VOLATILE ORGANIC COMPOUNDS AND FATTY ACID CONTENT OF GREEN MACROALGAE *Ulva lactuca*

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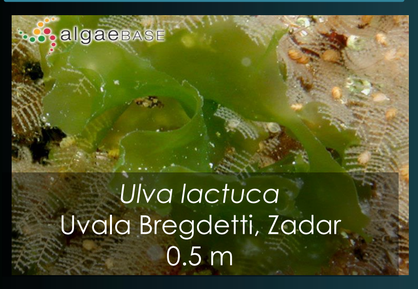
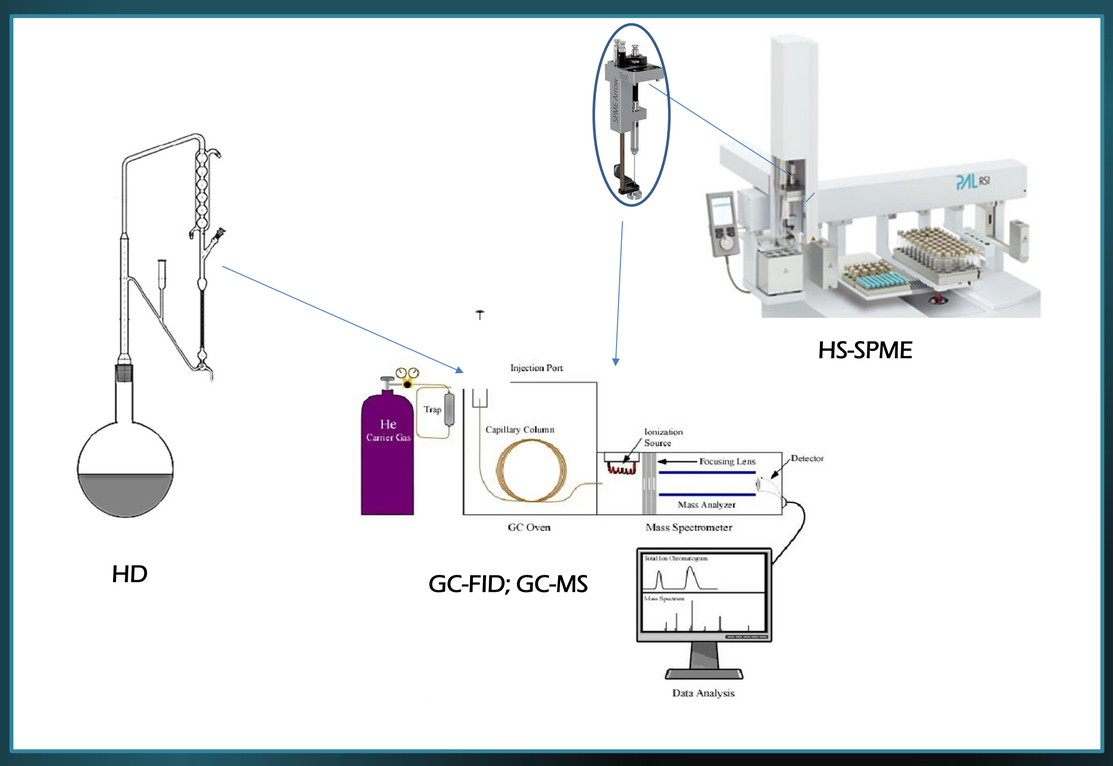
INTRODUCTION

Since macroalgae represent a valuable source of various bioactive compounds it is important to investigate their chemical profiles that will lead to the better understanding of marine algal biodiversity in the Adriatic Sea, in this case *Ulva lactuca*. Beyond its subtle appearance, *Ulva lactuca*, also referred to as sea lettuce, provides an abundance of chemical potential. This common green macroalgae is a prominent producer in marine ecosystems, flourishing in coastal waters that are both temperate and tropical worldwide. But *Ulva lactuca*'s significance extends far beyond its ecological function. Recent studies have examined the macroalgae's extraordinary adaptability and have found a multitude of important chemical components that have applications across a wide range of industries. Revealing the secrets buried in *Ulva lactuca*'s chemical composition has the potential to improve human health and environmental well-being while also contributing to the construction of a more sustainable future.

HYDRODISTILLATION (HD)

Hydrodistillation (HD) was performed in a European pharmacopeia apparatus for 2 h with the use of 1 mL of solvent trap (pentane : diethyl ether 1:2 v/v). The prepared sample (10 g) was cut into small pieces. The volatile oil dissolved in the solvent trap was removed with a pipette, passed through the layer of magnesium sulphate in a small glass funnel and carefully concentrated by the slow flow of nitrogen until the volume of 0.2 mL. One microlitre was used for GC-MS analyses.





HEADSPACE SOLID-PHASE MICROEXTRACTION (HS-SPME)

The headspace solid-phase microextraction was performed by PAL RSI 85 Autosampler System (CTC Analytics AG, Zwingen, Switzerland) with SPME fiber tool using the SPME fibre divinylbenzene / carboxene / polydimethylsiloxane (DVB/CAR/PDMS) obtained from Supelco Co. Each sample (2 g) was placed in 20 mL screw top clear vial and hermetically sealed with cap containing PTFE/silicone septum. The automated PAL RSI experimental procedure involved the following steps: conditioning SPME fiber, sample agitation (agitation speed: 250 rpm; agitator on time: 5 s; agitator off time: 2 s) and equilibration for 30 minutes on 40 °C, the extraction of volatiles for 40 min without agitation, and the injection of SPME fiber in GC injector for desorption (7 min).

GC-MS ANALYSIS

The GC-MS analyses were performed using an Agilent Technologies (Palo Alto, CA, USA) gas chromatograph model 7820A equipped with mass selective detector (MSD) model 5977E (Agilent Technologies) and PAL RSI 85 Autosampler System (CTC Analytics AG, Zwingen, Switzerland). HP-5MS capillary column was used for GC-MS analysis under the same conditions as for the GC-FID analysis. The MSD (EI mode) was operated at 70 eV, and the mass range was 30-300 amu. The identification of the volatile constituents was based on the comparison of their retention indices (RI) and their mass spectra compared with NIST17 database.

EXTRACTION OF TOTAL LIPIDS

Extraction of total lipids from macroalgal sample 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 sample 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. The volatiles from *Ulva lactuca* obtained by HS-SPME and HD after GC-MS analysis

No	Compound	RI	PDMS/DVB	DVB/CAR/PDMS	HD
1.	Dimethyl sulfide	< 900	4.46	5.81	-
2.	(*E*)-But-2-enal	< 900	0.05	3.48	-
3.	Pent-1-en-3-ol	< 900	2.10	3.48	-
4.	Pentanal	< 900	0.86	0.97	-
5.	3-Methylbut-2-enal	< 900	0.71	1.54	-
6.	Hexanal	< 900	2.05	2.04	-
7.	(*E*)-Hex-2-enal	< 900	0.49	0.31	-
8.	Hexan-1-ol	< 900	1.40	1.11	-
9.	Heptanal	904	20.17	16.35	0.33
10.	(*E*)-Hept-2-enal	961	0.84	1.23	-
11.	Benzaldehyde	968	2,61	2.61	0.44
12.	2-Pentylfuran	992	-	-	0.24
13.	(*E*,*Z*)-Hepta-2,4-dienal	999	0.11	0.35	1.45
14.	Octanal	1004	1.86	1.11	-
15.	2-(2-Ethoxyethoxy)- ethanol (Carbitol)	1007	-	0.70	-
16.	(*E*,*E*)-Hepta-2,4-dienal	1014	3.73	1.89	0.50
17.	Benzyl Alcohol	1043	8.53	9.71	-
18.	(*E*)-Oct-2-enal	1062	2.98	5.56	-
19.	Heptanoic acid	1087	0.05	0.74	-
20.	(*E*,*E*)-Octa-3,5-dien-2-one	1095	0,11	0.66	-
21.	Nonanal	1105	10.57	6.62	-
22.	2,6-Dimethylcyclohexanol	1111	1.58	0.76	0.18
23.	(*E*,*Z*)-Nona-2,6-dienal	1157	0.22	5.81	-
24.	(*E*)-Non-2-enal	1163	1.69	3.48	-
25.	Decanal	1206	2.12	1.13	-
26.	(*E*)-Dec-2-enal	1265	0.05	0.54	-
27.	(*E*,*Z*)-Deca-2,4-dienal	1294	-	-	0.29
28.	(*E*,*E*)-Deca-2,4-dienal	1318	-	-	3.15
29.	α-Ionone	1429	-	-	0.20
30.	(*E*)-6,10-Dimethylundeca-5,9-dien-2-one	1455	-	-	0.15
31.	Dodecan-1-ol	1478	-	-	1.01
32.	*trans*-β-Ionone	1486	-	-	3.04
33.	Pentadec-1-ene	1492	0.11	0.56	-
34.	Pentadecane	1500	2.69	4.24	-
35.	Tridecanal	1511	-	-	0.28
36.	Tridecan-1-ol	1580	-	-	0.23
37.	Tetradecanal	1613	-	-	0.99
38.	Heptadec-8-ene	1679	21.80	12.46	4.39
39.	(*Z*)-Pentadec-11-enal	1693	-	-	1.66
40.	Heptadecane	1700	-	0.49	-
41.	Pentadecanal	1715	-	-	10.61
42.	Tetradecanoic acid	1775	-	-	3.93
43.	(*Z*)-Hexadec-11-enal	1795	-	-	0.39
44.	Hexahydrofarnesyl acetone	1846	-	-	0.79
45.	Hexadecan-1-ol	1852	-	-	2.29
46.	(*Z*,*Z*,*Z*)-Hexadeca-7,10,13-trienal	1863	-	-	1.61
47.	(*Z*)-Hexadec-9-en-1-ol	1863	-	-	1.12
48.	Methyl hexadeca-4,7,10,13-tetraenoate	1879	-	-	7.50
49.	Hexadecan-1-ol	1884	-	-	1.34
50.	(*Z*,*Z*,*Z*)-Hexadeca-7,10,13-trienal	1893	-	-	22.67
51.	Dibutyl phthalate	1963	-	-	1.13
52.	Hexadecanoic acid	1978	-	-	13.18

Table 2. Fatty acid composition and nutritional indices of *Ulva lactuca*

No.	Fatty Acid	Av ± SD (%)
1.	Lauric acid (C12:0)	4.43 ± 0.19
2.	Myristic acid (C14:0)	2.76 ± 0.58
3.	Palmitic acid (C16:0)	45.16 ± 1.82
4.	Stearic acid (C18:0)	5.40 ± 0.55
5.	Arachidic acid (C20:0)	14.57 ± 0.84
6.	Behenic acid (C22:0)	1.03 ± 0.08
	Total saturated fatty acids (SFA)	**73.35**
7.	Palmitoleic acid (C16:1)	2.37 ± 0.17
8.	Oleic acid (C18:1n9*c*+*t*)	13.59 ± 1.47
	Total monounsaturated fatty acids (MUFA)	**15.96**
9.	*cis*-Linoleic acid (C18:2n6*c*)	4.69 ± 0.62
10.	α-Linolenic acid (C18:3n3)	5.82 ± 0.51
11.	Docosadienoic acid (C22:2n6)	1.06 ± 0.11
	Total polyunsaturated fatty acids (PUFA)	**11.57**
	Total ω6 fatty acids	**5.75**
	Total ω3 fatty acids	**5.82**
	Nutritional indices	
	PUFA/SFA	**0.16**
	Index of Atherogenicity (IA)	**2.20**
	Index of Thrombogenicity (IT)	**1.82**
	Hypocholesterolemic/Hypecholesterolemic ratio (HH)	**0.48**
	Unsaturation index (UI)	**44.92**

CONCLUSION

Total of 28 compounds were extracted by HS-SPME and identified by GC-MS with relatively different compounds abundance among the fibers. Unsaturated alkane heptadec-8-ene ranged in the headspace from 21.80% to 12.46%. Another abundant compounds were lower aldehydes heptanal and nonanal, followed by minor percentages of pentanal, hexanal, octanal and decanal. Benzyl alcohol and benzaldehyde were the main benzene derivatives in the headspace. Total lipids were extracted using the Folch method and analysed using GC-FID. Twelve (12) fatty acids were identified. The content of palmitic, oleic acid isomers and arachidic acid consist more than 70 % of total identified fatty acids (45.16 %, 13.59 % and 14.57 %, respectively). U. lactuca is macroalgae characterized by a high content of saturated fatty acids (SFA), which consequently indicates a low PUFA/SFA ratio and high index of atherogenicity (IA) and thrombogenicity (IT).

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