

# Journal of Biodiversity and Environmental Sciences (JBES) ISSN: 2220-6663 (Print) 2222-3045 (Online) Vol. 6, No. 4, p. 401-411, 2015 http://www.innspub.net

# OPEN ACCESS

Biochemical components of three marine macroalgae (*Padina pavonica*, *Ulva lactuca* and *Taonia atomaria*) from the levantine sea coast of antalya, Turkey

Fatma CAF<sup>1,\*</sup>, Ökkeş Yilmaz<sup>2</sup>, Furkan Durucan<sup>3</sup>, Nurgül Şen Özdemir<sup>4</sup>

<sup>1</sup>Bingöl University, Faculty of Art and Sciences, Department of Biology. 12000 Bingöl, Turkey <sup>2</sup>Fırat University, Faculty of Sciences, Department of Biology. 23119 Elazığ, Turkey <sup>3</sup>Süleyman Demirel University, Faculty of Fisheries, Department of Marine Biology, Isparta, Turkey <sup>4</sup>Bingöl University, Faculty of Agriculture, Department of Fisheries.12000 Bingöl, Turkey Article published on April 30, 2015

Key words: Edible seaweed, Fatty acids, Biochemical component, Levantine Sea, Turkey.

## Abstract

Green macroalgae Ulva lactuca, brown macroalgae Taonia atomaria and Padina pavonica are spread in the Turkish Levantine Sea. There is limited information about antioxidant activities and fatty acid composition of these species from Levantine Sea. In this study was to determine and compare antioxidant activities, vitamin and fatty acid (FA) composition of U. lactuca, T. atomaria and P. pavonica. The analysis was made with HPLC and GC device. g. Then, the results were analyzed using SPSS software. The results showed; palmitic acid (C16:0) as the most abundant saturate fatty acid (21-41%). The green algae was rich palmitic acid (C16:0) (41.68%). Monounsaturated fatty acids (MUFAs) were major components (39.81-42.89%). The total MUFA content for U. lactuca was 40.63%, P. pavonica 42.89% and for T. atomaria 38.81%. Oleic acid (C18:1 n-9) was the most abundant MUFA in all the species analyzed. Eicosapentaenoic acid (C20:5 n-3) and arahidonic acid (C20:4 n-6) were found in significant levels in T. atomaria. P. pavonica and T. atomaria showed similar amounts of C18 and C20 PUFAs contents. In T. atomaria eicosopentaenoic acid (EPA, C20:5n3) accounted 4.78% of total fatty acids. PUFA/SFA ratio in T. atomaria was 1.10%, U. lactuca; 0.26% and for P. pavonica 0.68%. The total phenolic contents ranged from 0.96 to 2.22 mg gallic acid equivalents per 1 g of dry macroalgae material. Phenolic content of the water extract of T. atomaria (2.22 mg GAE /g) was higher than that of the water extract of P. pavonica and U. lactuca. It has been thought that the amount of  $\alpha$ -tocoferol was higher than the other lipophilic vitamins in all the three species tested. In Conclusion; these species can be used as food and in food industry.

\*Corresponding Author: Fatma CAF 🖂 fcaf@bingol.edu.tr

### Introduction

Commercially available varieties of marine macroalgae are commonly referred to as "seaweeds". Macroalgae can be classified as red algae (Rhodophyta), Brown algae (Phaeophyta) or green macroalgae (Chlorophyta), depending on their nutrient and chemical composition. Red and brown macroalgae are mainly used as human food sources (Dawczynski *et al.*, 2007).

Marinealgae are considered to be a rich source of phenolic compouns with a number of important biological activities such as phlorotannins, which are polymers of phloroglucinol and constitune an extremely heteregeneous group of moleculer (Yu *et al.,* 2009).

Marine macroalgae are important constituents of aquatic ecosystems, accounting for more than half the total primary production at the base of the food chain worldwide. Algal lipids are major dietary components for primary consumers where they are a source of energy and essential nutrients. The role of algal polyunsaturated fatty acids (including the human essential fatty acids linoleic (LIN; 18:2 n -6) and alfa– linolenic (ALA; 18:3n-3) as well as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) in aquatic food webs is well documented. They provide a substantial contribution to the food quality for invertebrates and are vital for maintaining somatic and population growth, survival, and reproductive success (Arts *et al.*, 2009).

The n-3 PUFAs can not be synthesized by humans and are thus obtained through diet. In view of their promising medical and nutritional applications, they have been extensively investigated. At present, marine fishes and fish oils are the main commercial sources of PUFAs but their suitability for human consumption has been questioned from a biosafety perspective, raising the need to search for alternative sources of high quality PUFAs (Bhosale *et al.*, 2009). Turkish Mediterranean Sea coast is rich in marine macroalgae, regarding biomass and algal biodiversity. Three species of macroalgae - Ulva rigida and Padina pavonica, Taonia atomaria belonging to the two phyla Chlorophyta, and Phaeophyta, respectively, were chosen for the present study based on their wide distribution in the littoral zone of Turkey. There is limited information about the fatty acids contents of Turkish Mediterranean Sea macroalgae in literature. Because, fatty acids are essential compounds in the cell and are fats that are vital for life but they are also, substances that can not be produced by the human body (Tehlivets et al., 2007). Today, due to improper diet habits, these fats and vitamins are consumed as low as to threaten health and therefore, vital disorders occur in the body. Although, the fatty acid and vitamin content of macroalgae, which are commonly used in our traditional diet are not known exactly, a study towards analyzing these differences has not been conducted as yet. The aim of this study was to determine and compare antioxidant activities, vitamin and fatty acid (FA) composition of U. lactuca, T. atomaria and P. pavonica.

### Materials and methods

#### Study area and sampling

Marine macroalgae were from several rocky shores by hand in April 2014 from Lara coast (Antalya, Turkey) (Fig. 1-2). Washed to remove from epifits, sediment and another organic matter several times with sea water. These macroalgae moved to the laboratory in bags. Then, washed again with distiled water. The dried material was powdered (particle size <910  $\mu$ m) and kept in the dark, in a desiccator, until fatty acids extraction.

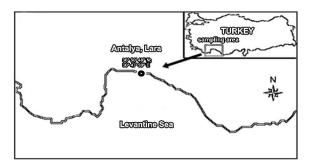


Fig. 1. Study Area and Collection of Specimens.



Fig. 2. Species used in the study.

Extraction of lipids: Macroalgae species were homogenized with 3/2 (v vG1) Hexaneisopropanol mixture. The homogenate was centrifuged at 5000 rpm for 5 min at 4°C. The supernatant part was used in the ADEK vitamin and fatty acid analysis (Hara and Radin, 1978).

Preparation of fatty acid methyl esters: An aliquot was taken from the supernatant part of the macroalgae pellet and 5 mL of 2% methanolic sulphuric acid was added. The mixture was vortexed and then kept at 50°C for 12 h. Then, after being cooled to room temperature, 5 mL of 5% sodium chloride was added and then it was vortexed. Fatty acid methyl esters were extracted with  $2\times5$  mL hexane. Fatty acid methyl esters were treated with 5 mL 2% KHCO<sub>3</sub> solution and then the hexane phase was evaporated by the nitrogen flow and then by dissolving in 1 mL fresh hexane (Christie, 1992) they were taken to auto sampler vials.

Gas chromatographic analysis of fatty acid methyl esters: Methyl esters were analyzed with the SHIMADZU GC 17 Ver. 3 Gas Chromatography (Kyoto, Japan). For this analysis, 25 m of long Machery-Nagel (Germany) capillary colon with an inner diameter of 0,25  $\mu$ m and a thickness of 25 micron film was used. During the analysis, the colon temperature was kept at 120-220°C, injection temperature was kept at 240°C and the detector temperature program was adjusted from 120-220 °C and the temperature increase was determined to be 5°C/ min until 200 and 4°C/min from 200-220°C. It

was kept at 220°C for 8 min and the total duration was set as 35 min and nitrogen gas was used as the carrier gas. During the analysis, before the analysis of fatty acid methyl esters, mixtures of standard fatty acid methyl esters were injected and the residence time of each fatty acid was determined. After this process, the necessary programming was made and the fatty acid methyl esters mixtures of the samples were analyzed (Christie, 1992).

HPLC analysis of ADEK vitamins and sterol amount: The 5 mL supernatant was taken to 25 mL tubes with caps and 5% KOH solution was added. After it was vortexed, it was kept at 85°C for 15 min. The tubes were then taken and cooled to room temperature and 5 mL of pure water was added and mixed. Lypophlic molecules that did not saponify were extracted with 2×5 mL hexane. The Hexane phase was evaporated with nitrogen flow. It was dissolved in 1 mL (50 + 50%, v vG1) acetonitril/methanol mixture and then was taken to auto sampler vials and was analyzed. The analysis was made with the Shimadzu brand HPLC device. In the device as the pump LC-10 ADVP UVvisible, as the detector SPD-10AVP, as column oven CTO- 10ASVP, as auto sampler SIL-10ADVP, as degasser unit DGU-14A and Class VP software (Shimadzu, Kyoto Japan) was used and during the mobile phase the acetonitril/ methanol (60+40% v vG1) mixture was used. The mobile phase flow rate was determined to be 1 mL A UV detector was used for the analysis and as a column the Supelcosil LC 18 (15×4.6 cm, 5 µm; Sigma, USA) column was used. For vitamin A detection of wave length 326 nm, for Determination of the flavonoid profile using HPLC: Chromatographic analysis was carried out using PREVAIL C 18 reversed-phase column (150x4.6 mm, 5 µm) diameter particles. The mobile phase was methanol/water/acetonitrile (46/46/8,v/v/v) containing 1.0% acetic acid (Zu et al., 2006). This mobile phase was filtered through a 0.45 µm membrane filter (millipore), then deaerated ultrasonically prior to use. Naringin, rutin, resveratrol, morin, myricetin, naringenin and kaempferol were quantified by DAD following RPHPLC separation at 280 nm for naringin, naringenin, 254 nm for rutin, morin, myricetin, 306 nm for resveratrol and 265 nm for kaempferol. Flow rate and injection volume were 1.05 mL/min and 10 µL, respectively. The chromatographic peaks of the analyses were confirmed by comparing their retention time and UV spectra with those of the reference standards. Quantification was carried out by the integration of the peak using the external standard method. All chromatographic operations were carried out at a temperature of 25 °C.

Determination of total phenolic compounds in the extracts: Generally, measurement of color occurred by reaction between Folin-Ciocalteu's phenol reagent (Kogure et al., 2004). Total contents of the phenolic compounds in the extracts were determined by Folin-Ciocalteu's method as gallic acid equivalents (GAE). Then 250 µL of Folin-Ciocalteu's phenol reagent was mixed with 50  $\mu$ L of the samples, and 500  $\mu$ L of 20% water solution of Na<sub>2</sub>CO<sub>3</sub> was added to the mixture. Mixtures were vortexed and completed with water to 5 mL. As control, reagent without adding extract was used. After incubation of the samples at room temperature for 30 min, their absorbance was measured at 765 nm. The calibration curve created by using fresh prepared gallic acid solutions was used as a base in calculations of total phenolic compound contents in the extracts. Experiments were repeated 3 times for every extract and the total phenolics were given in average values as GAE (mg gallic acid/g extract). Different dilutions of stock solution were prepared and were determined by Folin-Ciocalteu's method. Experiments were repeated 3 times for every dilution and a calibration curve was created. (y=0.0311x-0.1161,  $R^2=0.9949$ ).

Statistical analysis: Data were analyzed SPSS16.0 software. One-way ANOVA was used to compare the means of chemical composition data determined for the three groups of macroalgae.

## **Results and discussion**

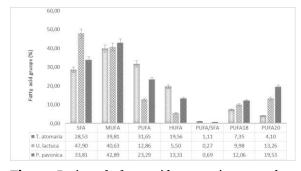
#### Fatty acids composition

The fatty acids compositions of investigated macroalgae are listed in Table-1 and Fig. 3. Although species analyzed in this work displayed high amounts of SFA, Saturated fatty acids (SFA) were major components accounting from 33.81% for Padina pavonica to 47.90 for Ulva lactuca. The total sum monounsaturated fatty acids (MUFAs) ranged from 39.81% to 42.89%, whereas total sum of PUFAs were 12.86-31.65%. Palmitic acid was the major fatty acid in all species tested. Fatty acids composition of algal lipids varies widely with species, habitat, light, salinity, pollution and environmental conditions (Ratana-Arporn and Chirapart, 2006) but in most studies palmitic acid (C16:0) is predominant (Gressler et al., 2010). The second major fatty acid were oleic acid (C18:1 n-9) in the all species. Oleic acid (C18:1 n-9) was the most abundant MUFA in species analyzed, followed by palmitoleic acid (C16:1). C14:1 was detected only in T. atomaria and C22:1n-9 only in Taonia atomaria and Padina pavonica. The amounts of polyunsaturated fatty asids (PUFA's) varied significantly among the species. The total PUFA content for Ulva lactuca was 12.86%, Taonia atomaria 31.65% and for Padina pavonica 23.29%. Important long-chain polyunsaturated fatty acids (PUFA's) such as eicosapentaenoic acid (EPA, C20:5 n-3), linoleic acid (LA, C18:2 n-6), α-linolenic (C18:3, n-3) and arahidonic acid (AA, C20:4 n-6) were found in significant levels. Arahidonic acid was found to be the most dominant fatty acid in phaeophyta species.

The obtained value of (C20:4, n-6) was 11.81% for *T. atomaria* and 6.36% for *Padina pavonica*.

**Table 1.** Fatty acids composition of *Ulva lactuca, Taonia atomaria and Padina pavonica* given in means  $\pm$  SD(% of total FAME)nd. – not detected; \*Significance level P < 0.05 (n=3).</td>

Fatty acids	T. atomaria	U. lactuca	P. pavonica	Fatty acids	T. atomaria	U. lactuca	P. pavonica
C 14:0	4.59±0.72	1.35±0.12	4.62±0.19	C 18:3 n-6	1.18±0.04	n.d.	n.d.
C 14:1	1.56±0.36	n.d.	n.d.	C 18:3 n-3	4.20±0.07	4.79±0.24	6.28±0.18
C 15:0	$1.29 \pm 0.13$	n.d.	n.d.	C 20:5 n-3	4.78±0.28*	1.86±0.30	$3.40 \pm 0.39^{*}$
C 16:0	21.43±0.96	41.67±1.01*	27.39±0.67	C 20:1 n-9	$8.53 \pm 0.25$	7.69±0.14	$11.80 \pm 0.41$
C 16:1 n-7	$12.23 \pm 0.52$	14.07±0.45	13.06±0.39	C 20:2 n-6	$1.58 \pm 0.16$	n.d.	n.d.
C 18:0	$1.20 \pm 0.12$	4.88±0.30	$1.79 \pm 0.15$	C 20:3 n-6	$1.38 \pm 0.04$	n.d.	$3.55 \pm 0.39$
C 18:1 n-9	$15.85 \pm 0.31$	17.46±0.20	15.46±0.42	C 20:4 n-6	$11.81 \pm 0.42^{*}$	2.24±0.20	6.36±0.42
C 18:1 n-6t	0.58±0.04	n.d.	n.d.	C 22:1	1.63±0.37	n.d.	$2.55 \pm 0.20$
C 18:2 n-6c	6.13±0.06*	2.56±0.26	3.70±0.14	C 24:1	n.d.	1.40±0.32	n.d.



**Fig. 3.** Ratios of fatty acid groups in macroalgae species.

Linoleic acid (C18:2, n-6) and  $\alpha$ -linolenic acid (C18:3, n-3) are two PUFAs which can not be synthesized by humans and other vertebrates. The PUFAs include two metabolic series of compounds: the n-6 and the n-3 FAs. Linoleic acid belongs to the n-6 series while linolenic acid refers to both  $\alpha$ -linolenic (C18:3, n-3) and  $\gamma$ -linolenic acid (C18:3, n-6). Within the body both can be converted to other PUFAs such as arachidonic acid (C20:4, n-6), eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3) (Ginneken *et al.*, 2011). In *Taonia atomaria* C20:5 n-3 accounted 4.78% of total fatty acids. To the best of our knowledge HUFA (High Unsaturated fatty Acids) synthesis pathways have not been studied in macroalgae/seaweeds. Based on fatty

acid compositions, seaweeds can produce ARA and EPA at quite high levels, but generally lack DHA (Sanchez-Machado *et al.*, 2004a; Dawczynski *et al.*, 2007). DHA was not detected in species macroalgae. In the literature, this FA is generally absent or exists in very small amounts in different phaeophytes (Li, 2002).

Marine macroalgae are essentially the only organisms that possess the enzymes necessary for producing long-chain polyunsaturated FA (PUFA), such as 20:5n-3 and 22:6n-3 (Sargent and Henderson 1995; Cook, 1996). Other fatty acids which are important in aquatic ecosystems are HUFA. The highest value of total highly unsaturated fatty acids (HUFA) was detected highest in Taonia atomaria. Ulva lactuca being a green alga was rich in C20 PUFAs while Padina pavonica and Taonia atomaria being brown alga was rich in both C18, C20 PUFAs. Such trends have already been established earlier in several studies (Kumari et al., 2011). In all studied brown alga species, the concentration of C20 PUFA was always generally higher than that of C<sub>18</sub> PUFA, which is consistent with the typical profile of other phaeophytes (Li et al., 2002). In this study, Phaeophyta species exhibited higher concentrations

of PUFA, and PUFA/SFA ratios higher than 1 (*Taonia atomaria*; 1.11). The lowest PUFA/SFA ratios were observed in *Ulva lactuca*. It appears that chlorophytes has a lower potential, comparing to the phaeophytes studied, as a nutritional source of PUFA for human consumption. The results presented herein are in agreement with previous studies in which *Taonia atomaria* and *Padina pavonica* displayed higher concentrations of unsaturated fatty acids as compared with *Ulva lactuca*. Several studies have found reverse correlation between the PUFA/SFA ratios and cardiovascular diseases and suggested that replacement of SFA with PUFA in the human diet will decrease similar health problems (Simopolous, 2000; Erkkila *et al.*, 2008)

Season and location directly effects on biochemical formation in macroalgae (Renaud & Luong-Van, 2006). Temperature has influenced on fatty acid composition of cell membranes in algae. Low temperature has an effectived on increased level of unsaturated fatty acid in polar lipid. This augmentation of unsaturation results in lower melting points and maintain lipid in liquid stat for normal protoplasmic viscosity (Nelson *et al.*, 2002). In this study, unsatured fatty acid contents are found higher than satured fatty acid level in spring.

#### Vitamins and phytosterols contents

Seaweeds are a good source of fat-soluble vitamins. Seaweed vitamins are important not only due to their biochemical functions and antioxidant activity but also due to other health benefits such as decreasing blood pressure, prevention of cardiovascular diseases, or reducing the risk of cancer (Skrovankova, 2011). There is limited imformation about fat soluable vitamin content of brown macroalgae. Panayotova *et al.* (2013), raported that *Cystoseira barbata* contained high amounts  $\alpha$ -tocopherol (15.77±0.21 mg per 100 DW). Additionally, Durmaz *et al.* (2008) informed that  $\alpha$ -tocopherol was 17.10±0.10 in *Cystoseira* spp., 9.10±0.50 in *Ulva* spp., and 9.90±0.10 *Zostera* spp., from brown macroalgae.

Sterols are essential for all eukaryotes. They are components of membranes and have a function in regulation of membrane fluidity and permeability. Sterols also play an important role as precursors of many steroid hormones including vitamin D and brassinosteroids as well as for a wide range of secondary metabolites such as saponins and glycoalkaloids (Piironen *et al.*, <u>2000</u>). Phytosterols are present in small amounts, and two common examples are stigmasterol and sitosterol (Abidi, 2001).

Sterols occur naturally in plants, animals and fungi, with the most familiar type of animal sterol being cholesterol. Cholesterol is vital to cellular function where it affects the fluidity of the animal cell membrane and serves as a secondary messenger in developmental signalling. Furthermore, cholesterol is a precursor to fat-soluble vitamins and steroid hormones. Content and type of sterols vary with the seaweed species (Sanchez-Machado et al., 2004b). In our work, cholesterol was the main compound in phaeophyta the highest level of cholesterol a was observed in the Padina pavonica  $(31.47\pm0.39 \mu g/g)$ DW). Macroalgae samples allowed the determination of four compounds: ergosterol, cholesterol. stigmasterol and  $\beta$ -sitosterol (Tablo 2). Cholesterol, ergosterol, stigmasterol, sitosterol in different ratio was found in all species. On the other hand, Padina species from brown macroalgae have significant differences in the sterol composition (Al Ease et al., 1995). In the present study was observed that main sterol of P. pavonica was cholestrol in spite of (Iatrides et al., 1983) different study by Kamenarska et al., 2002 observed that the main sterol of P. pavonia was focosterol in the Turkish Mediterranean Sea.

Phytosterols are bioactive compounds, which can be found in a great variety of plant-based foods (Brufau *et al.*, 2008). Many studies have demonstrated their ability to reduce blood cholesterol levels in hyper- and normocholesterolemic subjects. Most of the available studies demonstrate that foods enriched with phytosterols reduce intestinal cholesterol absorption (Brufau *et al.*, 2008). Other properties have been described for these compounds, including antiinflammatory, antipyretic, and antidiabetic activities. Thus, the consumption of macroalgae or their derived products containing bioactive compounds as an alternative or a supplement to prescription drugs is gaining popularity (Lagarda *et al.*, 2006).

To survive in a competitive environment, marine macroalgae have developed defense strategies that result in a significant diversity of compounds. They are a rich source of phytosterols, which can occur in

**Table 2.** Vitamins and sterols contents  $(\mu g/g)$ .

free form, esterified with fatty acids, or, in minor concentrations, involved in glycosylated conjugates (Moreau *et al.*, 2002). These compounds are important constituents of cell membranes and responsible for many of the cell functions (Kamenarska *et al.*, 2002; Moreau *et al.*, 2002).

It is reported that sterols such as  $\beta$ -sitosterol lead to the decrease of the concentration of cholesterol in the serum in experimental animals and humans (Whittaker *et al.*, 2000). In this study; the highest amount of  $\beta$ -sitosterol determined in *Ulva lactuca* species (Table 2).

Vitamins and sterols	U. lactuca	P. pavonica	T. atomaria		U. lactuca	P. pavonica	T. atomaria
Vitamin K1	0.004±0.0005	0.74±0.09	0.73±0.1	Ergosterol	62.13±1.85	33.27±0.94	502.04±4.82*
Vitamin K2	0.78±0.07	n.d.	4.34±0.36	Stigmasterol	0.046±0.02	5.41±0.2	$0.005 \pm 0.001$
Vitamin D2	$1.15 \pm 0.05$	0.41±0.11	$11.2 \pm 0.5$	β-sitositerol	72.72±1.55	11.91±0.35	7.57±0.51
Vitamin D3	n.d.	n.d.	0.04±0.005	Retinol	$0.15 \pm 0.01$	0.039±0.009	0.04±0.007
$\delta$ -Tocopherol	0.46±0.07	0.73±0.12	11.71±0.99	Retinol Acetate	<sup>9</sup> 0.01±0.004	0.013±0.001	$0.15 \pm 0.05$
α-Tocopherol	6.95±0.33	3.95±0.35	12.03±1.41	Cholesterol	18.28±0.96	31.47±0.39	17.63±0.71

Kapetanovic *et al.* (2005) raported that in the green alga *Ulva lactuca*, the principal sterols were cholesterol. In the present study, it was found low cholesterol and high ergesterol,  $\beta$ -sitosterol in *Ulva lactuca*. In fact, the ecological differences, geographic origins and developmental stage of the collected marine macroalgae contribute to the different phytosterol profiles. In particular, it has been reported that the salinity and temperature of seawater probably influence the differences in the phytosterol profiles of species in the Ulvacea (Kapetanovic' *et al.*, 2005).

Brown seaweed *Taonia atomaria* contained high amounts of ergesterol. In general, the chemical composition of these *Taonia atomaria*, *Ulva lactuca and Padina pavonica* species from the Turkish Mediterranean Sea showed that, *Taonia atomaria* was a good source of lipids with a good level of 20:5(n-3) and 20:4(n-6), and  $\alpha$ -tocopherol. This study results also showed that *Taonia atomria* could be used as a supplement of  $\alpha$ -tocopherol (Table 2). High levels of  $\alpha$ -tocopherol correlate with high levels of polyunsaturated fatty acids. As an antioxidant  $\alpha$ tocopherol preserves tissue PUFA from oxidation (Panayotova and Stancheva, 2013).

### Flavonoid and total phenolic contents

Phenols, sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is phenol, the parent compound used as a disinfectant and for chemical synthesis (Burtin, 2003). Green seaweed have low concentrations of phenols (Mabeau and Fleurence 1993) compared to brown seaweed species. Extracting solvents such as water, methanol significantly affected the total phenolic amounts determined using the Folin-Ciocalteu method. In this study; phenol content was higher in *Taonia atomaria* than *Ulva*  *lactuca* and the ratio varies from 0.90 to 2.20 mg/g of dry seaweed biomass (Table 3). Concentrations of polyphenols exhibit seasonal variations, but also vary within the different parts of thalli, such as old versus new thalli, basal part or frond (Johnson and Mann 1986). Polyphenol content shows a significantly temporal correlation with the reproductive state of the macroalgae.

**Table 3.** Flavonoids content in macroalgae water extracts (ug/g DW) and Total phenolic compounds of macroalgae extracts (mg/g DW).

Flavonoidler	T. atomaria	U. lactuca	P.pavonica		
Rutin	0.029±0,0065	n.d	n.d	Water extracts	
Mirisetin	0,033±0,0011	0.066±0,0005	0,034±0,0010	T. atomaria	$2.20{\pm}0.05^{***}$
Morin	0.029±0,005	0.065±0,0034	0,011±0,001	U.lactuca	$1.57 \pm 0.29$
Quersetin	0.017±0.003	0.011±0.001	0.013±0.0006	P. pavonica	$1.79 \pm 0.02$
Kamferol	0.011±0.001	$0.012 \pm 0.0005$	n.d	Metanol extract	
Naringin	n.d	0.011±0.001	n.d	T. atomaria	$1.43 \pm 0.01$
Naringenin	0.006±0.0002	0.0064±0,0002	$0.065 \pm 0.0012$	U.lactuca	$1.02 \pm 0.02$
Resveratrol	0.0027±0.0005	0.003±0.0002	0.11±0.08	P. pavonica	0.96±0.03

In this study, it was determined that total phenolic contents of algae water extracts were higher than algae metanol extracts and *Taonia atomaria* was observed the highest phenolic contents in water extracts. Research on concentrations of algal polyphenols has shown that these compounds vary according to season, habitat, and local environmental factors such as salinity, UV irradiation, light, and nutrient availability (Jormalainen and Honkanen 2008). Flavonoid content were detected in 1 g of extracts of macroalgae. Rutin, resveratrol, morin, naringin, naringenin, myricetin, quercetin and kaempferol were determined in the macroalgae extracts.

### Conclusion

Fatty acids composition,  $\alpha$ -tocopherol, ergesterol, cholesterol, flavonoid, total phenolic acids, in brown macroalgae *Padina pavonica, Taonia atomaria* and in green macroalgae *Ulva lactuca* were analyzed in the present study. The three macroalgae species studied exhibited different profiles and contained unsaturated fatty acids,  $\alpha$ -tocopherol, ergesterol in important amounts. Because, biochemical content of different marine macroalgae species can change depend on the physiological condition, nutritional status, light intensity, temperature and season (Dawczynski *et al.*, 2007). Palmitic acid (C16:0) was the most abundant fatty acid, followed by C18:1, n-9. The high concentrations of  $\alpha$ -tocopherol, polyunsaturated fatty acids and the presence of the powerful antioxidant  $\alpha$ -tocopherol, phenolic acids demonstrate possible application of this macroalgae as a supplement for use in food. In this study was made on macroalgae species which collected along the Mediternean Sea coasts exhibited. We revealed that these species can be used as the expensive food sources in the industry.

### References

**Abidi SL.** 2001. Chromatographic analysis of plant sterols in foods and vegetable oils. Journal of Chromatography A **935**, 173-201.

doi.org/10.1016/S0021-9673(01)00946-3.

Al Easa HS, Kornprobst J, Rizk AM. 1995. Major sterol composition of some algae from Qatar. Phytochemistry **39**, 373–374.

doi.org/10.1016/0031-9422(94)00968-Y.

Arts MT, Brett MT, Kainz M. 2009. Lipids in Aquatic Ecosystems. Springer, New York.

**Bhosale R, Velankar D, Chaugule B**. 2009. Fatty acid composition of the cold-water-inhabiting freshwater red alga Sirodotia Kylin. Journal of Applied Phycology **21**, 99–102. doi.org/10.1007/s10811-008-9333-5.

**Brufau G, Canela, MA and Rafecas M.** 2008. Phytosterols: physiologic and metabolic aspects related to cholesterol-lowering properties. Nutrition Research **28**, 217–25. doi.org/10.1016/j.nutres.2008.02.003.

**Burtin P.** 2003. Nutritional value of seaweeds. Electronic Journal of Environmental, Agricultural and Food Chemistry **2**, 498-503.

**Christie WW.** 1990. Gas chromatography and lipids: A practical guide. Bridgewater, Somerset: The Oily Press.

**Cook HW.** 1996. Fatty acid desaturation and chain elongation in eukaryotes, pp. 129–152. In D.E. Vance and J.E. Vance (eds.), Biochemistry of Lipids, Lipoproteins and Membranes. Elsevier, Amsterdam. doi.org/10.1016/S0167-7306(08)60512-8.

**Dawczynski C, Schibert R, Jahreis G**. 2007. Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chemistry **103**, 891 – 899. doi.org/10.1016/j.foodchem.2006.09.041.

**Durmaz Y, Duyar H, Gokpinar S, Taskaya L, Ogretmen Y, Bandarra N, Nunes M.** 2008. Fatty Acids,  $\alpha$ -tocopherol and Total Pigment Contents of Cystoseira spp., Ulva spp. and Zostera spp. from Sinop Bay (Turkey). International Journal of Natural and Engineering Sciences **2(3)**, 111-114.

**Erkkila A, de Mello V, Risirus U, Laaksonen D.** 2008. Dietary fatty acids and cardiovascular disease: An epidemiological approach. Progress in Lipid Research **47 (3)**, 172–187.

doi.org/10.1016/j.plipres.2008.01.004.

Ginneken V, Helsper J, de Visser W, van Keulen H, Brandenburg W. 2011. Polyunsaturated fatty acids in various macroalgal species from north Atlantic and tropical seas. Lipids in Health and Disease 10, 104–111. doi.org/10.1186/1476-511X-10-104

**Guschina IA, Harwood JL.** 2009. Algal lipids and effect of the environment on their biochemistry. M.T. Arts *et al.* (eds.), Lipids in Aquatic Ecosystems, 20-43 springer, New York.

Hara A, Radin NS. 1978. Lipid extraction of tissues with a low-toxicity solvent, analytical biochemistry, **90(1)**, 420-426.

**Iatrides MC, Artaud J, Vicente N.** 1983. Sterol com- position of Mediterranean marine plants. Oceanologica Acta **6**, 73–77.

**Johnson CR, Mann KH.** 1986. The importance of plant defence abilities to the structure of subtidal seaweed communities: the kelp Laminaria longicruris de la Pylaie survives grazing by the snail Lacuna vincta (Montagu) at high population densities. Journal of Experimental Marine Biology and Ecology **97**, 231–267.

doi.org/10.1016/0022-0981(86)90244-3.

**Jormalainen V, Honkanen T**. 2008. Macroalgal chemical defenses and their roles in structuring temperate marine communities. In: Algal Chemical Ecology (ed. C.D. Amsler), Springer-Verlag: Berlin, Heidelberg, Germany; pp. 57–89. doi.org/10.1007/978-3-540-74181-7\_3.

Kamenarska Z, Yalcin F, Ersöz T, Calis I, Stefanova K, Popov S. 2002. Chemical Composition of Cystoseira crinita Bory from the Eastern Mediterranean. Zeitschrift für Naturforschung 57, 584–590.

Kapetanović R, Sladić DM, Popov S, Zlatović MV, Kljajić Z, Gašić MJ. 2005. Sterol composition

of the Adriatic Sea algae Ulva lactuca, Codium dichotomum, Cystoseira adriatica and Fucus virsoides. Journal Of The Serbian Chemical Society **70(12)**, 1395-400. doi.org/10.2298/JSC0512395K.

**Katsanidi E, Addis PB.** 1999. Novel HPLC analysis of tocopherols and cholesterol in tissue. Free Radical Biology and Medicine **27**, 1137-1140. doi.org/10.1016/S0891-5849(99)00205-1.

Kogure K, Yamauchi I, Tokumura A. 2004. Novel antioxidants isolated from plants of the genera Ferula, Inula, Prangos and Rheum collected in Uzbekistan. Phytomed 1, 645-651. doi.org/10.1016/j.phymed.2003.09.004.

**Kumari P, Reddy C, Jha B.** 2011. Comparative evaluation and selection of a method for lipid and fatty acid extraction from macroalgae. Analytical Biochemistry **415**, 134–144. doi.org/10.1016/j.ab.2011.04.010.

Lagarda MJ, García-Llatas G, Farré R. 2006. Analysis of phytosterols in foods. Journal of Pharmaceutical and Biomedical Analysis **41**, 1486-1496. doi.org/10.1016/j.jpba.2006.02.052.

Li X, Fan X, Han L, Lou Q. 2002. Fatty acids of some algae from the Bohai Sea. Phytochemistry. **59**, 157–161. doi.org/10.1016/S0031-9422(01)00437-X.

**Mabeau S, Fleurence J.** 1993. Seaweed in food products: biochemical and nutritional aspects. Trends in Food Science & Technology **4**, 103–107. doi.org/10.1016/0924-2244(93)90091-N.

Moreau RA, Whitaker BD, Hicks KB. 2002. Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and healthpromoting uses. Progress in Lipid Research. **41**, 457-500.

doi.org/10.1016/S0163-7827(02)00006-1.

Nelson MM, Phleger CF, Nichols PD. 2002. Seasonal lipid composition in Macroalgae of the Northeastern Pacific Ocean. Botanica Marina **45**, 58-65. doi.org/10.1515/BOT.2002.007.

**Panayotova V, Stancheva M.** 2013. Fat soluble vitamins and fatty acids composition of black sea Cystoseira barbata, Cbu International Conference On Integration And Innovation In Science And Education, Prague, April **7-14**, 362-367 (in Czech Republic).

**Piironen V, Lindsay DG, Miettinen TA, Toivo J, Lampi AM.** 2000. Plant sterols: biosynthesis, biological function and their importance to human nutrition. Journal of the Science of Food and Agriculture **80**, 939–966.

doi.org/10.1002/(SICI)1097-0010(20000515)80:7.

**Ratana-arporn P, Chirapart A**. 2006. Nutritionalevaluation of tropical green seaweeds Caulerpa lentillifera and Ulva reticulate. Kasetsart Journal: Natural Sciences **40**, 75–83.

**Renaud SM, Luong-Van, JT.** 2006. Seasonal variation in the chemical composition of tropical Australian marine macroalgae. Journal of Applied Phycology **18(3-5)**, 381-387. doi.org/10.1007/s10811-006-9034-x.

Sanchez-Machado DI, Lopez-Cervantes J, Lopez-Hernandez J, Paseiro-Losada P. 2004a. Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. Food Chemistry **85**, 439– 444. doi.org/10.1016/j.foodchem.2003.08.001.

Sanchez-Machado DI, Lopez-Hernandez J, Paseiro-Losada P, Lopez-Cervantes J. 2004b. An HPLC method for the quantification of sterols in edible seaweeds. Biomedical Chromatography **18**, 183–190. doi.org/10.1002/bmc.316.

Sargent JR, Henderson RJ. 1995. Marine (n-3) polyunsaturated fatty acids, pp. 32–65. In R.J.

Hamilton (ed.), Developments in Oils and Fats. Blackie Academic and Professional, London. doi.org/10.1007/978-1-4615-2183-9\_2.

**Simopoulos A, Leaf A, Salem N.** 2000. Workshop statement on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. Prostaglandins Leukotrienes and Essential Fatty Acids **63(3)**, 119–121. doi.org/10.1054/plef.2000.0176.

**Skrovankova S.** 2011. Seaweed Vitamins as Nutraceuticals. In: Taylor, S. (Ed.). Marine Medicinal Foods: Implications and Applications, Macro and Microalgae. Advances in Food and Nutrition Research Series, **64**, 357-369. Waltham, MA: Academic Press Inc.

**Tehlivets O, Scheuringer K, Kohlwein SD.** 2007. Fatty acid synthesis and elongation in yeast Biochimica et Biophysica Acta **1771(3)**, 255-270.

**Watson SB, Cruz – Rivera E**. 2003. Algal chemical ecology; an introduction to the special issue.

Phycologia **42**, 319-323. doi.org/10.2216/i0031-8884-42-4-319.1.

Whittaker MH, Frankos VH, Wolterbeek AMP, Waalkens-Berendsen, DH. 2000. Effects of dietary phytosterols on cholesterol metabolism and atherosclerosis: clinical and experimental evidence. American Journal of Medicine **109**, 600–601. doi.org/10.1016/S0002-9343(00)00588-X

Yu SS, Hu YC, Wu XF, Liu J. 2009. Natural phloroglucinols-molecular diversity and bioactivity. Recent Progress in Medicinal Plants, **25**, 103-139.

**Zu YG, Li CY, Fu YJ, Zha CJ.** 2006. Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (Hippophae rhamnoides L.) leaves by RP-HPLC with DAD Journal of Pharmaceutical and Biomedical Analysis **41**, 714-719. doi.org/10.1016/j.jpba.2005.04.052.