


# The determination of the molecular weight profiles and biochemical compositions eight macroalgae species from Turkey

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**Abstract** Algae biomass could be one of the most important economic values in near future. For this reason, we determined the biochemical compositions and molecular weight profiles (MWP) of eight macroalgae; Green Algae (*Codium fragile*, *Ulva intestinalis*, *Chaetomorpha linum*, and *Codium bursa*), Red Algae (*Ellisolandia elongata*, *Jania rubens*, and *Amphiroa rigida*), Brown Algae (*Padina pavonica*) collected from different regions in Turkey. The differences measured between biochemical compositions such as ash, lipid, and protein of macroalgae species were statistically significant ( $P < 0.05$ ). The lowest and highest ash, lipid, and protein values of eight macroalgae tested were  $30.599 \pm 2.739$  % (*Ulva intestinalis*)-  $81.231 \pm 0.527$  % (*Amphiroa rigida*),  $0.190 \pm 0.063$  % (*Ellisolandia elongata*)-  $1.764 \pm 0.090$  % (*Chaetomorpha linum*), and  $2.057 \pm 0.093$  (*Amphiroa rigida*)-  $15.280 \pm 0.621$  (*Chaetomorpha linum*). The highest levels of MWP of macroalgae tested were determined in  $2532 \text{ Da} \geq$ . The lowest levels of MWP belonging to the macroalgae was observed in  $2532\text{-}13000 \text{ Da}$  except for *Ulva intestinalis* and *Padina pavonica* belonging to  $67000 \text{ Da} \leq$ . The values observed  $2532 \text{ Da} \geq$  were similar to each other except

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for *Ulva intestinalis*. The 13700 – 67000 Da and 2532-13000 Da levels belonging to *Ulva intestinalis* were higher than those of other tested macroalgae. However, level of *Ulva intestinalis* (67000 Da  $\leq$ ) was lower than those of tested macroalgae species, followed by *Padina pavonica*. In conclusion, biochemical compositions of tested macroalgae can make important contributions to feed formulations and functional foods. In addition, based on our biochemical composition analysis the specific macroalgae species are the most promising algal content for use in different industrial areas such as cosmetics, drugs, and functional foods.

**Keywords** Macroalgae . Biochemical composition . Blue bioeconomy . Circular economy . Marine science . Aquaculture

## Introduction

Algae are an important autotrophic group of organisms that have unicellular or multicellular structure (Pooja 2014). Algae can be classified into two categories according to several features, which is carried out in the form of macroalgae and microalgae. Macroalgae, also known as moss, are macroscopic and multicellular living creatures. They have contain different photosynthetic pigments and flagella. According to these characteristics, they are divided into Chlorophyta (green algae), Rhodophyta (red algae), Phaeophyta (brown algae), Chrysophyta (Diatoms), and Flagellata (Flagellates) (El Gamal 2014).

Algae are used in industrial fields such as food, agriculture, feed, cosmetics, medicine, and pharmacy. Also, algae have high biological activity and high levels of fatty acids, protein, and mineral substances (Durmaz et al. 2008). The biochemical content of macroalgae varies depending on the type, light, and temperature conditions (Durmaz et al. 2008).

About 70% of our world is covered by the sea (Visbeck 2018). The marine ecosystem and particularly marine algae draw attention as an important opportunity area in the use of biological innovations as a source of raw materials (Rotter et al. 2021). Biochemical compositions (ash, lipid, and protein) and molecular weight profiles (MWP) of soluble proteins of macroalgae are important to determine for industrial potentials. There is no knowledge about combining analysis of proximate compositions and the MWPs of soluble protein of macroalgae. Therefore, we aimed to determine the biochemical compositions (ash, lipid, and protein) and MWPs of soluble protein of eight macroalgae; Green Algae (*Codium fragile*, *Ulva intestinalis*, *Chaetomorpha linum*, and *Codium bursa*), Red Algae (*Ellisolandia elongata*, *Jania rubens*, and *Amphiroa rigida*), Brown Algae (*Padina pavonica*) collected from different regions in Turkey.

Recently, macroalgae have attracted the attention of the food industry due to their low calorie content for weight loss, high content of vitamins, minerals, and dietary fiber (Fatma et al. 2015; Taskin et al. 2020). Macroalgae consumed in Asian countries for centuries are considered promising sustainable alternatives to traditional terrestrial animal resources (Bleakley and Hayes 2017; Øverland et al. 2019; Yang et al. 2017). So, the abundance of algae in Turkey can also contribute to its economy. However, it is necessary to understand the chemical composition of these algae in order to find an effective use in the target industry. The current study aims to determine the biochemical characterization i.e (molecular weight, ash, lipid, and protein) content of eight different algal types (*Codium fragile*, *Ulva intestinalis*, *Chaetomorpha linum*, *Codium bursa*, *Ellisolandia elongata*, *Jania rubens*, *Amphiroa rigida*, and *Padina pavonica* species) which are commonly found on the coasts of the Mediterranean and Aegean Sea.

As a result of decreasing available land and water resources and increasing population, increasing demand for protein sources has led to increased interest in macroalgae as alternative protein sources.

## Materials and methods

Macroalgae were collected from the coasts of Çanakkale, Muğla, and Antalya (Fig.1). Sampling studies were carried out in the form of vertical and horizontal scans, with SCUBA diving and free diving between 0-40 m depths underwater. The sampling area has been reached by boat when necessary. Macroalgae were photographed underwater with the “Olympus OM-DE-M5” camera during the sampling (Fig. 2). Some of the collected materials have been determined in jars in 4-6% neutralized formaldehyde solution





**Fig. 1** Map of study area and location of sampling stations. Çanakkale; *Codium fragile*, Muğla; *Codium bursa*, Antalya; *Amphiroa rigida*, *Chaetomorpha linum*, *Ellisolandia elongata*, *Jania rubens*, *Padina pavonica*, *Ulva intestinalis*.

prepared with seawater, for later determination and definition in the laboratory. The identification studies of macroalgae were carried out with Olympus brand SZX16 model stereo zoom and BX51 model binocular light microscopes.

Macroalgae have been gathered in shirred bags which are made of plankton cloth underwater (70x50cm). The collected materials were cleaned with fresh water in order to remove the epiphytes, rocks, sand, and mud that may be present in them. The extracted materials have been dried in a shaded area in the laboratory for later analysis without being exposed to direct sunlight.

#### Biochemical compositions

Biochemical compositions, such as ash, lipid, and protein of eight macroalgae collected from the coasts of Çanakkale, Muğla, and Antalya were determined according to the AOAC (2000) and also, lipid analyses were performed according to the chloroform–methanol extraction method described by Bligh and Dyer (1959).

#### Molecular weight profiles

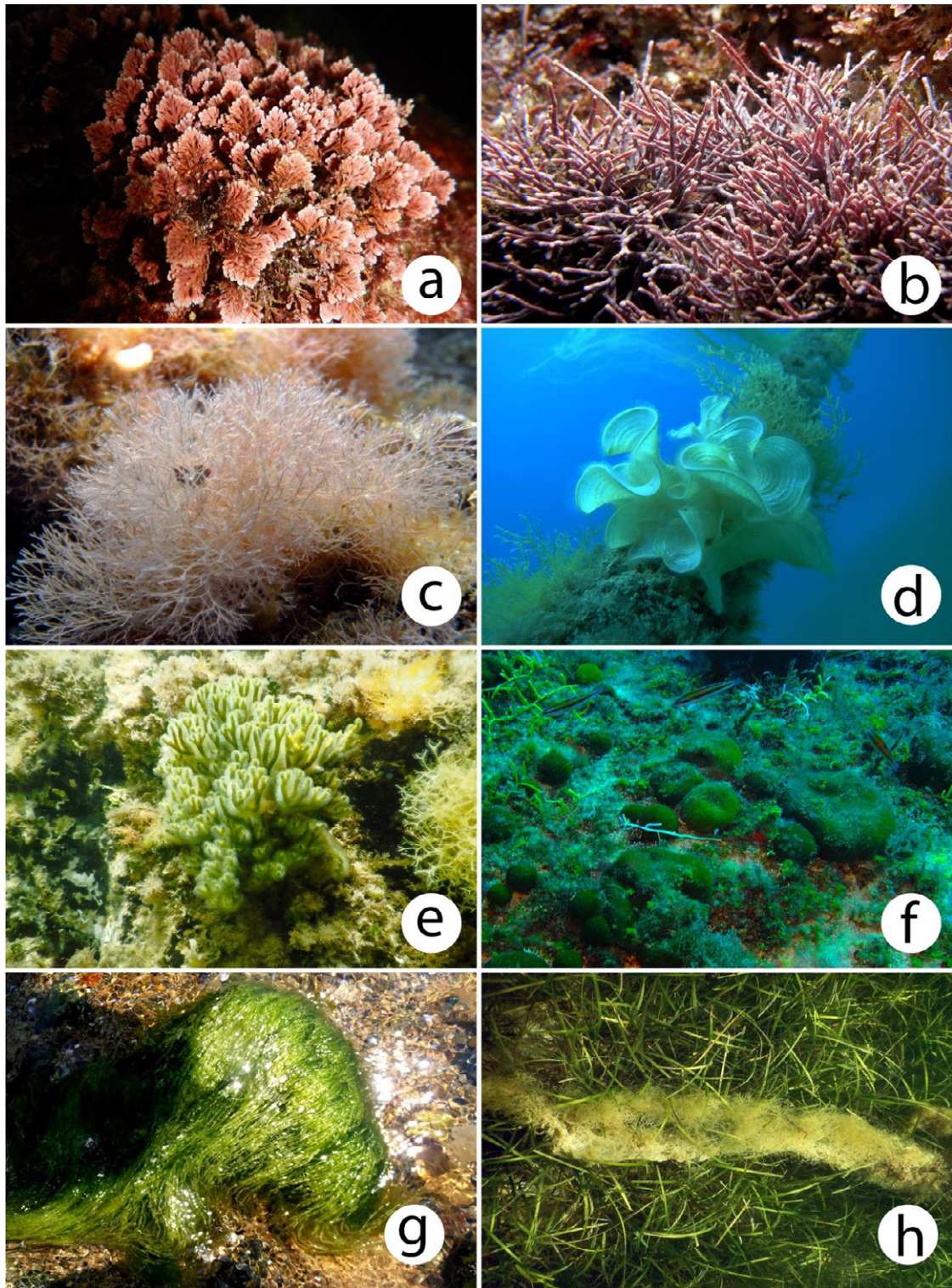
The MWPs of eight macroalgae were performed according to Boza et al. (1994). In the analysis, TSK–Gel 2000 SWXL column was used in the HPLC device and molecular weights were calculated according to the bovine albumin (67.000 Da), ribonuclease A (13.700 Da), insulin chain A (2532 Da), tyr–tyr–tyr (508 Da), tryptophan (204 Da), tyrosine (181 Da), and p–aminobenzoic acid (137 Da) standards. Retention order of the standards used in HPLC gel filtration chromatography method were bovine albumin (67.000 Da), ribonuclease A (13.700 Da), insulin chain A (2.532 Da), L–tyrosine (181 Da), 4–aminobenzoic acid (137 Da), Tyr–Tyr–Tyr (508 Da), and D–tryptophan (204 Da). The molecular weight classes of macroalgae were evaluated in 4 different sections depending on their retention times: 67.000 Da  $\leq$ , 67.000–13.700 Da, 13.700–2.532 Da, and 2.532 Da  $\geq$ . Calculating the total areas of the molecular weight classes and considering the areas of 67.000  $\leq$  Da, and 2.532 Da  $\geq$ , the areas of 67.000 Da  $\leq$ , 67.000–13.700 Da, 13.700–2.532 Da were determined as protein / polypeptide and areas of 2.532 Da  $\geq$  were determined as free amino acid / di+tri+oligopeptide.

#### Statistical analysis

Results were submitted as mean  $\pm$  standard error (SE). Statistical comparisons were made by OneWay Analysis (ANOVA) using SPSS 12. Differences were considered statistically significant when  $P < 0.05$  (Zar et al. 1999).







**Fig. 2** a; *Ellisolandia elongata*, b; *Amphiroa rigida*, c; *Jania rubens*, d; *Padina pavonica*, e; *Codium fragile*, f; *Codium bursa*, g; *Ulva intestinalis*, h; *Chaetomorpha linum* (Photography by Emine Şükran Okudan).

## Results and discussion

It is important to determine the relationship between algae biochemical composition differences and environmental conditions on Turkey's coasts. By using these biochemical ingredients, we think that consider algae is a special ecosystem. With these raw materials, could be obtained products with high

**Table 1** The stations where the macroalgae are collected in Turkey's coasts

Species Name	City	Coordinate	Depth	Date
<i>Codium fragile</i> (Suringar) Hariot 1889	Çanakkale	40° 1'35.90"K 26°19'49.49"D	0-1 m	9.07.2015
<i>Ulva intestinalis</i> Linnaeus 1753	Antalya	36°13'42.30"K 29°56'23.31"D	0-1m	7.08.2015
<i>Chaetomorpha linum</i> (O.F.Müller) Kützinger 1845	Antalya	36°13'42.30"K 29°56'23.31"D	0-1m	7.08.2015
<i>Codium bursa</i> (Olive) C.Agardh 1817	Muğla	36°47'21.46"K 28°15'18.69"D	5-25m	10.10.2015
<i>Ellisolandia elongata</i> (J.Ellis & Solander) K.R.Hind & G.W.Saunders 2013	Antalya	36°53'30.95"K 30°42'50.25"D	0-1 m	22.07.2015
<i>Jania rubens</i> (Linnaeus) J.V.Lamouroux 1816	Antalya	36°32'70.28"K 30°33'28.94"D	0,2m	29.07.2015
<i>Amphiroa rigida</i> J.V.Lamouroux 1816	Antalya	36°16'41.17"K 30° 8'28.51"D	1-3m	7.08.2015
<i>Padina pavonica</i> (Linnaeus) Thivy in W.R.Taylor 1960	Antalya	36°49'59.84"K 31° 7'28.67"D	1-2m	13.08.2015

**Table 2** Proximate compositions of macroalgae (mean±SD)

Green Algae	Proximate Compositions		
	Ash (%)	Lipid (%)	Protein (%)
<i>Codium fragile</i>	53.818 ± 1.750 <sup>c</sup>	1.536 ± 0.061 <sup>c</sup>	8.497 ± 0.044 <sup>d</sup>
<i>Ulva intestinalis</i>	30.599 ± 2.739 <sup>a</sup>	0.642 ± 0.060 <sup>c</sup>	13.100 ± 0.447 <sup>e</sup>
<i>Chaetomorpha linum</i>	45.382 ± 0.997 <sup>b</sup>	1.764 ± 0.090 <sup>f</sup>	15.280 ± 0.621 <sup>f</sup>
<i>Codium bursa</i>	69.728 ± 2.365 <sup>e</sup>	0.897 ± 0.030 <sup>d</sup>	2.488 ± 0.316 <sup>a</sup>
Red Algae			
<i>Ellisolandia elongata</i>	76.442 ± 0.094 <sup>f</sup>	0.190 ± 0.063 <sup>a</sup>	5.218 ± 0.082 <sup>c</sup>
<i>Jania rubens</i>	76.495 ± 0.813 <sup>f</sup>	0.329 ± 0.048 <sup>b</sup>	4.416 ± 0.169 <sup>b</sup>
<i>Amphiroa rigida</i>	81.231 ± 0.527 <sup>g</sup>	0.242 ± 0.033 <sup>ab</sup>	2.057 ± 0.093 <sup>a</sup>
Brown Algae			
<i>Padina pavonica</i>	61.993 ± 0.621 <sup>d</sup>	0.674 ± 0.110 <sup>c</sup>	4.299 ± 0.138 <sup>b</sup>

added value to ensure sustainable development efficiency. The application of algal bioactive contents in the pharmaceutical and nutraceutical industries will be important in the near future (Gomes-Dias et al. 2022; Mandalka et al. 2022).

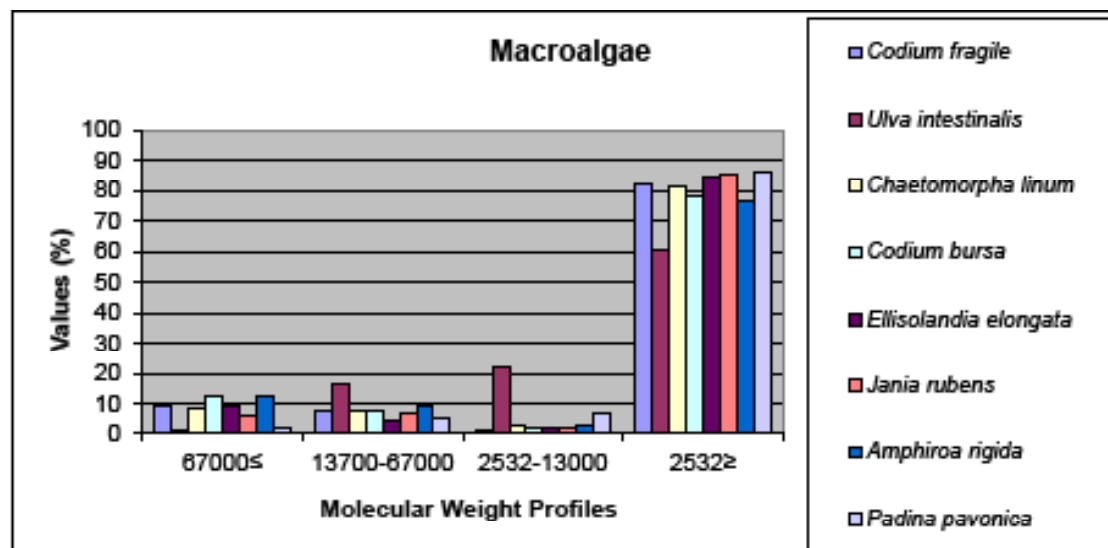
The aim of this study was to determine the biochemical compositions (ash, lipid, and protein), and MWPs of soluble protein of eight macroalgae; Green Algae (*Codium fragile*, *Ulva intestinalis*, *Chaetomorpha linum*, and *Codium bursa*), Red Algae (*Ellisolandia elongata*, *Jania rubens*, and *Amphiroa rigida*), Brown Algae (*Padina pavonica*) collected from stations mentioned in Table 1. The biochemical compositions, and MWPs (HPLC-Gel filtration chromatography) of eight macroalgae are summarized in Table 2, and Fig. 3, respectively.

The differences observed in the ash, lipid and protein values of the eight macroalgae tested were statistically significant ( $P < 0.05$ ). The protein contents of the eight macroalgae ranged from  $2.057 \pm 0.093\%$  (*Amphiroa rigida*) to  $15.280 \pm 0.621\%$  (*Chaetomorpha linum*). The highest protein levels were determined in green algae except for *Codium bursa* ( $2.488 \pm 0.316\%$ ). Protein values of red and brown algae were similar to each other except for *Amphiroa rigida* ( $2.057 \pm 0.093\%$ ).

It has been reported that the protein content is generally between 10-30% in red and green algae, and between 5-15% in brown algae (Burtin 2003). Dawczynski et al. (2007) revealed that protein levels of red algae were higher than those of brown algae. However, Aycan and Sayin (2020) showed that the protein ratio of *Ellisolandia elongata*, which is the only red algae species, is similar to *Sargassum vulgare* and lower than *Dictyota dichotoma* from brown algae. According to the results of our study, protein levels of green







**Fig. 3** Molecular weight profiles of macroalgae; Green Algae (*Codium fragile*, *Ulva intestinalis*, *Chaetomorpha linum*, and *Codium bursa*), Red Algae (*Ellisolandia elongata*, *Jania rubens*, and *Amphiroa rigida*), Brown Algae (*Padina pavonica*).

and red algae except for *Codium bursa* and *Amphiroa rigida* were higher than those of *Padina pavonica* from brown algae as mentioned by Burtin (2003) and Dawczynski et al. (2007). Olsson et al. (2020) were indicated that the protein content of seaweeds on the Swedish west coast which was most abundant in the red seaweeds but was generally low in all species (59–201 g kg<sup>-1</sup> dw). Besides, It were stated that the green macroalgae species *Ulva fasciata* was found to have the highest protein (20.66 ± 0.04%), nitrogen contents (3.31 ± 0.01%), and *Cladophora laetevirens* had the highest lipid contents (Haroon et al. 2018).

Wahbeh (1997) showed that the protein content of the macroalgae *Padina pavonica* collected at the beach in Aqaba, Jordan was 17.4%. Tabarsa et al. (2012) found a protein content of 11.83% in *Padina pavonica*, which they collected in April in southern Iran (Persian Gulf). Ozgun et al. (2015) showed that the protein levels of eight brown macroalgae collected from Iskenderun Bay was varied from 2.897±0.373% to 6.519±0.432%. The protein level of *Padina pavonica* tested in the study were lower than those of protein levels revealed by Wahbeh et al. (1997) and Tabarsa et al. (2012) but not Ozgun et al. (2015). Mazlum et al. (2021) revealed that the protein level of *Jania rubens* from red macroalgae was 5.99. The protein level of *Jania rubens* tested was similar to the level determined by Mazlum et al. (2021). Studies have shown that seasonal changes have caused changes in the biochemical compositions of macroalgae.

The protein content of macroalgae varies within seasonal cycles. A recent review lists the protein content of macroalgae species from different geographical regions (Koyande et al. 2019; Pimentel et al. 2019). Gordalina et al. (2021) studied on protein isolation and characterization using SDS-PAGE methods in different seaweed species.

Unlike microalgae, macroalgae have been used in human nutrition for many years, meaning that most of them do not need new approval for human consumption (Banach et al. 2020). It is known that, the amount of EFSA-approved macroalgae species is significantly higher than microalgae. Andreeva et al. (2021) showed that protein concentrates obtained from tested algae species in the study can be used as a protein source for both food products and feed additives.

The lowest and highest lipid amounts of eight macroalgae were found in *Ellisolandia elongata* (0.190 ± 0.063%) and *Chaetomorpha linum* (1.764 ± 0.090%), respectively. The highest lipid levels were observed in green macroalgae, followed by *Padina pavonica* which belongs to brown macroalgae. The lipid level of *Ulva intestinalis* belonging to green macroalgae was similar to *Padina pavonica*. The lipid levels of red macroalgae were lower than those of other macroalgae species.

Wahbeh et al. (1997) revealed that the lipid content of the macroalgae *Padina pavonica* collected at the beach in Aqaba, Jordan was 4.4%. McDermid and Stuercke (2003) reported that lipid content of macroalgae was less than 4%. Polat and Ozogul (2008) showed that lipid contents of red and brown algae were between 1.10-11.53%. Lipid contents are generally low in macroalgae, at between 1-5% (Singh and Singh 2015).



Gosch et al. (2012) showed that the lipid content of brown seaweeds (*Phaeophyceae*) typically had the highest total lipid content, followed by green (*Chlorophyta*) and red seaweeds (*Rhodophyta*).

Ahmad et al. (2012) stated that brown algae species have higher lipid content than red and green algae species. The results of our study were supported by Ahmad et al. (2012) but not green macroalgae. In our study, lipid contents except for *Codium fragile* and *Chaetomorpha linum* were lower than those of 1–5% contents stated by Singh and Singh (2015). Chakraborty and Bhattacharya (2012) mentioned that lipid contents of macroalgae may vary depending on the type and amount of nutritive elements in the environment. Also, Singh et al. (2015) indicated that lipid contents of macroalgae depends on light intensity, salinity, and temperature conditions. Mazlum et al. (2021) revealed that lipid content of *Jania rubens* from red macroalgae was  $0.39 \pm 0.103$ . Lipid content of *Jania rubens* tested was similar to the content determined by Mazlum et al. (2021).

Algal ash content is an important food, feed, and feedstock for biofuel production. Algae can contain as much as 70% dry matter ash in different locations. Algal ash content not only reduces the amount of ash-free matter, the valuable part of algae biomass but also leads to a concern for heavy metal toxicity and decreases inclusion levels for algae used in food and agriculture (Liu 2017; de Souza et al. 2019). The highest and lowest ash values were found in *Amphiroa rigida* ( $81.231 \pm 0.527\%$ ) and *Ulva intestinalis* ( $30.599 \pm 2.739\%$ ), respectively. The highest ash values were found in red macroalgae. *Padina pavonica* belonging to brown macroalgae was higher than those of green macroalgae species except for *Codium bursa*. Tabarsa et al. (2012) emphasized that the ash content of macroalgae varies between 8–40% of their dry weight. Polat and Ozogul (2008) determined that the ash contents varies between 2.28–51.63%. On the other hand, Liu (2017) reveal that algae can contain as high as 70% dry matter ash in different locations. Wahbeh (1997) indicated that the ash content of the macroalgae *Padina pavonica* collected at the beach in Aqaba, Jordan was 23.1%. Ozgun and Turan (2015) showed that the ash contents of eight brown macroalgae collected from Iskenderun Bay was varied from  $1.66 \pm 0.29\%$  to  $18.19 \pm 2.66\%$ . Uslu et al. (2021) determined that the ash amount of the macroalgae *Padina pavonica* collected from Antalya, Turkey was  $22 \pm 0.2\%$ . The results of Tabarsa et al. (2012) Polat and Ozogul (2013) and Ozgun and Turan (2015) were lower than those of the results of our study except for *Ulva intestinalis* and *Chaetomorpha linum*. *Padina pavonica* ash contents revealed by Wahbeh (1997) and Uslu et al. (2021) were lower than those of *Padina pavonica* tested in our study. Ash content results of macroalgae tested were supported by Liu (2017). Factors such as geographical location and season might be important factors in change the ash content of macroalgae (Renaud et al. 2006; Mohamed et al. 2012; Cabrita et al. 2016). Mazlum et al. (2020) revealed that ash content of *Jania rubens* from red macroalgae was  $78.740 \pm 0.066$ . Ash content of *Jania rubens* tested was similar to the content determined by Mazlum et al. (2020).

MWPs of eight macroalgae Green Algae (*Codium fragile*, *Ulva intestinalis*, *Chaetomorpha linum* and *Codium bursa*), Red Algae (*Ellisolandia elongata*, *Jania rubens* and *Amphiroa rigida*), Brown Algae (*Padina pavonica*) collected from Turkey were given in Fig. 4. The highest MWPs in macroalgae tested in the present study were determined in  $2532 \text{ Da} \geq$ . The lowest contents were found in  $2532\text{--}13000 \text{ Da}$  except for *Ulva intestinalis* and *Padina pavonica*. The highest MWPs belongs to  $67000 \leq \text{Da}$ ,  $13700\text{--}67000 \text{ Da}$  and  $2532\text{--}13000 \text{ Da}$  were observed in *Amphiroa rigida* ( $12.535 \pm 0.57\%$ ), *Ulva intestinalis* ( $16.16 \pm 0.73\%$ ), and *Ulva intestinalis* ( $21.94 \pm 0.29\%$ ). The MWPs belongs to  $67000 \leq \text{Da}$  of *Codium bursa* ( $12.44 \pm 1.08\%$ ) and *Amphiroa rigida* ( $12.535 \pm 0.57\%$ ) were similar to each other. On the other hand, the MWPs belongs to  $67000 \leq \text{Da}$  and  $2532 \text{ Da} \geq$  of *Ulva intestinalis* were lower than those of other tested macroalgae species. The MWPs belong to  $67000 \leq \text{Da}$ ,  $13700\text{--}67000 \text{ Da}$ , and  $2532\text{--}13000 \text{ Da}$  of macroalgae species were lower than 20% except for *Ulva intestinalis* ( $21.94 \pm 0.29\%$ ). Kolkovski (2004) and Kolkovski (2008) reported that in general, molecular weight classes between 1,000 and 10,000 daltons were positive effect on nutrition.

Diken et al. (2018) observed that the molecular weight profiles of animal and vegetable (except for soy protein concentrate–SPC) feed ingredients were high in the  $2.532 \text{ Da} \geq$  group. Also, the molecular weight profiles of *Ulva* sp. and *Sargassum* sp. tested in this study were the highest in the group of  $2.532 \text{ Da} \geq$ . The above-mentioned differences might be attributed to environmental conditions and algal species differences.

It has been known that protein forms used in feeds have an important role in the development of the larval digestive system (Cahu and Infante 1995; Zambonino Infante et al. 1997). Ronnestad et al. (1999) and Holt (2000) indicated that the identification of the protein forms may contribute towards the more appropriate feed formulation for critical larval stages. MWPs showed that macroalgae tested had an exogenous supply



of free aminoacids. On the other hand, the use of macroalgae in feed formulations may cause the high leaching ratios due to the high contents of  $2532 \geq$  Da MWP of eight macroalgae.

It is well known that biochemical compositions of algae species could be linked to cultivating conditions, algal developmental stages, species specificity, genetic differences, or differences in environmental conditions (Araujo et al. 2021; De Vicoose et al. 2012; Degeorges and Masquelet 2002; Khan et al. 2018).

## Conclusion

In conclusion, all tested macroalgae can make important contributions to feed formulations and be the basis for the development of functional foods. Considering these MWPs data results, it is cautioned that the use of macroalgae in feed formulations may cause the high leaching ratios containing  $2532 \geq$  Da MWP. In addition, based upon our research on biochemical composition in the macroalgae species, green, brown, and red macroalgae are the most promising algal species for use in different industrial applications such as cosmetics, drugs, and functional foods with great benefits for the human health and our society.

**Authors contribution** EIS & TD & SS and MG: Design and conceptualization of the study; EIS & ZC: finalization of the draft; MN & ESO: Collection of the sample; EIS & SS & MN: laboratory analysis of the sample and drafting the manuscript; PGK and SBB: Statistical analysis, and revision of the draft; Wet lab analysis: SS & EIS and MN; NB and EO: Data acquisition and interpretation; OCA & SV and AG: Material composition analysis and manuscript language editing; EIS: Coordination of the study and finalization of the manuscript.

**Conflicts of interest** The authors declare that they have no conflict of interest.

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