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Determination of Mineral, Fatty Acid, and Soluble Carbohydrate Profiles of Green Algae *Ulva rigida*, *Chaetomorpha linum*, *Codium fragile*, *Caulerpa prolifera* and *Caulerpa racemosa f. requienii* Collected from Türkiye Coasts

Aysun Yucetepe[1,*], Umit Altuntas[2], Eda Sensu[3], Yunus Izci[4], Beraat Ozcelik[5], Emine Sükran Okudan[6]

[1]Department of Food Engineering, Faculty of Engineering, Aksaray University, 68100 Aksaray, Türkiye.
[2]Department of Food Engineering, Faculty of Engineering and Natural Sciences, Gümüşhane University, 29100 Gümüşhane, Türkiye.
[3]Department of Food Technology, Istanbul Gelisim Higher Vocational School, Istanbul Gelisim University, Istanbul, Türkiye.
[4]Department of Food Engineering, Engineering Faculty, Inonu University, 44280, Malatya, Türkiye.
[5]Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, Istanbul Technical University, Maslak, 34469, Istanbul, Türkiye.
[6]Faculty of Aquatic Sciences and Fisheries, Akdeniz University, 07070 Antalya, Türkiye.

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Corresponding Author
E-mail: aysunyucetepe@aksaray.edu.tr

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Abstract

The nutritional properties of five different green macroalgae including *Ulva rigida*, *Chaetomorpha linum*, *Codium fragile*, *Caulerpa prolifera* and *Caulerpa racemosa* f. *requienii* from Turkey were investigated. The chemical composition of green macroalgae was varied, with ash, crude fiber, protein, lipid and carbohydrate ranging from 40.71 to 52.82%, 12.02-17.56%, 4.40-26.19%, 0.57-3.85% and 4.58-42.28% in dry weight, respectively. The fatty acid contents of the algae were quite variable and palmitic acid (C16) was found to be the primary fatty acid for all the samples with a value of more than 49.61%. Palmitic acid was followed by oleic acid, which is a monounsaturated fatty acid. This study revealed that green algae are rich in important soluble carbohydrates such as myo-inositol and glucose, health promoting unsaturated fatty acids (mainly oleic acid) and essential macroelements such as potassium, magnesium and microelements such as iron, zinc and selenium. The results of the current study contribute to a better understanding of macroalgae and encourage their use in food-related applications.

Introduction

Macroalgae have been a part of human diet as a food source since ancient times (Korzen et al., 2016; Sirbu et al., 2020). Several algae species are traditionally consumed as food in Asian countries especially in China, Japan, and Korea for a long time (Hao et al., 2019), and are also approved for consumption in Europe (Sirbu et al., 2020), not only for its taste but also for health (Hao et al., 2019). With the results of recent studies on their nutritional content and advantages as a functional food, algae are becoming more popular in many countries (İrkin & Erduğan, 2014). Moreover, they have recently been considered as a rich alternative supply of veggies (Hao et al., 2019; Sirbu et al., 2020).

Macroalgae are classified as brown (Phaeophyceae, Heterokontophyta), red (Rhodophyta) and green algae (Chlorophyta), according to their pigmentation (Mouritsen, 2013). Among these macroalgae, especially green algae are essential resources in the marine ecosystem, hence several studies on their usage have been done in recent years (Ruslan et al., 2021). Green macroalgae have been discovered to have a variety of bioactive and nutritious

components. These include natural colors, polyunsaturated fatty acids, lipids, proteins, and polysaccharides (Ruslan et al., 2021). Besides nutritional value, marine algal compounds exhibit different physiological activities and health benefits including anticoagulant, antiviral, antioxidant, anticancer and anti-inflammatory activities due to numerous primary and secondary metabolites they contain (Hao et al., 2019; Trigui et al., 2013).

Ulva rigida among the green algae is an excellent food source due to its high concentration of cell-wall polysaccharides (Ray & Lahaye, 1995), and low total lipid content and valuable proteins (Taboada et al., 2010). On the other hand, it also provides a wide variety of vitamins and trace elements (Godard et al., 2009). *U. rigida* is also rich in several functional micro-elements including iron, boron, and zinc, all of which are present in appropriate amounts (Berik & Çankırılıgil, 2019). *Chaetomorpha linum* is widely spread green macroalgae and well recognized for its possible ecological role as a nutrient regulator in estuarine ecosystems, as well as its therapeutic characteristics, which are thought to have a high phytochemical value (Hamzaoui et al., 2020; Stabili, Acquaviva, et al., 2019). This group of macroalgae comprises a wide range of bioactive compounds with antibacterial, antiviral, and antifungal characteristics, including macrolides, polysaccharides, minerals, vitamins, proteins, lipids, polyphenols, and fatty acids (Stabili , Acquaviva, et al., 2019). *Codium fragile* is a species of edible green seaweed belonging to the family Codiaceae and it has been traditionally used as a food ingredient in Asian countries (Wang et al., 2020). *C. fragile* has been valued as a health-promoting supplement in several countries like China and Japan (Li et al., 2021). These algae contain various amounts of nutrients as follows; polysaccharides (44.1-80.5%), sulfates (3.2-22.2%), proteins (3.0-15.7%) and uronic acid (1.1-4.2%) (Park et al., 2020). *Caulerpa* spp. is green seaweed, widely distributed in tropical areas (Chaves Filho et al., 2018). Despite the fact that *C. prolifera* is widely recognized as a valuable source of novel bioactive molecules, few studies have focused on their regenerative medicine potential (Chaves Filho et al., 2018). *Caulerpa racemosa is* large edible green algae and also invasive marine seaweed, widely distributed throughout tropical and subtropical areas (Klein & Verlaque, 2008).

Green macroalgae, like photosynthesizing plants, have antioxidant defense mechanisms. They also include polysaccharides, glycoproteins and PUFAs that contain chemicals with antioxidant, anti-inflammatory, anti-cancer, anti-coagulant properties and also known to prevent cardiovascular diseases and diabetes. Thus macroalgae hold significant potential as an alternative source of functional food due to their high nutritional and therapeutic value (Adarshan et al., 2023).

To sum up, nutritional value studies for green macroalgae are limited, and due to interest in functional ingredients to develop supplements or functional foods they need to be completed. Thus, the objective of the present study is to determine fatty acid, mineral and carbohydrate profiles of five selected green macroalgae including *Ulva rigida, Chaetomorpha linum, Codium fragile, Caulerpa prolifera* and *C. racemosa f. requienii* collected from different coasts from Türkiye.

Materials and Methods

Collection and Preparation of Macroalgal Biomass

The macroalgae used in this study were collected from two different coasts of Türkiye and five stations. These stations were Antalya (36°27'40.47"N 30°32'38.18"E) for *C. prolifera* and *C. racemosa* f. *requienii*, Çanakkale (40°14'27.03"N - 26°32'29.74"E) for *U. rigida* and *C. linum* and Çanakkale (40°1'35.90"N - 26°19'49.49"E) for *C. fragile*. The image of the selected green macroalgae is shown in Figure 1. The collected samples were put in a glass container containing neutralized formaldehyde solution (4-6%) for further analyses. The collected samples were washed with water for the elimination of possible sand, dust, and impurities. The cleaned materials were dried in controlled atmosphere and grounded into powder (<500 µm) by using a laboratory type grinder (ZM 100, Retsch), Then, the powdered samples were packaged appropriately and stored at -20°C until further analysis.

Chemicals

The chemical solutions used to prepare calibration curve was obtained from Sigma (St. Louis, USA) for mineral profile analysis. Fatty acid methyl esters (FAME) standard mixture (37 components) was purchased from Supelco (Bellefonte, USA). All the chemicals and reagents used for analysis were of either analytical or HPLC grade and they were purchased from Merck (Darmstadt, Germany).

Proximal Analyses

Determination of Total Carbohydrate Content

The phenol-sulfuric acid assay adapted by Dubois was used to determine total carbohydrate content of the green algae (DuBois et al., 1956). Total carbohydrate found in algae samples, was released into solution by concentrated hydrochloric acid (HCl), and rapidly dehydrated to furfural derivatives by concentrated sulfuric acid (H_2SO_4.). Then, a condensation reaction between the furfurals and phenols produced an orange-yellow compound. Briefly, 0.08 g dehydrated algae powder sample was extracted under reflux with 15 mL of water and 2 mL of the concentrated HCl on a shaking water bath (N-Biotek-303, South Korea) at 95°C for 1 h. Then, the extract was filtered through a filter paper and diluted to 50 mL with water in a volumetric flask. For the determination of the total carbohydrate content in the

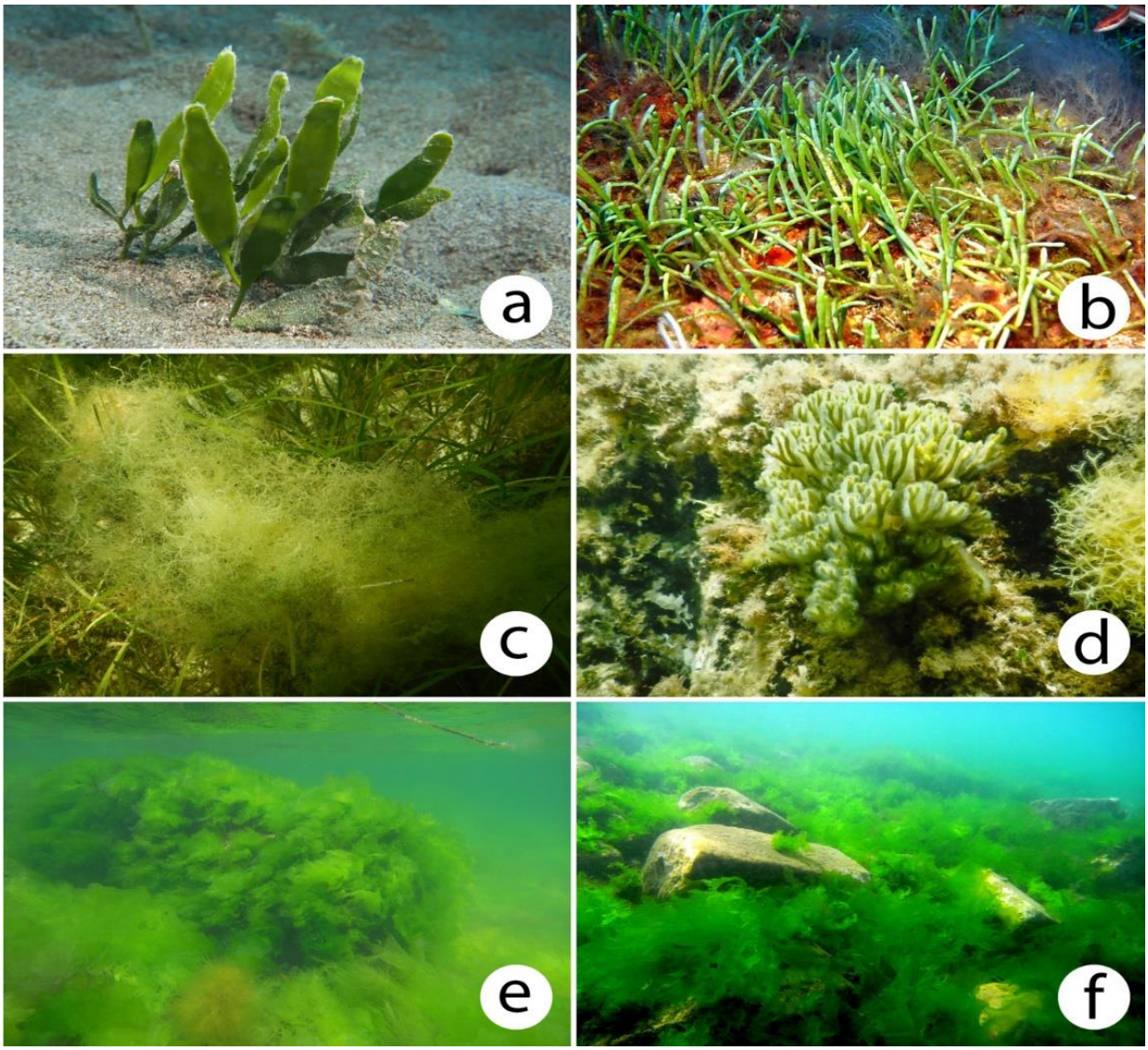

Figure 1. a) *Caulerpa prolifera* b) *Caulerpa racemosa* f. *requienii,* c) *Chaetomorpha linum,*d) *Codium fragile,* e-f) *Ulva rigida* (photographed by Dr. Emine Şükran Okudan).

sample, 0.1 mL of previously prepared extract was added to a 10 mL colorimetric tube and diluted to 1.0 mL with distilled water, this was followed by adding 1.0 mL of 5% phenol and 5.0 mL of the concentrated H_2SO_4. After a 30 min reaction under ambient temperature, the absorbance of the sample was recorded at 487 nm by using a UV-vis spectrophotometer (Sci-UV1000, Scilogex, USA). The total carbohydrate content was calculated with the use of a standard curve of absorbance against glucose concentration (mg/mL), and the results were expressed in g glucose/100 g of dry weight (g glucose/100 g dw).

Determination of Crude Fiber

Crude fiber of the algae samples was determined according to AOAC 962.09, (2010). 2 g of defatted sample were treated successively with boiling solution of H_2SO_4 of 0.26 N and potassium hydroxide (KOH) of 0.23 N. The residue was then separated by filtration, washed, and transferred into a crucible then placed into an oven adjusted to 105°C for 18-24 h. The crucible with the sample was weighed, ashed in a muffle furnace at 500°C and weighed and crude fiber amount was calculated gravimetrically.

Determination of Total Lipid Content

Soxhlet extraction method with some modifications was used to determine total lipid content of the algae samples (López-Bascón & de Castro, 2020). Briefly, 10 g of sample weighed into a cartridge filter and was mixed with adequate of *n*-hexane in the Soxhlet apparatus and the solvent was heated to reflux for a certain time. Then, pellet was left from the distillation flask. The remaining *n*-hexane was removed by using a rotary evaporator (IKA A11 basic analytical mill, Germany). The obtained lipid fraction was weighed, and lipid amount was calculated gravimetrically.

Determination of Ash Content

For the determination of ash content of green macroalgae an official method of analysis (AOAC, 1995) adapted from Jung et al. (2015) were applied. 5 gr of each macroalgae heated at 550°C for approximately 24 h in order to determine ash content. The obtained ash residue was weighed, and ash content was calculated gravimetrically.

Determination of Mineral Profile

For Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analysis of the green algae samples, approximately 0.2 g of each sample were weighed and taken into vessel tubes. Then, 10 mL of 65% HNO_3 was added, and they were kept open-top for 15-20 min under a fume hood. Afterwards, the samples were incinerated in the microwave combustion system (Mars 5 Microwave Accelerated Reaction System, CEM Corporation, Charlote, USA) under heating conditions (200°C of temperature, 800 psi of pressure, 900-1800 W of power, 15:00 (mm:ss) of ramp and 15:00 (mm:ss) of hold) (Ahamad et al., 2017; Pilarczyk et al., 2013). The analytics were taken microwave digestion for 15 min and then were passed through 0.45 µm syringe filters and stored at 4°C until ICP-MS analysis.

The samples, which were subjected to microwave digestion and filtered, were taken to the stage of determination of heavy metal contents in the ICP-MS (Agilent Company, Santa Clara, USA). For this purpose, firstly, blank, and standard solutions were prepared in order to set a calibration curve. Then, 2% Nitric acid (HNO_3) solution, prepared from 65% HNO_3 (suprapure) with ultrapure water, was used as blank. Subsequently, 1, 5, 6, 10, 30, 50, 100, 150, and 200 ppb standard concentrations were prepared from 10 ppm pure standard stock that has equal concentrations of heavy metals, and the calibration curve was drawn by analyzing these standards (Ahamad et al., 2017; Pilarczyk et al., 2013).

Determination of Fatty Acid Profile

Fatty acid (FAs) composition of the green macroalgae was analyzed by an Agilent 7820A Gas Chromatography-Flame Ionization Detection (GC-FID) system (Agilent Company, Santa Clara, USA) and each individual FAs of the samples were quantitatively determined. The FA compositions of the algae samples were determined according to the method described by Uluata et al. (2021). The sample preparation is based on transesterification of fatty acids to fatty acid methyl esters (FAMEs), and quantification and identification of FAMEs using gas chromatography an aliquot of total lipid extract was dissolved in hexane and methylated with methanolic boron trifluoride (BF_3). Fatty acid methyl esters were extracted into hexane. The carrier gas selected as hydrogen at flow rate of 40 mL/min and the split ratio of 1/50. The retention times of each FAs were compared against the standard mixture of FAME to identify the FA composition of the samples. The FA composition results were expressed as weight percentage (%).

Determination of Carbohydrate Profile

The method of Pfetzing et al. (2000) with some minor modifications, was used to perform carbohydrate profile analyses. High performance anion exchange chromatography (HPAEC) was used to quantify mannitol, myo-inositol and simple carbohydrates in the algae samples. To extract carbohydrates from the algae, 10 mg dried powder was added to 3 mL of ultra-pure water. The suspension was incubated at 80°C for 4 h in water bath, then the solution was centrifugated at 6000 *g* for 15 min. Aliquots of supernatant were sampled and stored at -20ºC for further analyses. The HPAEC (C5000, Dionex Corporation, Sunnyvale, USA) was performed using a Dionex ICS-5000 chromatography system equipped with a PA-200 column and a guard column (Dionex Corporation, Sunnyvale, USA). Simple sugars of the algae were separated using a binary gradient method: 0% B for 3 min, up to 11% B until 15 min, down to 0% again in 12 min and so on completing a total of 30 min whilst A was 600 mM NaOH and B was 100 mM NaAc in solvent A. The flow rate was 0.40 mL/min at room temperature. An electrochemical ED40 detector in the integrated amperometric mode was utilized for detection.

Statistical Analysis

Minitab 18.0 licensed program was used for the statistical analysis of the data obtained from samples. In order to identify differences among groups, all data were analyzed using one-way analysis of variance (ANOVA) and Tukey's *post hoc* test for multiple comparisons with statistical significance at a 95% confidence level ($p<0.05$).

Results

Proximate Composition

The proximate composition of *U. rigida, C. linum, C. fragile, C. prolifera* and *C. racemosa f. requienii* was shown in Table 1. The protein content for all macroalgae were ranged from 4.40 to 26.19% dw. *C. prolifera* had the highest protein content whereas *C. linum* had the lowest ($p<0.05$). The highest lipid content was observed in *C. prolifera* (3.85% dw), whereas *C. linum* had the lowest lipid content (0.57% dw) ($p<0.05$). Lipid contents of macroalgae samples were ranged between 0.67%, and 2.70% dw (Table 1). *C. linum* had the highest ash content among the macroalgae whereas *C. prolifera* had the lowest amount ($p<0.05$). Total ash content of the other macroalgae samples was as follows: 21.51% for *U.*

rigida, 35.90% for *C. fragile* and 21.21% for *C. racemosa f. requienii*. Total carbohydrate (soluble carbohydrate) content of the analyzed green macroalgae samples varied between 40.71 and 52.82% in dry weight (41.22%, 52.72% and 52.36% for *C. fragile, C. prolifera*, and *C. racemosa f. requienii*, respectively, as seen in Table 1). *U. rigida* had the highest value, whereas *C. linum* had the lowest total carbohydrate content ($p<0.05$). *U. rigida*, *C. fragile* and *C. racemosa* had the similar amounts of dietary fiber, approximately 17% by dry weight. *C. linum* and *C. prolifera* follow with ~12% dietary fiber content (Table 1).

Mineral Profile

Mineral composition of green macroalgae is shown in Table 2. Although magnesium (Mg) and potassium (K) were found at higher amounts than other minerals determined, mineral concentrations varied significantly between species at a 95% confidence level ($p<0.05$). Except for *C. linum*, Mg was found to be higher than K ranging 2.78 to 38.24 mg/kg. The highest Mg content (38.24 mg/kg) was found in *U. rigida*.

According to the results of the present study, 10 microelements; iron (Fe) and manganese (Mn), selenium (Se), silver (Ag), copper (Cu) and zinc (Zn) were detected in the samples (Table 2). Zn was found to be the most abundant microelement in macroalgae, except for *C. fragile*. Zn is followed by Cu and Fe. Fe concentrations in the five species of green algae were as follows: 1.64, 1.06, 0.92, 0.66 and 0.49 g/kg sample in dry weight for *C. fragile*, *C. racemosa f. requienii, C. linum*, *C. prolifera* and *U. rigida*, respectively ($p<0.05$, Table 2). *C. fragile*, especially, had the highest Se (4.20 µg/kg) among macroalgae. Heavy metals such as Al, Cd, Ag, Ni, and Pb were detected in green macroalgae (Table 2). *C. prolifera* contained 0.81 mg/kg of Pb with the lowest amount within all the samples.

Fatty Acid Composition

A total of 11 fatty acids (FAs) were identified in samples of *U. rigida*, *C. linum* and *C. prolifera* samples whereas 13 FAs were identified in samples of both *C. fragile* and *C. racemosa f. requienii* (Table 3). Results revealed that the saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) of the algae were ranged between 56.66-75.67%, 14.34- 24.05% and 4.12-19.29% in dry weight, respectively ($p<0.05$). The highest total SFAs was detected in *C. prolifera* (75.67%) ($p<0.05$). The primary saturated fatty acid was palmitic acid for all samples within the concentration range of 45.61 to 55.41% ($p<0.05$, Table 3). Total MUFAs were the most abundant fatty acids after total SFAs. The highest total MUFAs was detected in *C. fragile* (24.05%), while the lowest was detected in *U. rigida* (14.34%). Oleic acid as a MUFA was also abundant in *U. rigida* (12.56%), All the other macroalgae samples exhibited a comparative level of the green seaweeds. Unsaturated fraction of fatty

Table 1. Proximate composition (%, in dry weight) of selected green macroalgae from Türkiye coasts

Composition (%)	*U. rigida*	*C. linum*	*C. fragile*	*C. prolifera*	*C. racemosa* f. *requienii*
Protein	7.41±0.12^{b}	4.40±0.09^{c}	4.63±0.60^{c}	26.19±0.64^{a}	6.94±0.29^{b}
Lipid	0.67±0.03^{c}	0.57±0.10^{c}	1.02±0.10^{b}	3.85±0.31^{a}	2.70±0.07^{b}
Ash	21.51±0.63^{c}	42.28±0.70^{a}	35.90±0.04^{b}	4.58±0.09^{d}	21.21 ±0.69^{c}
Crude fiber	17.56±1.03^{a}	12.02±0.13^{b}	17.21±0.58^{a}	12.63±0.13^{b}	16.77±0.45^{a}
Total soluble carbohydrate	52.82±1.20^{a}	40.71±1.41^{b}	41.22±1.04^{b}	52.72±1.44^{a}	52.36±1.52^{a}

*Each value is given as mean±standard deviation (n=3). Different letters in the rows indicate statistically ($p<0.05$) differences by the Tukey test.

Table 2. Mineral composition of selected green macroalgae from Türkiye coasts by ICP-MS

	U. rigida	*C. linum*	*C. fragile*	*C. prolifera*	*C. racemose* f. *requienii*
Macro elements (mg/kg DW)					
Mg	38.24±0.70^{a}	2.49±0.05^{d}	10.64±0.29^{b}	4.36±0.04^{c}	2.78±0.05^{d}
K	1.25±0.01^{c}	16.36±0.24^{b}	0.69±0.02^{c}	0.11.±0.00^{a}	0.61±0.00^{c}
Micro elements (µg/kg DW)					
Mn	0.01±0.00^{c}	0.06±0.00^{a}	0.07±0.00^{a}	0.02±0.00^{b}	0.02±0.00^{b}
Fe	0.49±0.00^{e}	0.92±0.00^{c}	1.64±0.02^{a}	0.66±0.01^{d}	1.06±0.00^{b}
Cu	4.83±0.09^{a}	3.98±0.09^{b}	1.71±0.06^{c}	4.22±0.15^{b}	4.17±0.12^{b}
Zn	12.18±0.09^{a}	12.80±0.33^{a}	1.57±0.02^{d}	10.35±0.27^{c}	12.22±0.46^{b}
Se	0.57±0.01^{d}	0.81±0.01^{d}	4.20±0.04^{a}	2.22±0.041^{b}	1.20±0.03^{c}
Heavy metals (µg/kg DW)					
Cd	0.05±0.00^{b}	0.04±0.00^{c}	0.03±0.001^{d}	0.03±0.001^{d}	0.06±0.00^{a}
Pb	1.08±0.01^{c}	2.48±0.04^{a}	1.30±0.047^{b}	0.81±0.020^{d}	1.05±0.01^{c}
Ni	3.24±0.00^{c}	2.67±0.03^{d}	6.72±0.14^{a}	4.66±0.09^{b}	6.18±0.076^{a}
Ag	13.04±0.11^{a}	3.42±0.10^{c}	1.07±0.03^{d}	12.82±0.23^{b}	3.82±0.09^{c}
Al	0.15±0.01^{d*}	0.39±0.01^{c}	0.88±0.01^{a}	0.55±0.01^{b}	0.82±0.01^{a}

*Each value is given as mean±standard deviation (n=3). Different letters in the rows indicate statistically ($p<0.05$) differences by the Tukey test.

acids consisted of approximately 24-43% of total fatty acid content in all algae samples. *C. fragile* had the highest total MUFAs (24.05%) and PUFAs (19.29%) *U. rigida* was the species with the higher proportion of total PUFAs (16.34%) content as well as *C. fragile* (19.29%) as the highest. Linoleic acid was the major PUFA in all samples. *C. fragile* contained high percentages of linolenic acid (6.70%), eicosatrienoic acid (6.64%) and oleic acid (20.73%). Among all fatty acids, palmitic acid (55.41%) was the predominant one found in *Ulva rigida* followed by oleic acid (12.56%) and linoleic acid (10.75%).

Soluble Carbohydrate Composition

Carbohydrate composition of green algae was determined, and the results were given in Table 4. The major sugars in green algae varied based on the species of algae. Sucrose was found as the major sugar in the samples as 3586.18 and 4968.06 mg/kg in dw belong to the *U. rigida* and *C. linum*, respectively ($p<0.05$, Table 4). Glucose had the highest value within the sugar compounds of *C. fragile* and *C. prolifera* whereas myo-inositol was the primary component in the samples of *C. racemosa f. requienii*. As seen in Table 4, there were statistically significant differences ($p<0.05$) among samples. The concentration of myo-inositol was found as 3156.96 mg/kg in sample of *C. linum* as the highest sugar alcohol whereas the lowest value (228.10 mg/kg) was obtained for the *C. racemosa f. requienii* ($p<0.05$). On the other hand, mannitol was detected just in *C. prolifera* and *C. racemosa f. requienii* with the trace values of 4.61 and 2.42 mg/kg, respectively ($p<0.05$).

Discussion

Proximate Composition

The protein content of several green algae has been reported in the literature. The protein content of *Ulva rigida* and *Ulva lactuca* were found to be as 9.3% and 7.6%, respectively, (Neto et al., 2018; Wong & Cheung, 2000). Moreover, protein content of *C. racemosa var peltata*, species. were ranged from 11.39% to 17.36 (Hao et al., 2019; Nagappan & Vairappan, 2014). Magdugo et al. (2020) reported that protein content of *C. racemosa* and *Ulva fasciata* from Philippines were 8.8-19.9% and 8.0-11.1%, respectively. Berik et al. (2022) stated that the protein content of *Ulva rigida* collected from Türkiye were found between 15.78% and 25.98 depending on the season. Protein content in green macroalgae varied depending on the

Table 3. Fatty acid composition (%, in dry weight) of selected green macroalgae from Türkiye coasts by GC-FID

Fatty acids	*U. rigida*	*C. linum*	*C. fragile*	*C. prolifera*	*C. racemose* f. *requienii*
C10:0 (Capric acid)	2.06±0.02^{a}	0.72±0.01^{c}	1.77±0.02^{a}	-	1.07±0.01^{b}
C12:0 (Lauric acid)	0.67±0.01^{d}	0.52±0.01^{e}	0.96±0.01^{a}	0.78±0.02^{b}	0.71±0.01^{c}
C14:0 (Myristic acid)	2.73±0.10^{c}	17.18±0.30^{a}	2.58±0.06^{c}	18.34±0.08^{a}	12.94±0.06^{b}
C16:0 (Palmitic acid)	55.41±1.09^{a}	50.38±1.10^{c}	45.61±0.94^{d}	54.16±1.02^{b}	53.17±1.03^{b}
C18:0 (Stearic acid)	1.25±0.02^{c}	1.08±0.10^{d}	2.03±0.01^{b}	1.12±0.01^{d}	2.44±0.01^{c}
C21:0 (Heneicosanoic acid)	7.20±0.70^{a}	5.55±0.38^{b}	1.71±0.02^{c}	-	-
C22:0 (Behenic acid)	-	-	-	1.77±0.02^{a}	1.25±0.01^{b}
Total SFA	69.32±1.84^{c}	75.43±1.90^{a}	56.66±1.03^{d}	75.67±1.02^{a}	71.53±1.11^{b}
C15:1 (Pentadecenoic acid)	-	-	-	0.84±0.01^{b}	3.69±0.04^{a}
C16:1 (Palmitoleic acid)	1.78±0.04^{c}	1.87±0.20^{a}	1.75±0.01^{b}	1.67±0.01^{c}	1.72±0.01^{b}
C17:1 (Heptadecenoic acid)	-	0,86±0.01^{b}	1,57±0.02^{a}	-	-
C18:1 n-9 (Oleic acid)	12.56±0.81^{d}	17.72±0.74^{b}	20.73±0.78^{a}	16.51±0.82^{b}	14.76±0.04^{c}
Total MUFA	14.34±0.85^{d}	20.45±0.95^{c}	24.05±0.16^{a}	19.02±0.12^{c}	20.17±0.09^{b}
C18:2 n-6 (Lineloic acid)	10.75±0.75^{a}	3.30±0.34^{c}	4.25±0.26^{b}	3.50±0.02^{c}	4.75±0.02^{b}
C18:3 n-6 (Linolenic acid)	-	-	1.70±0.02^{a}	0.91±0.01^{b}	0.47±0.01^{c}
C18:3 (Linolenic acid)	2.35±0.02^{b}	-	6.70±0.03^{a}	0.90±0.01^{d}	1.17±0.01^{c}
C20:3 n-6 (Eicosatrienoic acid)	3.24±0.03^{b}	0.82±0.01^{d}	6.64±0.09^{a}	-	1.91±0.01^{c}
Total PUFA	16.34±0.80^{b}	4.12±0.02^{e}	19.29±0.13^{a}	5.31±0.02^{d}	8.3±0.22^{c}

*Each value is given as mean±standard deviation (n=3). Different letters in the rows indicate statistically ($p<0.05$) differences by the Tukey test.

Table 4. Soluble carbohydrate composition (mg/kg dw) of selected green macroalgae from Türkiye coasts

Carbohydrates	*U. rigida*	*C. linum*	*C. fragile*	*C. prolifera*	*C. racemose f. requienii*
Myo-inositol	867.85±1.49^{b}	3156.96±5.97^{a}	415.58±0.78^{c}	265.72±0.50^{c}	228.10±0.42^{c}
Mannitol	-	-	-	4.61±0.01^{a}	2.42±0.01^{b}
Glucose	745.93±1.28^{b}	2336.47±4.42^{a}	480.62±0.91^{c}	416.23±0.91^{c}	-
Fructose	375.98±0.65^{b}	1264.58±2.39^{a}	-	-	201.26±0.37^{c}
Sucrose	3586.18±6.20^{b}	4968.06±9.41^{a}	-	-	-

*Each value is given as mean±standard deviation (n=3). Different letters in the rows indicate statistically ($p<0.05$) differences by the Tukey test.

habitat, depth of the macroalgae and season of gathering (Ruslan et al., 2021). Macroalgae are known to have a relatively low lipid content, with considerable variability between species (Burtın, 2003). In the literature, the lipid content of *C. racemosa spp., Caulerpa lentillifera, U. lactuca* and *C. fragile* were reported as ~2.20, 1.57, 0.09 and 1.53, respectively (Nagappan & Vairappan, 2014; Nguyen et al., 2011; Oucif et al., 2020; Saygili et al., 2022). The total lipid content of the algae varied between 0.57 and 3.85% where similar results were obtained by Wong & Cheung (2000) (1.6%), Plaza et al. (2008) (0.5-3.2%) and Taboada et al. (2010) (0.9%). The ash content of *Ulva rigida* was found as 21.51% and this value was close to that of Plaza et al. (2008) and Berik et al. (2022), which were found 17% and of Paiva et al. (2017), which were found 20.60%. However, ash content of *U. rigida* was lower than the amount reported by Neto et al. (2018), which were found 31.7%. Moreover, the *C. racemosa var peltata* had 7% ash content, whereas *C. racemosa var Iv*. and *C. racemosa var cm*. had 26.74 and 23.81% ash content (Hao et al., 2019). The ash content of *C. linum* (42.28%) was found to be similar to that of Saygili et al. (2022) (45.38%).Previous investigations on Chlorophyta species indicated carbohydrate content in the range of 43.4-60.2% dw, which is consistent with our data. Taboada et al. (2010) found the concentration of soluble carbohydrates as 42.6% for *U. rigida,* which was slightly lower than our findings. Balar et al. (2019) reported that the carbohydrate content of *U. rigida* ranged from 16.63% to 65.93% in dry matter. Hamzaoui et al. (2020) reported that total carbohydrate (52.54%) was higher in *C. linum* compared to *Ulva pertusa* (33.4%). Moreover, according to Kasimala et al. (2017), green macroalgae contain more carbohydrates than other malcoalgae. According to reports, green macroalgae grow in relatively shallow waters, therefore increased exposure to sunlight may help them to manufacture more carbohydrates through photosynthesis (Kasimala et al., 2017). Dietary fibers reduce the risk of many diseases such as heart diseases, high blood pressure, obesity, diabetes and gastrointestinal disorders (Anderson et al., 2009). Only the dietary fiber content (36.6%) of *U. rigida* has been reported by Neto et al. (2018) in the literature. According to recent studies, the high fiber content of macroalgae can be included into low-fiber or fiber-free foods (such as meat and fish), hence increasing the nutritional qualities of these food formulations (Fernández-Segovia et al., 2018). With the results of the recent studies on their nutritional content and advantages as a functional food source, green algae are becoming more popular in many countries (Yucetepe et al., 2022).

Mineral Profile

Minerals are essential for both mental and physical health, as they are found in bones, teeth, soft tissues, hemoglobin, muscles, blood, and nerve cells (Kuda & Ikemori, 2009). The obtained mineral content results are similar to those obtained by Mabeau & Fleurence (1993), MacArtain et al. (2007) and the values are within the range reported by Rupérez (2002) and Dang & Hoang, (2004) for other algae Taboada et al. (2010). In the literature, Panayotova & Stancheva (2013) reported the amount of Mg and K in *C. linum* similar to this study. According to Oucif et al. (2020), green and red macroalgae have particularly high Mg content. Moreover, Mg is one of the most abundant minerals in seaweeds and helps to enhance nutrient absorption under stressful situations. Macroalgae require Mg for development and metabolic functions (Fouda et al., 2019). A daily Mg intake of 310-360 mg for women and 400-420 mg for men is recommended due to its numerous biological activities at the cellular level (Muñoz-Garach et al., 2020). Even at trace quantities, microminerals such as Fe, Cu, Zn, Mn, and Se play vital roles in the basic functioning of the human body (Fouda et al., 2019). According to microelement results, Zn was identified as the main microelement in macroalgae. Zn affects enzyme performance, protein stability, and gene regulation (Ismail, 2017; Smith et al., 2010). Moreover, *C. fragile* could be a suitable source of microelements for Fe supplement in food due to its rich iron content. Balar et al. (2019) reported that addition of *Ulva* subspecies to typical snack foods increased iron content by five-fold (26.4-126 mg/100 g). Green macroalgae had the trace amount of Se and Se has been identified as a health-promoting component in foods due to its interaction with an endogenous antioxidant mechanism (Fairweather-Tait et al., 2011). The mineral composition of macroalgae can be influenced by a variety of environmental factors, including mineral concentrations in water, element interactions, salinity, pH, and light intensity (Fouda et al., 2019).

Heavy metals are one of the main components of mineral profiles; because of their toxicity, endurance, and tendency to bioaccumulate, these heavy metals are regarded as one of the leading sources of environmental contamination (Tchounwou et al., 2012). This condition is caused by marine pollution and ability of macroalgae to absorb huge amounts of metal (Paz et al., 2018). The analysis of heavy metals revealed that the content of critical elements such as Cd and Pb, which were known for their toxic properties, was less than 2.5 µg/kg (Ustunada et al., 2011). The Acceptable Daily Intake, ADI, for Pb was estimated to be 0.63 mg/person/day, which represents 7.4% of the total intake of the heavy metals (Kacholi & Sahu, 2018). Thus, the consumption of the macroalgae studied is assumed to be below the limits since these are not consumed in higher proportions in a diet.

Fatty Acid Composition

Saturated fatty acid (SFA) content has been reported between 47.06% and 65.07% for *C. prolifera* by Terrados & Lopez-Jimenez (1996) and between 39.6%

and 67.9% for *C. racemosa* by Blažina et al., (2009), in the literature. While SFA of *U. rigida* was determined to be as 69.32%, Paiva et al. (2017) and Neto et al. (2018) reported it 46.79% and 75.8%, respectively. Berik et al. (2022) reported that SFA of *U. rigida* from Türkiye ranged between 39.72% and 64.24%. Xu et al. (1998) determined palmitic acid (C16:0) to be the most abundant fatty acid (25-45%), which is an agreement with our results. Moreover, Oucif et al. (2020), reported that, palmitic acid was the most saturated fatty acid found in the five macroalgae studied. Many investigations have shown that oleic acid (C18:1) is prominent in macroalgae (Dawczynski et al., 2007; El-Shenody et al., 2019; Neto et al., 2018). Similarly, in the study by Oucif et al. (2020), the linoleic acid (C18:2) content of macroalgae ranged from 2.35% to 8.69%, with green algae having the greatest concentration. According to Stabili, Cecere, et al. (2019) the main PUFAs were linoleic acid accounting for about 38.46% of total fatty acids in *C. linum*. Moreover, *C. fragile* had the highest linolenic acid (C18:3) content (6.70%). According to the Bittner et al. (2010) linolenic acid possible chemotaxonomic character of the species of *Codium*, was found in relatively high amounts (7.2 to 9.5%) in all species. In the study of Xu et al. (1998) found linolenic and eicosapentaenoic acid (EPA, C20:5 n-3) in six *Codium* species in concentrations ranging from 1 to 10% (Bittner et al., 2010; Xu et al., 1998). The fatty acid composition of macroalgae changes depending on the species, geographical region, season, climate, salinity, and light intensity, as well as the interactions between these variables (Miyashita et al., 2013). However, macroalgae contain a larger proportion of essential fatty acids, such as eicosapentaenoic (EPA) and docosahexaenoic (DHA), than terrestrial plants. Since a result, algae can be considered a natural source of significant interest, since they contain chemicals with a wide range of biological activities and may be used as a functional ingredient in a variety of technological applications to produce functional foods (Peñalver et al., 2020).

Soluble Carbohydrate Composition

According to the results of carbohydrate composition, the sugars and alditols (sugar alcohols) found in the green algae were mainly consisted of five carbohydrates including myo-inositol, mannitol, glucose, fructose and sucrose. The study of Ji et al. (2008), on analysis of neutral sugars, revealed that the predominant sugar was glucose at a level of 56.8%, followed by galactose at a level of 31.8% and mannose at a level of 11.4%. Our findings showed that glucose is an important part of sugar composition in the algae species investigated in this study. It has been found that mannans are the predominant skeletal wall polymers in several genera of green algae (Estevez et al., 2009). Moreover, the findings in respect of mannans are in parallel with the findings of Estevez et al. (2009).

Conclusion

Macroalgae are a viable food source, used in many different countries for their balanced nutritional composition, and have emerged as an alternative source of food and medicine in many countries. Being a part of human diet, green algae are recently considered as a food and a rich alternative supply of veggies. For all that, the findings of this study also showed that all the green macroalgae studied are rich in mainly important carbohydrates, which are myo-inositol, glucose, fructose and sucrose, unsaturated fatty acids (mainly oleic acid, linoleic acid, and linolenic acid) and essential minerals such as Mg, K, Cu and Fe. The nutritional value of algae, due to its high content of protein, minerals, dietary fiber, fatty acids, polysaccharides with large therapeutic potential, may contribute to the improvement of human life and the promotion of a balanced diet if consumed on a regular basis. Thus, in the light of the findings of this study, green macroalgae may suggest as an alternative functional food ingredient source for food industry. However, toxicologically properties of these algae should be investigated before their usage.

Ethical Statement

Not applicable.

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Author Contribution

Aysun Yücetepe: Investigation, experimental study, methodology, reviewing and editing, coordination. Ümit Altuntaş: Investigation, writing original draft preparation, reviewing and editing Eda Şensu: Investigation, experimental study, methodology, writing original draft preparation Yunus İzci: Investigation, experimental study, methodology Beraat Özçelik: Investigation, methodology Emine Şükran Okudan: Investigation, methodology.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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