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Volatile compounds of the green alga, *Capsosiphon fulvescens*

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Abstract Essential oils extracted by static vacuum simultaneous distillation–extraction (V-SDE) and conventional SDE from a green alga, *Capsosiphon fulvescens*, were analyzed by gas chromatography (GC) and GC-mass spectrometry. The essential oil extracted with V-SDE and SDE has totals of 151 and 140 compounds, respectively. A combined total of 208 compounds were identified and 81 volatiles were common in both extracts. These included 8 acids, 28 alcohols, 34 aldehydes, 11 esters, 25 ketones, 19 aliphatic hydrocarbons, 43 branched hydrocarbons, 6 unsaturated hydrocarbons, 19 cyclic hydrocarbons, and 15 miscellaneous. The major volatile compounds of the oil extracted with V-SDE were (*E*)-β-ionone, octane, (*E*,*E*)-2,4-heptadienal, hexadecanoic acid, and β-cyclocitral, while those extracted with SDE were hexadecanoic acid, (*Z*,*Z*)-1,5-octadien-3-ol, tetradecanoic acid, (*E*,*E*)-2,4-heptadienal, and benzaldehyde. The characteristics of the flavor of the green alga might be contributed by the presence of a large number of aldehydes and ketones. Many of the compounds extracted with SDE might originate from thermal degradation and/or thermal interactions among the constituents in the alga during steam distillation.

Keywords Chlorophyta · *Capsosiphon fulvescens* · Simultaneous distillation-extraction · Volatile compounds

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Introduction

For several centuries, there has been a traditional use of seaweeds as food in China, Japan, and Korea. In the Occident, seaweeds have been utilized mainly as raw material for extraction of phycocolloids. These are currently used in the pharmaceutical, cosmetic, and food industries (Armisen 1995). In recent years, there has been a strong movement in France to introduce seaweed into the European cuisine, with some success, although it is still regarded as an exotic component of the menu (McHugh 2003). Certain seaweeds consumed present a great nutritional value as source of proteins, carbohydrates, minerals, and vitamins. Many edible types of seaweeds can also be considered as functional foods. Demand for seaweed as food has also extended to North America, South America, and Europe (McHugh 2003).

The green alga *Capsosiphon fulvescens* (C. Agardh) Setchell and Gardner is a filamentous chlorophycean seaweed found in the upper intertidal regions of coastal sediments and rocky shore in Korea. Koreans have been consuming this seaweed as food since the ancient time because of its unique taste and flavor properties. Moreover, it has been successfully cultivated in Korea for many years on a commercial scale. Recently, much attention has been paid to this alga that has been used traditionally as food, especially soup with oysters, and several investigators have studied various activities of this alga and have found that it has not only nutritional value, but also beneficial properties to cure various diseases and maintain good health (Mun et al. 2005; Kwon and Nam 2006a, b; Lee et al. 2006).

The aromas of marine algae have attracted the attention of people. However, the content of essential oils in the algae is much lower than that of terrestrial plants (Kajiwara et al. 1993). The dehydrated *C. fulvescens* has peculiar aromatic characteristics compared with other green algae. It has been reported that the major volatile compounds in green algae are aldehydes and alcohols (Fujimura et al. 1990; Kajiwara et al. 1992). However, full information on the volatile compounds of this green alga is scarce and it would be informative to investigate the volatile components of essential oils in *C. fulvescens*. The objective of this study was to identify volatile compounds of essential oils in *C. fulvescens* and to compare the effects of steam and vacuum extraction methods.

Materials and methods

Fully grown *C. fulvescens* thalli were collected from a seaweed farm located in Daeduck, Janghung county, on the southwestern coast of South Korea on December 20, 2005. Freshly collected plants were washed several times in clean cold seawater, kept on ice, and transported to the laboratory immediately. The species was identified microscopically, manually separated from other algae, and washed with tap water and distilled water. Authentic standard compounds were purchased from Tokyo Kasei Kogyo Co., Ltd. (Japan) and Supelco Inc. (Bellefonte, USA).

Conventional steam distillation and extraction at atmospheric pressure A Likens and Nickerson (1964) type simultaneous steam distillation and extraction (SDE) apparatus (Model 523010-000, Kontes, USA) was used for the extraction of volatile components. Fresh green alga (100 g, 96% moisture, *w/w*) was cut into 5 cm pieces and placed in a 5-L-round bottom flask with 2 L of double-distilled water. One milliliter of 2,4,6-trimethylpyridine, used as an internal standard (I.S., 10 μg mL^{-1} in distilled water), was added to the sample before extraction. Each sample was extracted with 50 mL of redistilled diethyl ether. Extraction time was set for 3 h after the distilled water started to boil in the sample flask. Extract was then concentrated to 10 mL with a gentle stream of nitrogen gas (purity 99.99%), dried with anhydrous sodium sulfate, and further concentrated to 0.2 mL. Extract was kept in a freezer (−80°C) until further analyzed. Triplicate extractions were prepared for each sample.

Static vacuum SDE A SDE apparatus under vacuum described by Maignial et al. (1992) was used with some modifications. Fresh sample (100 g) and double-distilled water (600 mL) were placed in a 2.5-L double-jacketed flask. The internal standard was then added in the same manner as described above. The volatile compounds were extracted for 3 h with isooctane (3 mL, purity 99.7%) under static vacuum (49±1 mbar, Vacuum Controller 800, Buchi Corp., Switzerland). The temperatures of the sample flask, the double-jacketed solvent flask, and the condenser of the SDE apparatus were maintained at 36±0.2°C, 22±0.2°C, and −5°C, respectively, with circulating water baths. Before heating, the system was pumped with a vacuum pump and closed under the vacuum (49 mbar) with an extended-tip PTFE valve for the duration of the experiment. Both the sample and the solvent flask were vigorously magnetically stirred. Triplicate extractions were prepared for each sample.

Gas chromatography/mass spectrometry conditions Qualitative and quantitative analyses of extracts were carried out with a Shimadzu GC-2010 gas chromatography (GC) coupled with a Shimadzu GCMS QP-2010 mass detector (MD). Separations of the volatile compounds were performed on a DB-Wax column (60 m length×0.25 mm. i.d.×0.25 μm film thickness; J and W Scientific, USA). Three microliters of each extract was injected into the GC. Other GC conditions were as follows: pulse splitless with valve delay for 30 sec, injector temperature at 230°C, and helium carrier gas at 0.7 mL min^{-1}. Temperature program was as follows: initial temperature of 35°C for 5 min, ramp rate at 1°C min^{-1}, and final temperature of 220°C for 140 min. MD conditions were as follows: ion source temperature at 230°C, interface temperature at 220°C, ionization voltage at 70 eV, mass range at 40–350 amu, scan rate at 1.5 scans s^{-1}, and electron multiplier voltage at 1.20 kV.

Compound identification and quantification Compound identification and confirmation were made by comparison of the retention indices (van den Dool and Kratz 1963) and mass spectra of an unknown with those of authentic standards under identical analytical conditions. Tentative identification of compound was made by matching the mass spectra of an unknown with that in the Wiley Chemical Database (7th ed., Wiley, New York, NY) and/or with retention indices of published literatures (Jennings and Shibamoto 1980; Sadtler Research Laboratories 1985). The relative abundance of each compound was determined by the ratio of the peak area of the identified compound to the area of the internal standard.

Results

Volatiles of *C. fulvescens* were first extracted using static vacuum simultaneous distillation–extraction (V-SDE). Table 1 shows the identified volatile components and their mean area ratio. The mean area ratio of peaks was used to

Table 1 Volatile components in fresh green alga, *Capsosiphon fulvescens*

Compounds	RI[a] (DB-Wax)	V-SDE		SDE		Identification method[c]
		Mean ratio[b]	SD	Mean ratio	SD	
Acids (8)						
Nonanoic acid	2,203	11.74	1.90	3.88	0.94	MS, RI
Decanoic acid	2,298	20.56	3.09			MS, RI
Dodecanoic acid	2,409	54.14	1.84			MS, RI
Tetradecanoic acid	2,686	33.80	2.97	654.56	71.31	MS, RI
Pentadecanoic acid	2,816	35.92	6.12	45.62	4.01	MS, RI
Hexadecanoic acid	2,892	313.61	25.45	2,430.52	414.40	MS, RI
9-Hexadecenoic acid	2,925	30.16	1.51	349.16	28.33	MS
Octadecanoic acid	3,122	121.1	12.76	6.91	0.45	MS, RI
Alcohols (28)						
2-Methyl-2-propanol	1,004			12.03	0.98	MS, LRI
2-Propanol	930			3.04	0.42	MS, RI
Ethanol	944			84.39	12.68	MS, RI
1-Buten-3-ol	1,057	3.73	0.39			MS, LRI
Propanol	1,043			1.79	0.16	MS, RI
Butanol	1,176	5.48	0.26	0.54	0.02	MS, RI
1-Penten-3-ol	1,169			2.03	0.16	MS, RI
2-Ethoxyethanol	1,214			0.44	0.04	MS, LRI
2,2,4-Trimethyl-3-pentanol	1,235	2.11	0.21	1.15	0.10	MS, LRI
(*E*)-2-Penten-1-ol	1,318			1.47	0.13	MS, RI
(*Z*)-2-Penten-1-ol	1,327			48.44	4.63	MS, RI
(*E*)-3-Hexen-1-ol	1,370			19.29	2.72	MS, RI
2,2,4-Trimethyl-1-pentanol	1,381	4.27	0.13	15.68	0.61	MS, LRI
(*Z*)-3-Hexen-1-ol	1,385	2.99	0.29	36.45	3.78	MS, RI
1-Octen-3-ol	1,453	76.70	9.91	319.14	34.63	MS, RI
(*Z*,*Z*)-1,5-Octadien-3-ol	1,489	109.74	6.87	823.73	53.39	MS
isoPulegol	1,564	22.64	1.49	4.68	0.46	MS, RI
Nonanol	1,672	45.03	5.75	14.89	2.07	MS, RI
2-Propyl-1-heptanol	1,668			4.15	0.41	MS
Dodecanol	1,973	7.63	0.73	4.04	0.36	MS, RI
Tetradecanol	2,195			9.13	0.78	MS, LRI
isoPhytol	2,302			13.31	0.85	MS
2,4-Bis(1,1′-dimethylethyl)phenol	2,318	68.79	6.16	13.07	0.85	MS, LRI
Hexadecanol	2,379	23.92	2.54			MS, RI
Octadecanol	2,575	9.31	0.22			MS, RI
4-(1,1,3,3-Tetramethylbutyl)phenol	2,623	22.69	1.50	31.75	2.11	MS
(*E*)-Phytol	2,626	71.83	6.45	146.81	12.55	MS, RI
4-Nonylphenol	2,736			12.43	0.80	MS
Aldehydes (34)						
Propanal	784			4.92	0.32	MS, RI
2-Propenal	842			4.76	0.51	MS, RI
Butanal	880			1.33	0.13	MS, RI
3-Methyl butanal	926			0.52	0.03	MS, RI
2,4,4-Trimethylpentanal	1,069	18.95	1.54			MS
2,4-Pentadienal	1,103			10.64	0.42	MS
2-Methyl-(*E*)-2-butenal	1,119			0.58	0.05	MS, RI
4-Pentenal	1,128			5.66	0.48	MS, LRI
(*E*)-2-Pentenal	1,139			34.75	1.23	MS, RI

Table 1 (continued)

Compounds	RI[a] (DB-Wax)	V-SDE		SDE		Identification method[c]
		Mean ratio[b]	SD	Mean ratio	SD	
2-Methyl-2-pentenal	1,151			2.82	0.40	MS, RI
Heptanal	1,183	12.64	0.20	3.79	0.51	MS, RI
(*E*)-2-Hexenal	1,219	24.96	3.49	38.37	3.88	MS, RI
(*Z*)-4-Heptenal	1,249	13.40	1.39	3.85	0.46	MS, RI
Octanal	1,288	17.98	1.95	0.92	0.08	MS, RI
(*E*)-2-Heptenal	1,322	28.46	1.84	1.29	0.14	MS, RI
Nonanal	1,391	53.02	5.22	35.23	3.11	MS, RI
(*E*,*E*)-2,4-Hexadienal	1,398			61.74	5.95	MS, RI
(*E*)-2-Octenal	1,434	46.87	4.66	84.31	7.73	MS, RI
(*E*,*Z*)-2,4-Heptadienal	1,464	330.72	29.20	381.21	33.06	MS, RI
(*E*,*E*)-2,4-Heptadienal	1,494	467.96	5.14	521.27	3.33	MS, RI
Benzaldehyde	1,530	88.07	3.47	433.70	17.19	MS, RI
(*E*)-2-Nonenal	1,545			3.31	0.35	MS, RI
5-Methyl-2-furfural	1,550			5.91	0.56	MS, LRI
(*E*,*Z*)-2,6-Nonadienal	1,588	74.40	3.11	10.51	0.70	MS, RI
(*E*,*E*)-2,4-Octadienal	1,591	28.82	2.52	43.04	3.77	MS, RI
β-Cyclocitral	1,623	268.56	31.05	45.91	5.22	MS, LRI
(*E*)-2-Decenal	1,649	81.17	5.10	3.61	0.23	MS, RI
4-Ethylbenzaldehyde	1,716	20.81	1.94	46.04	4.73	MS, RI
2,5-Dimethylbenzaldehyde	1,745			7.57	0.48	MS, RI
(*E*,*Z*)-2,4-Decadienal	1,774	142.82	12.15	31.88	3.12	MS, RI
(*E*,*E*)-2,4-Decadienal	1,822	260.22	10.14	71.59	2.43	MS, RI
Tridecanal	1,830			168.91	14.50	MS, RI
Tetradecanal	1,935			7.48	0.48	MS, LRI
3,5-Di-tert-butyl-4-hydroxybenzaldehyde	2,514			67.78	4.39	MS
Ester (11)						
Ethyl formate	818			31.07	2.06	MS, RI
Ethyl acetate	891			69.84	6.04	MS, RI
Butyl acetate	1,074	6.07	0.14			MS, RI
Methyl hexanoate	1,187	1.49	0.09			MS, RI
Hexyl formate	1,361			15.07	1.31	MS, LRI
Methyl octanoate	1,378	1.88	0.19			MS, RI
Linalyl propionate	1,678	4.84	0.43			MS, LRI
Methyl hexadecanoate	2,206	3.43	0.34	2.55	0.21	MS, RI
Methyl (*Z*)-9-octadecenoate	2,459	8.13	0.73			MS
Dibutyl phthalate	2,688	54.11	4.65	9.91	0.37	MS, LRI
Dodecyl 2-ethylhexanoate	2,710	41.21	1.61			MS
Ketone (25)						
2-Butanone	901			2.07	0.13	MS, RI
3-Buten-2-one	952			1.01	0.12	MS, RI
3,3-Dimethyl-2-butanone	961			0.93	0.03	MS
1-Penten-3-one	1,027			29.97	3.29	MS, RI
2,2,4-Trimethyl-3-pentanone	1,006	3.18	0.03			MS
2,3-Pentadione	1,065			2.22	0.19	MS, RI
6-Methyl-2-heptanone	1,239	8.51	0.51	0.91	0.06	MS, LRI
2-Octanone	1,284	1.52	0.06			MS, RI
2,6,6-Trimethylcyclohexanone	1,315	39.13	1.44	3.62	0.13	MS, LRI
2-Methyl-3-octanone	1,328	6.43	0.39			MS, LRI

Table 1 (continued)

Compounds	RI[a] (DB-Wax)	V-SDE		SDE		Identification method[c]
		Mean ratio[b]	SD	Mean ratio	SD	
(*Z*)-6-Octen-2-one	1,330	6.22	0.25			MS, LRI
6-Methyl-5-hepten-2-one	1,335	11.91	1.68	4.45	0.44	MS, RI
2,2,6,6-Tetramethyl-3,5-heptanedione	1,341	9.89	0.35			MS
3,3,5-Trimethylcyclohexanone	1,355	8.86	0.56	15.54	0.60	MS, LRI
(*Z*)-1,5-Octadien-3-one	1,373	11.23	1.09			MS, RI
β-Isophorone	1,400	9.96	0.90			MS, LRI
(*E*,*E*)-3,5-Octadien-2-one	1,570	31.20	2.69	5.99	0.57	MS, RI
3,5,5-Trimethyl-2-cyclopenten-1-one	1,642	31.52	2.01	17.22	1.04	MS, RI
6,10-Dimethylundecan-2-one	1,701			17.53	1.83	MS, LRI
Propiophenone	1,739			9.36	0.59	MS, LRI
1-(4-Methylphenyl)-ethanone	1,791			6.05	0.38	MS
(*E*)-α-Ionone	1,862	98.68	6.54	25.76	1.25	MS, RI
(*E*)-β-Ionone	1,953	530.28	46.03	92.75	11.24	MS, RI
2,6-Di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	2,056	20.29	1.31	5.79	0.57	MS
6,10,14-Trimethylpentadecan-2-one	2,072	7.01	0.72	24.72	1.58	MS
Aliphatic hydrocarbons (19)						
Octane	800	482.16	41.33	0.38	0.02	MS, RI
Nonane	896	103.74	9.85			MS, RI
Decane	985	25.03	2.04	0.96	0.09	MS, RI
Undecane	1,099	98.41	13.59	1.12	0.16	MS, RI
Dodecane	1,203	41.51	1.66	3.19	0.12	MS, RI
Tridecane	1,301	8.03	0.87	1.02	0.11	MS, RI
Tetradecane	1,395	67.21	5.90	1.47	0.16	MS, RI
Pentadecane	1,500	26.30	4.48	251.89	37.32	MS, RI
Hexadecane	1,601	80.83	6.56	1.66	0.12	MS, RI
Heptadecane	1,703	4.74	0.31	1.58	0.12	MS, RI
Octadecane	1,804	28.41	2.99	7.39	0.73	MS, RI
Nonadecane	1,906	12.13	0.99			MS, RI
Eicosane	2,002	14.77	1.20	6.01	0.51	MS, RI
Heneicosane	2,095	22.17	3.06	2.04	0.28	MS, RI
Docosane	2,210	8.28	1.24	3.38	0.50	MS, RI
Tricosane	2,296	5.32	0.58			MS, RI
Tetracosane	2,407	9.11	0.80			MS, RI
Pentacosane	2,504	34.61	1.46			MS, RI
Hexacosane	2,603	4.13	0.34			MS, RI
Branched hydrocarbons (43)						
2,4-Dimethylhexane	759	9.34	0.03	0.87	0.04	MS
2,2,3-Trimethylpentane	766	212.69	20.33			MS
2,3,4-Trimethylpentane	774	31.81	4.49			MS
4-Methylheptane	778	1.25	0.05			MS
3-Methylheptane	791	0.73	0.08			MS
2,4-Dimethylheptane	807	2.34	0.25			MS
4-Methyloctane	840	5.96	0.39			MS, LRI
3-Methyloctane	849	4.39	0.43			MS
2-Methyloctane	857	0.58	0.08	0.41	0.06	MS, LRI
2,4-Octadiene	913	2.96	0.29			MS
2,2,4,6,6-Pentamethylheptane	946	7.76	0.69			MS, RI
3-Methylnonane	953	0.45	0.04			MS

Table 1 (continued)

Compounds	RI[a] (DB-Wax)	V-SDE		SDE		Identification method[c]
		Mean ratio[b]	SD	Mean ratio	SD	
2,4,6-Trimethyloctane	1,003	0.79	0.05			MS
6-Ethyl-2-methyl-octane	1,014	2.61	0.17			MS
2,5-Dimethylnonane	1,016	2.38	0.26	0.85	0.09	MS, RI
5-Ethyl-2-methyl-octane	1,019	26.08	0.24			MS
5-Methyldecane	1,032	1.34	0.09	0.45	0.03	MS
4-Methyldecane	1,036	1.46	0.12	0.33	0.03	MS
Trimethyloctane	1,038	1.63	0.11			MS
3-Methyldecane	1,047	1.95	0.12			MS
2-Methyldecane	1,061	9.52	0.79			MS
2,6-Dimethylnonane	1,067	31.19	2.01			MS
3,7-Dimethylnonane	1,073	10.05	1.07			MS
2,3,6,7-Tetramethyloctane	1,082	2.75	0.27			MS
5-Methylundecane	1,137	1.52	0.10			MS
4-Methylundecane	1,144	1.44	0.13			MS
2,3-Dimethyldecane	1,147	2.07	0.08			MS
3-Methylundecane	1,160	1.25	0.11			MS
4,8-Dimethylundecane	1,242	4.82	0.41	1.86	0.16	MS
4,7-Dimethylundecane	1,246	57.73	2.04	5.05	0.18	MS
5,7-Dimethylundecane	1,253	9.72	1.39	3.30	0.43	MS
5-Methyldodecane	1,259	9.17	1.24	4.64	0.49	MS
2,7-Dimethylundecane	1,266	13.49	1.36	0.48	0.05	MS
3,7-Dimethylundecane	1,291	7.83	0.93			MS
2,10-Dimethylundecane	1,297	49.13	4.36			MS
2,4-Dimethylundecane	1,306	11.06	1.18			MS
2,8-Dimethylundecane	1,308	5.62	0.50	1.88	0.17	MS
4,6-Dimethyldodecane	1,320	2.23	0.21			MS
2,6,10-Trimethyldodecane	1,440	4.97	0.46			MS, LRI
2,6,11-Trimethyldodecane	1,443	36.34	3.15			MS, LRI
5-Methyltetradecane	1,451	1.45	0.01	6.51	0.05	MS
8-*n*-Hexylpentadecane	2,041	3.07	0.12			MS
2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene (squalene)	3,105	53.59	5.73			MS
Unsaturated hydrocarbons (6)						
3-Methyl-1,4-heptadiene	922	2.19	0.19			MS
1,6-Dimethylhepta-1,3,5-triene	984			2.52	0.29	MS
3-Ethyl-1,5-octadiene(isomer)	998	6.11	0.39	1.76	0.11	MS
3-Ethyl-1,5-octadiene(isomer)	1,012	15.17	1.56	1.64	0.16	MS
1,3,5-Octatriene	1,120			1.18	0.08	MS
(*Z,Z,Z*)-4,6,9-Nonadecatriene	2,081			1.84	0.17	MS
Cyclic hydrocarbons (19)						
Ethylcyclopentane	782	16.06	1.03			MS
1-Ethyl-3-methylcyclopentane	814	0.92	0.06			MS
1,2-Dimethylcyclohexane	834	0.54	0.01			MS, LRI
Propylcyclopentane	871	0.86	0.08			MS
Ethylcyclohexane	882	0.37	0.02			MS, RI
3,3,5,5-Tetramethylcyclopentene	928	0.92	0.08			MS
Methylbenzene	1,023	12.76	0.51	18.32	0.65	MS, RI
Ethylbezene	1,117	4.65	0.31	1.19	0.08	MS, RI
p-Xylene	1,131	4.15	0.36	0.11	0.01	MS, RI

Table 1 (continued)

Compounds	RI[a] (DB-Wax)	V-SDE		SDE		Identification method[c]
		Mean ratio[b]	SD	Mean ratio	SD	
m-Xylene	1,143	9.93	0.64	2.21	0.16	MS, RI
o-Xylene	1,180	10.36	1.07	0.81	0.08	MS, RI
d-Limonene	1,201	1.52	0.13			MS, RI
4-Ethyltoluene	1,223	1.70	0.18	0.96	0.10	MS, RI
1,3,5-Trimethylbenzene	1,231	4.75	0.18	0.68	0.03	MS, RI
1,2,4-Trimethylbenzene	1,278	10.26	0.65	1.74	0.10	MS, RI
(*E*)-isoLimonene	1,303			19.50	1.33	MS, LRI
1,2,3-Trimethylbenzene	1,333	3.96	0.24			MS, RI
1,2,4,5-Tetramethylbenzene	1,432	2.32	0.15			MS, RI
1,2-Dihydro-1,1,6-trimethylnaphthalene	1,762			9.28	1.04	MS
Miscellaneous (15)						
Dimethyl sulfide	754			14.9	0.28	MS, RI
Ethoxybutane	789			4.28	0.37	MS, LRI
2-Methylfuran	873			1.53	0.10	MS, RI
1,1-Diethoxyethane	905			5.06	0.29	MS, RI
1-Ethoxy-1-propene	927			6.61	0.24	MS
Dichloromethane	941			2.50	0.22	MS, RI
3-Ethylfuran	966			27.29	1.10	MS
Trichloroethylene	1,011			0.63	0.07	MS
1,1-Diethoxybutane	1,017			4.95	0.27	MS
2,3-Biethoxybutane	1,068			2.05	0.09	MS
2-Pentylfuran	1,232	0.72	0.07	5.48	0.54	MS, RI
Bis(2-ethoxyethyl) ether	1,411	2.37	0.21			MS
Dimethyl sulfoxide	1,562			145.23	7.54	MS, RI
Dimethyl sulfone	1,909			10.02	0.37	MS, LRI
Epoxy-BHT	2,123	7.31	0.76			MS
Unknown compounds (5)						
Unknown 1	1,748	89.50	8.30	84.51	7.00	
Unknown 2	1,762	106.44	1.87	93.93	3.76	
Unknown 3	1,831	6,665.02	572.21	2,547.36	270.32	
Unknown 4	1,881	1,484.45	91.85	1,344.04	69.43	
Unknown 5	2,012	457.05	25.16	417.75	20.08	

SD standard deviation

[a] *RI*, retention indices determined on the basis of van den Dool and Kratz (1963) after injection of a *n*-alkane series (C_9–C_{32}) under the same conditions

[b] Mean area ratio was calculated by the ratio of the peak area ($n=3$) of the identified compound to the area of the internal standard

[c] MS, RI was identified with mass spectral data and retention indices (RI) of an authentic standard compound; MS, LRI was identified with mass spectral data and retention indices of published literatures (Jennings and Shibamoto 1980; Sadtler Research Laboratories 1985); MS was identified with only mass spectral data in the Wiley Chemical Database (8th ed., Wiley, New York, NY)

indicate relative amounts of individual compounds and was not considered any response factor corrections. *C. fulvescens* extracted with V-SDE and SDE has a total of 151 and 140 compounds, respectively. A combined total of 208 compounds were identified and 81 volatiles were common in both extraction technique. Out of 208 compounds, 106 compounds were positively identified with retention index of authentic standards and mass spectra, and 102 compounds were tentatively identified with published mass spectra and/or retention index. These included 8 acids, 28 alcohols, 34 aldehydes, 11 esters, 25 ketones, 19 aliphatic hydrocarbons, 43 branched hydrocarbons, 6 unsaturated hydrocarbons, 19 cyclic hydrocarbons, and 15 miscellaneous.

Eight acid compounds were identified, and six common ones were found in both extracts. Hexadecanoic acid had the highest amount of the identified compounds in the sample while nonanoic acid contained relatively low amount.

Twenty saturated and eight unsaturated alcohols were found in the sample. Fifteen and 25 alcohols were detected from V-SDE and SDE, respectively. (*Z*,*Z*)-1,5-Octadien-3-ol was the most abundant of these alcohols, followed by 1-octen-3-ol and (*E*)-phytol. Four unsaturated alcohols including 1-penten-3-ol, (*E*)-2-penten-1-ol, (*Z*)-2-penten-1-ol, and (*E*)-3-hexen-1-ol were detected only in the SDE extract. 1-Penten-3-ol and (*E*)-2-penten-1-ol were found at relatively low amount in the alga.

One of the most abundant volatile groups in the sample was aldehyde including straight chain alkanals, 2-alkenals, 4-alkenals, dienals, methyl alkenals, and alkyl benzaldehydes. Twenty-one of 34 aldehydes detected were unsaturated and may impart more important organoleptic properties than the saturated aldehydes (Mottram 1991). The SDE extract contained more aldehyde compounds (33 aldehydes) than the V-SDE extract (18 aldehydes).

Ketones are another large class containing 25 compounds. The ketones, which were found in both extracts, usually contained higher amounts in the V-SDE extract than in the SDE extract. The 6-methyl-2-heptanone and 6-methyl-5-hepten-2-one were found in relatively high amount in the V-SDE extract. β-Ionone was the most abundant compound in the ketone compounds. A ketone, 6,10,14-trimethylpentadecan-2-one (phytone) was detected in relatively low amounts in both extraction techniques.

The most abundant volatiles in *C. fulvescens* were hydrocarbons including 19 aliphatic hydrocarbons, 43 branched hydrocarbons, 6 unsaturated hydrocarbons, and 22 cyclic hydrocarbons. A series of aliphatic hydrocarbons were detected ranging from octane to hexacosane in the V-SDE extract. Out of 43 branched hydrocarbons, 15 mono-methyl hydrocarbons and 16 dimethyl hydrocarbons were identified in the V-SDE extract. Only 12 branched hydrocarbons were detected in the SDE extract. The SDE technique involves the possibility of forming thermally induced artifacts. Furthermore, losses of high-volatility components such as octane, nonane, and decane have been observed using the method. Three sulfur-containing compounds, dimethyl sulfide, dimethyl sulfoxide, and dimethyl sulfone, were identified in the SDE extract. Three alkylfurans such as 2-methylfuran, 3-ethylfuran, and 2-pentylfuran were identified in the green alga.

Discussion

Nonanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, and oleic acid were identified in a Japanese marine red alga, *Porphyra tenera*, and the hexadecanoic acid was one of the major compounds (Kajiwara et al. 1990). Those generally are minor contributors to alga flavor because of their high odor threshold values and low volatility. However, some long change aldehydes such as pentadecanal, (*Z*)-8-heptadecenal, (*Z*,*Z*)-8,11-heptadecadienal, and (*Z*,*Z*,*Z*)-8,11,14-heptadecatrienal had been demonstrated to produce enzymatically from hexadecanoic acid, oleic acid, linoleic acid, and α-linolenic acid, respectively, in a green alga, *Ulva pertusa* (Kajiwara et al. 1988, 1990).

(*Z*,*Z*)-1,5-Octadien-3-ol was detected in herring (Aro et al. 2002) and oyster (Piveteau et al. 2000). The compound is formed via pyrolysis of hydroperoxides of arachidonic acid, and in fungi and mushrooms, it is formed from 10-hydroperoxide of linoleic acid by homolytic hydroperoxide lyase (Wurzenberger and Grosch 1984, 1986). (*E*)-Phytol was found in brown alga (Shin 2003) and green alga (Kambourova et al. 2003; Fujimura et al. 1990). It has been reported that the compound is odorless and tasteless to most observers because it has an unusually low vapor pressure at normal temperature (Arctander 1969). 1-Penten-3-ol was the most noticeable compound detected in rancid sardine oil (Peterson and Chang 1982). 1-Penten-3-ol, observed in dried brown seaweed (*Laminaria* spp.), is derived from the degradation of ω-3 polyunsaturated fatty acids (Takahashi et al. 2002). Also, peroxidation products of unsaturated fatty acids result in formation of alcohols such as 1-hexanol, (*E*)-2-hexenol, (*E*)-3-hexenol, and (*Z*)-3-hexenol (Schreier 1984).

The lipid-degraded compounds were identified as (*E*)-2-hexenal, (*E*)-2-heptenal, (*E*)-2-decenal, (*E*,*E*)-2,4-heptadienal, and (*E*,*E*)-2,4-decadienal. It has been reported that flavor compounds of seaweeds such as (*E*)-2-hexenal and (*E*)-2-nonenal showed potent antimicrobial activities against *Escherichia coli* TG-1 and *Erwinia carotovora*. Both (*E*,*Z*)-2,4-heptadienal and (*E*,*E*)-2,4-heptadienal were detected at relatively low amounts in both the extraction methods. The heptadienals were found in a brown alga (Shin 2003; Kajiwara et al. 1991) and a red alga (Kajiwara et al. 1990). The increase in fishy, metallic, and rancid off-flavors has been correlated to high concentrations of (*E*,*E*)-2,4-heptadienal in fish oil enriched mayonnaise (Jacobsen 1999). The β-cyclocitral seems to be important constituents of some brown algae such as *Costaria costata* and *Alaria crassifolia* (Kajiwara et al. 2006). β-Cyclocitral can be formed from oxidative cleavage of the double bond between carbons seven and eight of β- and α-carotene and carbons 7′ and 8′ of α-cryptoxanthin (Mahattanatawee et al. 2005). The amount of benzaldehyde was five times higher in SDE extract. The compound was reported as one of the very desirable aromas (Aldrich 1998), which has a pleasant almond, nutty, and fruity aroma in Crustacea (Vejaphan et al. 1988). Hayashi et al. (1981) reported that benzaldehyde was thermally generated and contributed to food flavor. This compound and methylbutanals, probably derived from Strecker degradation of amino acids, have been identified as the major monocarbonyls in roasted peanuts (Mason et al. 1967). Long-chain aldehydes such as tridecanal and tetradecanal were found only in the SDE extract. The long-chain aldehydes were formed from unsaturated fatty acids by the enzymes of the thalli culture of the marine green alga. The long-chain aldehydes such as (*Z*)-8-heptadecenal, (*Z*,*Z*)-8,11-heptadecadienal, and (*Z*,*Z*,*Z*)-8,11,14-heptadecatrienal were identified as major volatile compounds in the essential oils of the green alga, *U. pertusa* (Fujimura et al. 1990). Short-chain aldehydes including (*E*,*E*)-2,4-octadienal, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-decadienal, and (*E*,*Z*)-2,4-decadienal were detected in both V-SDE and SDE. Those aldehydes were found in the red alga, *P. tenera* (Kajiwara et al. 1990).

Eleven esters were detected and believed to be products of an esterification of corresponding alcohols and carboxylic acids (Peterson and Chang 1982). In general, such esters give sweet fruity flavors. The most abundant component among these compounds was dibutyl phthalate that is regarded as toxic pollutants in industrial waste-water (Strier 1980). Dibutyl phthalate was identified in some blue-green algae such as *Phormidium*, *Aphanisomenon*, and *Anabaena* (Tsuchiya and Matsumoto 1988), and in the green alga, *Enteromorpha* (Kajiwara et al. 1992). The methyl esters of hexadecanoic and 9-octadecenoic acids have already been reported as major products in the cyanobacteria, *Oscillatoria* (Slater and Blok 1983), which are characterized by a strong odor.

Ketones can be generated by many ways. Ames and Macleod (1984) reported aliphatic ketones might be products of lipid oxidation or degradation. Methyl ketones (C_3–C_{17}), such as 6-methyl-2-heptanone, could result from beta-oxidation of the carbon chain followed by decarboxylation (Selke et al. 1975). Also, the 6-methyl-5-hepten-2-one could be formed from the oxidative cleavage of carotenoids such as lycopene and phytoene (Buttery et al. 1969). The β-ionone was one of the major compounds in sea mustard (*Undaria pinnatifida*) cultured in Japan (Kajiwara et al. 1993). Carotene and pro-vitamin A are derived from ionone as intermediate in the biosynthesis of vitamin A (Weeks 1986). β-Ionone was described to have warm, woody and dry odor with a fruity undertone. In addition, the compounds had a very low threshold value, which was responsible for its potent odor (Enzell 1981).

Phytone (6,10,14-trimethylpentadecan-2-one) was found in the green alga, *U. pertusa* (Fujimura et al. 1990), the red alga, *P. tenera* (Kajiwara et al. 1990), and the brown alga, *Scytosiphon lomentoria* (Kajiwara et al. 1991). This widely distributed isoprenoid component results mainly from the hydrolysis of chlorophyll or bacteriochlorophyll-a photoproducts (Marchand and Rontani 2003), or from phytol biodegradation (Rontani and Acquaviva 1993).

Sartin et al. (2001) suggested that seaweed is a source of long chain *n*-alkanes (C_9–C_{28}). Vacuum SDE appears to be a valuable alternative that avoids thermal degradation and formation of thermal artifacts (Maignial et al. 1992), whereas conventional SDE extracts medium- and high-boiling point components. However, using the SDE the branched hydrocarbons were lost or modified during heating. Squalene, a triterpene compound, which is not related to phytol, was detected in the sample. Squalene is present in high concentration in microbial mat samples (Sartin et al. 2001) and in the green alga, *Scenedesmus incrassatulus* (Kambourova et al. 2003). 1,3,5-Octatriene, an alkene, has been found in the red alga, *Palmaria palmata* (Le Pape et al. 2004), and the oyster, *Crassostrea gigas* (Le Guen et al. 2001).

Seven alkylbenzenes including methylbenzene, ethylbenzene, 4-ethyltoluene, 1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene, 1,2,3-trimethylbenzene, and 1,2,4,5-tetramethylbenzene were detected in the sample. Ethylbenzene and 1,2,4-trimethylbenzene were previously reported as volatile components in the blue-green alga, *Oscillatoria perornata* (Tellez et al. 2001) and the red alga, *P. palmata* (Le Pape et al. 2004), respectively. Also, 1,2,4-trimethylbenzene has been observed in oysters and crayfish (Pennarun et al. 2002; Tanchotikul and Hsieh 1989). It was described as coming from polysaccharide degradation by Pennarun et al. (2002), but Tanchotikul and Hsieh (1989) explained that carotenoids are thought to be precursors of benzene derivatives such as alkylbenzene. Three xylene isomers *p*-xylene, *m*-xylene, and *o*-xylene were found in both extracts. *p*-Xylene and *o*-xylene have already been identified in the green alga, *Enteromorpha* (Kajiwara et al. 1992), and the cyanobacterium, *O. perornata* (Tellez et al. 2001). Toluene and xylene are also commonly found as native constituents in plant materials (Buttery 1981) and as extraction solvent residual or environment contaminants (Scotter et al. 2000).

The aroma of dimethyl sulfide has been described as onion- or cabbage-like (Vejaphan et al. 1988) or as sulfurous and bad egg-like (MacLeod and Cave 1976). Dimethyl sulfide production from dimethylsulfoniopropionate (DMSP) has long been associated with marine algae (Challenger and Simpson 1948). DMSP is a tertiary sulfonium compound produced in high concentration by certain species of marine algae (Ackman, et al. 1966). Alkylfurans have been reported as a volatile component contributing significantly to an off-flavor of several fats and oils, imparting a beany, grassy odor characteristic of the reversion flavor of soybean oil (Krishnamurthy et al. 1967). 2-Pentylfuran was found to have a green bean, metallic, and vegetable aroma (Fors 1983). Furans could be formed by sugar dehydration or fragmentation from the Maillard reaction (Fors 1983). Five unknown compounds with relatively high peak area (Table 1) were detected. The unknown three (RI=1831) with mass spectral ions at m/s

79(100), 77(63), 91(48), 121(32), 103(15), 117(8.9), and 150(1.6) was the most abundant volatile compound in *C. fulvescens*.

Many of the compounds extracted with the SDE method were considered to be generated from thermal degradation of compounds and/or thermal interactions among the constituents in *C. fulvescens* during storage and steam distillation. Static V-SDE was considered to be a valuable alternative that avoids thermal degradation and formation of thermal artifacts. The present investigation is concerned with the volatile components without giving much consideration to the actual compounds that contributed to the aroma of the fresh *C. fulvescens*. Further research involving GC/olfactometry and sensory characteristics are necessary to screen out those important odorous components characterizing common aroma in *C. fulvescens*. Also, it is important to continue this study to identify those unknown compounds that may be responsible for specific aromas in the green alga and relate their contribution to the development of the characteristic *C. fulvescens* flavor.

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