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Volatile compound extraction using Solid Phase Micro Extraction coupled with Gas Chromatography Mass Spectrometry (SPME-GCMS) in local seaweeds of *Kappaphycus alvarezii*, *Caulerpa lentillifera* and *Sargassum polycystem*

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Abstract: A pioneer study with a new extraction method was applied to the volatile compounds of three local seaweeds from Borneo, *Caulerpa lentillifera*, *Kappaphycus alvarezii* and *Sagassum polycystum* using HS-SPME method. Dynamic headspace was optimized according to an experimental design, with appropriate temperature of 50^0C and 60^0C and extraction time as 20 min, 30 min, 40 min and 50 min by using PDMS and PDMS-DVB. Significantly, PDMS fibre extracted more compounds than PDMS-DVB. Thus PDMS is recommended as a better fibre comparatively to PDMS-DVB in volatile compounds extraction using SPME for these particular seaweeds. Qualitative study portray a total of 223 volatile constituents successfully extracted and identified with 82, 91 and 50 volatiles from each species of *K. alvarezii*, *C. lentillifera* and *S. polycystum* respectively. The volatile compounds are comprised of nine chemical compounds of hydrocarbons, aldehydes, ketones, esters, alcohols, halogenated compounds, acid compounds, aromatic compounds and some miscellaneous compounds. Comparison on the major volatile constituents among the seaweeds shows a similar percentage of volatile compounds in all seaweeds among the nine groups. Within them, hydrocarbon compounds were the most characteristic of all three algae, and more particularly, all three seaweeds mostly share the common constituents in their structures.

Keywords: Volatile compounds, SPME-GCMS, *Kappaphycus alvarezii*, *Caulerpa lentillifera*, *Sargassum polycystem*

Introduction

Malaysia has an extensive coastline with numerous islands form clusters along the coastlines of Peninsular Malaysia, and East Malaysia comprising of Sabah and Sarawak. All these provide niches for the variety of seaweed species found in Malaysian waters (Ahmad, 1995; Phang, 2006). The last two decades have seen an increase in seaweeds as a potential economic resource in the Asia-Pacific region, including Malaysia. Presently 375 taxa of seaweed are recorded in Malaysia (Phang, 2006). But until now there has been very limited research and investigation from the organic biochemistry aspect of these seaweeds. So far many researchers have been carried out in terms of its nutrient content, antioxidant properties, cultivation and processing, physiochemical properties and many more. Yet there is dearth of studies on the volatile constituents of these locally available seaweeds (Gardon and Sonia, 2000). Many scientifically interesting as well as commercially important species have been identified. Ecological information is scarce. Biomass assessments of natural seaweed areas, productivity determination and phenological studies of important species are also less, comparatively to other regions of world (Ahmad, 1995; Phang, 2006). So this present study is designed to contribute to the data on major group of volatile compounds of local seaweeds.

Caulerpa lentillifera is a species of seaweed which is commonly known as green macro alga under the family of *Caulerpaceae*. This species is also consumed under the names of sea grape or green caviar or umi-budo in other regions of the world. While *Eucheuma cottonii* or *Kappaphycus alvarezii* is a type of red algae from the class of Rhodophyceae (Ahmad, 1995; Phang, 2006). This seaweed accounts for the largest production worldwide. Today, the most important commercial seaweed for carrageenan production is the cultivated species *E. cottonii* (Chapman and Chapman, 1980).

In Sabah, cultivation of *E. cottonii* for export had been carried out for almost three decades, where it is normally exported as sun-dried seaweed or processed into semi-refined carrageenan (Ahmad, 1995). Meanwhile, *Sagassum polycystum* also known as brown algae or seaweed is from the genus of *Sargassum* and from the class of Phaeophyta. They grow and reproduce on substrates such as dead coral and masive material on the reef flats and are found

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in the coastal waters all of the years (Balaji *et al*., 2006). Even though it does not play an important role commercially, yet it exhibit major role in ecological balance.

For HS-SPME, it has gained increasing attention over traditional techniques like steam distillation and liquid solvent extraction. Moreover, commonly used methods for the extraction and preconcentration of analytes in solid samples are often too time consuming, involve multi-step procedures prone to loss of analytes, and use of toxic organic solvents (Antoniou *et al*., 2005). Modern trends in analytical chemistry are developed towards the simplification of sample's preparation, as well as minimization of organic solvent used. This has turned in recognition that classical methods can be replaced with procedures that are faster, cheaper, and equal or better than classical methods (Moreno *et al*., 2007). Thus a HS-SPME extraction method is used to analyze the volatile constituents from the seaweeds. The volatile compounds of *C. lentillifer*, *K. alvarezii* and *S. polycystum* are expected to be extracted and identified by above mentioned way, before the components may classifieds according to their chemical groups.

The study involves the extraction of volatile compounds from three different species of seaweeds namely *Caulerpa lentillifera*, *Kappaphycus alvarezii* and *Sagassum polycystum* which are also known as green, red and brown seaweed respectively. The extraction technique involves the usage of Head Space Solid Phase Micro Extraction, which is a solvent free method, with two different types of fibres being used: a 100 µm PDMS fibre and 65 µm PDMS-DVB to trap the volatile constituents. This will lead to the analysis of constituents by gas chromatography which is fused with mass spectrometer as a detector. Eventually a qualitative study on the major volatile groups from the local seaweed species would be obtained together with the identification on the better fibre for extraction. Besides, a clear comparison and distinction in volatile compounds among the three different species of seaweed would be able to be made.

Materials and Methods

Sample collection and preparation

Caulerpa Lentillifera (Green seaweed) and *Kappaphycus alvarezii* (Red seaweed) were purchased from local wet market in Kota Kinabalu, Sabah. While *Sagassum polycystum* (brown seaweed) was randomly harvested from the costal region of Kota Kinabalu. The samples were washed with tap water to remove salt, epiphytes and debris (Antoniou *et al*., 2005; Sivansankari *et al*., 2006). Finally the samples were rinsed in distilled water. The raw seaweeds were placed in a freezer at -20°C immediately after cleaning then sun dried for five days. Prior to analysis, seaweeds were grinded using a conventional blender till it turns to fine powder form. Green, red and brown seaweeds in powder form were weighted 5 g, 0.5 g and 2 g (50%) respectively and sealed in a 20ml Agilent Headspace glass vial (flat bottom, 100PK, made in US) using a PTFE/silicon septum 200 mm. The variations in weight occur due to the initial water content of the seaweeds (Hattab *et al*., 2006; Matanjun *et al*., 2007; Krishnaiah *et al*., 2008).

Solid Phase Microextraction condition

Solid Phase Microextraction (Supelco) method is used with 100 µm PDMS fibre and 65 µm PDMS-DVB silica coated fibre . Both fibres were conditioned prior to use, according to the manufacturer's instructions (Antoniou *et al*., 2005; Ferreira *et al*., 2008). For each extraction, the sealed samples in glass vial were incubated in water bath at 50^{0}C for 1 hour. Needle of syringe of SPME inserted and 1ml fibre was exposed in HS inside vial for 20 min. (Antoniou *et al*., 2005). Desorption allowed to occur by exposing the fibre for optimized time before injecting it to the GC port at 250^{0}C (Antoniou *et al*., 2005).

Optimization

Time varying from 20 min, 30 min, 40 min and 50 min for extraction of volatile compounds to HS-SPME using both PDMS and PDMS-DVB fibres was conducted. For temperature optimization, the SPME extraction was conducted with the water bath temperature of 50^{0}C and 60^{0}C. The optimized time and temperature combination is selected base on the high frequent yield on the number of peak with maintained consistency with highest peak area. Thermal desorption performed directly into the GC injection port at 250^{0}C for 3 min and flushed into the column (Zambonin *et al*., 2002; Hattab *et al*., 2006; Ferreira *et al*., 2008).

Qualitative volatile compound in seaweed is determined by GC which is attached to mass spectrometry. Mass spectrometry is widely used as a GC detector (G3440A Agilent). Analyses were performed in the electron ionisation (EI) mode at 70 eV, the mass range was m/z 40–550, transfer line and ion source temperatures of 280^{0}C and 250^{0}C were used, respectively. Samples were injected (1 µL) with a splitless condition and the injector temperature was set to 250^{0}C. Fused DB five Column with 60 m × 0.25 mm × 0.25 µm film thickness used as a capillary column. A continuous flow rate of 1 mL/min of

chromatographic grade helium was used. The GC oven was initially at 90^{0}C and was held for 3 min after injection, followed by temperature ramping at 3^{0}C/ min up to a final temperature of 220^{0}C with a hold time of 3 min. The total run time was approximately 46 min. Detector temperature was set to 230^{0}C in gas chromatography. Sample to be injected in splitless condition.

Volatile compound from the sample was determined with the refferal of molecular weight table of compounds with mass spectra literature reviews or libraries using MSD Chemstation software. The optimized samples for time and temperature of SPME extraction were chosen from the abundance of peak area obtained from the chromatography. The obtained volatile compounds were identified into their major groups using The Merck Index software.

Results and Discussion

Water content of sun dried seaweeds

Moisture content of all type of seaweeds was decreasing with days exposed to sun. *S. polycystum* exhibits a higher moisture content level (20.3%), comparative to *Kappaphycus alvarezii* and *Caulerpa lentillifera* (19.6% and 14.9%). This is due to the physical structure of the brown seaweeds with larger filamentous thallus and presence of kelp which is rich in moisture content retention. The removal of moisture content is very important in order to get good quality peaks in GC-MS analysis. High moisture content moisture may affect the extractions, thus restricting the yield of volatile compounds (Flament and Ohloff, 1984). Studies by Qiming *et al*. (2006), show that the yields of volatile compounds are better in dried seaweed rather than fresh seaweeds.

Optimization of samples for extraction

SPME requires a previous optimization of the extraction parameters that can affect extraction efficiency, in order to obtain high recoveries of volatiles. For analysis of volatile compounds, incubation temperature should not exceed 80^{o}C, over which the forming of artifacts may occur. Volatile compounds show an increased response upon heating to 40-45^{o}C, above these temperatures, the response goes down due to migration of analytes out of the fibre.

For analyzing very volatile compounds, the extraction time and temperature should be decreased. Previous studies by Qiming *et al*. (2006) and Hattab *et al*. (2006) showed that volatile compounds overload in the extraction above 60^{0}C and 70^{0}C for most seaweed types. In this study, the effects of heating temperature at 50^{0}C and 60^{0}C fluctuates the chromatographic response as well. It was seen, that aldehydes, hydrocarbons and higher molecular compounds increased with higher temperatures while smaller molecules, like isobutyraldehyde, 2-propanone and 2,3-butanedione would not depend on the temperature. The amount of analytes extracted depends also on the extraction time. Increasing temperature and time would not be always beneficial due to the high boiling compounds overloading the fibre and replacing the low boiling compounds as the active sites of the fibre are being filled up.

Concerning to *K. alvarezii*, capacity problems did not occur and the peaks have highest abundance with 60^{o}C incubation and 50 min extraction time. Therefore, temperature 60^{o}C and extraction time 50 minute was chosen for both PDMS and PDMS-DVB fibres. But for *S. polycystum* using PDMS only, the peaks exhibit maximum abundance at 60^{o}C with 50 min extraction time. While the rest, including *C. lentillifera* for both fibres and *S. polycystum* for PDMS-DVB fibre shows a better peak abundance with 50^{o}C. From the results it was seen that generally lower boiling compounds, like isobutyraldehyde, 2-propanone, 2,3-butanedione and others were not affected by extraction time. For tetratriacontane and other higher boiling compounds, it was seen that 20 minutes extraction gave better chromatographic response. *C. lentillifera* for both fibres and *S. polycystum* for PDMS-DVB fibre shows higher abundance peak areas comparatively to other extraction time periods (Kaseleht and Leitner, 2008).

Qualitative analysis

A total of 223 volatile compounds were identified in this study from all three types of seaweeds. This includes 82, 91 and 50 compounds from *K. alvarezii*, *C. lentillifera* and *S. Polystum* respectively. It comprises of nine major groups of compounds from hydrocarbon, esters, acid, aldehydes, ketones, alcohols, aromatic, halogenated and other miscellaneous compounds. So far there is no published studies have been done on these three seaweeds from the state of Sabah, Malaysia. But many teams have worked on volatile compounds of other seaweed species in different regions of the world. Figure 1 (a), (b) and (c) shows the chemical groups belongs to the volatile compounds obtained from all three types of seaweed.

Among the seaweed species, the green seaweed *C. lentillifera* seems to have a highest mean value, 52 for total number of volatile compounds obtained. In terms of overall highest volatile compounds, the brown seaweed *S. Polystum* tops the list, yet due to many overlapping compounds extracted by both

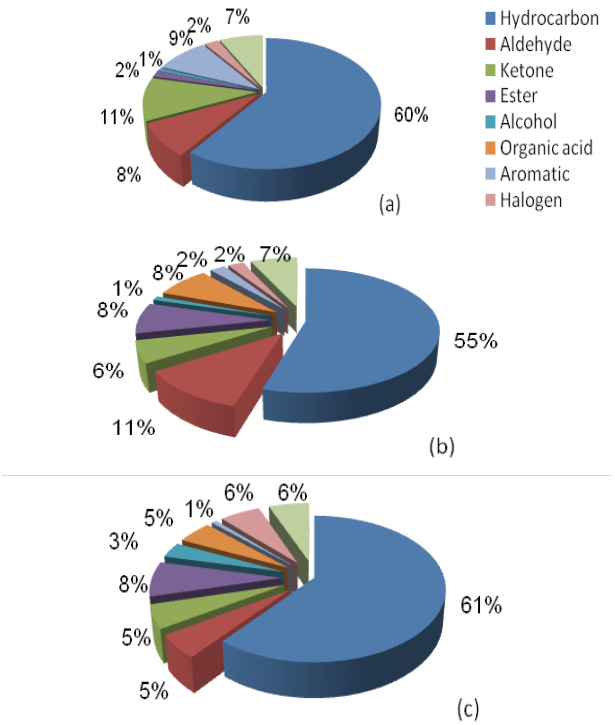

Figure 1. Volatile compound groups in (a) *K. alvarezii*, (b) *C. lentillifera* and (c) *S. Polystum*

fibres, the mean value for the volatile compounds was 50. Even though a decreasing patent can be seen among the seaweeds in the total volatile compounds detected, yet there is no significant difference among the type of seaweeds towards the volatile compounds obtained ($p>0.05$). This is due to the list of volatile compounds obtained, most of compounds overlap for all three species, which means the volatile constituents between the three species are similar. Thus, results indicate that volatile compounds among seaweeds do not vary on species basis for this study. But there is a distinctive significant different attained for the fibre used towards the volatile compounds ($p\leq0.05$). Whereby the total number of volatile compounds attained according to different fibres vary with large visible difference.

In 1990, Sugisawa and coworkers worked on the green edible algae *Ulva pertusa*. The volatiles were extracted by simultaneous distillation and extraction (SDE), and 66 compounds were found, of which many are in agreement with findings in these study for *C. lentillifera*, also edible green seaweed. 1-pentadecene, hexanal, azulene, heptanal, nonanal, tetradecane and many hydrocarbon compounds are those same compounds present in both studies.

In the same way, Takahashi *et al*. (2002), have studied the compounds of dried Kombu (*Laminaria* spp.) by Simultaneous distillation extraction (SDE) and the compounds they found; those of identical to *Sagassum Polystum* are dodecane 1-iodo, heptanal, 1-octen-3-ol and hydrocarbons such as heptadecane and others. But still many volatile compounds seem to be differing due to the two different extraction method utilized. This difference observed between the two extraction methods could be explained by the fact that dynamic headspace extracts low boiling- point components, whereas SDE extracts medium- and high-boiling-point components. Using this method, low-boiling point compounds are lost or modified during heating. Following tables stipulate the volatile compounds obtained according to their chemical groups and relevant species with the usage of both fibres.

Hydrocarbons

The volatile compounds extracted and identified in this study, shows hydrocarbon as the major group content with 53% for *K. alvarezii*, 60% and 62% for *C. lentillifera* and *S. Polystum* respectively. Most of the literature review on volatile extraction from seaweeds explains that the high percentage of hydrocarbon in seaweed is very common and it plays a vital role in sexual reproduction of the species. Flament and Ohloff in 1984 and many other studies also have shown that the brown species of seaweeds contains the highest amount of hydrocarbon. This can be seen through in this study as well. Whereby some of the hydrocarbons present in these algae are chemical messengers for male gametes, they are mainly highly unsaturated C_{11} aliphatic or cyclic hydrocarbons with saturated side chains. For example, undecane, 4,7-dimethyl and tetrasiloxane,3,5-diethoxy-1,1,1,7,7,7-hexamethyl-3,5-bis(trimethylsiloxy)-from *C. lentillifera* and heptadecane, 2,6,10,15-tetramethyl-from *S. Polystum* which acts as a sex hormone in the algae. Compound like trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]- for *Kappaphycus alvarezii* has the attributes to work as sex hormones to lure the gametes (Rzama *et al*., 1994; Pape *et al*., 2004).

Algal carotenoids are usually yellow-red, isoprenoid, polyene pigments where they serve important functions such as protection against photodynamic damage and auxiliary light absorption for photosynthesis and phototaxis by these seaweeds. In 1984, Flament and Ohloff reported that the thermal degradation of β,β-carotene from seaweeds produces polyenes and aromatic hydrocarbons such as p-xylene, naphthalene and heptadecane which are prominently found in this study too in all the species.

Aldehydes

Aldehydes are the second most abundant compound these three seaweeds with the content ranging from 5% to 11% with the highest percentage in red seaweed, *K. alvarezii.* Among these aldehyde

Table 1. Volatile compounds belong to hydrocarbon group in all seaweeds extracted by PDMS and PDMS-DVB fibres

RT	Volatile compounds from *K. alvarezii*	Peak area (%)
3.012	Cyclopentane, 1-ethyl-2-methyl-	1.87
3.527	D-Limonene	0.74
3.653	1,4-Hexadiene, 3-ethyl-	0.78
6.159	Cyclopentasiloxane, decamethyl-	3.92
6.966	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3bis[(trimethylsilyl)oxy]-	0.41
9.721	Dodecane,2,6,11-trimethyl-	0.95
11.215	Decane, 3,7-dimethyl	0.92
11.326	Cyclohexasiloxane, dodecamethyl	5.72
12.130	Heptadecane, 2,6,10,15-tetramethyl Decane, 2,3,5,8-tetramethyl-	0.21
15.660	6,10-dimethyl-(E)-	0.68
15.975	Heptadecane, 2,6,10,14-tetramethyl Tridecane, 6-propyl-Tetracosane	0.42
17.219	Heneicosane Dodecane, 2,6,11-trimethyl- Hexadecane	2.13
17.223	Dodecane, 4,6-dimethyl-	2.67
17.366	Eicosane	2.20
17.374	Pentadecane	1.91
17.528	Cycloheptasiloxane, tetradecamethyl- Pentasiloxane, dodecamethyl-	0.99
17.749	Undecane, 4,7,-dimethyl-	0.57
18.859	Pentadecane	2.44
19.205	Decane, 2,3,7-trimethyl-	0.68
19.540	Dodecane	2.44
20.381	Docosane	0.31
23.765	5-Octadecene	1.20
23.769	8-Heptadecene 3-Heptadecene, (Z)- 9-Eicosene, (E)-	2.02
24.072	2-Tetradecene, (E)- 1-Heptadecene	0.31
24.758	Heptadecane	60.67
24.964	Tridecane, 5-propyl-	2.02
25.222	Heptacosane Octacosane	0.19
26.392	Tridecane, 3-methyl- Hexadecane, 2,6,11,15-tetramethyl- Nonane, 5-butyl-	0.56
26.395	Tridecane	1.44
26.704	Pentadecane,8-hexyl-	0.27
27.968	Octadecane	0.20
32.037	Tetratriacontane	0.60
	Volatile compounds from C. lentillifera	
2.361	p-Xylene	0.65
3.654	1,4-Hexadiene, 3-ethyl-	0.58
6.155	Cyclopentasiloxane, decamethyl-	0.97
7.158	Azulene	0.35
7.746	Undecane, 2,6-dimethyl- Undecane, 3,6-dimethyl- Undecane, 4,6-dimethyl-	0.21
8.096	1-Cyclohexene-1-carboxaldehyde, 6,6-trimethyl-	0.32
8.100	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	0.86
9.725	Dodecane, 2,6,22-trimethyl	1.23
9.727	Dodecane, 2,6,11-trimethyl- Hexadecane	0.95
9.989	Heptadecane, 8-methyl- Decane, 2,3,7-trimethyl- Pentadecane, 2,6,10-trimethyl-	0.27
10.339	Tridecane	0.34
10.172	Eicosane	0.34
12.926	Dodecane, 2,6,10-trimethyl- Dodecane, 2,7,10-trimethyl-	0.34
15.974	Heptacosane Heptadecane, 2,6,10,15-tetramethyl	0.32
15.975	Tridecane, 6-propyl Hexadecane, 2,6,10,15-tetramethyl- Decane, 5-propyl-	0.38
16.639	2-Methyl-Z-4-tetracene 3-Tridecene	0.22
17.055	1-Tridecene, Cyclopentadecane 5-Octadecane	0.25
17.057	1-Pentadecene 6-Tridecene	0.31
17.217	Heneicosane	0.72
17.400	Pentadecane	9.55
17.526	Cycloheptasiloxane, tetradecamethyl- 3-isopropxy-1,1,1,7,7,7-hexamathyl-3-5,5,5-tris(trimethylsiloxy) tetrasiloxane Tetrasiloxane, 3,5-diethoxy-1,1,1,7,7,7-hexamethyl -3,5-bis(trimethylsiloxy)-	0.41
18.842	Pentacosane	0.75
19.203	Decane, 3,8-dimethyl-	0.29
19.534	Tetratriacontane Undecane, 4,7-dimethyl-	0.22
23.471	Cyclodecene Z-1,6-Undecadiene	3.27
23.883	8-Heptadecene 3-Heptadecene 9-Eicosene	37.74
24.650	Tetradecane	24.59
24.734	Heptadecane	19.07
24.907	10-Methylnonadecane	0.26
27.139	9-Octacene 5-Octadecene	0.29
27.143	9-Octadecene	0.23
30.182	Cyclododecene	0.29
30.183	Z-1,6-Tridecadiene	0.28
30.428	9-Nonadecene 1-Nonadecene	1.42
30.543	1-Nonadecene	0.55
31.269	Nonadecane	0.43
	Volatile compounds from S. Polystum	
6.15	Cyclopentasiloxane,decamethyl-	3.12
7.747	Undecane,2,6-dimethyl-	0.41
8.628	Dodecane,4,6-dimethyl-	0.72
8.845	Octane,2,3,6,7-tetramethyl- Decane,4methyl-	0.79
9.721	Dodecane,2,6,11-trimethyl- Hexadecane	4.14
11.212	Nonane,5-(2-methylpropyl)-	1.40
11.326	Cyclopentasiloxane, dodecamethyl-	0.99
11.758	Decane,3,7-dimethyl- Octane,3-ethyl- Hexane,3,3-dimethyl-	0.38
13.737	Tertadecane	0.58
17.05	5-Octadecene Cyclopentadecane	1.02
17.371	Eicosane	11.91
18.83	Hexacosane Nonane Heptacosane	1.1
19.196	Triacontane	0.4
23.745	2-Methyl-E-7-hexadecene	1.01
23.760	8-Heptadecene 3-Heptadecene 1-Pentadecene	2.90
24.058	1-Tridecene	0.38
24.262	1-Heptadecene 3-Eicosene 2-Tetradecene	1.81
24.899	Nonadecane, 2-methyl-	1.51
25.591	3-Tetradecene, (E)- 1-Undecene, 8-methyl- Cycloundecane, 1,1,2-trimethyl	0.53
27.966	Octadecane	0.57
28.275	Hexadecane, 2,6,10,14-tetramethyl- Heptadecane, 2,6,10,15-tetramethyl- Tetratetracontane	0.41
35.527	Nonadiene	0.75

Table 2. Volatile compounds belong to aldehyde group in all seaweeds extracted by PDMS and PDMS-DVB fibres

RT	Volatile compounds from *K. alvarezii*	Peak area (%)
1.959	Hexanal	2.37
2.222	2- Hexenal	0.63
3.012	2-Heptenal	1.87
3.653	2,4-Heptadienal, (E,E)-	0.78
5.180	Nonanal	3.14
6.399	2-Nonenal, (E)-	1.03
7.544	Decanal	0.52
7.801	2,4-Nonadienal, (E,E)-	0.43
10.937	2,4-Decadienal	2.96
	Volatile compounds from *C. lentillifera*	
1.961	Hexanal	0.46
3.654	2,4-Heptadienal, (E,E)-	0.58
5.194	Nonanal	4.94
7.551	Decanal	0.43
13.938	Undecanal, 2-methyl- Hexadecanal, 2-methyl-	0.17
	Volatile compounds from *S. Polystum*	
1.962	Hexanal	1.55
2.522	Heptanal	0.71
7.546	Decanal	0.58
8.096	1-Cyclohexene-1-carboxaldehyde,6,6-trimethyl	1.81
9.166	1-Cyclohexene-1-acetaldehyde,2,6,6-trimethyl	0.49
24.899	Tridecanal	1.51

compounds detected, many could arise from the degradation of polyunsaturated fatty acids (PUFA), either by autoxidation or by the action of enzymes such as lipoxygenases (Takahashi *et al*., 2002). These aldehydes which have very low odour thresholds are also produced during cell lysis by the action of lipoxygenase on linoleic acid, γ-linolenic and arachidonic acids (Flament and Ohloff, 1984). These aldehydes are widespread, as they have already been found in many other sea products, such as green and brown macroalgae lobsters and oysters (Pape *et al*., 2004). Other studies also show that aldehydes produced by lipid peroxidation, demonstrated that hexanal, hexenal, octanal, nonanal which are found mostly in all extraction, could come from linolenic acid. They also could arise from other PUFAs which have been reported as the most likely substrates for enzymatic oxidation, producing mainly aldehydes and alcohols. Other aldehydes origins are well known. It is frequently found in many sea products, such as crayfish and lobster. For instance, 2,4 Heptadienal, (E,E)-, and undecanal, 2-methyl- which are obtained from *C. lentillifera* (Takahashi *et al*., 2002).

Actually, these compounds are generally associated with products that have undergone heating processes. It is obtained from amino acids, and more particularly from leucine, during the Maillard reaction by the Strecker degradation. Nevertheless, in the case of the volatiles of *K. alvarezii*, *C. lentillifera* and *S. Polystum*, this route is not valid, as the product was not heated. According to Pape *et al*., 2004, a type of catabolism which normally occurs in cheese could occur in seaweeds too, with specific microorganisms which are responsible towards the emitting of stipulated compounds above.

Ketones

Besides hydrocarbon and aldehydes in seaweeds, ketones are other important volatiles too. It comes in a very close percentage to aldehydes with 6% for

brown seaweed and 7% for both red and green seaweeds in this study. It was suggested that both ketones compounds present in most of the seaweeds were originated from the degradation of unsaturated fatty acid and caretenoids (Rzama *et al*., 1994, Flament and Ohloff, 1984). An exact compound which is found to be overlapping in these two studies with this study is undecanone. Caretenoid degradation products have been reported as tobacco constituents and have frequently been observed in sea waters. Besides photooxygenation of β-damascol through the intermediate allylic hydroperoxide with subsequent oxidation, reduction, and hydration explains the yield of 5,9-undecadien-2-one, 6,and 10-dimethyl- which is present in all the extractions of volatile compound in this study (Rzama *et al*., 1994). Tridecanone definitely arose from fatty acid oxidation, but its exact origin remains unknown because it can be produced through the oxidation of various fatty acids (Pape *et al*., 2004).

Table 3. Volatile compounds belong to ketone group in all seaweeds extracted by PDMS and PDMS-DVB fibres

RT	Volatile compounds from *K. alvarezii*	Peak area (%)
15.660	5,9-Undecadien-2-one	0.68
15.663	5,9-Undecadien-2-one, 6,10-dimethyl-	0.71
16.879	3-Buten-2-one, 4-(2,6,6-trimethyl-1 -cyclohexen-1-yl),(E)-	1.77
29.464	2-Tridecanone	0.67
29.473	2-Pentadecanone, 6,10,14-trimethyl	1.09
	Volatile compounds from *C. lentillifera*	
8.100	3-Isopropylidene-5-methyl-hex-4-en-2-one	0.86
10.744	2,5-Pyrrolidinedione, 1-[(3,4-dimethylbenzoyl)oxy]-	1.69
13.938	2-Undecanone, 6,10-dimethyl-	0.17
14.802	3-Buten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	3.54
15.649	5,9-Undecadien-2-one, 6,10-dimethyl-	0.28
16.879	3-Buten-2-one, 4-(2,6,6-trimethyl -1-cyclohexen-1-yl)-	1.09
18.434	2(4H)-Benzofuranone 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	0.33
29.466	2-Pentadecanone, 6,10,14-trimethyl-	0.45
	Volatile compounds from *S. Polystum*	
8.096	3-Isopropylidene-5-methyl -hex-4-en-2-one	1.81
15.643	5,9-Undecadien-2-one, 6,10-dimethyl-1-	0.61
16.873	3-Buten-2-one	1.52

Alcohols

Previous studies using red seaweeds exhibits alcohols as the most abundant compounds detected. In this study, *C. lentillifera* has the high abundance in alcohol content (11%). Whereas, the other two seaweeds only has about 2% and 1% for red and brown seaweeds respectively. A study by Rzama *et al*. (1994) reveal that alcohol fractions from green seaweeds such as 1-octen-3-ol which is also found in *Caulerpa lentillifera* in this study was found in the volatile products from a synthetic meat flavour system. 1-Octen-3-ol has previously been detected in brown seaweeds by Takahashi *et al*., in 2002, but its origin was not discussed. Nevertheless, its formation may be due to the decomposition of secondary hydroperoxides of fatty acids or by the reduction of the corresponding aldehyde. The origin of 1-nonen-3-ol compound from *Sagassum Polystum* is more evident. This alcohol belonging compound was observed in oysters and in dried Kombu by Takahashi *et al*., 2002 is derived from the degradation of polyunsaturated fatty acids.

Esters and acids

The ester fraction was characterized mostly by esterified fatty acids ranging from C_{12} to C_{18} in this study. These compounds present as ester is quite high in both *K. alvarezii* and *S. Polystum*, 8%. But it's very low for *C. lentillifera* with only 2%. The same applies for the present of acid compounds with 8% and 5% in *K. alvarezii* and *S. Polystum*. But it's nil in *C. lentillifera*. Sometimes undesirable flavours are produced by the formation of volatile compounds resulting from the particular metabolism or the decomposition of certain varieties of algae (Pape *et al*., 2004). Some of the ester compounds gained including isobutyl nonyl ester, cis-7-dodecen-1-yl acetate and Z-8-tetradecen-1-yl acetate respectively from *Kappaphycus alvarezii*, *Caulerpa lentillifera* and *Sagassum Polystum* have obvious undesirable effects. It is because they are proven to be produced during the decomposition of algal mats by Flament and Ohloff in 1984.

Table 4. Volatile compounds belong to ester and acid group in all seaweeds extracted by PDMS and PDMS-DVB fibres

RT	Volatile compounds from *K. alvarezii*	Peak area (%)
11.323	Acetic acid, [bis[(trimethylsilyl)oxy] phosphinyl] Trimethylsilyl ester	1.00
11.326	Acetic acid, [bis[(trimethylsilyl)oxy] phosphinyl]-, trimethyl ester	5.72
17.549	Methoxyacetic acid, 2-tridecyl ester Methoxyacetic acid, 4-tetradecyl ester	0.92
20.810	Diethyl phthalate	0.62
20.813	Diethyl phthalate	0.41
30.211	1,2-Benzedicarboxylic acid, bis (2-methylpropyl) ester Phthalic acid, isobutyl nonyl ester Phthalic acid, (2-chlorocyclohexyl) methyl isobutyl ester	0.17
33.267	n-Hexadecanoic acid Tridecanoic acid	0.41
	Volatile compounds from *C. lentillifera*	
23.487	Cis-7-Dodecen-1-yl acetate	2.41
30.182	Cis-7-Tetradecen-1-yl acetate	0.29
	Volatile compounds from *S. Polystum*	
11.326	Acetic acid Trimethylsilyl ester	0.99
17.205	Hexyl octyl ester	1.08
	Sulfurous acid	
30.209	Phthalic acid, isobutyl nonyl ester 1,2-Benzenedicarboxylic acid, bis(2 methylpropyl) ester Phthallic acid, 2-cyclohexy lethyl butyl ester	0.33
35.527	Eicosatetraenoic acid	0.75

Halogenated and other compounds

The essential oil study of many previous seaweed studies including Rzama *et al*., 1994 and Pape *et al*., 2004 shows haloforms and other halogenated compounds mainly containing bromide and iodine from seaweeds especially from brown seaweed species. This property leads this seaweed to be labeled as carcinogenic or toxic seaweeds which are not suited for consumption. From this study, seaweeds species of *S. Polystum* observed with a highest number of halogenated compounds, 6% with compounds like octadecane,1-iodo, tridecane,1-

iodo and others. Yet other two species also contains halogenated compounds such as 2-bromododecane from *K. alvarezii* and dodecane, 1-iodo from *C. lentillifera*. According to Pape *et al*. (2004), marine macroalgae have a high ability to fix halide ions. By the action of haloperoxidase enzymes that have already been detected in seaweeds, and in the presence of hydrogen peroxide, these ions were oxidized and then could react with organic substrates. Naturally occurring fluorinated fatty acids or amino acids have been reported in seaweeds and could produce this compound too (Flament and Ohloff, 1984).

Besides above stated compound a very few compounds of aromatic and miscellaneous compounds obtained too. About 7%, 6% and 7% of miscellaneous compounds were found in red, brown and green seaweeds respectively. While aromatic compounds were present at 2%, 1% and 9% in above mention sequence of seaweeds. *C. lentiliifera* exhibits a high percentage of aromatic compounds among the species. The roles of the compounds and the origin with relevant biochemical path to yield those compounds are still in an infant research level globally.

Table 5. Volatile compounds belong to halogenated and other compounds group in all seaweeds extracted by PDMS and PDMS-DVB fibres

RT	Volatile compounds from *K. alvarezii*	Peak area (%)
9.990	Dodecane, 1-iodo- Tridecane,1-iodo-	0.17
19.540	2-Bromo dodecane	2.44
	Volatile compounds from *C. lentillifera*	
10.172	Dodecane, 1-iodo-	0.34
	Volatile compounds from *S. Polystum*	
	Volatile compounds from *S. Polystum*	
10.422	Dodecane, 1-iodo-	0.35
RT	**Volatile compounds from *K. alvarezii***	**Peak area (%)**
3.527	Trans-2-(2-Pentenyl) furan Cis-2-(2-Pentenyl) furan	0.74
4.196	Benzeneacetaldehyde	0.73
18.447	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	0.76
	Volatile compounds from *C. lentillifera*	
2.361	Benzene, 1,3-dimethyl-	0.65
6.864	Benzaldehyde, 2,4-dimethyl Benzaldehyde, 3,4-dimethyl Benzaldehyde, 3,5-dimethyl	0.32
7.168	Naphthalene	1.78
10.711	1,5,6,7-Tetramethylbicyclo[3.2.0] hepta-2,6-diene	1.25
10.744	3,4-Dimethylbenzamide 3-Isopropylbenzaldehyde	1.69
	Volatile compounds from *S. Polystum*	
3.404	Furan,2-pentyl- 2-n-Butyl furan	3.38
16.873	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-E	1.52

Qualitative difference among fibres

The figure below shows the total volatile compounds extracted using different fibres for all three types of seaweeds. Form the figure it can be seen that there is a significant difference between the volatile constituents extracted from all three type of seaweeds to the two different fibre used. PDMS extracted more volatile compounds in seaweeds comparetively to PDMS-DVB. With PDMS fibres, absorption is affected mainly by molecular size, polarity, and pH. It's because absorption is the relevant process, analytes are not retained on the active surface but partitioned when extracted by PDMS fibres. Meanwhile, PDMS-DVB fibres, for which the mechanism is adsorption, molecules with greater affinity tend to displace those with lower affinity,especially when the concentration of the latter are low. Thus absorption is by definition, a non-competitive process whereas adsorption is a competitive process in which the volatile molecules of *C. lentillifera*, *K. alvarezii* and *S. polycystum* 'fight' for the active site of the surface on fibre to be attached. Thus PDMS is more appropriate and gives a better yield in terms of volatile constituents when it comes to extraction. By avoiding the competition mechanism in PDMS-DVB fibre utilization, more volatiles can be extracted. More importantly, most of compounds obtained in this study were low boiling compounds such as 1-undecene, 8-methyl, dodecane, undecanal and others. Whereby PDMS is efficient in absorption of low boiling compounds volatile compounds compare to high boiling compounds which were more extracted by PDMS-DVB fibre. The natural presence of more low boiling compounds in all three seaweeds made better extraction by PDMS fibre rather than PDMS-DVB fibre (Zambonin *et al*., 2004).

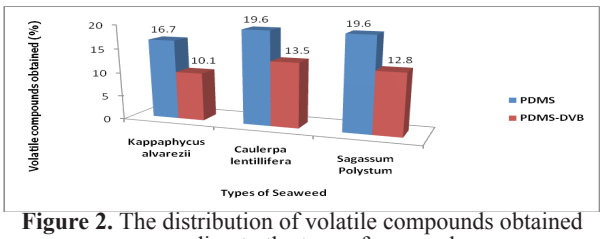

Figure 2. The distribution of volatile compounds obtained according to the type of seaweeds

Conclusion

The optimization result with temperature of 60^{0}C and extraction time of 50 minutes for both fibres for *K. alvarezii* and PDMS fibre extraction for *S. polycystum*. While for PDMS-DVB extraction for the same seaweed spesies, a 50^{0}C temperature with 20 min extraction time provided optimum peak areas. *Caulerpa lentillifera* shows peaks with highest abundance when extracted in 50^{0}C with 20 min for PDMS fibre and 50^{0}C with 40 min extraction for PDMS-DVB fibre. Whereby, no least significant difference is recorded for all the combination of factors applied in this study except for the fibre utilization. It's due to the mechanism of the fibre which differentiates the absorbed and adsorbed constituents and the presence of lower boiling point volatile compounds than high boiling point volatile compounds. No significant different with the yield of compounds among the seaweed shows that most of the volatile constituents were common among three species. Qualitative study portray a total of 223 volatile constituents successfully

extracted and identified with 82, 91 and 50 volatiles from each species of *K. alvarezii*, *C. lentillifera* and *S. polycystum* respectively. A quantitative data study on the chemical classes exhibits a total of 9 groups including hydrocarbon as the main group present in all three seaweeds followed by aldehydes, ketones, esters, acids, halogen, alcohol, aromatic and miscellaneous compounds. Thus HS-SPME extraction method with PDMS fibre is strongly recommended for volatile constituent's extraction from local seaweeds of Sabah.

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