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Research Article

Unlocking the Fatty Acid Profile of Macroalgae Species in Sea of Marmara, Türkiye: A Comprehensive Analysis of Extraction Methods

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Abstract

In the present study, the determination of proximate composition (moisture, ash, protein, and lipid) and fatty acid contents of three macroalgae (Ulva Lactuca, Cystoseira Barbata, and Ceramium Rubrum) collected from the southern coast of Istanbul in the Sea of Marmara in April 2021. The ash content was highest in Cystoseira Barbata (19.20%). The protein content ranged between 9.88% – 10.21%. The performances of soxhlet and cold press extraction methods were compared in determining the lipid content of macroalgae. The cold pressing extraction method was shown to have a higher yield of lipid extracts than the soxhlet extraction method. The most abundant fatty acid in the macroalgae species was palmitic acid (C16:0, 35.35 – 47.34%) from saturated fatty acids (SFAs). Oleic acid (C18:1, 18.78 – 25.25%) and palmitoleic acid (C16:1, 14.24 – 15.36%) were other plentiful fatty acids from monounsaturated fatty acids (MUFAs) in the studied species. Linoleic acid (C18:2ω6), α-linolenic acid (C18:3ω3), and docosahexaenoic acid (C22:6ω3) from polyunsaturated fatty acids (PUFAs) levels varied from 3.67 – 4.64%, 4.32 – 5.68%, 1.21 – 2.75%, respectively. It was concluded that the proximate composition and types and contents of fatty acids vary depending on species, season and geography.

Keywords: Proximate composition, fatty acids, macroalgae, Istanbul, Sea of Marmara

Introduction

Macroalgae constitute high levels of biomass in coastal areas and account for more than half of primary production worldwide (Caf, et al., 2015; Savun-Hekimoğlu, and Gazioğlu, 2021). Macroalgae provide food and habitat to many organisms in aquatic environments. Macroalgae are separated into three groups according to their pigmentation: green, brown, and red algae (Gür İ., 2023). Macroalgae have a wide range of uses such as food, animal feed, fertilizer, and as a source of bioactive compounds of biomedical and pharmaceutical (Chandran, 2015; Gür İ., 2023; Polat and Ozogul, 2008). These bioactive substances (proteins, polysaccharides, polyphenols, and lipids) have antiviral, antifungal, and antibacterial properties (Aslan, et al., 2019; Turan, S, Sayin, and Ozyilmaz, 2015). Additionally, macroalgae contain vitamins and minerals that are valuable nutritional sources (Gür İ., 2023; Kumar, et al., 2008). Algae, used as a resource in various industries, have many advantages. These advantages are the possibility of production that can be spread throughout the year, no dependence on soil and fertilizer, and even the ability to grow with wastewater (Wei, et al., 2013).

Recently, macroalgae lipids have attracted increasing attention due to their many health benefits (Miyashita, et al., 2013; Miyashita et al., 2011). Although macroalgae have low lipid content (1–5 wt% total lipid per dry weight), they are an important source of lipids due to their abundance in marine waters (Miyashita et al., 2013). The lipid content of macroalgae varies depending on geographical location, salinity, temperature, light intensity, type, and interactions between these factors (Miyashita et al., 2013; Sánchez-Machado, et al., 2004). Macroalgae generally contain high amounts of oleic, palmitic acids, and polyunsaturated fatty acids. C18 and C20 polyunsaturated fatty acids are essential in the diet of humans and all animals, especially due to their important roles in immune, cardiovascular, and reproductive functions (Yazici et al., 2007). Omega-3 (ω3) and omega-6 (ω6) are the two main groups of polyunsaturated fatty acids (van Ginneken, et al., 2011). Since ω3 polyunsaturated fatty acids do not exist in the natural structure of humans, they are taken into the body through food. Fish are the most important commercial source of polyunsaturated fatty acids, but alternative sources of polyunsaturated fatty acids continue to be explored (Caf et al., 2015). In recent years, macroalgae have become among the most remarkable resources for polyunsaturated fatty acids.

To benefit from macroalgae in many areas, a lot of studies are being carried out on the biochemical and proximate compositions of diverse macroalgae collected around the world (Ahmad, et al., Matanjun, 2012; Banerjee, et al., 2009; Bernert, 1989; Caf et al., 2015; Gressler et al., 2011; Miyashita et al., 2013; Renaud and Luong-Van, 2006; Rohani-Ghadikolaei, et al., 2012). Turkey's Sea of Marmara coasts are very rich in biomass and algal biodiversity. There is limited investigation about the proximate composition of the Sea of Marmara economically important macroalgae in literature (Koçer and Özçimen, 2021; Şükran, et al., 2003) This work is aim to investigate the proximate composition and fatty acid contents of macroalgae species found on the coasts of Istanbul, Sea of Marmara. In this study, three macroalgae species (Ulva Lactuca, Cystoseira Barbata, Ceramium Rubrum) that commonly found in this region were chosen for the investigation.

Fig. 1. Dominant macroalgae species on the shores of the Sea of Marmara.

Materials and Methods
Sampling

Macroalgae were collected and divided into species from the southern coast of Istanbul in the Sea of Marmara in April 2021. In addition, mixed algae samples collected as waste by ISTAC Inc., Istanbul Envıronment Management Industry and Trade Company were also used. Ulva Lactuca (green algae), Cystoseira Barbata (brown algae), Ceramium Rubrum (red algae) were the dominant species in this region. The dominant macroalgae species on the shores of the Sea of Marmara are given in Figure 1. The samples were rinsed with distilled water to remove unwanted particles. After the samples were brought to the laboratory, they were stored at 20°C until analysis.

Proximal analysis
The proximate compositions of macroalgae were determined: water and ash content by AOAC's method (AOAC, 1996). The amount of nitrogen is calculated on the CHN elemental analyzer (Carlo Erba NZ 1500) and multiplied by the coefficient of 6.25 to find the content of protein.

Lipid extraction
Two methods were used for lipid extraction. These methods are soxhlet extraction with hexane for 12 hours and cold press methods. 0.4 mL of the liquid fractions obtained as a result of the two extraction methods were transferred to tubes and completely dried in nitrogen gas. After that, 0.4 mL of concentrated H2SO4 was added and heated in a 60oC water bath for 10 minutes. After the content of the tube cooled down, 0.04 M phosphovanillin reagent was added, the content was mixed by vortex and waited for 30 minutes for color change. Consequently, The absorbances were measured at a wavelength of 520 nm by a UV-Vis spectrophotometer (AFNOR, 1996; Folch, Lees, and Sloane Stanley, 1957). Results are expressed as a percentage of the dry weight of algae. The efficiency of these two methods is compared and shown in Table 1.

Fatty acids analyses
Gas chromatography-mass spectrophotometry was used for the fatty acids analyses. 10 grams of dry algae samples are subjected to soxhlet extraction with a 3:1 mixture of ethanol: hexane. The extracts were also derivatized with 5 ml of 2% methanolic acid (sulfuric acid prepared in methanol), shaking occasionally and kept at 50°C for 12 hours. Then 5 ml of 5% NaCl was added. This mixture was extracted with hexane and 5 ml of 2% KHCO3 was added. It was evaporated to dryness by nitrogen gas. The eluent volume was completed to 1 ml hexane and analyzed with GC-MS (Bernert, 1989).

Results and Discussions
Moisture, ash, and protein

Three macroalgae were collected from the southern coast of Istanbul in April 2021 and analyzed their nutritional compositions. The moisture, total protein, and ash results of the three macroalgae species are shown in Table 1. The moisture content of macroalgae varied from 85.40 % to 90.34 %. The highest moisture content was found in red algae (Ceramium Rubrum (90.34 %)). The content of ash was the range of 18.45 % – 19.20 %. The ash contents of the macroalgae species in this study were similar to the studies of Turan et al. (2015), Gür (2023), Polat and Ozogul (2009), and Rohani-Ghadikolaei et al. (2012) (Gür İ., 2023; Polat and Ozogul, 2008; Rohani-Ghadikolaei et al., 2012; Turan et al., 2015). The high ash levels of macroalgae indicate that these species are rich in minerals. The protein content of macroalgae ranged from 9.88 % to 10.21 %. The maximum protein content was determined in brown algae (Cystoseira Barbata (10.21 %). The protein content of macroalgae in this study is slightly higher than different species reported by Gür (2023) but similar to other species reported by Ortiz et al. (2006). Ash moisture, and protein content of macroalgae varies depending on habitats, species and seasonal period (Fleurence, 1999; Gür İ., 2023; Turan et al., 2015).

Lipids

In this study, lipid contents of macroalgae were determined using two different extraction methods. Then, the performances of these two methods were compared according to the oil content results. The lipid contents of macroalgae vary between 7.81 % - 8.21 % with the soxhlet extraction method and 8.01 % - 8.99 % with the cold pressing method (Table 1). The lipid content of macroalgae is generally low (less than 4%) in the literature (Herbreteau, Coiffard, Derrien, and Roeck-Holtzhauer, 1997; Polat and Ozogul, 2008). The lipid levels in this work were higher than the various species determined Ortiz et al. (2006), Gür (2023). The lipid content obtained by the cold pressing method was higher than the soxhlet method. In this study, as in other studies in the literature, it was determined that the extraction method had an important effect on the extraction efficiency (Cvitković, Dragović-Uzelac, Dobrinčić, Čož-Rakovac, and Balbino, 2021; Najdek, IVEŠA, Paliaga, BLAŽINA, and ČELIG, 2014; Shen and Shao, 2005; Zhuang, McKague, Reeve, and Carey, 2004). The cold press method saves time and is an environmentally friendly extraction method as less solvent is used compared to the soxhlet method.

Table 1. Proximate compositions of macroalgae from Istanbul in the Sea of Marmara.

Macro algae species	Moisture (%)	Protein (%)	Ash (%)	Lipid (%) (With Soxhlet Method)	Lipid (%) (With cold pressing method)
Ulva Lactuca	85.40 ± 2.5	9.88 ± 1.1	18.45 ± 1.2	7.81 ± 1.9	8.01 ± 2.7
Cystoseira Barbata	88.20 ± 2.8	10.21 ± 1.3	19.20 ± 2.0	8.21 ± 1.8	8.99 ± 2.1
Ceramium Rubrum	90.34 ± 1.1	9.97 ± 1.5	18.98 ± 1.9	8.12 ± 2.8	8.84 ± 1.6
Mixed Algae	87.54 ± 1.4	9.98 ± 1.7	18.78 ± 2.2	7.99 ± 1.9	8.24 ± 1.7

Table 2. Fatty acids compositions of macroalgae species.

Fatty acids (%)	*Ulva Lactuca*	*Cystoseira Barbata*	*Ceramium Rubrum*	Mixed algae
C14:0	2.81 ± 0.82	4.94 ± 0.86	1.65 ± 0.41	2.00 ± 0.45
C16:0	35.35 ± 1.69	44.32 ± 1.11	47.34 ± 0.58	42.34 ± 0.82
C18:0	5.35 ± 1.61	4.34 ± 0.42	3.99 ± 0.28	4.45 ± 0.41
ΣSFA	43.51	53.60	52.98	48.79
C16:1	14.24 ± 1.1	15.36 ± 0.57	14.89 ± 0.80	14.85 ± 0.78
C18:1	25.25 ± 0.56	18.78 ± 1.29	19.02 ± 0.24	22.21 ± 0.59
C20:1	4.25 ± 0.84	2.86 ± 0.66	3.01 ± 0.44	3.55 ± 0.50
ΣMUFA	**43.74**	**37.00**	**36.92**	**40.61**
C18:2ω6	4.64 ± 0.39	3.87 ± 0.14	3.67 ± 0.23	4.01 ± 0.42
C18:3ω3	5.66 ± 0.58	4.32 ± 0.20	4.44 ± 0.25	4.56 ± 0.22
C22:6ω3	2.75 ± 0.02	1.21 ± 0.26	1.99 ± 0.44	2.03 ± 0.44
ΣPUFA	**13.05**	**9.40**	**10.10**	**10.60**
Σω6	4.64	3.87	3.67	4.01
Σω3	8.41	5.53	6.43	6.59
ω6/ω3	0.55	0.70	0.57	0.61

SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid.

Fatty acids

The fatty acids contents of macroalgae are shown in Table 2. The fatty acids determined in the macroalgae wereC14:0, C16:0, C16:1, C18:0, C18:1, C18:2ω6, C18:3ω3, C20:1, C22:6ω3. The total sum of fatty acid levels of each macroalgae species varied as follows: saturated fatty acids (SFAs) 43.51% – 53.60%, mono¬unsaturated fatty acids (MUFAs) 36.92% – 43.74%, polyunsaturated fatty acids (PUFAs) 9.40% – 13.05%. Palmitic acid (C16:0) was the highest content of fatty acid in all macroalgae species examined. Oleic acid (C18:1) is the second most abundant fatty acid in these species. Additionally, palmitoleic acid (C16:1) is another MUFA detected at high contents in all species. Total PUFA levels were 13.05%, 9.40%, 10.10% and 10.60% for Ulva Lactuca, Cystoseira Barbata, Ceramium Rubrum and mixed algae, respectively. Linoleic acid (C18:2ω6), α-linolenic acid (C18:3ω3), and docosahexaenoic acid (C22:6ω3) were detected in all species. Linoleic acid and α-linolenic acid, which cannot be synthesized by vertebrates and humans, are important for nutrition (Caf et al., 2015; Ortiz et al., 2006). PUFAs contents in macroalgae obtained in this study are compatible with the results reported by Caf et al. (2015) and Cvitković et al. (2021) (Caf et al., 2015; Cvitković et al., 2021). Macroalgae are single organisms that have enzymes that can produce long-chain PUFAs (e.g., 20:5n-3, 22:6n-3) (Hamilton, 1995). Polat and Ozogul 2009 reported that α-linolenic acid (C18:3ω3) is present ranged from 0.31% – 0.56% in macroalgae species. Polat and Özoğul 2009 reported that α-linolenic acid (C18:3ω3) varies between 0.31% and 0.56% in various macroalgae species. The α-linolenic acid contents in this study are higher than those of different macroalgae species reported by Polat and Özoğul 2009. According to the World Health Organization (WHO), the ω6/ω3 ratio in a diet should not be higher than 10 for human health. This ratio is necessary to aid in preventing cancer risk and coronary heart disease (WHO, 2003). The ω6/ω3 result determined by Panayotova et al. (2017) for Cystoseira Barbata was similar to the result in this study (Panayotova, et al., 2017). The ω6/ω3 ratio (2.4) found by Yazıcı et al. (2007) for the Cystoseira barbata species in the Black Sea and Dardanelles is higher than the ratio (0.57) determined in this study. The ω6/ω3 ratios found in this study are below the value recommended by the World Health Organization.

Conclusions

The proximate and fatty acid compositions of Ulva Lactuca, Cystoseira Barbata, Ceramium Rubrum from the southern coast of Istanbul in the Sea of Marmara in 2021 were investigated. In addition, the effectiveness of soxhlet and cold press extraction methods used to determine the lipid contents of macroalgae were compared. In this study, it was shown that the cold pressing method was more effective than the soxhlet method in determining lipid content. The cold press method is an environmentally friendly extraction method because less solvent is used compared to the soxhlet method. The macroalgae examined in this study have significant protein content and high ash and total lipid content. Palmitic acid (C16:0) and oleic acid (C18:1) were determined to be the most abundant fatty acids in these species. Linoleic acid and α-linolenic acid, which cannot be synthesized by humans and are important for nutrition, have been detected in all species. The ω6/ω3 ratio, which is important for human health, is below the 10 value determined by WHO. This study shows that Marmara Sea macroalgae can be used in cosmetics, the pharmaceutical industry, agriculture, and even as biofuel raw materials, thanks to the biologically active components they contain.

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