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Characterization of odorant compounds of mussels (*Mytilus edulis*) according to their origin using gas chromatography–olfactometry and gas chromatography–mass spectrometry

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Abstract

Gas chromatography–olfactometry consists of sniffing the effluent of a gas chromatograph and leads to the direct determination of potent odorants in food. GC–olfactometry and GC–MS were applied in order to identify volatile compounds, and to characterize potent odorants of cooked wild mussels and bouchot mussels. Eighty-five volatiles were identified by GC–MS, among those the majority were identified for the first time in mussels. Using GC–olfactometry, the main contributors of cooked mussels aroma were characterized. Of the 85 volatiles identified in the flavor, only 33 were odor-active and contribute to the overall aroma of mussels. Dimethyl disulfide (sulfury odor) was the odorant the most differently perceived between the two extracts and seems to be characteristic of wild mussels. Combined GC–MS and GC–olfactometry made it possible to point out odorants which actually contribute to the aroma of cooked mussels and those which showed typical dependence on the origin of mussels.

Keywords: *Mytilus edulis*; Food analysis; Olfactometry; Detection, GC; Volatile organic compounds; Aroma compounds

1. Introduction

In France, consumption of mussels is such that production does not meet the demands of French consumers, even though it was estimated to be around $60 \cdot 10^6$ kg in 1998. The French market is then characterized by competition among national and European products imported to meet the demand. In this competitive context, French producers wish to emphasize regional values in order to upgrade and differentiate their production.

Aroma compounds play a significant role in the quality of our food because aroma perception is one of the foremost criteria used by the consumer for the preference or acceptance of a food product. Aroma compounds could then be used as quality indicators.

Gas chromatography (GC)–olfactometry has been extensively used in aroma research. Considering the large differences between detection thresholds of volatile compounds, all compounds identified by instrumental techniques do not contribute equally to the overall aroma of the product. GC–olfactometry consists of sniffing the effluent of a gas chromatograph and leads to the direct determination of potent odorants in food. The interest in determining the individual contribution of volatile compounds present in foods, has led to a new generation of GC–olfactometry techniques. One of which is based on detection frequency [1,2]. Only one dilution level is used and GC–olfactometry is repeated by several

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panel members under the same conditions. Aromagrams of individuals are then summed, and peak heights correspond to their detection frequencies. This technique needs no training of the panel and provides an acceptable compromise between good reproducibility/repeatability and a restricted number of injections.

In contrast to many foods, very little information has been reported on the volatile flavor components of mussels. Yasuhara and Morita [3] have already studied the volatile organic components in mussels, for monitoring marine pollution. They identified more than 100 volatile compounds in cooked mussels, among which were many esters, carboxylic acids, phenylalkanes, and several alkylbenzenes. However, to our knowledge, no studies have been conducted where a panel of judges evaluated the odor quality of mussel extracts by GC–olfactometry.

The objectives of this study were: (1) to extract and identify volatile compounds of cooked mussels; (2) to characterize potent odorants in cooked mussels; (3) to point out compounds which allowed a characterization of the origin of mussels.

2. Experimental

2.1. Mussels

The sampling of mussels (*Mytilus edulis*) was performed in October 1999 on two different sites: one sample came from a wild production area in Eastern Normandy (France) and the other one was obtained from bouchot culture in Mont Saint Michel Bay (France). Once collected, mussels were immediately transported under refrigerated conditions to the laboratory and then stored at +4°C.

2.2. Chemicals

Dichloromethane (GC quality), collidine (99%) and all the standard compounds were purchased from Sigma–Aldrich, except dimethyl sulfide, toluene, xylene, heptanal, pyridine, octanal and 1-octanol which came from Merck. 1-Propanol and phenylethyl alcohol were obtained from Prolabo.

2.3. Vacuum hydrodistillation

Vacuum hydrodistillation was performed in a Forss and Holloway device [4] modified by Dumont and Adda [5] as previously described [6]. After rinsing, 1.6 kg of mussels was cooked in a vapor cooker (Magimix M050) for 20 min. A 350-g amount of decorticated mussels, 800 ml of purified water, and 1 ml of an aqueous solution of collidine (2,4,6-trimethylpyridine) at 14 μg/ml (used as an internal standard, I.S.) were transferred to a 6-l round bottom flask. Hydrodistillation was carried out for 3 h under a pressure of 5 mbar, with the 6-l round bottom flask maintained at 37°C. Most of the volatiles were collected with water into a 4-l round bottom flask by means of condensers. The more volatile compounds were collected in traps refrigerated with liquid nitrogen. After distillation, the contents of the 4-l round bottom flask and traps were pooled. The total amount of distillate was almost 870 ml which corresponded to the 800 ml added at the beginning of the experiment and intrinsic water of mussels. The distillate was successively extracted by 60, 40 and 30 ml of freshly distilled dichloromethane at 0°C with magnetic stirring and settling. After dehydration by anhydrous sodium sulfate, the organic extract was first reduced to 4 ml in a Kuderna–Danish concentrator, and then, it was concentrated to exactly 200 μl under a gentle stream of nitrogen. The whole process was repeated six times for each mussel batch. The extracts were stored at −20°C in glass vials before analysis.

2.4. Gas chromatography–mass spectrometry (GC–MS)

A 2-μl volume of the extracts was injected into a HP 5890 series II GC/HP 5971 mass-selective detector (Hewlett-Packard, Palo Alto, CA, USA) (splitless mode; 30 s valve delay; injector temperature 250°C; helium carrier gas at 1 ml/min) fitted with a capillary column (DB-Wax, 60 m×0.32 mm I.D., 0.5 μm film thickness; J&W Scientific, Folsom, CA, USA). The oven temperature was programmed from 40°C to 250°C at a rate of 4°C/min, with initial and final hold times of 2 and 10 min, respectively. The MS (electronic impact ionization) conditions were as follows: ionization energy, 70 eV; mass

range 33–300 u; scan rate, 2.0 scan/s; electron multiplier voltage, 2000 V. The detector interface temperature was set at 280°C, with the actual temperature in the MS source reaching 180°C.

2.5. Gas chromatography–flame ionization detector (FID)–olfactometry

The GC–FID–olfactometry system comprised a Varian 3400 GC system (Varian, Palo Alto, CA, USA) fitted with an FID system at 280°C and a sniffing port supplied with humidified air at 40°C. A 2-μl volume of each extract was injected (splitless mode) into a capillary column (DB-Wax 30 m×0.32 mm I.D., 0.5 μm film thickness; J&W Scientific). Effluent from the end of the GC column was split 1:1 between the FID system and the sniffing port. Oven temperature was programmed from 40°C to 250°C at a rate of 5°C/min with initial and final hold times of 2 and 10 min, respectively.

2.6. Olfactometry

A panel of 10 judges [7] who were trained in odor recognition, and who had experience in GC–olfactometry was selected. Sniffing was divided into two parts of 20 min. Each person participated in the sniffing of both parts, but during two distinct sessions to remain alert. The panelists were asked to assign odor properties for each odorant zone. Detection of an odor at the sniffing port by fewer than four assessors out of 10 was considered as noise [8]. A difference of perception of more than three assessors at the same retention index between two samples means that the volatile compound was differently perceived in the two samples [9]. The 10 aromagrams were summed yielding the final aromagram (detection frequency versus retention index, I).

2.7. Compound identification and quantification

Compound identifications were based on comparison of GC retention indices (I) [10], mass spectra (comparison with standard MS spectra databases: NBS 75K and internal library of the laboratory) and odor properties. For odorant compounds, chemical standards were also reinjected into the GC–sniffing system to check their odor quality.

Response surfaces were those obtained from FID chromatograms. Relative concentrations of positively identified compounds were the average of six extractions and were expressed in ng equivalent internal standard (collidine) per gram of mussels (ng/g).

2.8. Statistical treatment

Estimated concentrations of volatile compounds were compared using an F test (analysis of variance, ANOVA), carried out with Statgraphics Plus software (Manugistics, Rockville, MD, USA). When only traces were detected, they were considered as equal to zero for analysis of variance on volatile compound content.

3. Results

3.1. Identification of volatile compounds of mussels by GC–MS

A total of 85 volatile compounds were identified in both extracts of cooked mussels (Table 1), among those 22 aromatic hydrocarbons, 14 alcohols, 14 aldehydes, 12 ketones, eight sulfur-containing compounds, six alkanes, six pyrazines and three pyridines. Most of these compounds were identified by GC–MS, retention index and by injection of the chemical standard.

Yasuhara and Morita [1] identified 60 volatile compounds by vacuum distillation in a first batch of cooked mussels. One year later, they applied the same distillation/extraction process to another mussel batch of the same geographical origin. They again identified 60 volatile compounds of which only 11 were common to the two batches. Indeed, in the first batch, they mainly identified methyl esters or aliphatic carboxylic acids. Compounds from the second batch were fairly different from the ones in batch one. They identified certain methyl esters but, contrary to their first study, also a significant number of aliphatic hydrocarbons and halogenated compounds. We identified 85 volatile compounds in cooked mussels but we did not characterize any esters or halogenated compounds. Only 11 compounds were common between our study and the study of the two previous authors: hexanal, unde-

Table 1
Volatile compounds identified in cooked mussels

Compound	I^a	Methods of identification	Estimated concentration (ng equiv. I.S./g of mussel)		F test[b]
			Wild	Bouchot	
Aromatic hydrocarbons					
Ethylbenzene	1131	MS, *I*	0.2	0.4	**
p-Xylene	1139	MS, *I*, standard	0.1	0.5	*
m-Xylene	1146	MS, *I*, standard	0.4	1.2	***
o-Xylene	1192	MS, *I*, standard	0.3	0.8	***
Propylbenzene	1216	MS, *I*	tr	tr	
3-Ethyltoluene	1231	MS, *I*	0.3	0.6	*
1,3,5-Trimethylbenzene	1252	MS, *I*	tr	0.3	***
2-Ethyltoluene	1272	MS, *I*	tr	0.3	***
1,2,4-Trimethylbenzene	1291	MS, *I*, standard	0.3	1.3	***
Ethyldimethylbenzene	1335	MS	0.7	0.8	
2-Ethyl-1,4-dimethylbenzene	1364	MS, *I*	0.3	0.2	
1-Ethyl-2,3-dimethylbenzene	1369	MS, *I*	tr	tr	
4-Ethyl-1,2-dimethylbenzene	1379	MS, *I*	0.3	0.3	
2-Propenylbenzene	1385	MS, *I*	tr	0.3	***
1,2,4,5-Tetramethylbenzene	1438	MS, *I*	0.3	0.2	
Naphthalene	1763	MS, *I*, standard	0.6	1.9	***
2-Methylnaphthalene	1877	MS, *I*, standard	nd	nd	
1-Methylnaphthalene	1915	MS, *I*	0.2	0.2	
2,6-Dimethylnaphthalene	2031	MS, *I*, standard	nd	nd	
Alcohols					
1-Propanol (+toluene)	1040	MS, *I*, standard	0.7	1.8	***
1-Penten-3-ol	1170	MS, *I*, standard	0.1	0.3	*
3-Penten-2-ol	1181	MS, *I*, standard	0.7	1.9	**
3-Methyl-1-butanol	1221	MS, *I*, standard	1.0	0.7	*
1-Pentanol	1264	MS, *I*, standard	8.0	3.3	***
(*E*)-2-Penten-1-ol	1322	MS, *I*, standard	tr	0.2	***
(*Z*)-2-Penten-1-ol	1329	MS, *I*, standard	0.1	tr	**
1-Hexanol	1368	MS, *I*, standard	1.7	1.0	**
1-Octen-3-ol	1462	MS, *I*, standard	2.4	1.0	**
1-Heptanol	1470	MS, *I*, standard	3.6	4.1	
2-Ethyl-1-hexanol	1502	MS, *I*, standard	4.9	5.6	
2-Nonanol	1534	MS, *I*, standard	tr	tr	
1-Octanol	1566	MS, *I*, standard	1.8	1.2	**
Phenylethyl alcohol	1933	MS, *I*, standard	0.8	1.5	***
Aldehydes					
Pentanal	980	MS, *I*, standard	nd	nd	
Hexanal	1088	MS, *I*, standard	1.4	1.3	
(*E*)-2-Methyl-2-butenal	1101	MS, *I*	tr	tr	
Heptanal	1195	MS, *I*, standard	2.0	2.2	
3-Methyl-2-butenal	1212	MS, *I*,	0.5	0.7	
(*Z*)-4-Heptenal	1251	MS, *I*	tr	tr	
Octanal (+3-hydroxy-2-butanone)	1301	MS, *I*, standard	1.6	1.4	
(*E*)-2-Heptenal	1338	MS, *I*, standard	tr	tr	
Nonanal	1407	MS, *I*, standard	1.2	1.6	
Benzaldehyde	1539	MS, *I*, standard	10.3	7.7	
(*E*,*Z*)-2,6-Nonadienal	1594	MS, *I*, standard	0.5	1.4	***
(*E*,*E*)-2,4-Octadienal	1600	MS, *I*	1.7	tr	***
4-Ethylbenzaldehyde	1752	MS, *I*, standard	0.3	0.3	

Table 1. Continued

Compound	I[a]	Methods of identification	Estimated concentration (ng equiv. I.S./g of mussel)		F test[b]
			Wild	Bouchot	
Ketones					
2,3-Butanedione	985	MS, *I*, standard	9.5	3.0	***
3-Heptanone	1163	MS, *I*, standard	0.3	0.4	
Cyclohexanone	1311	MS, *I*, standard	0.3	1.3	***
6-Methyl-5-hepten-2-one	1352	MS, *I*, standard	0.4	0.3	
2-Nonanone	1403	MS, *I*, standard	1.4	0.9	
3,5,5-Trimethyl-3-cyclohexen-1-one	1429	MS, *I*	0.9	0.1	**
2-Decanone [+(*E*,*E*)-2,4-heptadienal)]	1508	MS, *I*, standard	1.7	2.7	
2-Undecanone	1606	MS, *I*, standard	1.0	0.6	
3,5,5-Trimethyl-2-cyclohexen-1-one	1621	MS, *I*, standard	21.0	7.4	*
Acetophenone	1669	MS, *I*, standard	2.3	2.6	
2,6,6-Trimethyl-2-cyclohexen-1,4-dione	1717	MS, *I*, standard	9.1	4.4	**
Sulfur-containing compounds					
Dimethyl sulfide	725	MS, *I*, standard	nd	nd	
Dimethyl disulfide	1074	MS, *I*, standard	tr	tr	
Dimethyl trisulfide	1394	MS, *I*	nd	nd	
Methional	1473	MS, *I*, standard	6.0	4.8	
2-Acetylthiazole	1662	MS, *I*, standard	2.8	1.7	
4-Methylthiazole	1675	MS, *I*	1.2	1.0	
2-Acetyl-2-thiazoline	1783	MS, *I*	1.3	0.5	
Benzothiazole	1991	MS, *I*	0.9	0.5	
Alkanes					
Undecane	1096	MS, *I*, standard	0.7	1.0	
Dodecane	1198	MS, *I*, standard	0.2	0.3	
Tridecane	1298	MS, *I*, standard	1.9	0.7	
Pentadecane	1497	MS, *I*, standard	tr	tr	
Hexadecane	1594	MS, *I*, standard	0.2	1.4	**
Heptadecane	1693	MS, *I*, standard	5.9	3.9	**
Pyrazines					
Methylpyrazine	1283	MS, *I*	0.8	0.5	**
2,6-Dimethylpyrazine	1343	MS, *I*	0.1	0.1	
1,2,3-Trimethylbenzene (+2-ethylpyrazine)	1349	MS, *I*, standard	0.4	0.2	
1-Methylethenylpyrazine	1514	MS	0.6	1.5	*
1-[3-Methyl-2-pyraziny]-1-ethanone	1640	MS	4.4	4.1	
1-Acetylpyrazine	1646	MS, *I*	1.5	1.1	
Pyridine-containing compounds					
Pyridine (+limonene)	1202	MS, *I*, standard	0.8	1.7	
2,6-Dimethylpyridine	1276	MS, *I*, standard	0.1	0.4	**
3-Methylpyridine	1319	MS, *I*, standard	0.3	0.5	*

[a] I on DB-Wax column.
[b] F test, significance level: ***, <1%; ** <5%; *, <10%.
I.S.: Internal standard.
tr: Trace.
nd: Not determined.

cane, 2-methyl-2-butenal, ethylbenzene, *o*-, *m*- and *p*-xylene, 1,3,5-trimethylbenzene, 2-penten-1-ol, octadienal and benzaldehyde. Consequently, most compounds we identified are being reported for the first time in cooked mussels.

Volatile flavor compounds identified in the two extracts were compared. Both extracts were rich in 1-heptanol, 2-ethyl-1-hexanol, benzaldehyde, 3,5,5-trimethyl-2-cyclohexen-1-one, 2,6,6-trimethyl-2-cyclohexen-1,4-dione, methional, heptadecane and 1-(3-methyl-2-pyrazinyl)-1-ethanone. Wild mussels also presented a great amount of 1-pentanol and 2,3-butanedione. There were no qualitative differences between the two batches. Indeed, the same compounds were detected in both extracts but in varying quantities. ANOVA was performed on quantities of each volatile extracted and it statistically showed significant differences for 34 compounds. Most of these differences were due to aromatic hydrocarbons, alcohols, dienals and to a less extent to certain ketones, alkanes, pyrazines and pyridines.

Aromatic hydrocarbons were numerous in both extracts but each of them was present in small quantities. Most of these compounds were present in significantly higher amounts in bouchot mussels than in wild mussels. This observation will require a more thorough study on other batches to confirm these results.

3.2. Identification of odorants by GC–olfactometry

The successful use of GC–olfactometry depends on the method used with the aim of developing an extraction procedure producing mussels extracts with odor as close as possible to that of the original cooked mussels. The quality of the odor of our extract was assessed and was shown to be very similar to that of cooked mussels.

Aroma-active compounds detected in the two extracts, their odor quality and their detection frequency are given in Table 2. Aromagrams of bouchot mussels (Fig. 1) and wild mussels (Fig. 2) were similar for predominant odorant compounds. A typical GC–FID chromatogram of bouchot mussels is shown in Fig. 3. The sum of detection frequencies was higher in wild mussels, which tallied with quantitative results showing that the overall estimated concentrations of volatile compounds were higher in wild mussels than in bouchot mussels.

Twenty-nine and 32 compounds were perceived by at least four panelists out of 10 in, respectively, bouchot mussels and wild mussels during olfactometry. In total, 23 positive identifications were made by comparing in addition to their retention index and mass spectrum, their odor properties (compared to literature and odor of the corresponding standards).

2,3-Butanedione (2), unknown 6 (*Z*)-4-heptenal (11), unknown 12, (*E*)-2-penten-1-ol (15), ethylpyrazine (17), dimethyl trisulfide (19), methional (21), 1-acetylpyrazine (26) and 2-acetyl-2-thiazoline (30) were detected by the majority of the panel in both extracts and may play a major role in the aroma of cooked mussels. Octanal (14) and unknowns 18, 20, 22 were also impact odorants of wild mussels whereas unknown 24 was important in the aroma of bouchot mussels. Five compounds were differently perceived between the two extracts. Four of them were detected by more assessors in wild mussels [dimethyl disulfide (4), unknown 18, (*E*,*E*)-2,4-octadienal (25) and unknown 32] and one compound was perceived by more judges in bouchot mussels [3-methyl-2-butenal (10)]. Quantitative results of four of these compounds were not significantly different. Indeed, compounds 4, 18 and 32 were present at trace state in both extracts. For compound 10, the amount was effectively higher in the bouchot extract but it was not significant. (*E*,*E*)-2,4-Octadienal was present in higher significant concentrations in wild mussels which complies with olfactometric results.

4. Discussion

Although 14 alcohols were identified by GC–MS in both extracts, only three of them were odorants. Indeed, alcohols generally do not contribute to the overall flavor because of their high threshold values [11], unless they are unsaturated. This is the case of (*E*)-2-penten-1-ol which was the only alcohol which contributed actively to the aroma of mussels. Alcohols may have been produced by lipid oxidation of polyunsaturated fatty acids (chemical or enzymatic) [12].

Table 2
Odor-active compounds in cooked mussels

Peak No.[a]	I[b]	Compound	Odor description[c]	Detection frequency[d]	
				Wild	Bouchot
1	930	Unknown	Fruity, pyrogenous	6	5
2	980	2,3-Butanedione	Buttery, caramel	9	7
3	1045	1-Propanol	Fruity, plastic	5	5
4	1074	Dimethyl disulfide	Sulfury	5	(1)
5	1092	Hexanal	Green	7	6
6	1113	Unknown	Sulfury, garlic	9	10
7	1150	*m*-Xylene	Plastic	4	5
8	1171	Unknown	Plastic	6	5
9	1197	Heptanal	Citrus fruit, green	4	4
10	1215	3-Methyl-2-butenal	–[e]	(1)	4
11	1253	(*Z*)-4-Heptenal	Boiled potato	10	9
12	1274	Unknown	Sulfury, garlic	8	8
13	1293	1,2,4-Trimethylbenzene	Plastic	4	4
14	1303	Octanal	Citrus fruit, orange	8	6
15	1316	(*E*)-2-Penten-1-ol	Mushroom	10	10
16	1336	(*E*)-2-Heptenal	Sulfury, grassy	6	6
17	1354	Ethylpyrazine	Nutty	10	10
18	1373	Unknown	Green, fruity	9	6
19	1390	Dimethyl trisulfide	Sulfury, green, marine	10	9
20	1451	Unknown	Nutty	8	6
21	1477	Methional	Boiled potato	10	9
22	1496	Unknown	Boiled potato, grassy	8	7
23	1532	2-Nonanol	Fruity, solvent	4	(3)
24	1576	Unknown	Moldy, earthy	6	8
25	1605	(*E,E*)-2,4-Octadienal	Cucumber	7	4
26	1650	1-Acetylpyrazine	Nutty	10	8
27	1660	2-Acetylthiazole	Grilled hazel nut	4	6
28	1681	4-Methylthiazole	Roasted, meaty	5	4
29	1753	4-Ethylbenzaldehyde	Fruity, anisic, minty	5	4
30	1790	2-Acetyl-2-thiazoline	Grilled hazel nut	10	9
31	1913	Unknown	Fruity, grassy	6	5
32	1935	Unknown	Rubber, roasted	4	(1)
33	2038	2,6-Dimethylnaphthalene	Grilled, grassy	4	(2)

[a] Numbers correspond to those in Figs. 1 and 2.
[b] Retention index on DB-Wax column.
[c] Odor description as perceived by panelists during olfactometry.
[d] Detection frequency (/10 panelists).
[e] Odor detected without a common descriptor for most of the judges.

More than half of aldehydes identified by GC–MS (eight of 14) were odor-active. Aldehydes are known to play a major role in many food products and are responsible for a wide range of oxidized flavors. (*E,E*)-2,4-Octadienal (cucumber-like odor) was differently perceived between the two extracts. This corroborated with quantitative results which showed significant differences between both extracts for this aldehyde. Hexanal, heptanal and octanal, polyunsaturated fatty acids oxidation products, were perceived with green and/or citrus fruits-like odor which are generally considered to be off-flavors of seafood products. On the basis of its high detection frequency, (*Z*)-4-heptenal appeared as an impact odorant in mussel flavor although it was only present at trace state. This can be explained by its low detection threshold (0.04 ppb [13]). There may be a requirement for the presence of (*Z*)-4-heptenal in freshly cooked mussels through its boiled potato-like odor (in accordance with Josephson and Lindsay

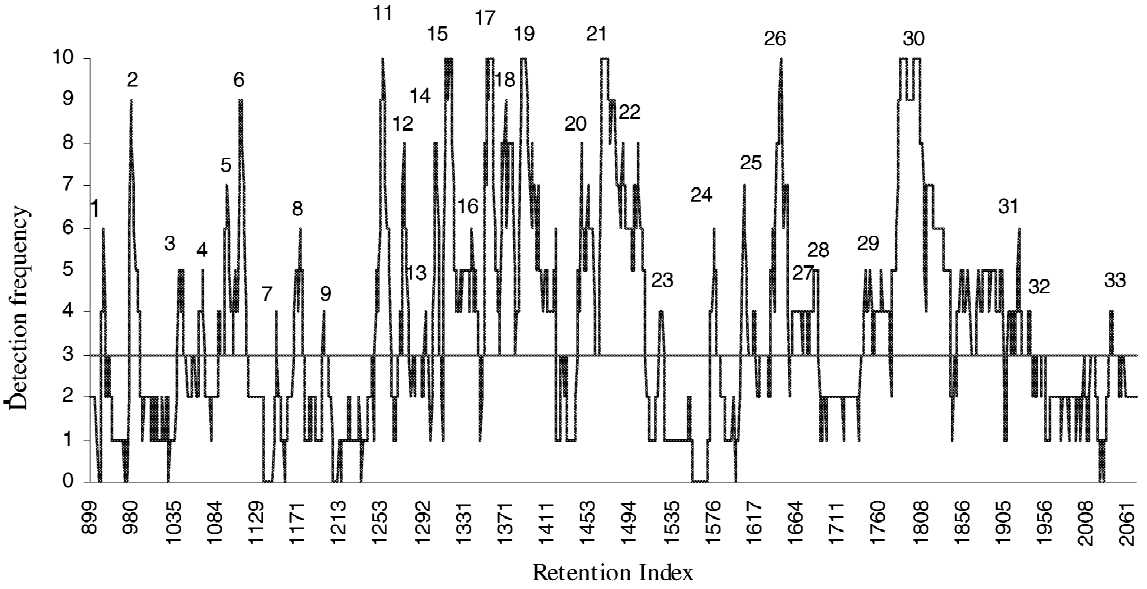

Fig. 1. Aromagram of wild mussels. Peak numbers correspond to those listed in Table 2.

[14] which reported that (*Z*)-4-heptenal was reminiscent of boiled potatoes).

Twelve ketones were identified in mussel extracts but only one was an odorant. 2,3-Butanedione was detected by two more assessors in wild mussels extract and was in significantly higher amounts in this extract. This tallied with the results of Prost et al. [9] who made the same observation between wild and farmed cooked turbot. 2,3-Butanedione was among the strongest notes detected in both extracts,

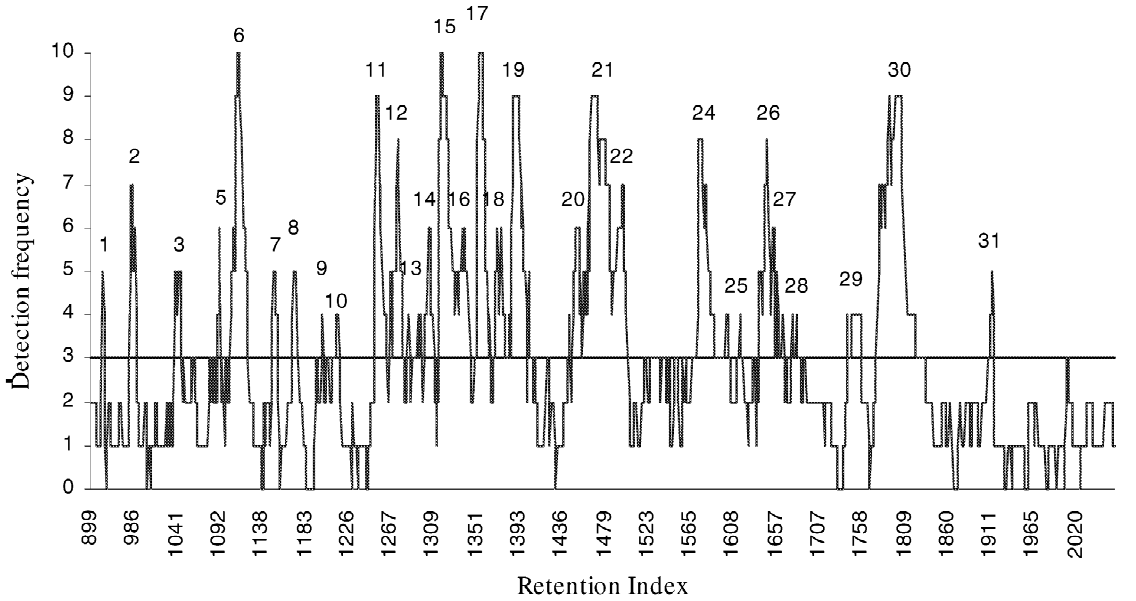

Fig. 2. Aromagram of bouchot mussels. Peak numbers correspond to those listed in Table 2.

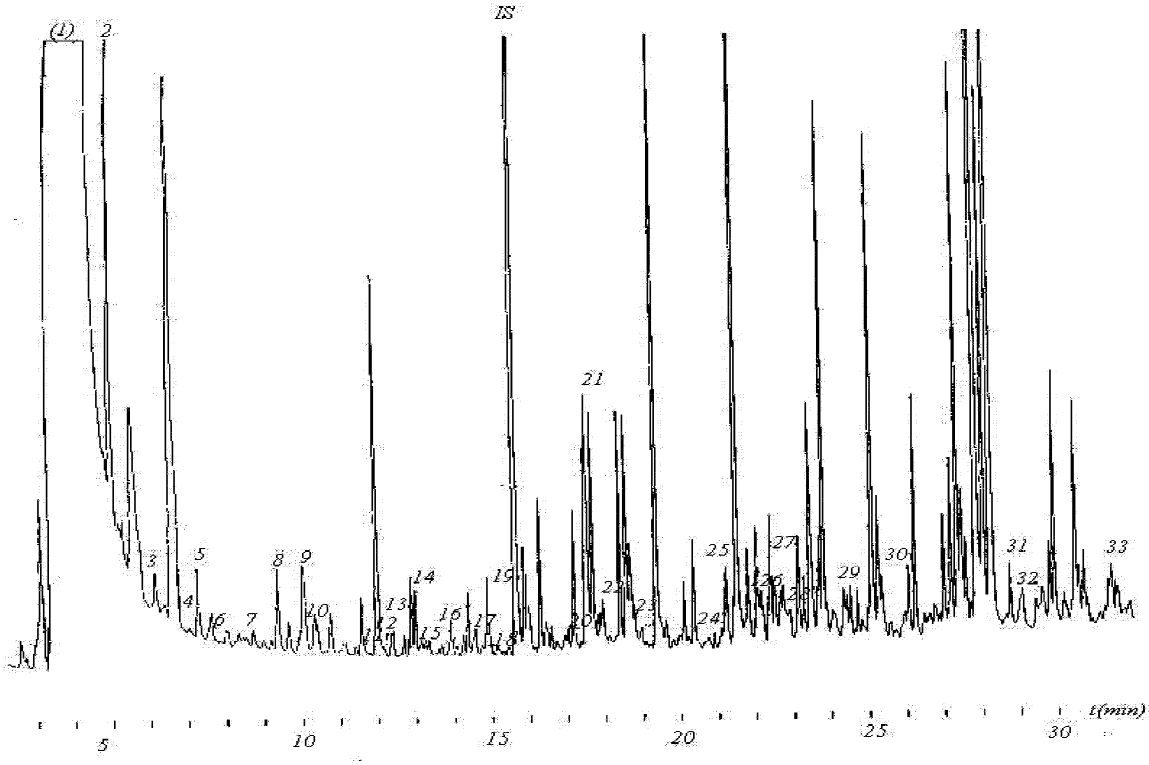

Fig. 3. Gas chromatogram (GC–FID) of volatile compounds of bouchot mussels. Peak numbers refer to those listed in Table 2. (I.S.: Internal standard).

with a threshold of 2.6 ppb [15]. It is a characteristic product in cooked food and is thermally generated through the Maillard reaction [16]. It may contribute to the desirable flavor of cooked mussels through its characteristic buttery, caramel-like odor.

Sulfur compounds play a major role in the aroma of cooked mussels. Indeed, six sulfur-containing compounds were odorant in cooked mussels which means that only two sulfur-containing compounds out of the eight identified by GC–MS were not odor-active. Moreover, three of them were impact odorants of cooked mussels (dimethyl trisulfide, methional and 2-acetyl-2-thiazoline). Dimethyl disulfide and trisulfide were reported to affect overall food aroma because of their low threshold values [17], even if they were in very low amounts. These two compounds were thermally generated from amino acids. Dimethyl disulfide was the compound with the most significant difference of perception between the two extracts. It could be considered as a characteristic odorant of wild mussels. Methional, whose odor threshold is 0.2 ppb [18] was considered as an important component of the desirable aroma of cooked mussels, through its strong boiled potato-like odor.

Only three aromatic hydrocarbons were odor-active although 21 were earlier characterized by GC–MS. Indeed, these compounds were present only at low concentrations in both extracts and usually have a high detection threshold. They did not contribute much to the global aroma of mussels but may have a foreground undesirable odor. The presence of alkylbenzene and alkylnaphthalene in mussels could be due to a petroleum contamination. The uptake of aromatic hydrocarbons has been previously reported in many fish or shellfish [19] or crustacea [20,21].

Two pyrazines out of the five identified by MS were odorants. Ethylpyrazine and 1-acetylpyrazine were perceived by the majority of the panel although they were not present in high concentrations in either

extract. These pyrazines, with a characteristic nutty odor, may contribute to the desirable flavor of cooked mussels. They could be formed by Maillard reactions and pyrolysis reactions through Strecker degradations in heat processes food from various sources such as amino acids [22].

No alkanes or pyridines were odor-active probably because of their high detection thresholds.

There were 10 unknown odor-active components. Some of them were not identified because they were at trace state (12, 18, 20, 22, 24, 32), coeluted (6, 8), masked by the solvent (1) or because of higher MS background at the higher temperature of GC separation (31). On the basis of their high detection frequencies, unknowns 6, 12, 18, 20, 22 and unknowns 6, 12, 24 may contribute actively to the aroma of, respectively, wild mussels and bouchot mussels. Unknowns 6 and 12 (sulfury, garlic-like odor), and unknown 24 (moldy, earthy odor) were believed to have a negative impact on the aroma of cooked mussels. Unknown 20 (nutty odor) which could be a pyrazine, imparted desirable aromas.

5. Conclusion

Eighty-five volatile compounds have been identified in extracts of cooked mussels by GC–MS. Many of these compounds were identified for the first time in mussels. No qualitative differences were observed between wild mussels and bouchot mussels, but 34 compounds, among those especially aromatic hydrocarbons and alcohols, presented significant quantitative differences. Using GC–olfactometry, the main contributors of cooked mussels aroma were characterized. Of the 85 volatiles identified in the flavor, only 33 were odor-active and contribute to the overall aroma of mussels. On the basis of the results of this study, the volatile flavor of cooked mussels could be defined and based principally on 10 individual compounds including 2,3-butanedione (buttery, caramel-like odor); unknown 6 (sulfury, garlic-like odor); (*Z*)-4-heptenal (boiled potato-like odor); unknown 12 (sulfury, garlic-like odor); (*E*)-2-penten-1-ol (mushroom-like odor); ethylpyrazine (nutty odor); dimethyl trisulfide (sulfury, green, marine-like odor); methional (boiled potato-like odor); 1-acetylpyrazine (nutty odor) and 2-acetyl-2-thiazoline (grilled hazel nut-like odor). Five compounds were differently perceived between wild mussels and bouchot mussels by olfactometry. Dimethyl disulfide (sulfury odor) was the odorant the most differently perceived between the two extracts and seems to be characteristic of wild mussels.

Comparison of the aromas of wild mussels and bouchot mussels by instrumental techniques is not sufficient to detect differences between the two samples. Indeed, most of these odorants have extremely low threshold values, and are more effectively detected by GC–olfactometry. GC–MS and GC–FID combined with olfactometry allows one to point out impact odorants from cooked mussels and to differentiate odor-active compounds of mussels according to their origin. In addition, GC–olfactometry makes it possible to characterize compounds which may have a positive impact on the aroma of mussels and those which could be considered as off-flavor.

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