ORIGINAL SCIENTIFIC PAPER

CROATICA CHEMICA ACTA

Croat. Chem. Acta **2021**, *94*(4), 223–232
Published online: August 9, 2022
DOI: 10.5562/cca3902

HKD 1926

Volatile Organic Compounds of Marine Sponge *Petrosia ficiformis* from the Adriatic Sea

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RECEIVED: July 8, 2022 ✶ REVISED: July 28, 2022 ✶ ACCEPTED: July 29, 2022

Abstract: Volatile organic compounds (VOCs) of *Petrosia ficiformis* were investigated for the first time. The VOCs from fresh and air-dried sponge were obtained by hydrodistillation and headspace-solid phase microextraction and they were analysed by coupled gas chromatography-mass spectrometry. Aliphatic compounds with octan-3-one (up to 24.02 %), oct-1-en-3-ol (up to 8.65 %) and heptadecane (up to 39.37 %) were the most abundant in the fresh sponge along with benzaldehyde (up to 18.59 %) and diisobutyl phthalate (up to 8.44 %). Higher percentage of *N*,*N*-dimethylmethanamine (up to 19.08 %) was found in dried sample headspace and the loss of octan-3-one and benzaldehyde and increase of benzyl alcohol (up to 18.90 %) were noted. The great difference among the fresh and dried sponge VOCs obtained by hydrodistillation was noticed for fatty acids and derivatives abundance and 1H-indole increased (up to 6.00 %) in the dried sponge. Both methods enabled obtaining more complete VOCs profile and drying significantly changed their composition.

Keywords: *Petrosia ficiformis* (Poiret, 1789), GC-MS, hydrodistillation, headspace, heptadecane, oct-1-en-3-ol, octan-3-one, benzyl alcohol, benzaldehyde, diisobutyl phthalate, *N*,*N*-dimethylmethanamine, dimethyl trisulphide, 1H-indole.

INTRODUCTION

Petrosia is one of 4 genera of Petrosiidae family belonging to the order Haplosclerida, which is known among sponges as the most prolific source of secondary metabolites.[1] *Petrosia* genus includes 122 species belonging to two subgenera (*Petrosia* and *Strongylophora*) according to the Word Register of Marine Species (WoRMS). A recent review paper compared secondary metabolites isolated from the sponges of the genus *Petrosia*.[2] It was reported that associated microorganisms can account for up to 60 % of the fresh weight of marine sponges and it is believed that these sponge-associated microorganisms (such as bacteria, fungi, cyanobacteria, and unicellular algae) may be involved in the biosynthesis of natural products.[3]

Petrosia ficiformis (Poiret, 1789; Haplosclerida, Petrosiidae) is a marine sponge found across the Mediterranean and in the Eastern Atlantic.[4] The mixtures of high-molecular weight polyacetylenes with 46-55 carbons were isolated from *P. ficiformis*.[5] Five additional polyacetylenes up to 52 carbons, isolated from *P. ficiformis* from the Mediterranean Sea, were also reported.[6] Polyacetylenes with two terminal 1-yn-3-ol-4-ene moieties and 46 total carbons isolated from Mediterranean *P. ficiformis* were identified and evaluated for their biological activity. It was found that they inhibit sea urchin egg development and act as a potent toxin against *Artemia salina*.[7,8] Petroformynes, isopetroformynes, and various oxidized or isomerized analogues were reported in *P. ficiformis* and most of these analogues exhibited lethality against brine shrimp.[7–10] Sterols have rarely been isolated from *Petrosia* sponges in temperate regions, but *P. ficiformis* contains sterol compounds with cyclopropane in the branch at C-17.[2] Petrosterol (26,27-cycloaplysterol), a steroid with a cyclopropane ring at C-25 and C-26, was isolated from *P. ficiformis* collected in the Bay of Naples and later ficisterol (23-ethyl-24-methyl-27-norcholesta-5,25-dien-3β-ol) was found as the minor component.[11–13] The relative abundance of phospholipids classes varies with sponge species, but amino phospholipids, especially phosphatidylethanolamine and phosphatidylcholine were found in *P. ficiformis*.[14] Its phospholipids also included numerous branched fatty acids (e.g. (*Z*,*Z*)-25-methylhexa-

cosa-5,9-dienoic acid and (*Z*,*Z*)-24-methylhexacosa-5,9-dienoic acid).[14]

There were no data about volatile organic compounds (VOCs) of this marine sponge that are the focus of this investigation. The present research has the following key goals: a) isolate VOCs of fresh *P. ficiformis* (FrPF) and air-dried *P. ficicormis* (DrPF) retrieved by both headspace solid-phase microextraction (HS-SPME) and hydrodistillation (HD); b) identify the isolated VOCs by gas chromatography–mass spectrometry analysis (GC-MS); b) compare VOCs of FrPF and DrPF.

EXPERIMENTAL

Marine Sponge Sample

P. ficiformis sample was collected in the Adriatic Sea near Rtina peninsula (Island of Pag), with the exact geographical location being: 44° 19' 10'' N; 15° 15' 37'' E. The sampling depth was 6 m with a sea temperature of 18 °C. Upon collection, the sample was placed in an airtight container filled with seawater and immediately delivered to the laboratory. Until processing, the fresh sample was stored and kept in the laboratory freezer, while the air-dried sample was kept in the dark at room temperature for 10 days. Before the analysis, the used sponge sample was cleaned of stones and other visible foreign matters. Both fresh and air-dried sponge samples were sliced into small pieces and used for headspace solid-phase microextraction (HS-SPME) and hydrodistillation (HD).

Headspace Solid-Phase Microextraction (HS-SPME)

HS-SPME was performed with PAL Auto Sampler System (PAL, RSI 85, CTC Analytics AG, Switzerland) using SPME fibres with PDMS/DVB (polydimethylsiloxane/divinylbenzene) and DVB/CAR/PDMS (divinylbenzene/carboxene/polydimethylsiloxane) coatings. Both fibres were obtained from Agilent Technologies (Santa Clara, CA, USA). Prior to the use, the fibres were conditioned according to the manufacturer's instructions. 1 g of fresh or dry sponge sample was placed into 20 mL glass vials and hermetically sealed with a screw cap containing polytetrafluoroethylene (PTFE)/silicone septum. The samples were equilibrated for 15 min at 60 °C and then extracted for 45 min. The injector temperature was set to 250 °C and thermal desorption was carried out for 6 min directly to the GC column. HS-SPME was performed in triplicate.

Hydrodistillation (HD)

Modified Clevenger apparatus with pentane (Kemika, Zagreb, Croatia) and diethyl ether (Kemika, Zagreb, Croatia) solvent trap (*v*/*v* ratio 1:2) was used for distillation. 100 g of fresh sample and 30 g of air-dried sample were used separately. HD was carried out for 2 hours. After HD, the solvent trap layer was carefully separated using a glass pipette, concentrated by the slow flow of nitrogen up to 0.05 mL and later used for GC-MS analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of VOCs

GC-MS analysis was carried out on Agilent Technologies (Palo Alto, CA, USA) gas chromatograph model 8890A equipped with mass selective detector model 5977E. Separation of the compounds was achieved on an HP-5MS column (Agilent Technologies, Santa Clara, CA, USA) 30 m x 0.25 mm containing a non-polar stationary phase (5 % diphenyl / 95 % dimethylpolysiloxane) and 0.25 µm film thickness. The following operating conditions for the gas chromatograph were used: 250 °C injector temperature; 300 °C detector temperature; column temperature program: 2 min isothermally at 70 ° C, then temperature gradient from 70 °C to 200 °C at 3 °C/min and further retention for 15 min.

RESULTS AND DISCUSSIONS

Headspace Composition of VOCs isolated by HS-SPME

Headspace composition of both FrPF (HS-FrPF) and DrPF (HS-DrPF) were analysed using solid-phase microextraction (HS-SPME). Two fibres of different polarities were used: divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS, f1) and polydimethylsiloxane/divinylbenzene (PDMS/DVB, f2) to get more profound headspace profile. In HS-FrPF 88.36 % (f1) and 89.22 % (f2) and in Hs-DrPF 95.46 % (f1) and 94.65 % (f2) of total VOCs were identified (Table 1).

Aliphatic compounds were dominant in both HS-FrPF (56.24 %, f1; 66.00 %, f2) and HS-DrPF (65.16 %, f1; 37.38 %, f2) (Figure 1) samples analysed with both fibres with octan-3-one (HS-FrPF, f1) and heptadecane (HS-FrPF, f2; HS-DrPF, both fibres) as two most abundant compounds (Figure 2). Octan-3-one, fatty acid derivative known as C_8-oxylipin,[15] was identified only in HS-FrPF (24.02 %, f1; 15.39 %, f2). This compound was not detected after air-drying probably because of its high volatility. Several more C_8-oxylipins were identified, such as oct-1-en-3-ol, oct-4-en-3-ol, octan-2,3-dione, octan-2-one, octanal, (*E*)-oct-2-en-1-al and volatile pheromone (3*E*,5*Z*)-octa-1,3,5-triene (fucoserratene) (Table 1). Heptadecane was the most abundant hydrocarbon. It is known that hydrocarbons are characteristic compounds for marine invertebrates and algae and form part of their defence system.[16] It can be observed that the air-dried sponge sample analysed with f1 contains the largest share of this compound, 2.65 times higher than the analysed fresh sample on the same fibre

Table 1. The volatile organic compounds isolated by headspace solid-phase microextraction (HS-SPME) and analysed by gas chromatography–mass spectrometry (GC-MS) from *P. ficiformis* samples: (I-fresh *P. ficiformis* extracted by DVB/CAR/PDMS fibre, II-air-dried *P. ficiformis* extracted by DVB/CAR/PDMS fibre, III-fresh *P. ficiformis* extracted by PDMS/DVB fibre, IV-air-dried *P. ficiformis* extracted by PDMS/DVB fibre).

No.	Compound	RI	Area (%) ± SD*			
			I	II	III	IV
1	2-Aminopropane (Isopropylamine)	470	1.56±0.00	–	–	–
2	*N*,*N*-Dimethylmethanamine	482	1.66±0.21	3.50±1.82	–	19.08±2.99
3	Propan-2-ol	510	–	1.82±0.77	–	–
4	Methylsulfanylmethane	515	–	–	–	2.38±0.10
5	3-Methylbutanal (Isovaleraldehyde)	652	2.03±0.38	–	1.18±0.45	0.32±0.04
6	Pentanal	698	1.04±0.20	–	0.27±0.00	–
7	3-Methylbutan-1-ol (Isoamyl alcohol)	734	–	–	0.49±0.00	–
8	N-Methylpyrrole	750	–	0.43±0.06	–	–
9	Pyridine	753	5.08±0.56	3.06±0.46	1.50±0.44	1.44±0.31
10	Pentan-1-ol	765	–	–	–	0.53±0.03
11	2-Methylpropanoic acid (Isobutyric acid)	785	–	–	–	3.88±0.05
12	Hexanal	800	–	0.24±0.07	–	–
13	3-Methylbutanoic acid (Isovaleric acid)	834	–	0.83±0.03	–	–
14	(3*E*,5*Z*)-Octa-1,3,5-triene (Fucoserraten)	880	1.15±0.25	–	0.86±0.10	–
15	2,5-Diethylfuran	888	–	–	–	0.60±0.05
16	Bromoform	892	–	–	2.01±0.31	–
17	Heptanal	901	–	1.11±0.15	–	0.80±0.02
18	2,6-Dimethylpyrazine	922	–	–	–	0.82±0.05
19	2-Ethylhexan-1-al	954	–	–	0.75±0.56	–
20	Benzaldehyde	961	18.59±1.87	2.68±0.02	10.82±4.56	5.33±0.29
21	3-Methylbutyl propanoate (Isoamyl propionate)	969	–	–	0.49±0.00	–
22	(Methyltrisulfanyl)methane (Dimethyl trisulfide)	972	–	–	–	0.66±0.04
23	Oct-1-en-3-ol	980	–	–	2.13±0.52	–
24	Oct-4-en-3-one	981	1.12±0.43	–	–	0.40±0.07
25	Octan-3-one	984	24.02±0.38	–	15.39±12.65	–
26	Phenol	984	–	0.77±0.22	–	1.98±0.12
27	Octan-2,3-dione	987	–	–	–	0.78±0.10
28	Octan-2-one	992	–	–	0.78±0.12	–
29	Octanal	1005	–	–	–	0.87±0.09
30	1-Methyl-4-(propan-2-yl)benzene (*p*-Cymene)	1026	–	–	0.24±0.05	–
31	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane (Eucalyptole)	1031	–	–	1.07±0.49	–
32	Phenylmethanol (Benzyl alcohol)	1042	1.61±0.15	14.78±1.62	–	18.90±0.46
33	2-Phenylacetaldehyde (Benzenacetaldehyde)	1050	1.8±0.90	–	1.50±1.22	–
34	(*E*)-Oct-2-enal	1063	0.49±0.18	–	0.76±0.22	–
35	1-Phenylethanone (Acetophenone)	1072	–	–	0.55±0.08	–
36	Nonan-2-one	1091	0.66±0.06	–	0.34±0.00	–
37	Nonan-3-one	1091	–	–	0.42±0.00	–
38	1,3,3-Trimethylbicyclo[2.2.1]heptan-2-one (Fenchone)	1096	–	–	0.34±0.00	–
39	3,7-Dimethylocta-1,6-dien-3-ol (Linalool)	1102	–	–	1.16±0.00	–
40	Nonanal	1102	–	–	–	2.41±0.13
41	2,6-Dimethylcyclohexan-1-ol	1110	–	0.97±0.21	–	1.78±0.15
42	4-Methyl-1-(1-methylethyl)-bicyclo[3.1.0]hexan-3-one (β-Thujone)	1110	–	–	0.34±0.00	–
43	2-Phenylethanol (Phenethyl alcohol)	1118	1.82±0.74	–	1.41±0.00	–
44	Dodecane	1203	–	–	–	1.00±0.26
45	Dodec-2-ene	1213	–	–	–	1.44±0.05
46	2,6,6-Trimethylcyclohexene-1-carbaldehyde (β-Cyclocitral)	1226	–	0.77±0.18	–	–
47	(2*E*)-3,7-Dimethylocta-2,6-dienal (β-Citral)	1243	–	1.65±0.16	–	–
48	1H-Indole	1292	–	0.95±0.10	–	1.56±0.79
49	Undecan-2-one	1294	0.55±0.07	–	–	–
50	4-(2,6,6-Trimethylcyclohexen-1-yl)but-3-en-2-one (β-Ionone)	1485	–	0.88±0.00	0.88±0.00	0.65±0.12
51	Pentadecane	1503	1.86±0.08	–	1.34±0.16	0.51±0.06
52	2,4-Di*tert*-butylphenol	1513	–	–	0.89±0.00	–
53	Hexadecane	1602	0.81±0.01	1.15±0.18	1.20±0.25	2.44±0.27
54	Heptadec-8-ene	1680	–	–	0.74±0.28	–
55	Heptadecane	1703	22.51±0.36	59.85±2.48	39.37±6.66	24.03±0.89

* SD is the standard deviation of sample triplicate; RI – retention index.

(Table 1, Figure 2). A higher percentage of heptadecane in the dry sample can be attributed to the presence of cyanobacteria living symbiotically with sponges. It has been discovered that the biosynthetic pathway from cyanobacteria consists of the acyl-acyl carrier protein reductase and the aldehyde decarbonylase, which together convert the intermediates of fatty acid metabolism into alkanes and alkenes.[17] Several other saturated hydrocarbons such as hexadecane and pentadecane were identified in smaller proportions in almost all samples (Table 1).

The second most dominant group of compounds were benzene derivatives (Figure 1). In HS-FrPF higher presence of benzaldehyde was found compared to HS-DrPF (6.9 times, f1; 2.0 times, f2) (Figure 2). As already explained in the previously published research[18–20] the loss of benzaldehyde during air drying could be due to its high volatility. On the other hand, benzyl alcohol percentage increased in the dry samples (9.2 (f1) – 18.9 (f2) times). Phenylpropane derivatives are derived from phenylalanine with the side-chain shortened by two carbon atoms which can take place β-oxidatively or non-oxidatively.[21]

The compounds containing nitrogen showed similar abundance when analysed with f1 (8.30 %, HS-FrPF; 7.94 %, HS-DrPF), but when analysed with f2 they were 15.3 times higher in HS-DrPF (1.50 %, HS-FrPF; 22.90 %, HS-DrPF) (Figure 1). The volatile amine, *N*,*N*-dimethylmethanamine (trimethylamine) has been frequently used as a freshness parameter of marine organisms. Trimethylamine is formed from trimethylamine oxide (TMAO) as the result of the action of bacteria that cause spoilage,[22] which may explain the higher percentage of this amine in dry samples (2.1 (f1) – 19.1 (f2) times). The aromatic heterocyclic compound pyridine was found in all samples and its percentage decreased 1.0 (f2) – 1.7 (f2) times after air-drying. Pyridine derivatives are often part of biomolecules in marine sponges such as alkaloids.[23]

Among terpenes, *p*-cymene, eucalyptol, fenchone, linalool, β-thujone and β-citral were detected in a low percentage. Other compounds such as norisoprenoids, organohalogen compounds and fatty acid derivatives were detected with minor abundance in the total headspace composition.

Composition of the VOCs Obtained by Hydrodistillation

Analysis of the VOCs in the hydrodistillate resulted in 88.53 % in FrPF (HD-FrPF) and 77.02 % in DrPF (HD-DrPF) of the total identified compounds (Table 2).

In both samples, the group of aliphatic compounds predominated with 44.99 % in HD-FrPF and 36.67 % in HD-DrPF (Figure 3). Oct-1-en-3-ol, oxylipin known as global metabolome that induces the defence of marine invertebrates and algae,[15] had the greatest abundance in HD-FrPF. It decreased 30.1 times after drying probably because of its high volatility (Figure 4). Nine more oxylipins were detected and identified (Table 2). Heptadecane, as the second most abundant aliphatic compound in HD-FrPF, was present with lower abundance than in the headspace of the samples extracted by HS-SPME (Figure 4).

The second most represented group of identified compounds was benzene derivatives (17.27 %, HD-FrPF; 12.09 %, HD-DrPF) (Figure 3) with diisobutyl phthalate as the dominant compound (8.44 %, HD-FrPF; 1.80 %, HD-DrPF) (Figure 4). Di-*n*-phthalates were found in the marine sponges *Cinachyrella cavernosa*[24] and *Halidcondria* sp.,[25] and their presence in sponges potentially originates from cyanobacteria on sponges. Two more benzene derivatives in higher abundance were identified: 1,4-xylene (2.87 %, HD-FrPF; 2.37 %, HD-DrPF) and benzaldehyde (2.43 %, HD-FrPF; 2.36 %, HD-DrPF).

In HD-FrPF 15.06 % of the total identified compounds belonged to the group of fatty acids and derivatives (Figure 3). In HD-DrPF, the portion of this group decreased 41.1 times. Two hexadecanoic acid esters, methyl (*Z*)-hexadec-7-enoate (3.77 %) and ethyl (*E*)-hexadec-9-enoate (3.66 %), were identified in HD-FrPF with higher abundance, but were not detected in HD-DrPF (Figure 4). The reason for fatty acid esters decrement after the drying may be oxidation reactions and lipid decomposition that can occurred during the drying.

Among the compounds containing sulphur, dimethyl trisulphide was quantitatively important, and its proportion increased after the drying (5.31 %, HD-FrPF; 7.50 %, HD-DrPF) (Figure 3). It was also the most abundant compound in HD-DrPF. Dimethyl trisulphide, along with other compounds, could be responsible for the strong, unpleasant smell that this sponge produced. The role of sulphur compounds in marine sponges is probably the defence against predators.[26,27]

The second most abundant compound in HD-DrPF was 1H-indole with 6.00 % (Figure 4). The indoles alkaloids are broadly present in the metabolism of marine organisms, especially sponges.[28] Indole alkaloids containing a benzopyrrole skeleton exhibit antibacterial, antimicrobial, cytotoxic and antineoplastic properties.[29,30]

Carotenoid degradation products, C_{13}-norisoprenoides, were more abundant in the hydrodistillate of the dry sample. β-Ionone increased 3.6 times after the drying, and β-cyclohomocitral, was detected only in the dry sample. Chlorophyll derivatives phytane and hexahydrofarnesyl acetone (phytone) were found. After the drying, their content increased, probably due to the degradation of chlorophyll.[31]

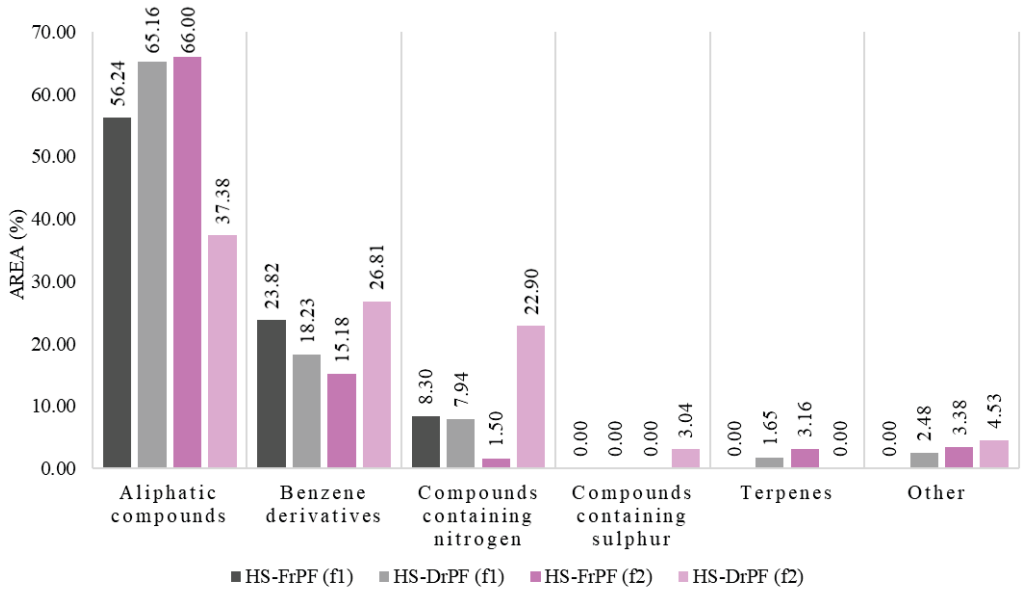

Figure 1. The volatile organic compounds of *P. ficiformis* extracted by headspace solid-phase microextraction (HS-SPME), analysed by gas chromatography-mass spectrometry (GC-MS) and sorted by structural groups. Extraction by DVB/CAR/PDMS fibre (f1): HS-FrPF (f1) – fresh *P. ficiformis*; HS-DrPF (f1) – air-dried *P. ficiformis*. Extraction by PDMS/DVB fibre (f2): HS-FrPF (f2) – fresh *P. ficiformis*; HS-DrPF (f2) – air-dried *P. ficiformis.*

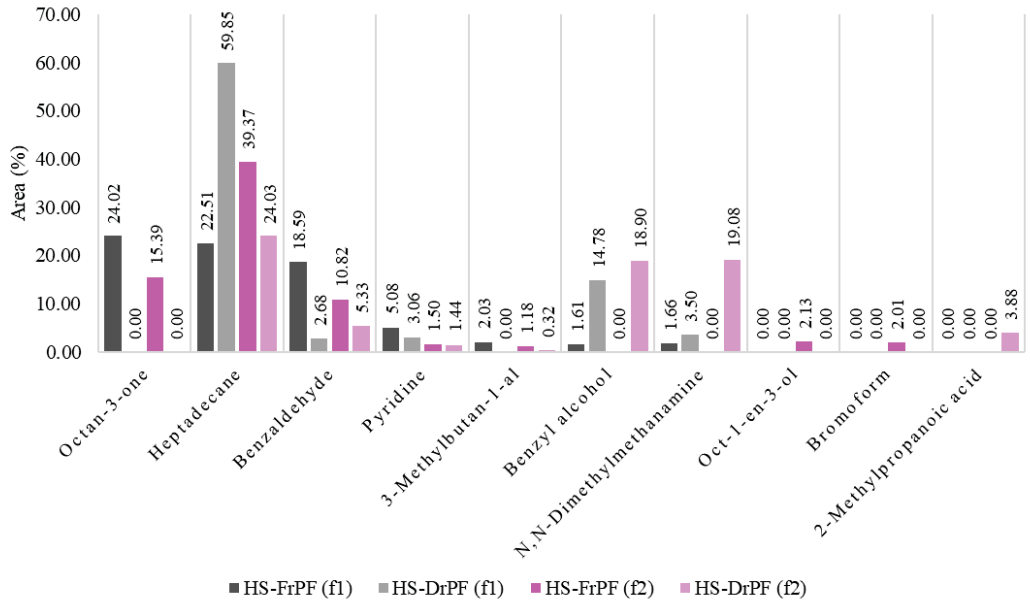

Figure 2. The most abundant compounds from *P. ficiformis* samples extracted by HS-SPME and analysed by GC-MS. The volatile organic compounds of *P. ficiformis* extracted by headspace solid-phase microextraction (HS-SPME), analysed by gas chromatography-mass spectrometry (GC-MS) and sorted by structural groups. Extraction by DVB/CAR/PDMS fibre (f1): HS-FrPF (f1) – fresh *P. ficiformis*; HS-DrPF (f1) – air-dried *P. ficiformis*. Extraction by PDMS/DVB fibre (f2): HS-FrPF (f2) – fresh *P. ficiformis*; HS-DrPF (f2) – air-dried *P. ficiformis.*

Table 2. The VOCs from *P. ficiformis* isolated by HD and analysed by GC-MS: (VI - HD of fresh *P. ficiformis*, VII - HD of air-dried *P. ficiformis*).SD is standard deviation of sample triplicate; RI – retention index

No.	Compound	RI	Area (%) ± SD*	
			V	VI
1	2-Methyl-1H-pyrrole	750	0.32±0.07	0.16±0.10
2	Furan-2-carbaldehyde (Furfural)	830	0.16±0.04	0.16±0.09
3	(*E*)-Hex-2-enal	857	0.39±0.01	0.41±0.10
4	4-Methyloctane	863	–	0.03±0.03
5	Ethylbenzene	868	1.00±0.01	0.67±0.03
6	1,4-Xylene (*p*-Xylene)	878	2.87±0.15	2.37+–0.08
7	(3*E*,5*Z*)-Octa-1,3,5-triene (Fucoserratene)	879	0.19±0.02	–
8	Cycloocta-1,3,5,7-tetraene	880	–	0.08±0.03
9	Heptan-2-one	889	0.71±0.04	0.28±0.07
10	Styrene	890	0.10±0.00	–
11	1,2-Xylene (*o*-Xylene)	894	–	0.61±0.03
12	Nonane	900	–	0.58±0.01
13	(*Z*)-Hept-4-enal	901	2.48±0.16	–
14	Heptanal	902	0.61±0.01	0.22±0.07
15	3-(Methylsulfanyl)propanal (Methional)	906	0.19±0.01	0.22±0.07
16	Benzaldehyde	961	2.43±0.16	2.38±0.68
17	(Methyltrisulfanyl)methane (Dimethyl trisulfide)	972	5.31±0.10	7.50±2.34
18	Heptan-1-ol	975	0.30±0.01	–
19	Oct-1-en-3-ol	982	8.65±0.58	0.29±0.04
20	Octan-2,5-dione	983	–	0.28±0.09
21	Phenol	984	–	0.22±0.01
22	Octan-2,3-dione	987	0.18±0.05	–
23	Benzonitrile	986	–	0.20±0.01
24	2,4,6-Trimethylpyridine	987	–	0.45±0.01
25	Octan-3-one	988	1.60±0.03	–
26	Octan-2-one	992	0.38±0.03	–
27	Octanal	1005	0.18±0.01	0.12±0.01
28	1-Methylpyrrole-2-carbaldehyde	1010	0.25±0.00	0.17±0.03
29	(*E*,*E*)-Hepta-2,4-dienal	1012	0.07±0.00	–
30	Bis(1-Methylethyl) disulphide (Diisopropyl disulphide)	1016	–	0.05±0.05
31	2-Ethylhexan-1-ol	1029	0.13±0.00	0.12±0.02
32	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane (Eucalyptole)	1035	0.26±0.08	–
33	Phenylmethanol (Benzyl alcohol)	1040	0.59±0.20	0.61±0.07
34	2-Phenylacetaldehyde (Benzenacetaldehyde)	1050	1.17±0.30	1.75±0.66
35	2-Methyldecane	1061	0.15+–0.02	–
36	(*E*)-Oct-2-enal	1063	0.66±0.06	0.22±0.03
37	1-Phenylethanone (Acetophenone)	1066	–	0.40±0.10
38	(*E*)-Oct-2-en-1-ol	1071	2.12±0.07	–
39	Octan-1-ol	1074	–	0.03±0.01
40	Octylcyclopropane	1075	2.65±0.18	0.11±0.00
41	(*E*,*E*)-Octa-3,5-dien-2-one	1083	0.33±0.05	–
42	Nonan-2-one	1091	0.10±0.02	0.20±0.04
43	Nonanal	1102	0.15±0.01	0.32±0.07
44	3,7-Dimethylocta-1,6-dien-3-ol (Linalool)	1104	0.40±0.01	0.45±0.12
45	2,6-Dimethylcyclohexan-1-ol	1110	0.17±0.03	0.24±0.05
46	2-Phenylethanol (Phenethyl alcohol)	1117	0.28±0.15	–
47	Phenylpropan-2-one (Phenylacetone)	1124	–	0.10±0.01
48	1-(4-Methylcyclohex-3-en-1-yl)ethanone	1130	–	0.34±0.05
49	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one (Camphor)	1139	0.12±0.04	0.04±0.04
50	(*E*,*Z*)-Nona-2,6-dienal	1156	1.02±0.10	0.45±0.18
51	(*Z*)-Non-2-en-1-al	1162	0.44±0.04	0.28±0.11
52	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol (Borneol)	1167	–	0.03±0.03
53	Decan-2-one	1194	0.15±0.00	0.28±0.02
54	Decanal	1200	0.50±0.02	0.33±0.03
55	1-Methoxy-4-(prop-2-en-1-yl)benzene (Estragole)	1201	–	0.17±0.03
56	(3*Z*,5*Z*)-2,6-Dimethylocta-3,5,7-trien-2-ol	1209	–	0.20±0.02
57	2-Methoxy-1-methyl-4-(1-methylethyl)-benzene (Carvacrol methyl ether)	1250	0.09±0.00	–

* SD is the standard deviation of sample triplicate; RI – retention index.

Table 2. Continued.

No.	Compound	RI	Area (%) ± SD*	
			V	VI
58	2-(2,6,6-Trimethylcyclohexen-1-yl)acetaldehyde (β-Cyclohomocitral)	1261	–	0.20±0.03
59	Dec-2-en-1-ol	1273	1.32±0.10	–
60	2,6,11-Trimethyldodecane	1275	0.33±0.00	–
61	1H-Indole	1292	0.52±0.02	6.00±0.83
62	2-Decyloxirane	1307	0.28±0.01	–
63	Undecanal	1308	–	0.12±0.02
64	4,6-Dimethyldodecane	1325	0.39±0.02	–
65	Octyl 2-methylpropanoate (Caprylyl isobutyrate)	1348	0.44±0.02	–
66	Dodecan-2-one	1349	0.28±0.03	0.42±0.05
67	Dodecan-3-one	1387	–	0.27±0.03
68	Phenoxybenzene (Diphenyl ether)	1396	0.25±0.00	0.26±0.02
69	Dodecanal	1409	1.17±0.12	0.97±0.04
70	3-Methyldecanoic acid	1417	–	0.26±0.26
71	(5*E*)-6,10-Dimethylundeca-5,9-dien-2-one ((*E*)-Geranylacetone)	1452	0.19±0.00	0.59±0.01
72	(*Z*)-Dodec-8-en-1-ol	1468	–	0.48±0.01
73	Dodecan-1-ol	1468	–	1.20±0.03
74	(*E*)-Dodec-2-en-1-ol	1473	0.62±0.00	–
75	1-Methyl-4-[6-methylhept-5-en-2-yl]cyclohexa-1,3-diene (γ-Curcumene)	1475	–	0.19±0.19
76	(*E*)-4-(2,6,6-Trimethylcyclohexen-1-yl)but-3-en-2-one (β-Ionone)	1485	0.94±0.08	3.38±0.18
77	Tridecan-2-one	1497	–	1.36±0.04
78	Pentadecane	1503	0.48±0.05	–
79	2-Bromododecane	1505	0.69±0.02	–
80	Tridecanal	1511	1.33±0.16	1.22±0.05
81	2,5-Di*tert*-butylphenol	1514	–	0.55±0.03
82	1-(4-Hydroxy-3-methoxyphenyl)propan-2-one (Guaiacyl acetone)	1531	0.45±0.01	1.96±0.09
83	1-Bromododecane	1552	–	0.70±0.03
84	(6*E*)-3,7,11-Trimethyldodeca-1,6,10-trien-3-ol (Nerolidol)	1564	0.13±0.02	0.31±0.02
85	Tetradecan-2-one	1569	0.30±0.15	1.18±0.04
86	Hexadec-1-ene	1579	1.29±0.17	1.39±0.03
87	Tridecanenitrile	1592	–	0.29±0.05
88	Hexadecane	1600	0.45±0.02	1.07±0.07
89	(*Z*)-Tetradec-9-en-1-al	1603	1.58±0.06	4.06±0.01
90	Tetradecanal	1614	3.17±0.52	2.68±0.06
91	3-[1-Hydroxy-2-(methylamino)ethyl]phenol (Phenylephrine)	1660	0.11±0.02	0.55±0.07
92	1-(4-Isopropylphenyl)-2-methylpropyl acetate	1667	–	0.45±0.15
93	(*Z*)-Tetradec-11-en-1-ol	1678	–	3.89±0.12
94	Tetradecanenitrile	1679	–	0.51±0.04
95	13-Methyltetradecanal	1681	0.40±0.11	–
96	(*Z*)-Heptadec-3-ene	1687	0.91±0.06	–
97	Heptadecane	1703	5.85±0.26	2.45±0.01
98	Pentadecanal	1711	0.63±0.10	1.08±0.06
99	Methyl tetradecanoate (Methyl myristate)	1726	0.29±0.01	–
100	8-Methylheptadecane	1758	0.73±0.04	0.88±0.05
101	4-Methylheptadecane	1760	0.53±0.04	1.53±0.03
102	Methyl 12-methyltetradecanoate	1786	0.68±0.03	–
103	Octadec-1-ene	1790	–	0.35±0.17
104	Pentadecanenitrile	1792	–	1.24±0.11
105	Methyl 9-methyltetradecanoate	1793	–	–
106	Octadecane	1804	0.14±0.05	–
107	2,6,10,14-Tetramethylhexadecane (Phytane)	1809	–	0.32±0.05
108	2-Phenoxyethoxybenzene (Diphenyl glycol)	1811	–	0.21±0.07
109	Hexadecanal	1822	–	0.31±0.07
110	3,7,11-Trimethyldodeca-2,6,10-trienyl acetate (Farnesyl acetate)	1844	–	0.27±0.27
111	6,10,14-Trimethylpentadecan-2-one (Hexahydrofarnesyl acetone)	1846	0.14±0.01	0.45±0.27
112	(9*Z*)-Hexadeca-1,9-diene	1863	–	0.47±0.19
113	(*Z*)-Hexadec-11-enal	1871	–	0.15±0.15
114	bis(2-Methylpropyl)benzene-1,2-dicarboxylate (Diisobutyl phthalate)	1872	8.44±1.12	1.80±0.57
115	Hexadecan-1-ol	1883	0.37±0.08	2.04±0.52

* SD is the standard deviation of sample triplicate; RI – retention index.

Table 2. Continued.

No.	Compound	RI	Area (%) ± SD*	
			V	VI
116	Nonadec-1-ene	1895	0.41±0.01	0.35±0.17
117	Methyl (*Z*)-hexadec-7-enoate	1899	3.77±0.16	–
118	(5*E*,9*E*)-6,10,14-Trimethylpentadeca-5,9,13-trien-2-one (Farnesyl acetone)	1919	0.17±0.03	0.31±0.10
119	Methyl hexadecanoate (Methyl palmitate)	1926	0.63±0.05	–
120	1-(Pentyloxy)tetradecane	1971	–	0.79±0.18
121	Methyl 14-methylhexadecanoate	1975	1.22±0.10	–
122	Ethyl (*E*)-hexadec-9-enoate	1979	3.66±0.26	–
123	Icos-1-ene	1992	–	0.15±0.09
124	Icosane	2000	–	0.12±0.12
125	(*Z*)-Methyl heptadec-10-enoate	2015	0.21±0.01	–
126	(6*E*,10*E*)-3,7,11,15-Tetramethylhexadeca-1,6,10,14-tetraen-3-ol	2032	0.21±0.03	0.64±0.21
127	3,8-Dimethyl-5-[6-methylhept-5-en-2-yl]-(1,3a,4,5,6,8a)-hexahydroazulen-4-ol (Isopachydictyol A)	2130	0.73±0.04	–

* SD is the standard deviation of sample triplicate; RI – retention index.

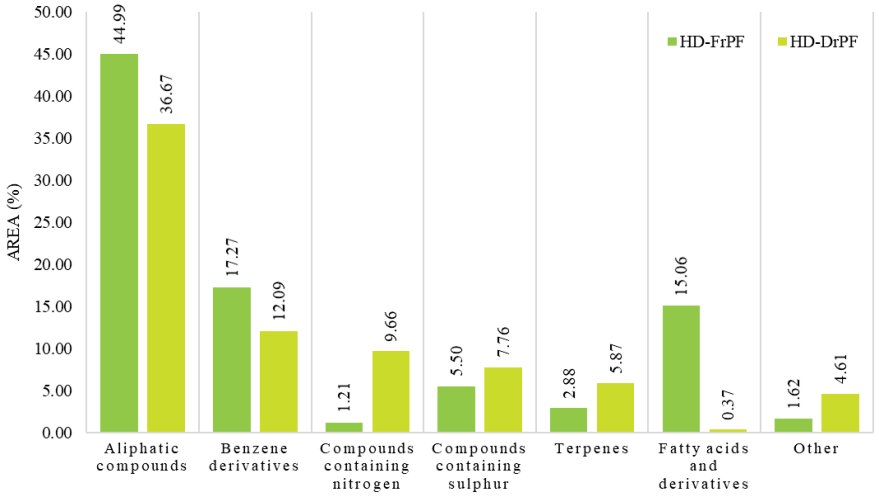

Figure 3. The volatile organic compounds (VOCs) of *P. ficiformis* obtained by hydrodistillation (HD), analysed by gas chromatography–mass spectrometry (GC-MS) and sorted by structural groups. HD-FrPF – hydrodistillate of fresh *P. ficiformis*; HD-DrPF – hydrodistillate of air-dried *P. ficiformis.*

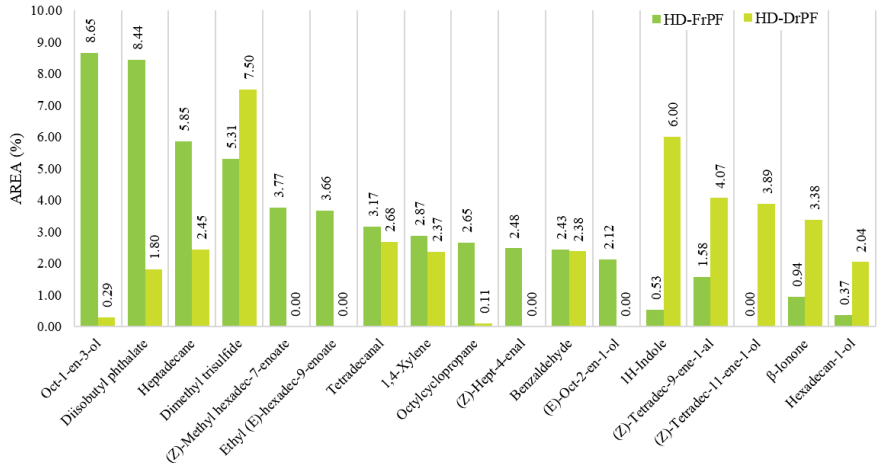

Figure 4. The most abundant compounds of *P. ficiformis* samples obtained by hydrodistillation (HD) and analysed by gas chromatography–mass spectrometry (GC-MS). HD-FrPF – hydrodistillate of fresh *P. ficiformis*; HD-DrPF – hydrodistillate of air-dried *P. ficiformis.*

CONCLUSION

Since there is no data available on *P. ficiformis* VOCs in the literature and only scarce data regarding polyacetylenes, sterols and phospholipids are present, this paper offers a detailed research on *P. ficiformis* VOCs profile for the first time. Aliphatic compounds were dominant in both FrPH and DrPF samples extracted by HS-SPME and in HD. Heptadecane was the most abundant hydrocarbon in the headspace samples with higher abundance in HS-DrPH and the second most abundant aliphatic compound in HD-FrPH, but with much lower abundance than in the headspace of the samples extracted by HS-SPME. The second most dominant group of compounds were benzene derivatives in both samples. In the headspace of the samples, benzene derivatives with the highest abundance were benzaldehyde and benzyl alcohol and in HD diisobutyl phthalate, 1,4-xylene and benzaldehyde. The great difference between fresh and air-dried samples was noticed in terms of fatty acids and derivatives abundance. Two hexadecanoic acid esters, methyl (*Z*)-hexadec-7-enoate and ethyl (*E*)-hexadec-9-enoate, were identified in HD-FrPF in higher abundance but were not detected in HD-DrPF. The compounds containing sulphur, especially dimethyl trisulphide, were mostly detected in HD-DrPF, probably causing an unpleasant smell. The compounds containing nitrogen were present in a higher portion in the samples after air-drying in both types of isolation, particularly *N*,*N*-dimethylmethanamine in the headspace. The second most abundant compound in HD-DrPF was 1H-indole. Few terpenes, C_{13}-norisoprenoides and chlorophyll derivatives were detected mostly in HD-DrPF. By choosing different methods of isolation of *P. ficiformis* VOCs their more complete profile was obtained for the first time.

List Of Abbrevations

VOCs ..... volatile organic compounds
WoRMS ..... Word Register of Marine Species
GC-MS ..... gas chromatography–mass spectrometry
HS-SPME ..... headspace solid-phase microextraction
HD ..... hydrodistillation
FrPF ..... fresh *P. ficiformis*
DrPF ..... dry *P. ficiformis*
DVB/CAR/PDMS .. divinylbenzene/carboxene/polydimethylsiloxane
PDMS/DVB ..... polydimethylsiloxane/divinylbenzene
f1 ..... DVB/CAR/PDMS fibre
f2 ..... PDMS/DVB fibre
HS-FrPF ..... headspace composition of fresh *P. ficiformis*
HS-DrPF ..... headspace composition of dry *P. ficiformis*
HD-FrPF ..... hydrodistillate of fresh *P. ficiformis*
HD-DrPF ..... hydrodistillate of dry *P. ficiformis*

Acknowledgements: We would like to thank the Croatian Government and the European Union (European Regional Development Fund—the Competitiveness and Cohesion Operational Programme KK.01.1.1.01) granted to The Scientific Centre of Excellence for Marine Bioprospecting-BioProCro. We thank also Donat Petricioli for the sample collection and identification.

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