

## Volatile Components of the Phaeophyceae *Hormophysa cuneiformis* Growing Along Qatar Coasts

M. El Hattab<sup>1</sup>, Hala S. Al Easa<sup>2</sup>, Annie Tabariès<sup>1</sup>, Jean-Michel Kornprobst<sup>3</sup>, Louis Pioveti<sup>1</sup>

<sup>1</sup> Equipe Produits Naturel Marins, Laboratoire MFS/PCM3, Université du Sud, BP 20132, 83957 La Garde cedex, France.

<sup>2</sup> Chemistry and Earth Sciences Department, College of Arts and Science, University of Qatar, PO Box 2713, Doha, State of Qatar.

<sup>3</sup> Groupe SMAB, Institut Substances et Organismes de la Mer (ISOMer), 2 rue de la Houssinière, BP, 53508, 44322 Nantes cedex 3, France.

المواد المتطايرة من الطحلب البني هورموفيسا كينوفورميس الذي ينمو على السواحل القطرية

محمد الحطاب\*، هاله سلطان العيسى\*\*، أني تيريس\*، جين ميشيل كورنبروبست\*\*\*، لويس بيوفيتي\*

\* معمل المنتجات البحرية الطبيعية ☒ جامعة الجنوب ☒ فرنسا .  
\*\* قسم الكيمياء وعلوم الأرض ☒ جامعة قطر ☒ ص.ب: 2713 .  
\*\*\* مجموعة سماب ☒ معهد الكائنات البحرية ☒ نانت ☒ فرنسا .

تمت دراسة المواد المتطايرة من الطحلب البني هورموفيسا كينوفورميس المجموع من شواطئ قطر في مايو 2002 وذلك باستخدام طريقتين للاستخلاص وهما الطريقة التقليدية للاستخلاص ( التقطير البخاري ) لمستخلص الايثر والاستخلاص باستخدام ثاني أكسيد الكربون. وأظهرت الدراسة باستخدام كروماتوجرافيا الغاز وطيف الكتلة أن المكونات الأساسية للزيت الطيار هي الأحماض الدهنية والاسترات المشتقة منها وقلّة وجود C-11 هيدروكربونات متطايرة. وربما يكون ذلك بسبب الفترة التي تم فيها جمع الطحلب والتي تكزن مناسبة لأبيض الأحماض الدهنية غير المشبعة التي تتكون منها هذه الهيدروكربونات ويؤيد هذا الافتراض وجود كميات قليلة من C-11 هيدروكربونات كبريتية.

**Keywords:** Brown alga; Cystoseiraceae; *Hormophysa cuneiformis*; Hydrodistillation; Supercritical CO<sub>2</sub> extraction; Volatiles compounds

## ABSTRACT

The present work deals with the study on volatile compounds from the brown alga *Hormophysa cuneiformis* collected from the Northwest coast of Qatar on May 2002. Two extraction methods have been used: conventional hydrodistillation of the crude diethyl ether extract of alga and supercritical CO<sub>2</sub> extraction of the same crude extract with two different stationary phases for the trapping.

The obtained oils were examined by GC/MS coupling experiments and the chemical compositions obtained from both methods were compared. Their major constituents were: squalene described for the first time from a macroalga, fatty acids and corresponding esters. It must be pointed out absence of volatile C-11 hydrocarbons which are commonly presents in brown algae might be due to the collecting period of the alga (May) which probably favors the metabolism of unsaturated fatty acids precursors of these C-11 hydrocarbons. The presence, in a small amount, of a C-11 sulphur compound with a near related biogenesis of the volatile C-11 hydrocarbons is in agreement with this hypothesis.

## Introduction

Supercritical fluid extraction (SFE) is an advanced separation technique based on the enhanced solvating power of substances such as carbon dioxide above their critical point. Its useful method for sample extraction is due to the combination of gas-like mass transfer properties and liquid-like solvating characteristics with diffusion coefficients greater than that of a liquid [1]. For conventional extraction methods such as hydrodistillation (steam distillation) and solvent extraction, there are few adjustable parameters to control the selectivity of the extraction processes. Hence, developing alternative extraction techniques with better selectivity and efficiency are highly desirable.

The use of supercritical CO<sub>2</sub> (SC-CO<sub>2</sub>) extraction for essential oils has become an important application in the supercritical fluid area [2]. The modification produced as a consequence of the distillation process on thermolabile compounds present in the essential oils could be considered as a problem in the production of natural fragrances. By comparison, the SC-CO<sub>2</sub> extraction represents an alternative to solve this problem by improving the organoleptic properties of the end product.

Conventional fragrance is performed in two steps. The first step consists of solvent extraction, usually by hexane or diethyl ether which gives a crude extract or an intermediate product called «concrete». It is mainly composed of fragrance-related compounds, but contains large quantities of hydrocarbons, fatty acids, fatty acids methyl esters, diterpenoids and triterpenoids, pigments... etc. In the second step, the concrete (or the crude extract) is post-processed by steam distillation or solubilization in a large excess of alcohol to obtain a volatile oil containing the fragrance.

Reverchon developed a process to fractionate jasmine [3] and rose [4] concretes by SC-CO<sub>2</sub>. The process is based on increasing of supercritical solvent density and by using multistage separation technique.

In this study, we applied the supercritical carbon dioxide fractionation to prepare volatile fraction from *Hormophysa cuneiformis* concrete. This was later obtained by solvent extraction using diethyl ether. A volatile fraction has been prepared by classical

hydrodistillation. Both volatile fractions were analyzed by coupling gas chromatography/mass spectrometry. This analysis allowed the comparison of their chemical composition.

## Experimental

The alga was collected off the North-West coast of Qatar on May 2002 [5], along the rocky shore near Al-Zubarah Castle. This specimen belongs to: class Phaeophyceae, order Fucales, family Cystoseiraceae, genus *Hormophysa* Kützing and *cuneiformis* (C. Agardh) Kützing species. *Hormophysa cuneiformis* is a perennial species with maximum development of the annual branches in summer which are largely covered by epiphytes in fall. Most of the specimens lose their annual axes in winter [6]. The collected specimen was then air-dried under shade without any other treatment, and then was powdered. The latter was extracted with diethyl ether as solvent in a Soxhlet yielding crude extract (1.5 %) after freeing it from solvent.

Both hydrodistillation and Supercritical carbon dioxide extraction were used. Hydrodistillation: The experiment was carried on a Dean Stark modified system [7]. The crude extract was crossed by a stream of steam. A heteroazeotropic oil-water was then formed, the following condensation allowed to obtain two phases. Liquid-liquid extraction with diethyl ether was used to extract oils (yield 4.7%). The supercritical carbon dioxide extraction: The experiment was carried on HP apparatus 7680 A SFE Module equipped with extractor vessel of 7 ml volume. A sample of 300 mg of *Hormophysa* concrete was heated to 40° C, mixed with about 5 g of diameter glass beads and then filled into the extractor. The aims of the mixing were performed to obtain a thin layer of concrete around the glass beads, and also offer the maximum of contact surface between the concrete and the supercritical solvent. The solution at the exit of the extraction vessel flowed into a trap collector. This later is a cylindrical tube charged with a stationary phase. The volatile oils were collected on two different stationary trap phases: Tenax GC and Florisil. The carbon dioxide used as solvent had a purity of 99.999 %. The experimental conditions were: Extraction Pressure: 97 bars; Tenax trap (yield 13.87%); Density (CO<sub>2</sub>): 0.6 g/ml; Florisil trap (yield 13.25%); Flow (CO<sub>2</sub>): 1 ml/min; Equilibrium time: 5 min; Experiment temperature: 40° C; Trap temperature: 0° C; Extraction time: 30 min.

The GC-MS apparatus was gas chromatograph HP-6890 coupled to mass spectrometer HP-5972, equipped with capillary column Chromapack CP-Sil 8CB(CP5860) fused silica low bleed/MS, 30m x 250 µm, phase thickness 0.25 µm, split 1/90. The GC apparatus was interfaced with a mass detector (70 eV). The GC conditions were: oven temperature of 90° C for 2 min to 250° C at 3 °/min and a fixed isothermal hold for 10 min. The area percentage of compounds was calculated from the gas chromatographic traces (automatic integrator). The eluted compounds were identified by comparing the mass spectra using the mass spectral databases [8].

## Results And Discussion

The analysis of the three volatile fractions (A: volatile fraction obtained by hydrodistillation, B: volatile fraction obtained by SFE and trapped on Tenax GC, C: volatile fraction obtained by SFE and trapped on Florisil) was performed by GC/MS coupling experiments and has allowed the characterization of 33 compounds with percentage upper than 0.05 % and 2 others lower than 0.05 % (Table 1)

**Table 1. Percentage composition of the fractions A, B, and C.**

#	Rt (min)	Compound	Fraction*		
			A	B	C
1	2.68	ethylbenzene	tr.	tr.	tr.
2	2.73	<i>p</i> -xylene	0.87	0.12	0.15
3	3.90	hexanoic acid	3.78	1.27	0.35
4	3.95	2-octene	0.23	tr.	tr.
5	4.02	6-methyl-5-hepten-2-one	tr.	tr.	0.10
6	5.80	heptanoic acid	2.29	0.27	tr.
7	8.36	octanoic acid	0.35	tr.	tr.
8	11.64	nonanoic acid	0.30	tr.	tr.
9	12.40	2,4-decadienal	0.39	tr.	tr.
10	14.47	5-pentyl-2(5H)-furanone	0.21	tr.	tr.
11	15.45	decanoic acid	0.25	0.10	tr.
12	18.55	6,10-dimethyl-5,9-undecadien-2-one	1.80	0.35	0.35
13	21.76	2(4H)-benzofuranone-5,6,7,7 $\alpha$ -tetrahydro	0.22	tr.	tr.
14	23.04	dodecanoic acid	0.59	0.13	tr.
15	24.29	hexadecane	0.75	0.11	tr.
16	28.83	tetradecanoic acid methyl ester	0.17	tr.	tr.
17	30.74	tetradecanoic acid	20.80	4.59	3.15
18	31.45	octadecane	0.58	tr.	tr.
19	32.57	5,9,13-trimethyl-4,8,12-tetradecatrienal	0.38	0.10	tr.
20	32.85	6,10,14-trimethylpentadecan-2-one	0.38	0.10	0.19
21	33.88	pentadecanoic acid	0.88	0.24	0.14
22	34.35	3-hexyl-4,5-dithiacloheptanone	tr.	0.13	tr.
23	34.93	9-hexadecanoic acid methyl ester	0.81	0.10	0.14
24	35.66	14-methylpentadecanoic acid methyl ester	0.81	0.10	tr.
25	36.39	9-hexadecenoic acid	8.10	4.29	3.35
26	37.34	hexadecanoic acid	18.70	9.07	6.15
27	39.35	3,13-octadecadiene	0.58	0.39	tr.
28	40.86	9,12-octadecadienoic acid methyl ester	0.44	0.63	0.35
29	42.58	9,12-octadecadienoic acid	14.92	22.02	16.28
30	42.72	9-octadecenoic acid	5.75	5.02	3.94
31	43.18	octadecanoic acid	1.03	0.60	tr.
32	45.59	5,8,11,14-eicosatetraenoic acid methyl ester	0.19	0.85	0.77
33	47.12	5,8,11,14-eicosatetraenoic acid ethyl ester	5.98	16.40	16.10
34	47.43	8,11,14-eicosatrienoic acid	2.53	6.61	6.50
35	60.81	squalene	3.09	21.59	35.24
		unidentified	1.85	4.82	6.75

\* Fraction A: volatile fraction obtained by hydrodistillation; Fraction B: volatile fraction obtained by SC-CO<sub>2</sub> with a Tenax GC trap; Fraction C: volatile fraction obtained by SC-CO<sub>2</sub> with a Florisil trap. tr. (% < 0.05)

. The majority of compounds were fatty acids [9] and derivatives which are probably the precursors of volatile C-11 hydrocarbons and other sulfur derivatives [10, 11] which are the main compounds in volatile oils of brown algae. The lack of C-11 hydrocarbons in the

volatile oils was probably a consequence of the collecting period (May, 2002). This hypothesis was confirmed by the presence of the sulfur compound (22) as trace which might be biosynthesized from fatty acids by the same way as C-11 hydrocarbons (figure 1).

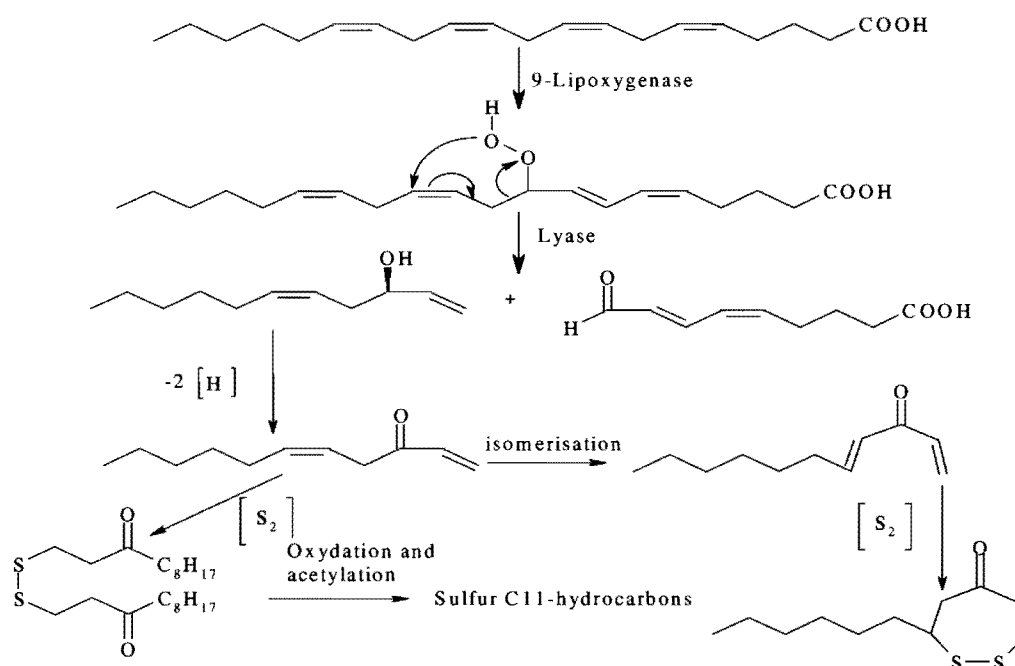


Figure 1: biosynthesis of sulphur hydrocarbons

The large amount of fatty acids in supercritical carbon dioxide oils could be due to the high solubility of these compounds at the experimental pressure and temperature [12, 13]. It must be also pointed out the presence of squalene (35) in the volatile compounds composition of *Hormophysa cuneiformis* which reached a particularly high level for the SFE extraction method (percentage upper than 35 % for the fraction C). From our knowledge, that is the first time this compound is found in a macroalga species. In microalga, it has been only described from a Cyanophyta (*Spirulina* species) [14].

Concerning the comparison between the three methods used to obtain the volatile oils from the crude extract, table 1 shows that methods B and C give approximately the same chemical composition, except the yield of squalene (35) which is clearly higher in method C. On the other hand, method A clearly differs from those of B and C with: (i) higher amounts of C-6 to C-16 carboxylic acids, (ii) a lower amount of C-20 unsaturated fatty acids and ester derivatives, (iii) a lower amount of squalene. In the last case, the higher amount of squalene in B and C oils could be due, like for fatty acids, to its high solubility in SC-CO<sub>2</sub> at the experimental conditions.

The conclusion of this study lead us to identify the volatile compounds of the brown alga *Hormophysa cuneiformis* showing the presence of squalene, described for the first time from a macroalga, and many fatty acids precursors of C-11 hydrocarbons. On the other hand, it shows the interest of the SC-CO<sub>2</sub> extraction method, not often used to obtain the volatile oils from algae, by comparison to hydrodistillation. In particular, the highly soluble compounds in SC-CO<sub>2</sub>, like C-20 unsaturated fatty acids or squalene, are extracted in a large

amount. We think that this method could be optimized to the production, in a commercial scale, of EPA and/or squalene (especially with the use of a florisisl trap in the last case) from marine sources.

### Acknowledgements

The authors thank Mr. Olivier Bottzeck (Laboratoire MFS/PCM3, Université du Sud, F83957 La Garde cedex, France) for technical assistance in supercritical CO<sub>2</sub> extraction.

### REFERENCES

- [1]. McHugh, M.A. and Krukonis, V.J., 1986. *Supercritical fluid extraction, Principles and Practice*, Butterworth, Stonham, MA, 2<sup>nd</sup> Ed., 512 p.
- [2] Reverchon, E., 1997. Supercritical fluid extraction and fractionation of essential oils and related products. *Journal of Supercritical Fluids* **10**: 1.
- [3] Reverchon, E. and Della Porta, G., 1995. Supercritical CO<sub>2</sub> fractionation of Jasmine. *The Journal of Supercritical Fluids* **8**: 60.
- [4] Reverchon, E. and Della Porta, G., 1999. Rose concrete fractionation by supercritical CO<sub>2</sub>. *The Journal of Supercritical Fluids* **9**: 199.
- [5]. Rizk, A.M., Al Easa, H.S. and Kornprobst, J.M., 1999. *The phytochemistry of the Macro and Blue-Green Algae of the Arabian Gulf*, Faculty of Science, University of Qatar, 745 pages.
- [6]. De Clerck, O. and Coppejans, E., 1996. Marine algae of the Jubail marine Wildlife Sanctuary, Saudi Arabia, in: *A Marine Wildlife sanctuary for the Arabian Gulf. Environmental Research and Conservation following the 1991 Gulf War Oil Spill*, F. Krupp, A.H. Abuzinada and I.A. Nader, Eds., NCWCD, Riyadh and Senckenberg Research Institute, Frankfurt a.M., pp. 199-289
- [7]. El Hattab, M., Culioli, G., Ortalo-Magné, A., Piovetti, L. and Chitour, S.E., 2002. Isolation of the volatile compounds from the brown alga *Dictyopteris membranacea* by focused microwave-assisted hydrodistillation. *Journal of Essential Oils Research* **14**:422.
- [8]. McHugh, M.A. and Krukonis, V.J., 1986. *Supercritical fluid extraction, Principles and Practice*, Butterworth, Stonham, MA, 2<sup>nd</sup> Ed., 512 p.
- [9]. Heiba, H.I., Al-Easa, H.S and Rizk, A. M., 1997. Fatty acid composition of twelve algae from thecoastal zones of Qatar. *Plant Foods for Human Nutrition* **51**: 27..
- [10] Pohnert, G. and Boland, W., 2002. The oxylipin chemistry of attraction and defense in brown algae and diatoms. *Natural Products Report* **19**: 108..
- [11] Moore, B.S., 1999. Biosynthesis of marine natural products: microorganisms and macroalgae. *Natural Products Report* **16**: 653.
- [12] Iwai, Y., Fakuda, T., Koga, Y. and Arai, Y., 1991. Solubilities of myristic acid, palmitic acid, and cetyl alcohol in supercritical carbon dioxide at 35° C. *J. Chem. Eng. Data* **36**: 430.
- [13]. Madras, G., Kulkarni, C. and Modak, J., 2003. Modeling the solubilities of fatty acids in supercritical carbon dioxide. *Fluid phase equilibria* **209**: 207.
- [14]. Forin, M.C., Maume, B. and Baron, C., 1975. Presence of squalene and sterols in a Cyanophyta *Spirulina*. *Comptes Rendus de l'Académie des Sciences, Série D* **281**: 195.