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THE FATTY ACID PROFILES OF SELECTED MACROALGAL SPECIES FROM THE ADRIATIC SEA

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INTRODUCTION

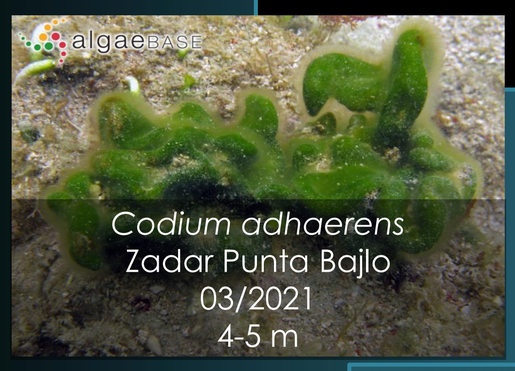
Macroalgae have drawn attention for being utilized as a source of bioactive compounds due to their biological and chemical diversity. The presence of bioactive compounds in algae depend on season, growth conditions, location and environmental changes. Algae are divided into three groups (green (Chlorophyta), brown (Ochrophyta) and red (Rhodophyta) containing specific bioactive compounds characteristic for particular group.

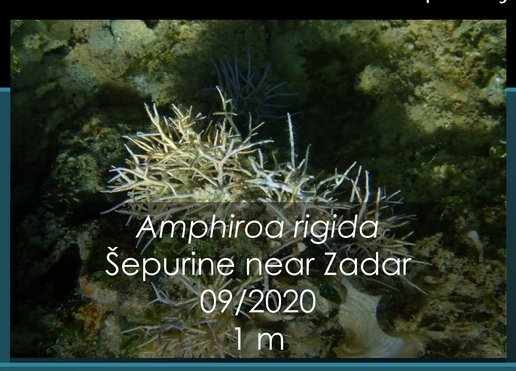
During the growth and adaptation to abiotic stress, algae are producing different small molecules (volatile organic compounds, polyunsaturated fatty acids, polyphenols, pigments, etc.) which serve for the communication and interaction with the surrounding environment. Lately, there is an increasing demand for alternative natural sources of polyunsaturated fatty acids (PUFAs) due to their health benefits of reducing the risk of heart diseases. Macroalgae were shown as potential sources of ω3 and ω6 fatty acids and to assess their suitability, species collected from the Adriatic Sea were investigated for their fatty acid profiles by the gas chromatography with flame ionization detector (GC-FID). Macroalgae included in this study were as follow; *Amphiroa rigida* and *Asparagopsis taxiformis* (red species), *Cystoseira compressa* and *Cystoseira amentacea* (brown species), *Codium adhaerens* and *Ulva lactuca* (green species).

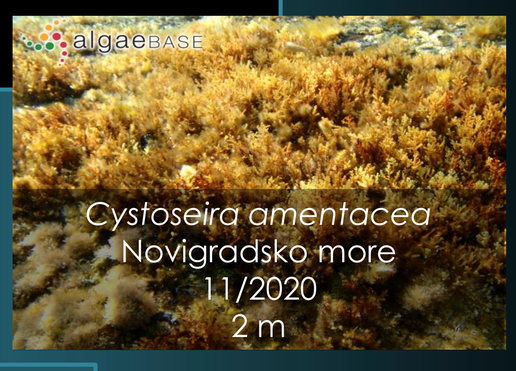
MARINE MACROALGAE SAMPLES

Macroalge were collected from different locations at different time and from certain depths that are placed on pictures of each species. All of the samples were collected with surrounding seawater and placed in an airtight plastic bags and immediately transported to the laboratory.

For the extraction of fatty acids, fresh macroalgae samples were freeze-dried. Before the freeze-drying, the samples were washed five times in water and twice in deionized water, then they were cut in slices (5–10 mm) and frozen at -60°C for 24 h in an ultra-low freezer. Five trays of frozen samples were placed in a laboratory freeze dryer (CoolSafe PRO, Labogene, Denmark). The freeze-drying was performed under a high vacuum (0.13–0.55 hPa) for 24 h with -30°C and 20°C as the primary and secondary drying temperatures.







EXTRACTION OF TOTAL LIPIDS

Extraction of total lipids from macroalgal samples was performed by the Folch method. Briefly, 1.00 g of the sample was mixed with 20 mL chloroform/MeOH (2:1, *v/v*) solvent mixture. The mixture was stirred for 20 min at 400 rpm (IKA, KS 260 Basic, Staufen, Germany), filtered and then washed with 4 mL of 0.9% NaCl solution. The upper phase was removed, and lower chloroform phase containing lipids was evaporated in a rotary evaporator (Laborota 4010, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 60°C. The samples were then dried in an oven (105°C until constant weight). The extraction was performed in three repetitions. Afterwards, the fatty acids methyl esters (FAMEs) were prepared with cold methanolic potassium hydroxide solution.

GC-FID ANALYSIS OF FATTY ACIDS

FAMEs were afterwards separated on a Shimadzu GC-2010 Plus gas chromatograph equipped with a flame ionization detector (FID) and fitted with a SH-Rtx-Wax capillary column (30 m, 0.25 mm i.d. and 0.25 µm thick stationary phase). Nitrogen was used as the carrier gas, flowing at the constant linear velocity of 1.33 mL min^{-1}. The split/splitless injector was set at 250°C, split ratio was 1:10, and the injection volume 2 µL. Initial column temperature of 110°C was held for 2 min, then gradually increased 10 °C min^{-1} until temperature of 175 °C that was hold for 8 min, followed by gradual increase 5 °C min−1 until 210 °C held for 5 min, and a temperature increase to a final temperature of 230 °C by a rate of 5 °C min^{-1}. Final temperature was held for 7 min. Total analysis time was 42.5 min. Flame ionization detector temperature was 300 °C. Hydrogen flow rate was 40 mL min^{-1}, air flow rate 400 mL min^{-1} and make-up gas (nitrogen) flow was 30 mL min^{-1}. Identification of separated FAMEs in samples was achieved based on the comparison of retention times with the retention times of certified reference standard (Supelco F.A.M.E. Mix, C4–C24) analyzed under the same conditions. The results were expressed as the percentage of identified fatty acid on total fatty acids (%).

RESULTS

Table 1. Fatty acid profile of six different macroalgal species

No.	Fatty Acid	*Codium adhaerens*	*Ulva lactuca*	*Amphiroa rigida*	*Asparagopsis taxiformis*	*Cystoseira compressa*	*Cystoseira amentacea*
		Av ± SD (%) *					
1.	Butyric acid (C4:0)	-	-	0.20 ± 0.02	-	-	-
2.	Caproic acid (C6:0)	-	-	0.91 ± 0.08	-	-	-
3.	Lauric acid (C12:0)	4.02 ± 1.16	4.43 ± 0.19	-	-	3.22 ±0.57	-
4.	Myristic acid (C14:0)	4.10 ± 0.28	2.76 ± 0.58	3.34 ± 0.1	16.90 ± 0.80	3.06 ± 0.41	6.87 ± 0.10
5.	Pentadecyclic acid (C15:0)	-	-	0.69 ± 0.15	0.53 ± 0.01	-	0.31 ± 0.01
6.	Palmitic acid (C16:0)	25.50 ± 0.13	45.16 ± 1.82	42.86 ± 0.26	56.47 ± 0.81	30.36 ± 1.50	21.58 ± 0.20
7.	Margaric acid (C17:0)	-	-	-	-	-	0.12 ± 0.00
8.	Stearic acid (C18:0)	7.12 ± 0.26	5.40 ± 0.55	11.65 ± 0.10	8.74 ± 1.05	8.20 ± 2.32	4.14 ± 0.52
9.	Arachidic acid (C20:0)	22.48 ± 0.49	14.57 ± 0.84	-	0.75 ± 0.30	4.16 ± 1.01	0.18 ± 0.01
10.	Heneicosanoic acid (C21:0)	-	-	-	0.78 ± 0.19	-	2.94 ± 0.05
11.	Behenic acid (C22:0)	1.72 ± 0.19	1.03 ± 0.08	-	-	1.24 ± 0.36	-
12.	Lignoceric acid (C24:0)	-	-	-	-	-	1.51 ± 0.06
	Total saturated fatty acids (SFA)	**64.92**	**73.35**	**59.65**	**84.17**	**49.38**	**37.65**
13.	Myristoleic acid (C14:1)	-	-	-	-	-	0.27 ± 0.01
14.	Palmitoleic acid (C16:1)	5.79 ± 0.65	2.37 ± 0.17	1.73 ± 0.09	3.88 ± 0.16	1.22 ± 0.29	2.29 ± 0.01
15.	Heptadecenoic acid (C17:1)	1.96 ± 0.24	-	-	-	-	0.18 ± 0.00
16.	Oleic acid (C18:1n9*c+t*)	16.91 ± 1.46	13.59 ± 1.47	5.46 ± 0.03	8.73 ± 0.29	16.52 ± 0.01	21.38 ± 0.29
17.	Paullinic acid (C20:1)	-	-	-	-	-	0.41 ± 0.04
18.	Erucic acid (C22:1)	-	-	-	1.60 ± 0.28	-	0.60 ± 0.09
19.	Nervonic acid (C24:1)	-	-	-	-	2.17 ± 0.50	-
	Total monounsaturated fatty acids (MUFA)	**24.66**	**15.96**	**7.19**	**14.21**	**24.11**	**25.12**
20.	*cis*-Linoleic acid (C18:2n6*c*)	4.67 ± 0.33	4.69 ± 0.62	3.03 ± 0.03	1.23 ± 0.34	11.39 ± 1.19	6.46 ± 0.13
21.	*trans*-Linoleic acid (C18:2n6t)	-	-	2.22 ± 0.02	-	-	-
22.	γ-Linolenic acid (C18:3n6)	-	-	0.46 ± 0.05	-	-	0.58 ± 0.01
23.	α-Linolenic acid (C18:3n3)	2.77 ± 0.41	5.82 ± 0.51	0.41 ± 0.06	-	5.75 ± 0.31	5.48 ± 0.09
24.	Eicosadienoic acid (C20:2n6)	-	-	7.90 ± 0.11	-	-	0.75 ± 0.02
25.	Eicosatrienoic acid (C20:3n3)	-	-	-	-	0.74 ± 0.38	3.85 ± 0.08
26.	Dihomo- γ-linolenic acid (C20:3n6)	-	-	-	0.48 ± 0.10	-	-
27.	Arachidonic acid (C20:4n6)	1.78 ± 0.32	-	-	-	7.08 ± 0.44	19.51 ± 0.21
28.	Eicosapentaenoic acid (C20:5n3)	-	-	19.14 ± 0.32	-	4.14 ± 0.07	0.64 ± 0.01
29.	Docosadienoic acid (C22:2n6)	1.63 ± 0.49	1.06 ± 0.11	-	-	-	-
	Total polyunsaturated fatty acids (PUFA)	**10.85**	**11.57**	**33.16**	**1.71**	**26.52**	**37.27**
	Total ω6 fatty acids	**8.08**	**5.75**	**13.61**	**1.71**	**17.32**	**27.30**
	Total ω3 fatty acids	**2.77**	**5.82**	**21.96**	**0.00**	**9.20**	**9.97**

* - Av—average area of 3 replicates expressed in percentage (%) with standard deviation (SD)

CONCLUSION

The obtained results indicated the significant differences in fatty acid profiles between and within algal groups. Red and brown macroalgae were generally richer in ω3 and ω6 fatty acids with eicosapentaenoic (C20:5, EPA) and arachidonic acids (C20:4, AA) as the most represented, while green macroalgae indicated higher levels of saturated fatty acids such as palmitic acid (C16:0). *Amphiroa rigida* was identified as the most promising source of ω3 fatty acid EPA, while *Cystoseira amentacea* had the highest content of ω6 fatty acid AA. There is a possibility of using these algae as good sources of these essential fatty acids.

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