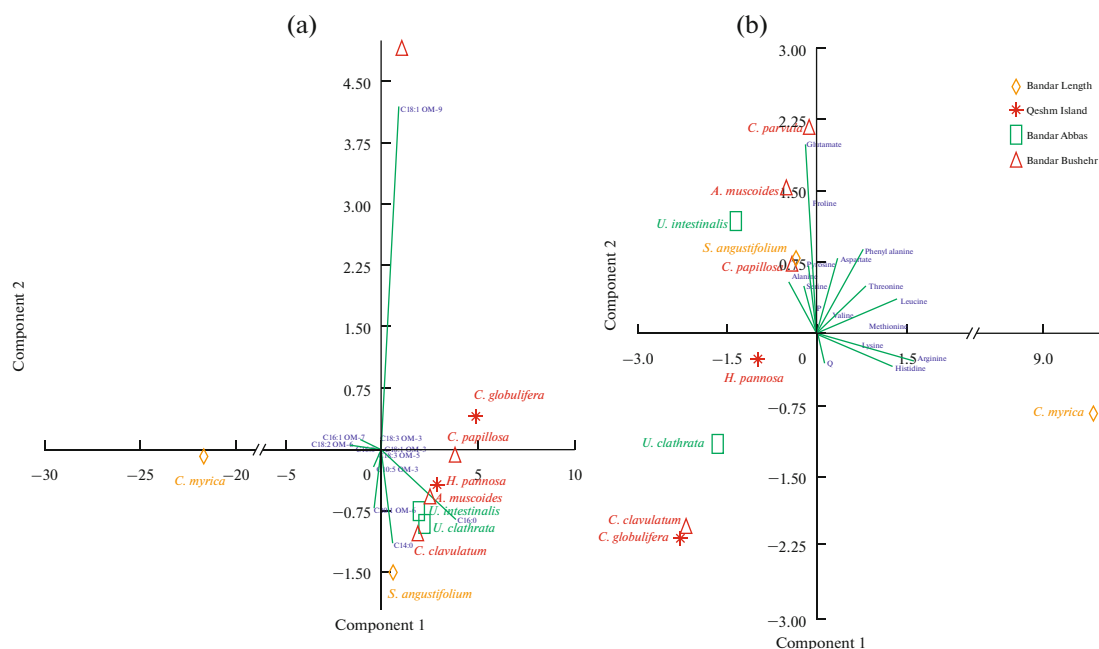

Fig. 4. Principal component analysis (PCA) of seaweed species based on (a) fatty acids and (b) amino acids profiles.

22.71−48.44%, while PUFA were in the range of 19.40−32.56% and monounsaturated fatty acids (MUFA) ranged from 29.21−35.66% (Fig. 2). Among the FAs, palmitic acid (C16:0) followed by oleic acid (C18:1) were most abundant. The amount of palmitic acid in red seaweeds was higher than the other macroalgae. In all of the samples, the level of MUFAs was higher than the PUFAs. The MUFA and PUFA content in *C. myrica* were about the same (approx. 32% of FAME). High proportions of PUFAs were observed in the investigated species. The sum of the PUFAs was the highest in *C. myrica* (32.56%) and the lowest in *C. globulifera* (19.40%). The ratio of SFA to UFA

(Unsaturated fatty acid) was <1 for all of the analyzed seaweeds. The highest ratio (1.3) of n-6/n-3 FAs was obtained in *C. myrica* due to high values of C18:2 n-6 (LA).

The range of protein recorded was 3.5−3.6% D.W. (green seaweed), 2.9−3.5% D.W. (red seaweed), and 3.7−4.3% D.W. (brown seaweed). The total protein contents for *S. angustifolium* and *C. myrica* (4.3 and 3.7% D.W. respectively) were higher than the other species. The general pattern of protein content in species is as follows: brown seaweed > green seaweed > red seaweed (Fig. 1). The EAAs, leucine and phenyl alanine, and non-essential amino acids (NEAA), aspartate and glutamate, were the most abundant AAs
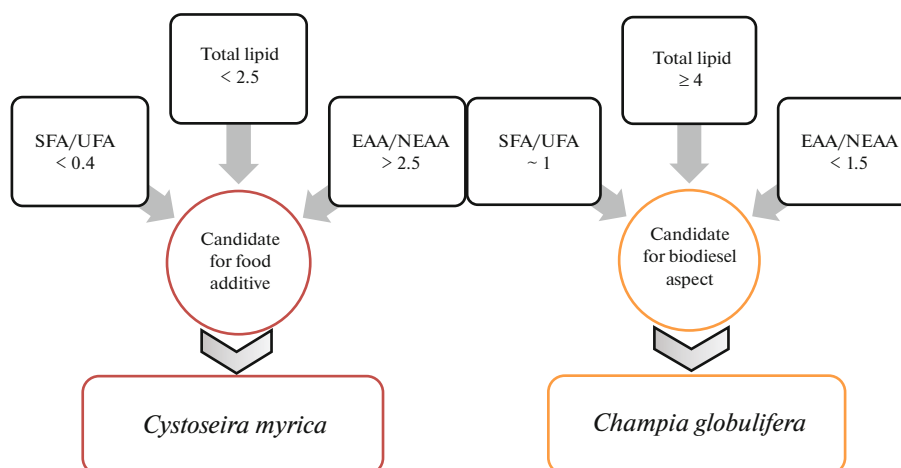
Fig. 5. Schematic diagram of seaweed exploitation after processing.

amongst samples (Table 3). In addition, the level of arginine was the highest in *C. myrica*. All samples were rich in EAAs and the ratios of EAA/NEAA were all above 1, which indicated a higher EAA content in the assessed samples. *C. myrica* (brown) had a significantly greater EAA/NEAA ratio (~ 2.6), whereas this ratio was more homogeneous in the other tested seaweeds. Histidine and cysteine were the first limiting AAs for all of the evaluated seaweed species. Other limiting AAs that were present at low concentrations included glycine and serine. Serine, glycine, cysteine, and alanine were present at low concentrations in *C. myrica* (brown algae) (Table 3).

The PCA of FAs and AAs showed that a greater than 90% variation between samples could be explained by two principal components (Fig. 4). The first component (PCA1) separates the *C. myrica* from other species primarily based on C16:0, C18:2 and C18:1 FAs (Fig. 4a) and arginine, leucine, and histidine AAs (x axis). *C. myrica* and *C. globulifera* had the highest PCA1, whereas *C. parvula* and *S. angustifolium* (*C. globulifera* in case of AAs) had the highest PCA2 scores. The second component (PCA2) separated *C. parvula* from the other species, which was mainly based on C18:1 and the y-axis in FAs. The second component (PCA2) separated *C. globulifera, C. clavulatum, U. clathrata*, and *H. pannosa* from the other samples according to glutamate and proline (y-axis).

DISCUSSION

The nutritional composition of marine seaweeds can be highly variable between divisions, genus and species as well as terrestrial plants. Seasonality and geographic locality can play a vital role in influencing the nutritional composition found in the seaweeds (Wan et al., 2019). For example, crude protein levels in red macroalgae *Hypnea flagelliformis* are much higher during the winter (Zarei Jeliani et al., 2021).

The ash content of the analyzed seaweeds was considerably higher than previously reported results for some subtropical seaweeds (Rodrigues et al., 2015; Siddique et al., 2013). There is a correlation between elevated levels of ash content and higher amounts of mineral elements and vitamins. This increased ash content might be due to the drying process, species type, season, or location (Rodrigues et al., 2015).

Favorable FAs profile and lipids contribute to the overall potential for seaweeds as important functional foods. This study demonstrated that the diverse seaweed flora may yield species that have potential commercial importance as food items. According to the limited information available, especially for marine algae from Iran, seaweeds can provide mainly FAs that consist of PUFAs. Although seaweeds have a low lipid content (approx. < 5%), the essential PUFAs content is much higher than traditional vegetables (Biris-Dorhoi et al., 2020). In the present study, all of the analyzed specimens had high compositions of PUFAs. Marine n-3 PUFAs have the most important role in anti-inflammatory effects because of their biological activities (Calder, 2012). The seaweeds are characterized by a high content of PUFAs, specifically eicosapentaenoic acid (20:5n-3, EPA), arachidonic acid (20:4n-6, ARA), and other n-3 and n-6 PUFAs in a balanced mix; because of these reasons they attract considerable interest (Bruni et al., 2020). Further, surveys such as those conducted by Biris-Dorhoi et al. (2020) have shown that the red and brown seaweeds are rich in EPA and ARA, that the findings of the current study are consistent with their findings. Researchers believes that the warm water seaweed species have higher SFAs, oleic acid and lower PUFA contents than the cold water species (Khotimchenko and Gusarova, 2004), which can be reason for the absence of C16 PUFA markers of green algae *Ulva* sp. in the present study, that is good agreement with previously reported values for *Ulva* spp. and the other

green algae (Abomohra et al., 2018; Chinthaka et al., 2018; Pirian et al., 2016, 2017, 2020). Additionally, Kelly and Scheibling (2012), reviewed that the seasonal variability has a major effect on FA composition in macroalgae (Kelly and Scheibling, 2012). According to the results, *C. globulifera* showed a highest value in sum of SFA (48.44% FAME) and *C. myrica* showed a high value in sum of PUFA (32.56% FAME) ($p \leq 0.05$) can be used as biodiesel and nutritional resources, respectively (Table 2 and Fig. 5) (Kamariah Bakar and Herng, 2017). The findings also indicated that the ratios of SFAs/PUFAs were <1 in all assayed species and *C. myrica* had the lowest value of this ratio; however, the other seaweeds were more homogeneous (Fig. 3). Scientists have suggested that the ratio of n-6/n-3 PUFAs should be 4 : 1 in the diet. In general, seaweeds have a favorable ratio of n-6/n-3 PUFA of ≤1. This ratio is of increasing interest as a food supplement nutrition (Ortiz et al., 2006; Rustan & Drevon, 2005). We noted that all of the examined seaweeds followed similar patterns. They had suitable ratios of PUFAs compared with the other detected FAs (Table 2).

Although oily fish is known as the most effective source of dietary n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) for humans, marine seaweeds can be considered as a new non-fish source. The current guidelines recommend an intake of n-3 LC-PUFAs for each person, namely 0.25−0.5 g/day (EFSA, 2010b; WHO, 2008). According to our findings, those doses can be fully obtained from the analyzed seaweeds by consumption of 100 g dry seaweed per day. In this line, due to differences in total lipids amongst species (Fig. 1), tissue concentrations of n-3 LC-PUFA were higher in red seaweeds specially *H. pannosa* and *C. clavulatm*. These findings of the current study are consistent with those of Skrzypczyk et al. (2018) who found that a consumer may regularly consume 100 g dry seaweed per day.

Proteins are an important component of the daily diet in terms of survival, suitable growth, and improved health of animals and humans. Daily protein intake is important because, unlike FAs, proteins are not stored in the body (Černá, 2011). Many seaweed species that are used as ingredients or foods have different benefits. Some seaweeds have a high proportion of proteins and are a considerable source of all AAs (glycine, alanine, arginine, proline, glutamic, and aspartic acids) at levels similar to those recommended by the FAO/WHO (Mohamed et al., 2012). The protein content of seaweeds differs according to nitrate levels in sea water and the season in which they are harvested (Zarei Jeliani et al., 2021; Zarei Jeliani et al., 2017). According to several researches, red seaweeds contain high levels of protein, followed by green seaweeds, and brown seaweeds (Fleurence et al., 2012; Wan et al., 2019). However, in the current study, we noted that the brown seaweeds, especially *S. angustifolium* (4.3% D.W.), had the highest amount of protein (Fig. 1). Generally, due to the insufficient amounts of

sulfur AAs in plants, they are considered to be limiting AAs. Some authors have reported that higher amounts of sulfur AAs, especially Met, were analyzed in seaweeds (Misurcova et al., 2014; Pirian et al., 2017). In addition, the highest content of Met (5.89/100 g total protein) was found in *C. myrica*. With respect to the ratio of EAAs/NEAAs, which ranged from 1.17 to 2.65, the distribution of EAAs and NEAAs in algal proteins imply that the content of EAAs were higher than NEAAs in all species in the present study. Interestingly, the EAA/NEAA ratios in *S. angustifolium* and *C. myrica* (brown seaweeds) were higher than all of the investigated samples (Fig. 3). Mišurcová et al. (2014) reported a ratio between 0.5 and 0.9 in other species from red, green, and brown seaweeds and microalgae products. According to present data, the analyzed seaweeds seem to be the most suitable sources of EAAs for human nutrition. The protein content differs based on species, season, geographic distribution, temperature, salinity, harvesting time and etc. Furthermore, sunlight is the main factor influencing the amount of nitrogen content in algae, consequently affects the protein content and photosynthetic capacity (Verma et al., 2016).

CONCLUSIONS

The increasing population growth will cause an expected increase in the demand for food; however, the availability of agricultural land and fresh water are limiting factors for food production. Therefore, seaweeds have become increasingly popular as valuable food and biodiesel sources to help meet human demand. Currently a limited data on the nutritional value of the Persian Gulf macralgae is available. Our study indicate that the Persian Gulf seaweeds can be collected and used as a supplementary dietary source because of beneficial FAs, especially EPA, suitable ratios of n-6/n-3 PUFA and EAA/NEAA, according to the FAO/WHO recommendations, and they have high ash content. According to our findings, 250 mg of omega-3 fatty acids can be fully obtained from the analyzed seaweeds by consumption of 100 g dry seaweed per day. In addition, *C. myrica* was characterized by a highest EPA content compared to other species. Interestingly, all examined seaweeds which were belong to different groups of macroalgae -with the exception of *C. myrica* -showed similar pattern of FAs. This may be related to the environmental effect of the Persian Gulf as one of the warmest sea water bodies in the world and among those with the highest salinity. Larger scale studies needed to elucidate the effect of physicochemical properties of water bodies on biochemical characteristics of seaweeds at global level. However, further research must also examine the potentially perceived quality, palatability, and toxic effects with regular consumption of seaweeds in these quantities.

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