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Comparison of Two Microalgal Diets. 2. Influence on Odorant Composition and Organoleptic Qualities of Raw Oysters (*Crassostrea gigas*)

ANNE-LAURE PENNARUN,*,† CAROLE PROST,† JOËL HAURE,‡ AND MICHEL DEMAIMAY†

Laboratoire de Biochimie Alimentaire et Industrielle, Ecole Nationale des Ingénieurs des Techniques des Industries Agro-Alimentaire, Rue de la Géraudière, B.P. 82225, 44322 Nantes Cedex 3, France, and IFREMER, Polder des Champs, 85230 Bouin, France

Oyster farming is of real economic interest in France. Oyster farmers attach more and more importance to improving the growth and the quality of their oysters. Some fatty acids known to be aroma precursors originate from microalgae such as *Skeletonema costatum* and *Tahitian isochrysis* clone. These microalgae were used to fatten oysters in order to observe their role in the development of oysters' aroma. This study shows that the profile of fatty acids of oysters is influenced by the contribution of fatty acids from the two microalgae (as reported in the first paper in this series: Pennarun, A.-L.; Prost, C.; Haure, J.; Demaimay, M. Comparison of Two Microalgal Diets. 1. Influence on the Biochemical and Fatty Acid Compositions of Raw Oysters (*Crassostrea gigas*). *J. Agric. Food Chem.* **2003**, *51*, 2006−2010 (in this issue)]. As a consequence, a microalgal diet causes changes in oysters' aroma composition. Aroma concentration depends on the content of fatty acids that are aroma precursors in oysters. Some aromas are characteristic of the diet of *S. costatum*, such as 6-methyl-5-hepten-2-one (ether odor), and others are characteristic of *T. isochrysis*, such as 3-nonyne (cucumber, marine odor), 6-(*E*)-nonen-1-ol (green and fresh odor), and 4-ethylbenzaldehyde (aniseed odor). Moreover, the organoleptic qualities (odor, taste, and texture) of oysters are modified by the diet of microalgae.

KEYWORDS: ***Crassostrea gigas oyster*; *Skeletonema costatum*; *Tahitian isochrysis* clone; lipid composition; odor-active compounds; organoleptic qualities**

INTRODUCTION

The French are great consumers of oysters, which they eat fresh and raw at the beginning of the meal, especially during New Year celebrations. In fact, France is the greatest producer of oysters (*Crassostrea gigas*) in Europe, and oyster farming is a well-established tradition (*2*). As consumers become more demanding about the quality of their food, oyster farmers, aided by scientists, are trying to develop fattening techniques to improve the growth, odor, and taste of oysters. The visual and odorant characteristics of oysters are the most important parameters indicative of their quality and freshness. Aroma can be used to study the traceability and quality of oysters.

The first step in the study of aroma is the choice of an extraction method for the volatile compounds which gives an extract as close as possible to the original product (*3*−*6*). This is essential because the extraction method could produce some side effects, such as oxidation or enzymatic hydrolysis, that generate off-flavors. Vacuum hydrodistillation is the best extraction method for oysters from the point of view of odor authenticity (*7*). This method is particularly well-adapted for raw products such as oysters, but also for wine (*8*) or for tomatoes (*9*). Odor-active compounds of oysters (*C. gigas*) have already been studied by Piveteau et al. (*10*), using dynamic headspace as the extraction method, but not by vacuum hydrodistillation. This technique is, in our case, the more reliable for the extraction of the odorants of oysters. Once a reliable method was found, the sniffing analysis was applied to characterize the potent odorants. Gas chromatography−olfactometry (GC-O) is a powerful method to detect the more active odorant compounds in an extract. The detection frequency method was applied to assess the odorants of raw oysters. This method is known to be reliable in the determination of odorants within a minimum time and with no specific training of the panel (*3*).

A series of nonvolatile compounds designated as aroma precursors could lead to volatile compounds through chemical or enzymatic action (*11*). The specific aroma precursors of

* To whom correspondence should be addressed (telephone 33-2-51-78-55-18; fax 33-2-51-78-55-20; E-mail pennarun@enitiaa-nantes.fr).
† Ecole Nationale des Ingénieurs des Techniques des Industries Agro-Alimentaire.
‡ IFREMER.

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oysters are lipids, particularly unsaturated fatty acids (PUFAs) which are sensitive to oxidation (*12*−*14*). Josephson (*12*) has shown the importance of some PUFAs as aroma precursors and their biosynthetic pathway. Our study aimed to enrich the fatty acid content of the diet of oysters, especially in aroma precursors, by means of two microalgae, *Skeletonema costatum* and *Tahitian isochrysis* clone (*1*). Knowledge of the biochemical composition of oysters could lead to the identification of the origin of some volatile compounds. The correlation between the aroma and lipid composition of oysters fattened by microalgae could enable us to understand the metabolism of aroma precursors. No comparison between different microalgal diets, such as *S. costatum* and *T. isochrysis*, was made in order to assess the role of fatty acids as aroma precursors or to study the aroma composition and organoleptic modifications of oysters (*C. gigas*).

Our study deals with the comparison and the influence of microalgae on the aroma composition of oysters and on their organoleptic qualities, according to the fatty acid content of the microalgae.

MATERIALS AND METHODS

Most experimental procedures were as described in the accompanying paper (*1*).

Reagents. All water was purified by using a MilliporeQ system (Millipore Corp., France). Dichloromethane and 3-hexanone were obtained from Sigma-Aldrich (Germany).

Oysters. Oysters (*C. gigas*) came from the Bay of Bourgneuf in Vendée on the Atlantic coast in western France. Adult oysters were collected from January to March 2001, outside the maturation period. After collection, they were stored alive at 4 °C for 6 days. This period did not lead to any organoleptic adulteration of the oysters. All the analyses were performed on live oysters within 6 days.

Microalgae. The culture of microalgae was performed as described in the Materials and Methods section of the accompanying paper (*1*).

Oysters' Diet. Three tanks, each containing 400 oysters, were supplied with seawater at 14 °C. The first tank contained oysters fed phytoplankton contained in the natural seawater, which represented 2.0 μg of the particulate organic matter (POM)/L. The second and the third tanks contained oysters fed with *Skeletonema costatum* and *T. isochrysis*, containing respectively 4.8 and 5.1 mg POM/L. The calculation of the content of POM is detailed in the accompanying paper (*1*).The experiment lasted 6 weeks. Oysters were sampled at time 0 and after 6 weeks of experimentation.

Volatile Compounds. *Extraction*. Volatile compounds were extracted by vacuum hydrodistillation using the method of Forss and Holloway (*15*). A 6-L flask containing 200 g of raw oyster flesh, obtained from about 40 oysters, 300 mL of ultrapure water (Millipore), and 1 mL of internal standard (3-hexanone, 10 μg/mL) was placed in a thermostated water bath at 20 °C. The residual pressure in the equipment was set at 600 Pa for 4 h 30 min. This time is necessary to evaporate the 300 mL of water and 70% of intrinsic water in oysters. Condenser columns were set at −1 °C. The volatile compounds were collected in a 4-L flask (set at 2 °C) and in three traps placed in Dewars containing liquid nitrogen at −196 °C. The contents of all the collectors were pooled and extracted by 3 × 30 mL of fresh dichloromethane. The organic extract was concentrated to 10 mL using a Kuderna-Danish apparatus at 45 °C, and to 1 mL under a gentle stream of nitrogen. The extractions were done in triplicate. The three extracts obtained by vacuum hydrodistillation were pooled and concentrated to 0.5 mL. This extract was stored at −20 °C prior to use.

A control extraction (blank) was carried out in triplicate using exactly the same experimental procedure, except that the 6 L-flask contained only 300 mL of ultrapure water and 10 μg of 3-hexanone.

Quantification. The quantification was achieved with a gas chromatograph (Star Varian 3400, Palo Alto, CA) equipped with a flame ionization detector (FID). The volatile compounds of the 3-μL extracts were separated on a capillary column (DB-Wax, 30 m length × 0.32 mm i.d. × 0.5 μm thickness, J&W Scientific, Folsom, CA). The oven temperature program for the oyster extracts was as follows: 50 °C to 70 °C at 6 °C/min, followed by a temperature increase to 150 °C at 4 °C/min and then to 250 °C at 10 °C/min for 10 min. For the blank, the oven was set at 50 °C and increased to 250 °C at 14 °C/min for 10 min. The injector and the FID were set at 250 °C. The helium carrier gas flow was 1 mL/min. The quantity of each volatile compound was calculated according to 3-hexanone.

Identification. Volatile compounds were identified by using a gas chromatograph (GC, HP 5890 II, Hewlett-Packard Co., Palo Alto, CA) coupled with a mass spectrometer (MS), equipped with an electronic impact source (HP 5971 II mass-selective detector (MSD), Hewlett-Packard Co.). The oven temperature program, the helium carrier gas flow, the injected volume, and the injector temperature were the same as descrobed previously. The interface was set at 180 °C. The parameters of the MSD were as follows: ionization energy, 70 eV; mass range, 33−300 u; scan rate, 2.0 scan/s; electron multiplier voltage, 200 V.

The volatile compounds were identified by comparing their spectra to those of two libraries: a commercial one (NBS 75k) and an internal one in our laboratory. Their retention indices, calculated according to Van Den Dool and Kratz (*16*), were matched to those found in the literature. If there was a chemical standard, it was analyzed by GC/MS.

Olfactometry. Three microliters of the extract was injected into a gas chromatograph (using the same apparatus and the same analysis conditions as described in the Quantification part). In addition to being plugged into the FID, the column was connected to a sniffing port supplied with humidified air at 40 °C. The panel was composed of 10 people, all trained in seafood aroma recognition. When an odor was detected, the judge had to give its descriptor (*3*) and its odor intensity. The global analysis corresponds to the detection frequency, that is to say, the number of judges who detected an odor at the same retention time. The average intensity is the mean of the intensities given by the judges for one compound at a precise time. The duration of an analysis for the oyster extracts was 40 min. A complete analysis was performed by two judges who each sniffed for 20 min to stay alert during all this time. For the blank, one judge smelled a complete analysis. An odor had to be smelled by at least three judges to be taken into account. If not, it was considered as noise (*17*). The 10 individual aromagrams were summed to yield a final aromagram (detection frequency versus retention index). The volatiles were identified by matching their mass spectra and their retention indexes with those found in the literature, as well as their odors and their chemical standards if they were available.

Sensory Analysis. Nine judges, all trained in seafood aroma recognition, took part in the sensory analysis. A preliminary session was held in an ordinary room to generate the odor, taste, and texture descriptors of oysters. Odor descriptors were generated on an oyster control group and on oysters fattened by microalgae. Taste and texture descriptors were generated on an oyster control group. After discussion with the judges, redundant and unsuitable descriptors were eliminated to keep only the more relevant ones for the analysis. Five descriptors were chosen to evaluate the odor of oysters: global odor intensity, seaside, grass, fresh fish, and mud. The descriptors for taste were salty, bitter, and astringent. Firm and melting were the descriptors for texture. Evaluation sessions took place in a sensory room (AFNOR V-09-105, 1987) at ambient temperature under daylight in isolated booths. During this session, judges were asked to evaluate the odor, taste, and texture of three oysters (oyster control group, oysters supplemented by *S. costatum*, and oysters supplemented by *T. isochrysis)*. For each oyster, judges evaluated the intensity of each descriptor on a horizontal unstructured scale of 10 cm. On this scale, the 0 on the left-hand anchor corresponds to no intensity and the 10 on the right-hand anchor corresponds to a very strong intensity. Each intensity was represented by a mark out of 10. The marks given for each descriptor by all the judges were summed. A factorial correspondence analysis was made with these data.

Statistical Treatment. Data acquisition was performed on Statgraph 4.0 software. The data acquisition for the factorial correspondence analysis (FCA) was carried out using Statgraph 4.0 software, and graphic representation was done on Sgwin software.

Table 1. Volatile Compounds Identified in Oysters of the Control Group and in Oysters Supplemented by *S. costatum* and *T. isochrysis* and Their Origin[a]

peak	compound	RI	identification method	OCG-S0	OCG-S6	OS-S6	OI-S6
			n–3 Fatty Acid Degradation				
1	1,3-(*E*)-5-(*Z*)-octatriene	1108	RI, odor	*	*	*	*
2	2-(*E*)-pentenal	1124	RI, MS, odor, std	*	*	*	*
3	1-penten-3-ol	1170	RI, MS	*	*	*	*
4	2-(*E*)-hexenal	1221	RI, MS	*			
5	4-(*Z*)-heptenal	1236	RI, MS, odor	*	*	*	*
6	2-(*E*)-penten-1-ol	1321	RI, MS, odor, std	*	*	*	*
7	2,4-(*E*,*E*)-heptadienal	1488	RI, MS, odor	*	*	*	*
8	3,5-(*E*,*E*)-octadien-2-one	1570	RI, MS	*			
9	2,6-(*E*,*Z*)-nonadienal	1584	RI, MS, odor, std	*	*	*	*
			n–6 Fatty Acid Degradation				
10	1-pentanol	1303	RI, MS, std	*			
11	2-(*Z*)-octenal	1413	RI, MS, odor	*	*		*
12	2-(*E*)-octenal	1429	RI, MS, odor, std			*	*
13	1-octen-3-ol	1451	RI, MS, std	*	*	*	*
14	2-(*E*)-octen-1-ol	1616	RI, MS	*	*		*
15	pentanoic acid	1734	RI, MS	*	*		*
16	octanoic acid	1845	RI, MS, odor	*	*	*	*
17	nonanoic acid	2157	RI, MS				*
			n–9 Fatty Acid Degradation				
18	3-hexanol	1211	RI, MS	*	*		
19	octanal	1289	RI, MS, odor	*	*	*	*
20	1-hepten-3-ol	1351	RI, MS, std	*	*	*	*
21	nonanal	1392	RI, MS, std	*	*	*	*
22	heptanol	1457	RI, MS, std	*		*	
23	decanal	1497	RI, MS, odor, std	*	*	*	*
24	1-octanol	1553	RI, odor, std	*	*		*
			Unidentified Fatty Acid Degradation				
25	3-octanone	1256	RI, MS, std	*	*	*	*
			Polysaccharide Degradation				
26	ethylbenzene	1132	RI, MS	*			
27	*p*-xylene	1140	RI, MS	*	*	*	*
28	*m*-xylene	1190	RI, MS			*	
29	*o*-xylene	1191	RI, MS				*
30	1,2,4-trimethylbenzene	1280	RI, MS	*			
31	phenol	1996	RI, MS, std	*	*		*
			Carotenoid Degradation				
32	limonene	1201	RI, MS, odor, std	*	*	*	*
33	tridecane	1290	RI, MS, std	*			
34	6-methyl-5-hepten-2-one	1338	RI, MS, odor, std	*	*	*	*
35	tetradecane	1390	RI, MS, std	*	*	*	*
36	2-undecanone	1596	RI, MS, odor, std	*	*	*	*
37	4-ethylbenzaldehyde	1745	RI, MS, odor, std				*
			Maillard Reaction				
38	ethylpyrazine	1354	RI, odor, std	*			
39	acetylpyrazine	1647	RI, odor	*		*	
			Strecker Reaction				
40	methional	1451	RI, MS, odor, std	*	*	*	*
			Reduction Product of the Strecker Reaction				
41	3-methylthio-1-propanol	1645	RI, odor	*	*	*	*
			Unknown Origin				
42	dimethyl sulfide	773	RI, MS, std	*			*
43	3-penten-2-ol	1181	RI, MS, std	*	*	*	*
44	dodecane	1200	RI, MS, std	*	*		
45	1-dodecene	1236	RI, MS, std	*	*	*	*
46	3-methyl-1-butanol	1250	RI, MS				*
47	3-nonanone	1357	RI, MS	*	*		
48	2,4,6-trimethylpyridine	1378	RI, MS, std	*			
49	3-(*E*)-hexen-1-ol	1386	RI, MS, odor, std	*	*	*	*
50	3-octanol	1395	RI, MS, odor	*	*	*	*
51	2-butoxyethanol	1406	RI, MS	*	*	*	*
52	7-(*Z*)-tetradecene	1465	RI, MS, std	*	*		
53	2-ethyl-1-hexanol	1490	RI, MS, std	*	*	*	*
54	3-nonyne	1514	RI, MS, odor				*
55	propanoic acid	1534	RI, MS		*	*	*
56	2-nonanol	1535	RI, MS, odor, std	*	*	*	*
57	hexadecane	1590	RI, MS, std	*	*	*	*
58	1-hexadecene	1630	RI, MS	*	*	*	*
59	acetophenone	1645	RI, MS, std	*	*	*	*
60	2,4,6,10-tetramethylpentadecane	1655	RI, MS, std	*	*	*	*

Table 1 (Continued)

peak	compound	RI	identification method	OCG-S0	OCG-S6	OS-S6	OI-S6
61	3-methylbutanoic acid	1666	RI, MS				*
62	heptadecane	1688	RI, MS, std	*	*	*	*
63	6-(*E*)-nonen-1-ol	1714	RI, MS, odor				*
64	3,6-(*E*,*Z*)-nonadien-1-ol	1731	RI, MS, odor	*	*	*	*
65	octadecane	1789	RI, MS, std	*	*	*	*
66	2-(2-butoxyethoxy)ethanol	1796	RI, MS				*
67	1-octadecene	1823	RI, MS	*	*		*
68	nonadecane	1889	RI, MS, std	*	*	*	*
69	butylated hydroxytoluene	1902	RI, MS, std	*	*	*	*
70	dodecanol	1964	RI, MS		*		*
71	eicosane	1966	RI, MS, std	*		*	
72	heneicosane	2083	RI, MS, std			*	
73	decanoic acid	2264	RI, MS				*

[a] RI, retention index on a DB-Wax capillary column; MS, mass spectrometry; std, chemical standard; OCG, oyster control group; OS, oysters supplemented by *S. costatum*; OI, oysters supplemented by *T. isochrysis*; S0, control point; S6, level after 6 weeks of experimentation.

Table 2. Some Fatty Acids of Oysters of the Control Group and Those Supplemented by *S. costatum* and *T. isochrysis* after 6 Weeks of Experimentation [Mean (Standard Deviation), $n = 6$][a]

fatty acid	OCG-S0	OCG-S6	OS-S6	OI-S6
n–3 PUFA	56.1 (3.6)	46.9 (3.2)	49.3 (1.2)	37.4 (1.4)
C18:3 *n*–3	0.1 (0.1)	2.2 (0.2)	1.5 (0.2)	5.6 (0.2)
C20:5 *n*–3	28.9 (1.4)	22.1 (1.2)	32.4 (0.2)	10.6 (0.7)
n–6 PUFA	0.4 (0.1)	6.8 (0.7)	2.9 (0.2)	9.3 (0.4)
18:2 *n*–6	0.1 (0.1)	0.8 (0.1)	0.8 (0.0)	5.7 (0.2)
n–9 MUFA	13.2 (0.9)	6.1 (1.6)	2.7 (0.7)	7.3 (0.5)

[a] MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; OCG, oyster control group; OS, oysters supplemented by *S. costatum*; OI, oysters supplemented by *T. isochrysis*; S0, control point; S6, level after 6 weeks of experimentation. The details of the fatty acid composition are given in the accompanying paper (*1*).

RESULTS AND DISCUSSION

Volatile Compounds and the Potent Odorants. Seventy-three different volatiles were identified in either the oyster control group or those fed microalgae (**Table 1**). The volatile compounds in oysters are mainly ketones, alcohols, and aldehydes. At the beginning of the experiment, 59 volatiles were extracted and identified in the oyster control group, and only 49 after 6 weeks of experimentation. The difference observed is probably due to the bad weather conditions affecting the biochemical composition of the oysters (*1*). As lipids, proteins, and carbohydrates could be aroma precursors of oysters (*12*), the biochemical composition of the oysters is related to the composition of volatiles. Respectively 44 and 56 volatile compounds were detected in oysters supplemented by *S. costatum* and *T. isochrysis*. Among the identified volatiles, 41 had a known origin. Most of them (24 compounds) came from the degradation of fatty acids found in great quantity in oysters (**Table 2**). Nine compounds came from the degradation of $n-3$ fatty acids (*10*, *12*−*14*, *17*, *18*). They were present in all groups of oysters, except for 2-(*E*)-hexenal and 3,5-(*E*,*E*)-octadien-2-one, which were present only in the oyster control group at week 0. This is in good agreement with the high level of $n-3$ fatty acids in these groups of oysters. The volatile compounds formed from $n-6$ fatty acids (*13*) were less present in oysters supplemented by *S. costatum*, due to a lower level of $n-6$ fatty acids in these oysters (2.9% compared to 6.8% for the oyster control group after 6 weeks of experimentation and 9.3% for oysters supplemented by *T. isochrysis*). Indeed, 2-(*Z*)-octenal, 2-(*E*)-octen-1-ol, and pentanoic acid were missing in oysters supplemented by *S. costatum* compared with oyster control group and with oysters fed *T. isochrysis*. As for $n-9$ fatty acid precursors of volatile compounds, the trends were less marked. However, all the volatiles issuing from $n-9$ fatty acid degradation (*13*, *14*) were present in the oyster control group at the beginning of the experiment, in correlation with their high level (13.2% versus about 2.7−7.3% for the other groups of oysters). Some volatiles, such as ethylbenzene, *p*-xylene, *m*-xylene, *o*-xylene, 1,2,4-trimethylbenzene, and phenol, came from the degradation of polysaccharides (*19*). Limonene, tridecane, 6-methyl-5-hepten-2-one, tetradecane, 2-undecanone, and 4-ethylbenzaldehyde derive from carotenoid degradation (*10*, *20*). Ethylpyrazine and acetylpyrazine, which were detected in the oyster control group at the beginning of the experiment and, for the latter compound, in oysters supplemented by *S. costatum*, were generated by the Maillard reaction (*21*). These compounds are generally formed through heat. For example, they were identified by Le Guen et al. (*3*) in cooked mussels. In our case, they were probably formed during the vacuum hydrodistillation extraction and/or the concentration step. They were found at low concentrations (respectively 0.8 and 2.6 ng equiv of 3-hexanone/g of dry matter of oysters for ethylpyrazine and acetylpyrazine in the oyster control group at the beginning of the experiment and 0.1 ng equiv of 3-hexanone/g of dry matter of oysters for acetylpyrazine in oysters fattened with *S. costatum*). This is probably the reason they were not found in the other groups of oysters extracted in the same conditions. Methional generated by the Strecker reaction (*17*) was detected in the four groups of oysters. It is generally found in cooked products such as cooked oysters *C. gigas* effluent (*22*) and cooked mussels (*3*), thus showing its formation through heat. The reduction product of methional, 3-methylthio-1-propanol (*23*), was also found in the four groups of oysters. Thirty-two compounds were of unknown origin. Most of them were found in the four groups of oysters.

GC-O Results. Odorants are presented in **Table 3**. Twenty-three odorous compounds were detected in the oyster control groups and in oysters supplemented by *S. costatum*. Twenty-five were detected in oysters supplemented by *T. isochrysis*. Most of the compounds identified were common to all the groups. Nine compounds could not be identified. Some of them were coeluted with the solvent (unknown, RI = 1118) or coeluted with another compound (unknowns, RI = 1196, 1417, 1419). Some of them were in trace quantities and too difficult to identify (unknown, RI = 1792). The others could not be identified due to a high MS background at high GC temperature.

We took care to perform a control extraction (blank) to ensure that there was no taint due to the extraction method or to the

Table 3. Aroma-Active Compounds in Oysters of the Control Groups and Those Supplemented by *S. costatum* and *T. isochrysis*

			OCG-S0			OCG-S6			OS-S6			OI-S6		
compound	RI[a]	odor[b]	qty	AI	GA	qty	AI	GA	qty	AI	GA	qty	AI	GA
1,3-(*E*)-5-(*Z*)-octatriene	1108	plastic, green	72.9	2.1	4	12.8	2.1	5	16.1	2.1	6	7.2	1.9	3
unknown	1118	garlic, sulfury	2.1	3.8	8	trace	2.3	5	trace	3.0	7	trace	2.1	6
2-(*E*)-pentenal	1124	grass	1.3	4.0	9	10.3	4.7	9	3.9	4.3	9	21.0	4.1	9
unknown	1196	ether	0.0		(0)	0.0		(0)	17.6	1.2	4	0.0		(0)
limonene	1201	moss, green	6.4	2.8	7	12.3	1.3	3	6.6	0.7	3	7.1	1.1	3
4-(*Z*)-heptenal	1236	cooked white fish	5.3	2.8	6	14.6	3.3	7	3.3	3.2	7	4.8	6.1	5
octanal	1289	citrus fruit	trace		(1)	3.2	1.3	3	2.1	1.5	4	1.5		(1)
2-(E)-penten-1-ol	*1321*	*mushroom*	*19.3*	*7.4*	*10*	*17.7*	*7.5*	*10*	*22.1*	*7.1*	*10*	*28.5*	*6.1*	*9*
6-methyl-5-hepten-2-one	1338	ether, alcohol	1.9		(1)	2.9		(1)	40.4	0.9	3	5.7		(1)
ethylpyrazine	1354	grilled	0.8	1.2	3	0.0		(0)	0.0		(0)	0.0		(0)
3-(*E*)-hexen-1-ol	1386	moss, green, fresh	9.5	7.9	10	3.5	7.9	10	trace	7.4	10	trace	6.9	9
3-octanol	*1395*	*moss, sulfury*	*trace*	*4.2*	*6*	*34.3*	*5.2*	*8*	*29.2*	*5.6*	*9*	*28.8*	*4.4*	*7*
2-(*Z*)-octenal	1413	mushroom, marine	trace		(2)	8.2	1.9	4	0.0		(0)	5.0	2.1	5
unknown	1417	mushroom	9.9	1.1	3	0.0		(0)	0.0		(0)	0.0		(0)
unknown	1419	fresh	0.0		(0)	0.0		(0)	0.0		(0)	19.2	0.9	3
2-(*E*)-octenal	1429	citrus, cucumber	0.0		(0)	0.0		(0)	7.7	1.3	3	trace	1.4	4
1-octen-3-ol + methional	1451	boiled potato	346.4	1.4	3	401.1	1.2	3	134.7	3.8	8	397.0	2.3	6
7-(*Z*)-tetradecene	1465	nc	7.1		(1)	25.6	1.6	4	0.0		(0)	0.0		(0)
2,4-(*E*,*E*)-heptadienal	1488	mushroom, moss	0.8	5.3	9	trace	3.4	8	4.0	3.4	8	19.5	4.1	9
decanal	1497	marine, cucumber	15.8	5.8	10	10.5	6.0	10	5.0	2.6	8	11.8	5.1	9
3-nonyne	1514	cucumber, marine	0.0		(0)	0.0		(0)	0.0		(0)	26.6	3.9	7
2-nonanol	1535	cucumber	2.0	3.6	6	2.7	3.5	5	1.4	2.6	5	1.6	2.6	6
1-octanol	1553	cucumber	1.5	2.3	4	3.4	2.6	5	0.0		(0)	3.1	1.7	3
2,6-(*E*,*Z*)-nonadienal	1584	cucumber	3.5	1.7	3	2.1	1.0	3	1.4	0.7	3	0.3		(0)
2-undecanone	1596	cucumber, fresh	47.8	5.9	10	34.7	4.7	8	27.9	2.2	5	29.7	4.2	8
acetophenone + 3-methylthio-1-propanol	1645	animal	0.3	0.9	3	39.3	6.2	9	trace	2.6	5	1.3	6.9	10
acetylpyrazine	1647	grilled	2.6	2.7	5	0.0		(0)	0.1	0.9	3	0.0		(0)
6-(*E*)-nonen-1-ol	1714	green, fresh	0.0		(0)	0.0		(0)	0.0		(0)	2.6	1.3	3
3,6-(*E*,*Z*)-nonadienol	1731	marine, cucumber	90.8	1.1	4	11.5	1.2	3	2.1		(1)	5.5	2.1	4
4-ethylbenzaldehyde	1745	minty, aniseed	0.0		(0)	0.0		(0)	0.0		(0)	25.8	1.9	3
unknown	1792	nc	0.0		(0)	0.0		(0)	0.2	0.8	3	trace	2.1	5
unknown	1806	green	27.1	1.6	4	0.0		(0)	0.0		(0)	0.0		(0)
octanoic acid	1845	fresh, moss	9.9	2.5	4	125.1	3.3	6	11.5	2.6	5	91.7	4.0	8
unknown	1858	moss, rubber, green	44.1	3.3	7	184.2	1.8	3	0.0		(0)	71.5	1.5	3
unknown	1947	nc	0.0		(0)	0.0		(0)	8.0	2.6	3	0.0		(0)
unknown	2159	nc	0.0		(0)	7.1	1.6	3	0.0		(0)	0.0		(0)

[a] Retention index on a DB-Wax 30m capillary column. [b] Odor descriptor given by our panel. OCG, oyster control group; OS, oysters supplemented by *S. costatum*; OI, oysters supplemented by *T. isochrysis*; S0, control point; S6, level after 6 weeks of experimentation; qty, nanogram equivalent of 3-hexanone per gram of dry matter of oysters; GA, global analysis, number of judges out of 10 who detected an odor; AI, average intensity, mean of intensities given by the judges for each compound; nc, noncommon descriptor to define odor. Values given in parentheses are those detected by fewer than three judges, so the odor is not significant. Compounds found in the control extract are given in italics.

analysis method. The aromagram of this extract is shown in **Figure 1**. Three compounds were significantly detected in the blank: *p*-xylene, 2-(*E*)-penten-1-ol, and 3-octanol. *p*-Xylene was detected by five judges and had an average intensity of 1.5. This compound, although found in the four groups of oysters, was not detected as an odorant in them (compare **Tables 1** and **3**). 2-(*E*)-Penten-1-ol, with a mushroom odor was detected by eight judges at trace concentrations. In the oyster extract, it was smelled by 9 or 10 judges at concentrations varying from 17.7 to 28.5 ng equiv of 3-hexanone/g of dry matter of oysters. The odor detection threshold of this compound may be low, thus explaining the difference in concentrations in the blank and in oyster extracts compared to the number of judges who detected this compound. 2-(*E*)-Penten-1-ol, which had an average intensity of 2.4 in the blank extract and of about 7.0 in the oyster extract, contributed a lot to the oysters' odor. The average intensity is in agreement with the quantitative data. The last compound identified in the blank was 3-octanol, smelled by 3 judges and having a moss and sulfury odor. In oyster extracts, it was smelled by 6, 8, 9, and 7 judges respectively for the oyster control group at week 0, at week 6, and for oysters fattened by *S. costatum* and *T. isochrysis*. The average intensity varied from 4.2 to 5.6 for oyster extracts and was 0.6 for the blank. This compound may participate a great deal in the global odor of oysters, considering the global analysis and the average intensity. Although these compounds were found at low average intensity in the blank compared to the average intensity in oyster extracts, they had to be taken into account in oyster extracts with care (and are noted in italics in **Table 3**). They could be considered as a taint due to the ultrapure water, the degradation of the internal standard, or the apparatus.

Table 3 gives the quantitative data, the olfactometry global analysis, and the average intensity. On the whole, the quantitative analysis is difficult because of the low concentrations of volatile compounds due to the extraction technique or because of the high background of the chromatograph. Thus, it is sometimes difficult to correlate the quantitative data and the global analysis. 2-(*E*)-Pentenal, characterized by a grass odor confirmed by Tanchotikul and Hsieh (*24*), was smelled by 9 judges in the four groups of oysters, whatever its concentration. The odor detection threshold was probably reached even at low concentration. The quantitative data for limonene did not correlate with the global analysis. Limonene, which was characterized by a green odor as reported by Sugisawa et al. (*25*) and by a moss odor, has an odor detection threshold of 10 ppb (*26*). This compound was detected by 3 judges in the oyster control group after 6 weeks of experimentation and in oysters supplemented by *S. costatum* and *T. isochrysis*. However, it was

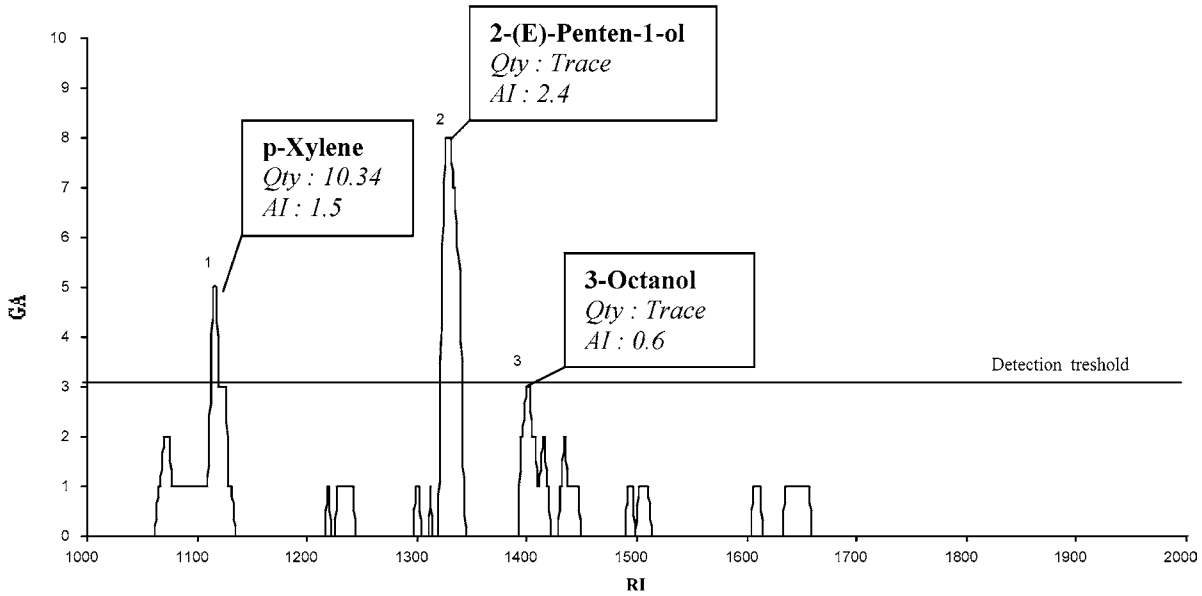

Figure 1. Aromagram of volatile compounds of the blank obtained by the olfactometry global method. RI, retention index on a capillary column DB-Wax 30m; GA, global intensity; Qty, nanogram equivalent of 3-hexanone per gram of dry matter of oysters; AI, average intensity.

smelled by 7 judges in the oyster control group at the beginning of the experiment at about the same concentration as in the other oysters. One hypothesis is that there was another compound probably smelling of moss, with a low detection threshold but present at trace concentration in the oyster control group at the beginning of the experiment.

2-(*E*)-Penten-1-ol and 3-(*E*)-hexen-1-ol were detected by 9 or 10 judges in the four groups of oysters whatever their concentration, showing that these compounds probably have a low odor detection threshold. 2,4-(*E*,*E*)-Heptadienal, characterized by a mushroom and moss odor as reported by Piveteau et al. (*10*), was smelled in all the four groups of oysters by 8 or 9 judges. This compound is odorant even at trace concentrations, showing a low odor detection threshold. Despite the high odor detection threshold of decanal (10 000 ppb) (*26*), this compound was smelled by 8, 9, or 10 judges in the four groups of oysters, showing that the optimal concentration to allow its detection is reached.

4-(*Z*)-Heptenal, characterized by a cooked white fish odor, was present in the four groups of oysters. The global analysis was not well correlated with the quantitative data, probably due to some experimental errors arising from the low concentration of this compound that led to imprecision in the integration software. The same conclusion could be made for 2-(*Z*)-octenal, which was detected in the oyster control group after 6 weeks of experimentation and in oysters supplemented by *T. isochrysis*, but for which there was no correlation between the global analysis and the quantitative data. In the same way, 2-(*E*)-octenal was only detected in oysters supplemented by the two microalgae, but there was no correlation between the quantitative data and global analysis.

Problems in quantification occurred when several compounds were coeluted. For instance, the quantitative data for 1-octen-3-ol with methional and for acetophenone with 3-methylthio-1-propanol did not correlate with the global analysis. In the case of 1-octen-3-ol and methional, 1-octen-3-ol is known to have a mushroom odor, and methional is known for its boiled potato odor. They have odor detection thresholds of 1 and 0.2 ppb, respectively (*26*). Apparently, the judges detected the boiled potato odor but not the mushroom odor. However, to explain the high quantity found in the oyster control group at the beginning of the experiment and after 6 weeks of experimentation, and for oysters fattened with *T. isochrysis*, there was probably a non-odorant or odorless compound that coeluted with the other two, which disrupted the quantitative analysis. The boiled potato odor is higher in oysters fed the two microalgae than in oysters of the control group.

With regard to acetophenone and 3-methylthio-1-propanol, the animal odor resulted from the latter compound and was predominant in the oyster control group after 6 weeks of experimentation and in oysters fattened by *T. isochrysis*, according to the global analysis. The difficulty in doing the quantitative analysis is due to the coelution of these two compounds. For 3,6-(*E*,*Z*)-nonadienol, octanoic acid, and the unknown compound at RI = 1858, the quantitative analysis was very difficult because of the high background of the chromatogram resulting from the high GC temperature.

These results show the difficulty in analyzing quantitative data and the importance of studying the concentration and the global olfactometry analysis together. The correlation between the quantitative data and the global olfactometry analysis allowed the relative contribution of each odor to be measured and enabled the odor detection threshold of some odors to be evaluated.

Some odorants are found only in oysters supplemented by one or the other of the microalgae. Some volatiles are characteristic of the diet. The unknown compound at RI = 1196 and 6-methyl-5-hepten-2-one were smelled only in oysters supplemented by *S. costatum*. The unknown compound at RI = 1196, with an ether odor, was smelled by 4 judges. The 6-methyl-5-hepten-2-one odor (odor detection threshold 50 ppb) (*26*) was found in all groups of oysters at a low concentration, except in oysters fattened with *S. costatum*, where it was found in high concentration, allowing its detection by the panel. This compound, derived from carotenoid degradation (*10*, *20*), was detected by 3 judges and had an ether and alcohol odor.

Four odorant compounds, the unknown compound at RI = 1419, 3-nonyne, 6-(*E*)-nonen-1-ol, and 4-ethylbenzaldehyde, were characteristic of oysters supplemented by *T. isochrysis*. The unknown compound at RI = 1419, detected by 3 judges, had a fresh odor. 3-Nonyne, with a cucumber and marine odor, was smelled by 7 judges. 6-(*E*)-Nonen-1-ol, characterized by a

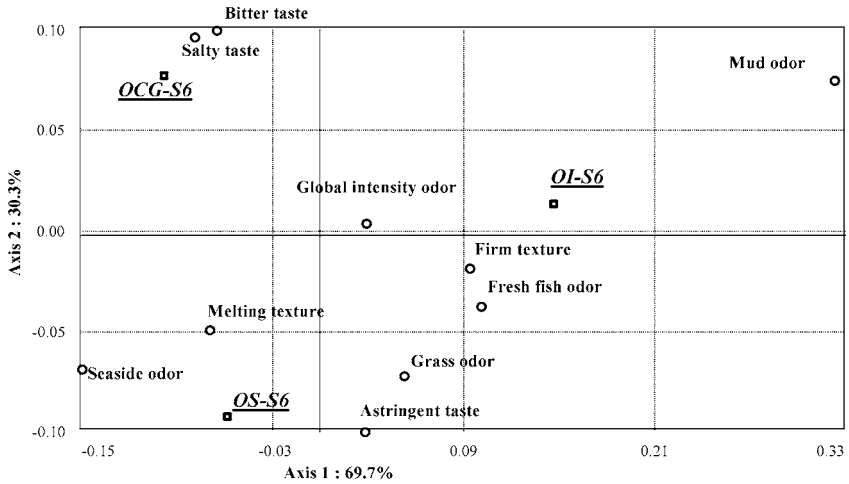

Figure 2. Factorial correspondence analysis of the three batches of oysters (oyster control group, OCG-S6; oysters supplemented by *S. costatum*, OS-S6; oysters supplemented by *T. isochrysis*, OI-S6) and their sensory descriptors.

green and fresh odor, was identified by 3 judges. Finally, 4-ethylbenzaldehyde, derived from carotenoid degradation, was detected by 3 judges and was characterized by a minty and aniseed odor.

Some odorants came from $n-3$ fatty acid oxidation; for example, 1,3-(*E*)-5-(*Z*)-octatriene, characterized by plastic and green odor (*10*). A significant difference exists, however, between oysters supplemented by *S. costatum* and oysters supplemented by *T. isochrysis*, as we considered a difference in perception between extracts by at least 3 judges as significant (*3*). Indeed, 1,3-(*E*)-5-(*Z*)-octatriene was detected by 6 judges in oysters supplemented by *S. costatum* and by 3 judges in oysters supplemented by *T. isochrysis*. This is in agreement with the content of $n-3$ fatty acid (49.3% of total fatty acids for oysters supplemented by *S. costatum* and 37.4% of total fatty acids for oysters supplemented by *T. isochrysis*) (**Table 2**). The concentration of this compound in the oyster control group at week 0 was very high compared to the average intensity, probably due to coelution with the solvent, leading to imprecision in the measurement of the concentration. 2-(*E*)-Pentenal, resulting from the degradation of linolenic acid C18:3 $n-3$ (*14*) via 15-lipoxygenase, had a grass odor. Nine judges smelled it. Its concentration was greater in oysters supplemented by *T. isochrysis*, in agreement with the high level of this fatty acid in these oysters (5.6% of the total fatty acids) (**Table 2**). 2,6-(*E*,*Z*)-Nonadienal came from the degradation of C20:5 $n-3$ (*12*). This compound is characterized by a cucumber note and was identified in all the four groups of oysters but in too low concentration in the oysters fed *T. isochrysis* to be smelled by the panel, although it has a weak detection threshold (0.09 ppb) (*26*). This is related to the low level of C20:5 $n-3$ in the oysters fattened with this microalgae.

Other odorant compounds, such as 2-(*Z*)-octenal and 2-(*E*)-octenal, came from the oxidation of $n-6$ fatty acids (*13*). These compounds, with respectively mushroom, marine odor and citrus, cucumber odor, were not detected by the panel in the oyster control group at the beginning of the experiment. This is in agreement with the low level of $n-6$ fatty acid in these oysters (0.4% of the total fatty acids) (**Table 2**). 2-(*Z*)-Octenal was not detected in oysters supplemented by *S. costatum*, in agreement with its level of $n-6$ fatty acid. Surprisingly, 2-(*E*)-octenal was smelled in oysters supplemented by *S. costatum*, even though its level of $n-6$ fatty acid is low. Octanoic acid, characterized by fresh and moss odor, came from the degradation of the linoleic acid C18:2 $n-6$ (*13*), found in great quantity in oysters fed *T. isochrysis*. Eight judges smelled these compounds in oysters fed *T. isochrysis* versus 4, 6, and 5 for the other groups (respectively the oyster control group at week 0 and at week 6 and in oysters supplemented by *S. costatum*).

1-Octanol came from the oxidation of $n-9$ fatty acid. This compound was not present in oysters fattened by *S. costatum*, probably due to its low concentration of $n-9$ fatty acid. Among the other odorants, some of them had a known origin but their quantity did not correlate with the fatty acid profile, while others were of unknown origin.

These results show the tendency of PUFAs to play a real part in the aroma composition of oysters. The aroma composition of oysters was influenced by the fatty acid profiles of the microalgae.

Organoleptic Changes in Oysters following Supplementation with Microalgae. A factorial correspondence analysis of the oyster control group and oysters supplemented by *S. costatum* and *T. isochrysis* after 6 weeks of experimentation was carried out, studying odor, texture, and taste (**Figure 2**). It was interesting to note a good inertia of the axes. All the information is presented graphically. **Figure 2** shows that the three groups of oysters had different sensory properties. Oysters supplemented by *S. costatum* and *T. isochrysis* were characterized by a strong grass odor. These groups were distinguished by a fresh fish odor for oysters supplemented by *T. isochrysis* and by a seaside odor for oysters supplemented by *S. costatum.* The mud odor is weak in oysters. Nevertheless, oysters fed *T. isochrysis* were closest to it. A correlation between the odor sensory parameters and odorants was difficult to make because the sensory qualities were assessed on whole oysters whereas odorants were analyzed in oyster extracts obtained by vacuum hydrodistillation.

As for the taste, the oyster control group was greatly characterized by a salty and bitter taste. Oysters supplemented by *S. costatum* and *T. isochrysis* were distant from the salty descriptor, thus leading toward a sweet taste. This was probably due to the increase in carbohydrates in oysters fed a microalgal diet (*1*). Oysters supplemented by *S. costatum* were nearer to having an astringent taste.

Supplementation by microalgae tended to modify the texture of oysters. The texture of oysters supplemented by *T. isochrysis* was firmer than that of oysters supplemented by *S. costatum*, whose texture is melting. For the judges, this mark was due to the milky character induced by the glycogen and lipid, which

was significantly higher in oysters supplemented by *S. costatum* than in other oysters (*1*).

The microalgal diet modified the biochemical and the fatty acid composition (*1*), the organoleptic qualities, and, to a lesser extent, the volatile composition of oysters. The use of a microalgal diet also increased the growth of oysters. The taste was sweeter due to the increase in carbohydrates. The texture in the mouth had more character. As for the volatile compounds and odorants, some compounds were characteristic of one or the other of the microalgae. These results show the influence of fatty acids in the aroma composition of oysters. However, microalgae contain many PUFAs as aroma precursors, so it is difficult to attribute an odorant precisely to its aroma precursor. Nevertheless, the use of microalgae in the diet of oysters indicates the role of PUFAs as aroma precursors. In subsequent work, a molecular study will be carried out using directly in the diet only PUFAs known to be aroma precursors. Thus, interference between PUFAs will be limited, and the role of some PUFAs as aroma precursors could be described more precisely.

ABBREVIATIONS USED

FCA, factorial correspondence analysis; GC-MS, gas chromatography−mass spectrometry; GC-O, gas chromatography−olfactometry; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

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