

Aroma of Fresh Oysters *Crassostrea gigas*: Composition and Aroma Notes

F. Piveteau,[†] S. Le Guen,[†] G. Gandemer,^{‡,§} J.-P. Baud,[#] C. Prost,[†] and M. Demaimay^{*,†}

Ecole Nationale d'Ingénieurs des Techniques et Industries Agro-Alimentaires, Laboratoire de Biochimie Industrielle et Alimentaire, B.P. 82225, 44072 Nantes Cedex 3, France; Institut National de la Recherche Agronomique, Laboratoire d'Etude des Interactions des Molécules Alimentaires, Equipe Lipides, B.P. 71627, 44326 Nantes Cedex 3 France; and IFREMER, Laboratoire Conchylicole des Pays de la Loire, Polder des Champs, 85230 Bouin, France

In contrast to many foods, very little is known about the aroma of fresh oysters. This study deals with the relationship between extracted volatiles of oysters and their olfactory properties. Nearly 50 volatiles were identified: most of them were principally related to fatty acid oxidation (86%) and particularly to *n*-3 polyunsaturated fatty acid oxidation (66%). Only one volatile arose from amino acid degradation. Panelists detected 42 odors by sniffing. Among them, only 12 odors were definitely attributed to identified volatile. These odors were green/sulfur/crustacean, mushroom/citrus, and marine/cucumber notes and were attributed to dimethyl sulfide, 1-penten-3-one, hexanal, (2,4)-*E,E*-heptadienal, 1-octen-3-one, 1-octen-3-ol, 6-methyl-5-hepten-3-one, octanal, (*E,Z*)-2,6-nonadienal, (*E*)-2-octenal, and decanal, respectively.

Keywords: *Oyster (Crassostrea gigas); aroma; volatiles; sniffing*

INTRODUCTION

In France, oysters are eaten fresh at the beginning of meals. As for many foods, aroma is one of the main quality traits of fresh oysters because it determines the acceptance of the product for the consumer before the act of consumption. In contrast to many foods, very little is known about the aroma of fresh oysters. On the one hand, sensory analyses reveal that oysters mainly have mild fresh planty and/or seaweed and also cucumber and melon-like aroma notes (Josephson et al., 1985). It was established that most of the volatiles extracted from fresh oysters arise from enzymatic oxidation of polyunsaturated fatty acids (PUFAs) (Josephson et al., 1985; Josephson, 1991). Among them, 2,6-nonadienal and 3,6-nonadien-1-ol were supposed to be responsible for the cucumber and melon-like aroma of fresh oysters because these molecules are often described in the literature as volatiles having these aroma notes and low odor threshold values. Similarly, sulfur-containing volatiles such as dimethyl sulfide found in fresh oysters were supposed to have a marked influence on aroma because of their typical aroma of onion and sulfur (Ronald and Thompson, 1964) and their low odor threshold values. However, sensory and volatile analyses were reported in separate papers. Consequently, the relationship between aroma notes and volatiles remains to be established. This study deals with the identification and quantification of volatiles extracted from fresh oysters by a purge and trap

method and with the description of aroma notes by sniffing the aroma extracts at the GC port to tentatively identify the most potent molecules involved in fresh oyster aroma.

MATERIALS AND METHODS

Oysters. Oysters (*Crassostrea gigas*) of commercial size were purchased from a private oyster farmer on Bourgneuf Bay in January 1997. They were stored at 2–4 °C before analysis (up to 7 days). The whole weight of oysters and the weight of flesh were measured from 30 oysters. The condition index (CI) was calculated using the formula $CI = (w_d \times 1000) / (w_t - w_s)$, where w_d = flesh dry weight, w_t = oyster total weight, and w_s = shell dry weight (Lawrence and Scott, 1982). This index is a ratio of dry flesh weight to the internal cavity volume of the shell. It is a prime indicator of how well an oyster has utilized the volume available for tissue growth. It is routinely analyzed to provide estimates of factors such as flesh quality and yield. The whole weight, the weight of flesh, and the CI were found to be 63.5 ± 10.6 g, 9.9 ± 0.4 g, and 50.5 ± 10.6 , respectively.

Chemical Analyses of Flesh. Protein, carbohydrate, and glycogen contents of 30 freeze-dried oysters were determined as described by Lowry et al. (1951) and Dubois et al. (1956), respectively. Ash and lipid contents were determined in triplicate on a mixture of 10 fresh oysters ground using an Ultra-Turrax homogenizer. Ash content was determined by incineration of 1 g of this mixture in a muffle furnace at 500 °C. Lipids were extracted from 10 g of the mixture according to the procedure of Folch et al. (1957). A chloroform/methanol mixture (2:1, v/v) containing 0.005% butylated hydroxytoluene (BHT) was used to prevent lipid oxidation. Total lipid content was estimated by gravimetric techniques after the solvent was evaporated under vacuum.

Fatty Acid Composition. Total lipid extracts were transesterified using boron trifluoride/methanol (14%) as described by Morrison and Smith (1964). The fatty acid methyl esters (FAME) were separated by gas-liquid chromatography (GLC) using an HP model 5890 series II chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a split/splitless

* Author to whom correspondence should be addressed (telephone 33-02-51-78-55-11; fax 33-02-51-78-55-20; e-mail demaimay@entiaa.nantes.fr).

[†] Ecole Nationale d'Ingénieurs des Techniques et Industries Agro-Alimentaires.

[‡] Institut National de la Recherche Agronomique.

[§] Present address: Centre Poitou-Charentes, 86600 Luzignan, France.

[#] IFREMER.

injector and a flame ionization detector (FID). The capillary column was 30 m in length and 0.25 mm in i.d. with 0.25 μm film thickness (DB-23, J&W Scientific, Folsom, CA). Helium was used as carrier gas with the head pressure set at 103 kPa (15 psi), and the split ratio was 1:20. The temperatures of the injector and the detector were set at 250 and 280 $^{\circ}\text{C}$, respectively. The oven temperature program involved an initial hold at 150 $^{\circ}\text{C}$ for 3 min, followed by an increase from 150 to 180 $^{\circ}\text{C}$ at the rate of 10 $^{\circ}\text{C min}^{-1}$, then a hold at 180 $^{\circ}\text{C}$ for 7 min, followed by another increase to 215 $^{\circ}\text{C}$ at the rate of 5 $^{\circ}\text{C min}^{-1}$, before a final hold at 215 $^{\circ}\text{C}$ for 15 min.

FAME were identified by comparing their retention times with those of standard mixtures (Sigma Chemical Co., Saint Louis, MO). The identification of each compound was confirmed by gas chromatography–mass spectrometry (GC-MS) performed on selected samples. GC-MS analyses were performed with an HP model 5890 series II chromatograph fitted with an HP5971 mass selective detector (MSD). The capillary column, injector, and chromatography conditions were the same as those described above. The conditions for the MSD were as follows: source temperature, 180 $^{\circ}\text{C}$; mass range, m/z 33–350 amu; and scan rate, 1.9 s^{-1} . FAME of oyster samples were identified by comparing their spectra with those of a commercial spectra database (NBS 75k and internal library of the laboratory).

Analysis of Volatile Compounds. *Dynamic Headspace Volatile Concentration.* A purge-and-trap concentrator (model LSC 2000; Tekmar Inc., Cincinnati, OH) equipped with a capillary interface for cryofocusing was connected to a gas chromatograph Varian Star 3400 (Varian, Palo Alto, CA). Ten fresh oysters were ground using an Ultra-Turrax homogenizer: 400 μL of an aqueous solution of 1 $\mu\text{g } \mu\text{L}^{-1}$ of *p*-cymene (Aldrich Chemical Co.) was added as internal standard. Fifteen grams of this mixture was immediately transferred into a flask. The headspace of the sample was purged with helium at 60 mL min^{-1} and swept into a porous adsorbent polymer (Tenax trap) at room temperature. Volatile compounds were thermally desorbed by heating the trap at 200 $^{\circ}\text{C}$. Backflushed volatile compounds were cryofocused at -40 $^{\circ}\text{C}$ using carbon dioxide.

GC-FID. A gas chromatograph (Star 3400, Varian) equipped with a flame ionization detector was used. Volatile compounds were separated on a capillary column coated with DB-Wax stationary phase (60 $\text{m} \times 0.32$ mm, 0.5 μm film thickness) (J&W Scientific). Helium was used as carrier gas with the head pressure set at 72 kPa (1 mL min^{-1}). The temperature of the detector was set at 250 $^{\circ}\text{C}$. The program of oven temperature involved an initial hold at 50 $^{\circ}\text{C}$ for 6 min, followed by an increase from 50 to 130 $^{\circ}\text{C}$ at rate of 1 $^{\circ}\text{C min}^{-1}$. The oven temperature was further increased from 130 to 240 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C min}^{-1}$ before a final hold at 240 $^{\circ}\text{C}$ for 15 min. The content of each compound was expressed as nanograms of an internal standard (*p*-cymene).

GC-MS. Extraction, desorption, and chromatographic conditions of volatile compounds were performed as described above. GC-MS consisted of an HP5890 II gas chromatograph and an HP5971 II MSD (Hewlett-Packard Co.). Helium was the carrier gas (34 kPa, 1 mL min^{-1}), and the capillary column was coated with a DB-Wax stationary phase (60 $\text{m} \times 0.32$ mm, 0.5 μm film thickness). The conditions for the MSD were as follows: electron impact mode, 70 eV; temperature of interface, 250 $^{\circ}\text{C}$; source temperature, 180 $^{\circ}\text{C}$; mass range, m/z 33–300 amu; scan rate, 1.9 s^{-1} .

The volatile compounds were identified by comparing their spectra with those of a commercial spectra database (NBS 75k and internal library of the laboratory) or standard molecules (Sigma Chemical Co.) and by matching their retention indices with those of the literature (Jennings and Shibamoto, 1980; Tanchotikul and Hsieh, 1989, 1991).

GC-FID–Olfactometry (GC-FID–O). Extraction and desorption of volatile compounds were performed as described above. A gas chromatograph (Star 3400 Varian, Palo Alto, CA) equipped with a capillary column coated with DB-Wax stationary phase (30 $\text{m} \times 0.32$ mm, 0.5 μm film thickness) (J&W Scientific, Folsom, CA) was used. Helium was used as carrier

Table 1. Fatty Acid Composition^a of Oyster Total Lipids

oysters		oysters	
16:0	18.7 \pm 0.1	18:2 <i>n</i> -6	1.3 \pm 0.1
18:0	4.1 \pm 0.3	20:4 <i>n</i> -6	2.6 \pm 0.1
other saturated	5.6 \pm 0.4	PUFAs <i>n</i> -6	4.4 \pm 0.2
total saturated	28.3 \pm 0.7		
		20:5 <i>n</i> -3	18.2 \pm 0.5
16:1 <i>n</i> -7	2.9 \pm 0.1	22:6 <i>n</i> -3	17.8 \pm 0.7
18:1 <i>n</i> -7	4.7 \pm 0.4	PUFA <i>n</i> -3	41.5 \pm 1.5
20:1 <i>n</i> -7	4.1 \pm 0.0	other PUFAs	12.0 \pm 0.4
MUFAs <i>n</i> -7	11.7 \pm 0.3	total PUFAs	51.8 \pm 1.4
16:1 <i>n</i> -9	0.4 \pm 0.4		
18:1 <i>n</i> -9	3.4 \pm 0.3		
20:1 <i>n</i> -9	0.5 \pm 0.0		
MUFAs <i>n</i> -9	4.4 \pm 0.6		
other MUFAs	3.1 \pm 0.0		
total MUFAs	19.1 \pm 0.3		

^a Results are expressed as the percentage of total fatty acids (mean of three determination \pm confidence interval at 95%).

gas with the head pressure set at 69 kPa (2 mL min^{-1}). The oven temperature program involved an initial hold at 50 $^{\circ}\text{C}$ for 5 min, followed by an increase from 50 to 80 $^{\circ}\text{C}$ at rate of 1 $^{\circ}\text{C min}^{-1}$. The oven temperature was further increased from 80 to 250 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C min}^{-1}$. CG effluent was split (1:1, v/v) into the FID at 250 $^{\circ}\text{C}$ and a sniffing port supplied with humidified air at 40 $^{\circ}\text{C}$ using deactivated and uncoated fused silica capillaries (30 $\text{cm} \times 0.3$ μm).

Olfactometry Global Analysis. According to the guidelines of Pollien et al. (1997), a panel of nine judges was trained for odor detection and description using numerous standard compounds with various odors and thresholds. Each person sniffed the 50 min chromatogram but during two distinct 25 min sessions to remain alert. Each panelist sniffed the first 25 min of the chromatogram followed by another person until all 9 had sniffed a given CG run. The results of the nine aromagrams were summed. Response values are the number of assessors who detected an odor at a given retention time of the GC effluent. An odor detected by fewer than three assessors is considered as noise (Van Ruth and Roozen, 1995); consequently, only odors perceived by at least four panelists were reported. The molecule supposed to be responsible for the odor was chosen among the molecules identified in the aroma extracts according to the following criteria:

- The retention index (RI) of the molecule was close to that of the odor reported by the panelist.
- The words used to describe the molecule were similar in the literature and in the present study.
- The odor reported by the panelists for the corresponding standard molecule is similar to that reported in the present study.

RESULTS AND DISCUSSION

Chemical Composition. Proteins and ashes were the predominant constituents of the flesh of oysters (35.3 and 19.3% of dry matter, respectively), whereas lipids, carbohydrates, and glycogen were in lower amounts (8.4, 7.5, and 5.2% respectively). The fatty acid composition of oysters was characterized by a high proportion of PUFAs (51.8% of total lipids) (Table 1). The major PUFAs were eicosapentaenoic acid (20:5 *n*-3) and docohexaenoic acid (22:6 *n*-3). These two fatty acids represented 36.0%. The proportion of *n*-6 PUFAs was found to be low (4.4%). The saturated and monounsaturated fatty acids amounted for 28.3 and 19.1%, respectively. Palmitic acid (16:0) and stearic acid (18:0) were the main saturated fatty acids, whereas the main monounsaturated fatty acids (MUFAs) were found to be 18:1 and 20:1. The results of chemical composition are quite similar to those reported in the literature for oysters reared in similar environmental conditions

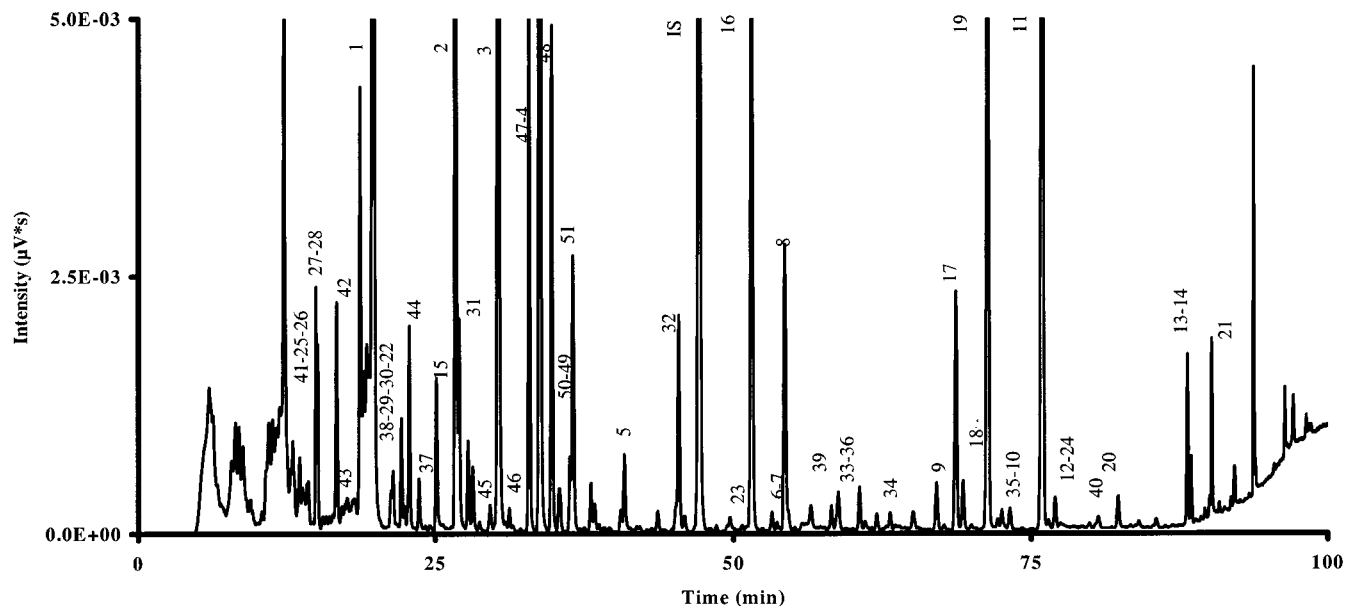


Figure 1. Gas chromatogram of volatile compounds from oysters after purge-and-trap extraction (numbers refer to the compounds in Table 2; IS, internal standard).

(Holland and Hannal, 1974; Tagaki et al., 1986; Josephson et al., 1985; Deslou-Paoli, 1988).

Volatile Compounds. Fifty-two volatile compounds were identified (Figure 1). The average amount of total volatiles was 4.000 ng equiv of *p*-cymene g^{-1} . Alcohols (11), hydrocarbons (19), aldehydes (14), and ketones (7) predominated, whereas only one sulfur-containing compound was identified (Table 2). Two-thirds (2754 ng equiv of *p*-cymene g^{-1}) of the total desorbed volatile compounds arose from *n*-3 PUFA oxidation. Alcohols and ketones were in the highest proportions. The main ones were 1-penten-3-one (900 ng equiv of *p*-cymene g^{-1}), 1-penten-3-ol (500 ng equiv of *p*-cymene g^{-1}), and (*Z,Z*)-1,5-octadien-3-ol (450 ng equiv of *p*-cymene g^{-1}). This result is consistent with the large amount of *n*-3 PUFAs found in oysters and with the fact that *n*-3 PUFAs are very sensitive to oxidation. In oysters most of the volatiles arising from *n*-3 PUFAs are formed through enzymic processes (Josephson, 1991). The 20:5 *n*-3 and 22:6 *n*-3, which are the main *n*-3 PUFAs in oysters, have been reported to be the most likely substrates for enzymatic oxidation producing mainly aldehyde and alcohol volatile compounds (Josephson et al., 1985).

The second group included the volatile compounds arising from the oxidation of *n*-6 PUFAs, and they accounted for 13% (545 ng equiv of *p*-cymene g^{-1}). They mainly were eight- and nine-carbon compounds. Among them, 1-octen-3-ol and 1-octen-3-one were detected at the highest amounts (241.2 and 154.5 ng equiv of *p*-cymene g^{-1} , respectively). These volatiles are formed from linoleic acid oxidation (Grosch, 1987). The proportion of oxidation products arising from *n*-6 PUFAs was high with regard to the low proportion of these fatty acids in oysters (4%). This result corroborates previous ones which indicated that these volatiles are widely represented in seafood aroma (Josephson, 1991).

The volatile compounds arising from the oxidation of *n*-9 MUFAs were aldehydes. They were present in small amounts (29.6 ng equiv of *p*-cymene g^{-1}). This is consistent with the low amount of these fatty acids in oysters and the low sensitivity of these fatty acids to

oxidation. The most abundant was 2-ethylpropanal (21.7 ng equiv of *p*-cymene g^{-1}).

Other volatiles definitely arose from fatty acid oxidation, but the exact origin remains unknown because these volatiles can be formed through the oxidation of various fatty acids. They were mainly 3-octanone (60 ng equiv of *p*-cymene g^{-1}), two isomers of 2,4-octadiene (28–44 ng equiv of *p*-cymene g^{-1}), two isomers of 1,3-octadiene (27–37 ng equiv of *p*-cymene g^{-1}), and (*Z,Z,Z*)-1,3,5-octatriene (52 ng equiv of *p*-cymene g^{-1}). These aliphatic hydrocarbons have already been found in oysters and fish (Hirano et al., 1982; Josephson, 1991). According to Josephson (1991), 1,3,5-octatriene could arise from the dehydration from (*E,Z*)-1,5-octadien-3-ol.

A small amount of the desorbed volatile compounds came from carotenoid oxidation (21.5 ng equiv of *p*-cymene g^{-1}). They are toluene and 6-methyl-5-hepten-3-one, which were previously found in oysters and fish (Josephson, 1991). The carotenoids may have originated from foods consumed by oysters; they were present in algae liable to be eaten by oysters (Groth-Nard, 1994).

Only one volatile arising from amino acid degradation was identified. It was dimethyl disulfide, which accounted for only 0.3% of the total volatile compounds. Dimethyl disulfide is reported to arise from the oxidation of methanethiol, a bacterial degradation product of methionine (Tanchotikul and Hsieh, 1989).

A last group included the volatile compounds having miscellaneous origins. Benzaldehyde was present in trace amount and could arise from amino acid degradation as described in other foods (Thierry et al., 1999). Nonane, pentanediene, and 3-pentanone synthesis could be possible in environments rich in lipids (Grosch, 1987). Aromatic hydrocarbons accounted for 21.6 ng equiv of *p*-cymene g^{-1} , and cyclic alkenes with five carbon atoms were present (380 ng equiv of *p*-cymene g^{-1}), but their sources are unknown.

Oyster Aroma Notes and Related Volatile Compounds. Forty-two aroma notes were detected (Figure 2). Among them, 22 were perceived by at least 7 of 9 assessors. Odors described as green/garlic/sulfur were

Table 2. Volatile Compounds Identified in Oyster *C. gigas* According to Their Most Likely Origin

peak ^g	compound	method of identification [*]	RI ^f	origin	quantity extracted (ng equiv of <i>p</i> -cymene g ⁻¹)	
					mean	range
<i>n</i>-3 PUFA oxidation					2754.5	
1	1-penten-3-one	MS, standard, RI ^{b,d}	1026	<i>n</i> -3	900.3	791–1061
2	(<i>E,E,Z</i>)-1,3,5-octatriene	MS tentatively	1109	<i>n</i> -3	265.9	193–368
3	(<i>E</i>)-2-pentenal	MS, standard, RI ^{c,d}	1138	<i>n</i> -3	423.6	377–499
4	1-penten-3-ol	MS, standard, RI ^{a,c,d}	1173	<i>n</i> -3	500.2	362–593
5	(<i>E</i>)-2-hexenal	MS, standard, RI ^{a,c,d}	1228	<i>n</i> -3	27.0	13–47
6	cyclopentanol	MS, standard, RI ^{a,d}	1327	<i>n</i> -3	6.6	5–8
7	(<i>E</i>)-2-penten-1-ol	MS, RI ^{c,d}	1333	<i>n</i> -3	4.2	2–6
8	(<i>Z</i>)-2-penten-1-ol	MS, standard, RI ^{c,d}	1334	<i>n</i> -3	105.2	82–120
9	(<i>Z</i>)-3-hexen-1-ol	MS, standard, RI ^a	1416	<i>n</i> -3	5.6	3–7
10	2,4-heptadienal ^e	MS tentatively	1470	<i>n</i> -3	6.4	4–10
11	(<i>E,E</i>)-2,4-heptadienal	MS, standard, RI ^{c,d}	1496	<i>n</i> -3	10.2	5–18
12	(<i>Z,Z</i>)-1,5-octadien-3-ol	MS tentatively	1504	<i>n</i> -3	450.4	308–622
13	(<i>E,Z</i>)-2,6-nonadienal	MS, standard, RI ^{b,d}	1591	<i>n</i> -3	39.7	19–71
14	(<i>E,E</i>)-2,4-octadienal	MS, standard, RI ^c	1595	<i>n</i> -3	14.0	11–18
<i>n</i>-6 PUFA oxidation					544.8	
15	hexanal	MS, standard, RI ^{a,c,d}	1091	<i>n</i> -6	28.7	15–40
16	1-octen-3-one	MS, standard	1311	<i>n</i> -6	154.5	132–178
17	(<i>E</i>)-2-octenal	MS, standard, RI ^{a,b,d}	1434	<i>n</i> -6	60.5	34–88
18	(<i>Z</i>)-2-octenal	MS tentatively	1439	<i>n</i> -6	16.6	12–19
19	1-octen-3-ol	MS, standard, RI ^{a,c,d}	1460	<i>n</i> -6	241.2	200–313
20	(<i>E</i>)-2-nonenal	MS, standard, RI ^{a,b,d}	1541	<i>n</i> -6	9.1	5–15
21	(<i>E</i>)-2-octen-1-ol	MS, standard	1626	<i>n</i> -6	34.2	23–54
<i>n</i>-9 MUFA oxidation					29.6	
22	2-ethylpropenal	MS tentatively	1055	<i>n</i> -9	21.7	20–24
23	octanal	MS, standard, RI ^{a,b,d}	1298	<i>n</i> -9	4.5	3–6
24	decanal	MS, standard, RI ^{a,c,d}	1510	<i>n</i> -9	4.9	4–5
fatty acid oxidation					293.7	
25	2,4-octadiene	MS tentatively	919	lipids	28.4	14–55
26	2,4-octadiene ^e	MS tentatively	930	lipids	44.5	14–84
27	1,3-octadiene ^e	MS tentatively	954	lipids	37.2	28–51
28	1,3-octadiene ^e	MS tentatively	958	lipids	27.3	21–38
29	(<i>E,E,E</i>)-1,3,6-octatriene	MS tentatively	1048	lipids	15.1	12–20
30	1,3,6-octatriene ^e	MS tentatively	1055	lipids	4.2	3–6
31	(<i>Z,Z,Z</i>)-1,3,5-octatriene	MS tentatively	1121	lipids	52.4	40–75
32	3-octanone	MS, standard, RI ^{b,d}	1265	lipids	59.5	28–94
33	hexanol	MS, standard, RI ^{a,b,d}	1365	lipids	12.9	10–15
34	2-nonanone	MS, standard, RI ^{a,b,d}	1395	lipids	4.8	4.6–5.0
35	heptanol	MS, standard, RI ^{a,c,d}	1467	lipids	7.3	5–12
36	1-hepten-3-ol	MS, standard, RI ^a	1362	lipids	6.4	5–8
amino acid degradation					10.4	
37	dimethyl disulfide	MS, standards, RI ^{a,b,d}	1085	amino acid	10.4	10–11
carotenoid degradation					17.1	
38	toluene	MS, standard, RI ^b	1043	carotenoids	8.4	6–12
39	6-methyl-5-hepten-2-one	MS, standard, RI ^{a,b,d}	1346	carotenoids	8.7	8–9
unknown origin					536.6	
40	benzaldehyde	MS, standard, RI ^{a,c,d}	1525	unkown	4.5	4–5
41	nonane	MS, standard, RI ^a	895	unkown	10.2	8–12
42	3-pentanone	MS tentatively	986	unkown	60.9	41–91
43	pentanediene ^e	MS, standard	994	unkown	12.7	2–30
44	2,3-pentanedione	MS, standard, RI ^{a,c,d}	1061	unkown	49.1	36–62
45	ethylbenzene	MS, RI ^b	1131	unkown	10.8	7–15
46	<i>p</i> -xylene	MS, RI ^{a,b}	1145	unkown	4.4	3–6
47	3-ethylidene-1-methylcyclopentene ^e	MS tentatively	1168	contaminant	138.9	103–205
48	3-ethylidene-1-methylcyclopentene ^e	MS tentatively	1178	contaminant	138.5	104–203
49	methylthiocyclopentane	MS tentatively	1184	contaminant	16.3	12–20
50	3-ethylidene-1-methylcyclopentene ^e	MS tentatively	1195	contaminant	74.8	59–104
51	3-ethylidene-1-methylcyclopentene ^e	MS tentatively	1207	contaminant	12.4	10–17
52	styrene	MS, RI ^{b,d}	1262	unkown	6.4	

*Retention index: ^aJennings and Shibamoto (1980); ^bTanchotikul and Hsieh (1989); ^cTanchotikul and Hsieh (1991); ^dENITBIO (internal library of the laboratory). ^e Configuration of isomer not determined. ^f Retention index on column DB-Wax (60 m). ^g Numbers correspond to those in Figure 1.

mainly perceived at the beginning of the aromagram [retention index (RI) <1280]. Then, aroma notes described as citrus and mushroom-like were detected in the RI region between 1180 and 1497. At the end of the aromagram (RI region >1400), several odors were described as green/cucumber and marine. Among these

aroma notes, sulfur, cucumber, and marine notes were previously reported in oysters (Ronald and Thompson, 1964; Josephson et al., 1985) and could be considered as typical aroma notes of oysters. Mushroom and citrus aroma notes were not described in the previous works on oysters. However, it was not surprising to find them

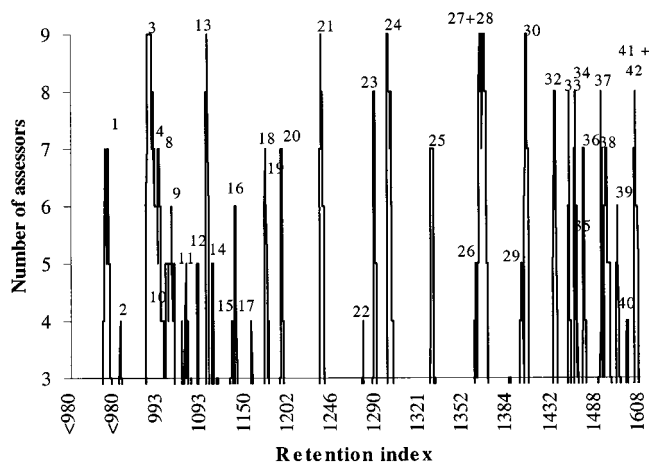


Figure 2. Sniffing chromatograms of volatile compounds of oyster (numbers refer to the compounds in Table 3).

in the aroma of oysters because these notes, mainly mushroom-like, are generally associated with volatile compounds arising from lipid oxidation such as 1-octen-3-ol, 1-octen-3-one, and (*E,Z*)-1,5-octadien-3-ol (Whitfield, 1982), which were abundant in the aroma extracts in the present study.

Among the 42 detected odors (Table 3), only 12 odors were definitely attributed to volatile compounds. This gap had two main reasons. First, some odors were detected at an RI at which no compound was identified, probably because the compound was present in minute amount. Second, in some cases, a volatile was identified at an RI close to that of aroma notes but the word used to describe the aroma note differed from that of the corresponding pure molecule at the sniffing port.

Three odors were definitely attributed to *n*-3 PUFA oxidation products. They included major compounds such as 1-penten-3-one and also minor compounds such as (*E,E*)-2,4-heptadienal and (*E,Z*)-2,6-nonadienal. In the present study, 1-penten-3-one was described as green. This result corroborates previous results on several seafoods (Tanchotikul and Hsieh, 1989, 1991; Prost et al., 1998) in which 1-penten-3-one was an abundant volatile compound and was described as green and grassy. (*E,E*)-2,4-Heptadienal was perceived as having a green and mushroom-like odor, and we found it in low amount, suggesting that this compound has a very low odor threshold. This compound has been currently described in the aroma of oysters (Josephson, 1991), fish (Milo and Grosch, 1996), crayfish (Tanchotikul and Hsieh, 1989), and marine green algae (Sugisawa et al., 1980), but its odor was reported to be green and grass. To take up this ambiguity, the pure (*E,E*)-2,4-heptadienal was injected in the GC. Our panelists described the pure molecule as both green and mushroom, which confirmed the possibility to associate mushroom description to this molecule. One odor described as green and cucumber was attributed to (*E,Z*)-2,6-nonadienal in the present study. (*E,Z*)-2,6-Nonadienal was currently reported in the aroma of food because of its very low threshold (0.01–1 ppb) (Furia, 1980; Leffingwell and Leffingwell, 1991; Josephson, 1991). This molecule could play a key role in oyster aroma (Josephson et al., 1985) and in various fish aromas (Hirano et al., 1992; Milo and Grosch, 1995, 1996; Triqui and Reineccius, 1995; Prost et al., 1998).

Four odors were definitely attributed to oxidation products of *n*-6 PUFAs: grass to hexanal, cucumber

and almond to (*E*)-2-octenal, and mushroom to 1-octen-3-ol and 1-octen-3-one. The odor of hexanal is currently described as green freshly cut grass and aldehydic, and generally 1-octen-3-ol and 1-octen-3-one were associated with a mushroom-like odor (Tanchotikul and Hsieh, 1989; Josephson, 1991). These molecules are often found in the aroma of many seafoods such as fish and crustacean (Whitfield, 1982; Milo and Grosch, 1996; Cadwallader et al., 1995; Prost et al., 1998). The authors attributed a key role in seafood aroma and fish because to these molecules because of their low threshold of perception [between 0.05 and 10 ppb in water (Josephson, 1991; Leffingwell and Leffingwell, 1991)]. (*E*)-2-Octenal was described as cucumber and almond in the present study. (*E*)-2-Octenal was currently found in the aroma of seafoods (Hirano et al., 1982; Milo and Grosch, 1993; Baek et al., 1996; Prost et al., 1998), and it was described under various terms such as lemon or cucumber, seaweed, or also nut-like odor (Furia, 1980; Josephson, 1991; Milo and Grosch, 1993). To take up this ambiguity, the pure (*E*)-2-octenal was injected in the GC and described as cucumber by our panelists.

Three odors were associated with volatile compounds arising from others lipids. Two odors could be related to *n*-9 MUFA oxidation. One described as citrus-like was attributed to octanal, which possessed an RI and an odor in the literature close to those of the odor smelled. Generally, octanal was associated with citrus odor and described in the aroma of fish and crustacean (Milo and Grosch, 1993, 1995, 1996; Tanchotikul and Hsieh, 1991). One odor described as marine was attributed to decanal according to Furia (1980) and its retention index. This compound has been already found in molluscs such as clam (Tanchotikul and Hsieh, 1991) and was a potent aroma of green algae with fatty attributes (Sugisawa et al., 1980). One odor described as citrus-like was attributed to 6-methyl-5-hepten-3-one, which was also found for crayfish and clam (Tanchotikul and Hsieh, 1989, 1991). 6-Methyl-5-hepten-3-one came from carotenoid degradation (Josephson, 1991), and it was described as citrus-like (Furia, 1980).

One odor perceived as having green and cucumber notes was tentatively attributed to (*E,E*)-2,4-octadienal, the origin of which was unknown. In a previous study, 2,4-octadienal was described as a potent aroma of fresh fish such as anchovy (*Engraulis encrasicolus* L.) and turbot with deep-fried fat and pine/resin attributes (Triqui and Reineccius, 1995; Prost et al., 1998). The apparent discrepancy in odor description could be explained by the configuration of isomers that was not well-defined. Indeed, the configuration of isomers could affect the specific odor of 2,4-octadienal, as was observed for 2-heptenal. (*Z*)-2-Heptenal has a typical odor of fish meal and cardboard when (*E*)-2-heptenal exhibits a typical odor of green and turnip (McGill et al., 1974).

Several green and garlic odors were described by panelists at the beginning of the aromagram (900 < RI < 1000). According to the literature, these odors could be associated with sulfur-containing molecules, which were found in the aroma of garlic or onion (Maarse et al., 1989). In a previous study, these garlic odors were described in the aroma of prawns (Whitfield, 1990) and were attributed to bis(methylthio)methane and trimethylarsine. However, we have not found these compounds in volatile extracts from oysters.

One odor described as sulfur, crustacean, and green was attributed to an amino acid degradation product,

Table 3. Odor-Active in Purge-and-Trap Extract of Oyster

peak ^a	RI ^b	detection frequency ^c	odor descriptor ^d	identified compound ^f	tentative identification ^m	origin
1	<900	7	crustacean, green, sulfur	dimethyl sulfide		amino acid
2	<900	4	<i>e</i>			
3	928	9	green, garlic-like, sulfur			
4	946	7	green, sulfur			
5	939–971	8	green, garlic-like, sulfur			
6	983	7	garlic-like, sulfur			
7	999	7	green, garlic-like, sulfur			
8	1009	5	garlic-like, green			
9	1018	6	green	1-penten-3-one ^g		<i>n</i> -3
10	1048	4	green, zested			
11	1056	5	buttery, milky	2,3-pentanedione ^g		unknown
12	1083	5	green	hexanal ^{g,i}		<i>n</i> -6
13	1103	9	phenol, green			
14	1110	5	alcohol			
15	1137	4	green, marine			
16	1141	6	green			
17	1160	4	boiled potato			
18	1180	7	alcohol			
19	1183	4	orange			
20	1200	7	garlic-like, sulfur, green			
21	1239	9	boiled potato			
22	1278	4	sulfur			
23	1289	8	citrus-like	octanal ^{k,l}		<i>n</i> -9
24	1302	9	mushroom	1-octen-3-one ^{g,i}		<i>n</i> -6
25	1334	7	lemon, citrus-like	6-methyl-5-hepten-3-one ^g		carotenoid
26	1367	5	mushroom, mossy-earthy			
27	1370	9	mushroom			
28	1372	9	musty, mossy-earthy, mushroom			
29	1402	5	cucumber			
30	1406	9	mushroom			
31	1408	6	mushroom			
32	1441	8	cucumber, almond	(<i>E</i>)-2-octenal ^{i,j}		<i>n</i> -6
33	1458	8	marine, roasted			
34	1467	8	green, marine, boiled potato			
35	1469	6	fruity, boiled potato			
36	1476	7	mushroom	1-octen-3-ol ^{g,h,i}		<i>n</i> -6
37	1497	8	green, mushroom	(<i>E,E</i>)-2,4-heptadienal ^{g,j}		<i>n</i> -3
38	1510	7	marine, cucumber	decanal ^g		<i>n</i> -9
39	1543	6	roasted, plastic			
40	1572	4	green, cucumber			
41 +	1590	8	green, cucumber	(<i>E,Z</i>)-2,6-nonadienal ^{i,j}		<i>n</i> -3
42					(<i>E,E</i>)-2,4-octadienal	unknown

^a Numbers correspond those in Figure 2. ^b Retention index on column DB-Wax (30 m). ^c Detection frequencies (number of panelists who detect an odor at this time of GC effluent). ^d Odor description as perceived by panelists during olfactometry global analysis. ^e Odor detected without common descriptor for most of the panelists. ^f Odorant that possessed a retention index and an odor in the literature close to those of the odor smelled. ^g Furia (1980). ^h Tanchotikul and Hsieh (1989). ⁱ Josephson (1991). ^j standard. ^k Milo and Grosch (1995). ^l Milo and Grosch (1996). ^m Odorant that possessed a retention index in the literature close to that of the odor smelled.

dimethyl sulfide. This compound was previously found in seafood aroma and often described as the most odorant compound of fresh oyster (Ronald and Thompson, 1964), clam (Tanchotikul and Hsieh, 1991), and boiled fish (Milo and Grosch, 1996). In the present study, dimethyl sulfide was present in minute amount, and its contribution to the overall aroma could be explained by its low threshold value [0.3–1 ppb in water (Furia, 1980)]. To confirm the presence of dimethyl sulfide, the pure molecule was injected in the GC. Our panelists have described the pure molecule as both crustacean and sulfur at the same RI as the odor smelled.

Conclusion. This work has established that the main part of the volatiles of oysters were volatiles arising from the fatty acid oxidation, mainly *n*-3 PUFAs. This is consistent with the large proportion of these fatty acids in oysters. Forty-two odors were described by the panelists sniffing the aroma extract at the GC port. The main aroma notes were green/sulfur/crustacean, mushroom/citrus-like, and marine/cucumber. These aroma notes were previously reported in various seafoods. Some of them such as mushroom and green can be

considered as atypical aroma notes because they are reported in many foods. Aroma notes such as crustacean, cucumber, and marine can be considered typical aroma notes of oysters and were previously described in the aroma of oysters by several authors. Unfortunately, only 12 aroma notes were definitely attributed to identified volatile compounds. Further studies on aroma dilution analysis are needed to assess the relative intensity of these notes and compounds associated with the oyster aroma. Further investigations could be also required to elucidate the identification of unidentified compounds. Probably, higher amounts of these compounds should be needed in these studies.

ACKNOWLEDGMENT

We thank the panelists of the ENITIAA laboratory, who spent a good deal of time describing the volatile compounds of oysters.

LITERATURE CITED

Baek, H. H.; Cadwallader, K. R. Volatile compounds in flavor concentrates produced from crayfish-processing byproducts

- with and without protease treatment. *J. Agric. Food Chem.* **1996**, *44*, 3262–3267.
- Cadwallader, K. R.; Tan, Q.; Chen, F.; Meyers, S. P. Evaluation of the aroma of cooked spiny lobster tail meat by aroma extract dilution analysis. *J. Agric. Food Chem.* **1995**, *43*, 2432–2437.
- Deslous-Paoli, J.-M.; Héral, M. Biochemical composition and energy value of *Crassostrea gigas* (Thunberg) cultured in the bay of Marennes—Oléron. *Aquat. Living Resour.* **1988**, *1*, 239–249.
- Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *18*, 350–356.
- Folch, J.; Lees, M.; Sloane Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509.
- Furia, T. E. In *CRC Handbook of Food Additives*, 2nd ed.; CRC Press: Boca Raton, FL, 1980; Vol. II, pp 259–302.
- Grosch, W. Reactions of hydroperoxides-products of low weight. In *Autoxidation of Unsaturated Lipids*; Chan, H. W.-S., Ed.; Academic Press: London, U.K., 1987; pp 95–139.
- Groth-Nard, C. Les lipides des diatomées: exemple d'*Haslea ostrearia* (Simonsen), responsable du verdissement des huîtres. Ph.D. Dissertation, The University of Nantes (France), 1994.
- Hirano, T.; Zhang, C.-H.; Morishita, A.; Susuki, T.; Shirai, T. Identification of volatile compounds in Ayu fish and its feeds. *Nippon Suisan Gakkaishi* **1992**, *58*, 547–557.
- Holland, D. L.; Hannant, P. J. Biochemical changes during growth of the spat of the oyster *Ostrea edulis* L. *J. Mar. Biol. Assoc. U.K.* **1974**, *54*, 1007–1016.
- Jennings, W.; Shibamoto, T. *A Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*; Academic Press: London, U.K., 1980; 472 pp.
- Josephson, D. B. Mechanisms for the formation of volatiles in seafood flavors. Ph.D. Dissertation, University of Wisconsin, 1987; 450 pp.
- Josephson, D. B. Seafood. In *Volatile Compounds in Foods and Beverages*; Dekker: New York, 1991; pp 179–202.
- Josephson, D. B.; Lindsay, R. C.; Stuibler, D. A. Volatile compounds characterizing the aroma of fresh Atlantic and Pacific oysters. *J. Food Sci.* **1985**, *50*, 5–9.
- Lawrence, D. R.; Scott, G. I. The determination and use of condition index of oysters. *Estuaries* **1982**, *5*, 23–27.
- Leffingwell, J. C.; Leffingwell, D. Gas flavor chemicals-detections threshold. *Perfum. Flavor.* **1991**, *16*, 1–19.
- Lowry, O. H.; Rosenbrough, N. T.; Fau, A. L.; Randall, R. J. Protein measurement with the Folin reagent. *J. Biol. Chem.* **1951**, *193*, 256–275.
- Maarse, H.; Visscher, C. A. Volatile compounds in food. *Qualitative and Quantitative Data*; TNO Biotechnology and Chemistry Institute: Zeist, The Netherlands, 1991; Supplement 2, pp 1–20.
- McGill, A. S.; Hardy, R.; Burt, J. R. Hept-*cis*-4-enal and its contribution to the off-flavour in cold stored cod. *J. Sci. Food Agric.* **1974**, *25*, 1477–1489.
- Milo, C.; Grosch, W. Changes in the odorants of boiled trout (*Salmo fario*) as affected by the storage of the raw material. *J. Agric. Food Chem.* **1993**, *41*, 2076–2081.
- Milo, C.; Grosch, W. Detection of odor in boiled cod and trout by gas chromatography-olfactometry of headspace samples. *J. Agric. Food Chem.* **1995**, *43*, 459–462.
- Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem.* **1996**, *43*, 2366–2371.
- Morrison, W. R.; Smith, L. M. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipids Res.* **1964**, *5*, 600–608.
- Pollien, P.; Ott, A.; Montignon, F.; Baumgartner, M.; Munoz-Box, R.; Chaintreau, A. Hyphenated headspace-gas chromatography-sniffing technique screening of impact odorants and quantitative aromagram comparaisons. *J. Agric. Food Chem.* **1997**, *45*, 2630–2637.
- Prost, C.; Sérot, T.; Demaimay, M. Identification of the most odorants in wild and farmed turbot (*Scophthalmus maximus* L.). *J. Agric. Food Chem.* **1998**, *46*, 3214–3219.
- Ronald, A. P.; Thompson, W. A. B. The volatile sulfur compounds of oysters. *J. Fish. Res. Board Can.* **1964**, *21*, 1481–1487.
- Sugisawa, H.; Nakamura, K.; Tamura, H. The aroma profile of the volatiles in marine green algae (*Ulva pertusa*). *Food Rev. Int.* **1990**, *6*, 573–589.
- Tagaki, T.; Itabashi, Y.; Kaneniva, M. Fatty acid composition of bivalves from Japanese waters. *J. Jpn. Oil Chem. Soc.* **1986**, *35*, 517–521.
- Tanchotikul, U.; Hsieh, T. C.-Y. Volatile flavor components in crayfish waste. *J. Food Sci.* **1989**, *54*, 1515–1520.
- Tanchotikul, U.; Hsieh, T. C.-Y. Analysis of volatile flavor components in steamed rangia clam by dynamic headspace sampling and simultaneous distillation and extraction. *J. Food Sci.* **1991**, *56*, 327–331.
- Thierry, A.; Maillard, M. B.; Le Quéré, J.-L. Dynamic headspace analysis of emmental aqueous phase as a method to quantify changes in volatile flavour compounds during ripening. *Int. Dairy J.* **1999**, *9*, 453–463.
- Triqui, R.; Reineccius, G. A. Flavor development in the ripening of anchovy (*Engraulis encrasischolus* L.). *J. Agric. Food Chem.* **1995**, *43*, 453–458.
- Van Ruth, S. M.; Roozen, J. P.; Posthumus, M. A. Instrumental and sensory evaluation of the flavour of dried french beans (*Phaseolus vulgaris*) influenced by storage conditions. *J. Agric. Food Chem.* **1995**, *69*, 393–401.
- Whitfield, F. B. Flavour of prawns and lobsters. *Food Rev. Int.* **1990**, *6*, 505–519.
- Whitfield, F. B.; Freeman, D. J.; Last, J. H.; Bannister, P. A.; Kennett, B. H. Oct-1-en-3-ol and (5*Z*)-octa-1,5-dien-3-ol, compounds important in the flavour of prawns and sand-lobsters. *Aust. J. Chem.* **1982**, *35*, 373–383.

Received for review December 22, 1999. Revised manuscript received June 30, 2000. Accepted July 21, 2000.

JF991394K