Evaluation of the Representativeness of the Odor of Cooked Mussel Extracts and the Relationship between Sensory Descriptors and Potent Odorants

Sophie Le Guen,* Carole Prost, and Michel Demaimay

ENITIAA, Laboratoire de Biochimie Alimentaire, Rue de la Géraudière, B.P. 82225, 44322 Nantes Cedex 3, France

The representativeness of the odor of mussel extract was assessed after each step of the distillation– extraction–concentration process. Results showed that the whole process was convenient for cooked mussels, but the extract was representative only when it was reincorporated into a suitable matrix such as water. Sensory and gas chromatography–olfactometry (GC-O) analyses were then performed on representative extracts of wild and bouchot mussels. Most of the sensory attributes were related to odors detected during olfactometry. Methional and (Z)-4-heptenal were two of the most potent odorants of mussels and, thus, were identified as the major contributors to the characteristic boiled potato-like odor of cooked mussels distinguished during sensory analysis. The sulfury note, highlighted for wild mussels during sensory analysis, could be linked to dimethyl disulfide, which was significantly more perceived in wild mussels by GC-O. Dimethyl disulfide could then be considered to be a characteristic compound of wild mussels.

Keywords: Representativeness; aroma; detection frequency; olfactometry; mussel

INTRODUCTION

Consumption of mussels in France is very high. Mussels from bouchot culture account for most of the French production. However, for some years, production of wild mussels from natural deposits has been on the increase.

Volatile compounds of mussels have already been studied (1, 2), but the authors did not study aromaactive compounds. In a previous work (3), we compared three olfactometric techniques applied to bouchot mussels, but we did not make a detailed study of the representativeness of the extract, and correlation between sensory attributes and odors detected by gas chromatography–olfactometry (GC-O) was not developed.

To determine which compounds contribute significantly to the odor of a product or which compounds are responsible for the differences between the odor of two products, it is necessary to ensure that the method of extraction provides an extract with an odor that is representative of the original product (4). This representativeness study should be a prerequisite to further aroma analyses such as GC-O. Moio et al. (5) emphasized that the aroma of the wine may change either during the solvent extraction step or during the concentration process and does not necessarily reproduce the characteristics of the wine from which it was obtained. They demonstrated that the odor of an extract prepared according to a common solvent extraction, and involving concentration by distillation of the solvent, lacked several characteristic wine odors and exhibited additional cooked odors. Many authors also highlighted the importance of testing the representativeness of the extracts in a matrix with characteristics similar to those of original products. Thus, Etievant et al. (6) showed that a cheese extract was more similar to the reference cheese when it was added in an emulsion than when it was evaluated in water. Guyot et al. (7) found that aqueous extracts of butter obtained by vacuum distillation were representative of the original butter only if they were reincorporated into a model emulsion similar to the one they came from. Abott et al. (4) and Bernet et al. (8) tested the representativeness of, respectively, beer extracts and Gewurtztraminer wine extracts, in hydroalcoholic solutions. Guillard et al. (9) and Charles et al. (10) assessed the odor quality of, respectively, cooked cured ham extracts and wine vinegar extracts by redilution in water. Charles et al. (10) explained that their dichloromethane extracts were rediluted in water, which is one of the main components of vinegar, in such a concentration that dichloromethane was not detected in the odor. To our knowledge, no studies have been published on the representativeness of seafood extracts.

Once the representativeness of the extracts has been assessed, GC-O analyses may be applied. GC-O proved to be a powerful method leading to the characterization of key compounds of food aroma. Recently (3), we compared three types of olfactometric techniques (detection frequency, OSME, and AEDA) applied to cooked mussels. We concluded that the three methods were well correlated and that the key compounds contributing mainly to the aroma were identical whatever the method considered. The choice of a method depends on the objective of the study, the quality of the panel, and the time scheduled for the analyses. The detection frequency method makes it possible to determine aromaactive compounds within a minimum of time, with no specific training of the panelists.

^{*} Author to whom correspondence should be addressed (telephone +33-2-51-78-55-18; fax +33-2-51-78-55-20; e-mail leguen@enitiaa-nantes.fr).

Consequently, the aims of this study were (i) to assess the representativeness of mussel extracts at each step of the whole extraction process of volatile compounds (i.e., after hydrodistillation, after liquid–liquid extraction, and after concentration) and after incorporation of the extract in a suitable matrix; (ii) to compare odor quality of bouchot and wild mussels and their corresponding extracts; and (iii) to apply the detection frequency method on representative extracts of both mussel batches in order to relate sensory attributes and odors detected during GC-O.

MATERIALS AND METHODS

Materials. Mussels (*Mytilus edulis*) were obtained from 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).

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

Vacuum Hydrodistillation. Vacuum hydrodistillation was performed in a low-pressure distillation apparatus modified from the one designed by Forss and Holloway (11) as previously described by Etievant and Bayonove (12). After rinsing, 1.6 kg of mussels was cooked in a vapor cooker (Magimix M050) for 20 min; 350 g of shelled mussels, 800 mL of purified water, and 1 mL of an aqueous solution of collidine (2,4,6trimethylpyridine) at 14 μ g/mL (used as an internal standard) were transferred into a 6-L round-bottom flask maintained at 37 °C during the distillation. Hydrodistillation was continued for 3 h under a pressure of 5 mbar. 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 receiving 4-L round-bottom flask and traps were pooled. 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 reduced to 4 mL in a Kuderna-Danish concentrator. 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 then stored at -20 °C in glass vials before analysis.

Sensory Analysis/Representativeness of the Extracts. *Panel.* The panel was composed of 10 assessors from our laboratory, previously trained to describe cooked mussel aroma over one year. Panelists were trained to generate descriptors of mussels and mussel extracts and to describe mussels and mussel extract aroma by descriptive analysis.

Sample Preparation and Presentation. As a first step, sample presentation was optimized. Cooked mussels (bouchot) and four extracts corresponding to different states of extraction and/or concentration were presented to the panelists. These extracts were as follows:

Extract A, in water, directly recovered after hydrodistillation, was contained in almost 870 mL of water (which corresponds to the 800 mL of water added in the 6-L round-bottom flask at the beginning of the extraction plus the intrinsic water of mussels).

Extract B, in solvent, obtained after liquid–liquid extraction of extract A by dichloromethane, was contained in almost 100 mL of solvent.

Extract C, in solvent, obtained after concentration of extract B and redilution in dichloromethane 10 times, was chosen by the panelists as a compromise between a more diluted extract leading to an excessively low intensity and a less diluted extract that had a very strong and aggressive odor preventing judges from describing it correctly.

Extract D was obtained after concentration of extract B (as extract C) but rediluted 5 times in purified water (optimal dilution).

As a second step, wild and bouchot mussels and their corresponding extracts were presented to the panel members, with the same presentation as extract D.

An aliquot of each mussel extract was adsorbed on coded smelling strips and presented to the judges after 30 s (the time necessary for solvent evaporation). Dichloromethane is very volatile and was evaporated in the atmosphere during 30 s. No judges detected the odor of the solvent. Cooked mussels were presented in brown flasks, at 30 °C.

Similarity Test. A similarity test was performed to assess the odor of extracts A–D compared to the odor of cooked mussels (reference sample). The extracts were presented in random order. The panelists were instructed to sniff and memorize the aroma of the reference sample and for each extract, to sniff smelling strips, and to determine the similarity of their odors. A 100 mm unstructured scale was used, anchored with "very different from the reference" on the left and "identical to the reference" on the right. The position of the sample on the unstructured scale was read as the distance in millimeters from the left anchor. Results were analyzed with a two-way analysis of variance with Statgraphics Plus software (Manugistics, Inc., Rockville, MD). A Student–Newsmans– Keuls test was used to perform a multiple comparison of means.

Odor Intensity Evaluation. The panelists were instructed to assess the odor intensity of extracts A-D. A 100 mm unstructured scale was used, anchored with "no odor" on the left and "very strong odor" on the right. The position of the sample on the unstructured scale was read as the distance in millimeters from the left anchor. Statistical analysis was performed as described above.

Descriptive Analysis of Cooked Mussels and Extracts. Two different sessions were organized: the first one, for extracts presented in dichloromethane and water at different concentrations (A-D); and the second session to describe wild and bouchot mussels and their corresponding extracts. Therefore, four samples were assessed at each sensory session. A list of 11 descriptors previously determined by the judges as being necessary to describe the odor of cooked mussels was used. This list was constituted by the whole panel by describing cooked mussel aroma by free vocabulary. The final list of descriptors was realized by adding all generated descriptors and by deleting redundant and nonconsensual descriptors. The panelists had then to smell each real food product corresponding to each descriptor in order to validate these descriptors. Panelists were instructed to describe each sample by choosing no more than five attributes as described by Moio et al. (5). Each sensory descriptor had to be cited in the order of significance. A weight of 5 was attributed to the first cited descriptor, 4 for the second, etc....

Results of the first session were processed using a factorial correspondence analysis (Statgraphics Plus), performed on the total weight of each sensory attribute to mussels and extracts.

During the second session, panel members described the odor of wild and bouchot mussels and of their corresponding extracts. Data were compared using analysis of variance for each descriptor.

Gas Chromatography–Olfactometry (GC-O). GC-O analyses were performed on wild and bouchot mussel dichloromethane extracts, concentrated to 200 μ L.

GC-O Conditions. The gas chromatography–flame ionization detector–olfactometry (GC-FID-O) system comprised a Varian 3400 GC (Varian, Palo Alto, CA) fitted with an FID at 280 °C and a sniffing port supplied with humidified air at 40 °C. Two microliters from each extract was injected (splitless mode) into a capillary column (DB-Wax, 30 m length × 0.32 mm i.d. × 0.5 μ m film thickness, J&W Scientific Inc., Folsom, CA). Effluent from the end of the GC column was split 1:1 between the FID and the sniffing port. The oven temperature was programmed from 40 to 250 °C at a rate of 5 °C/min with initial and final hold times of 2 and 10 min, respectively. The injector temperature was maintained at 250 °C. A solution of

hydrocarbons (C_6-C_{26}) was injected daily under the same conditions to calculate retention indices (RI).

Odor Detection Frequency. The detection frequency method was applied (13). A panel of 10 judges trained in odor recognition and with experience in GC-O 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 4 of 10 assessors was considered to be noise (14). The final aromagram was obtained by summation of the 10 individual sniffings.

Gas Chromatography—Mass Spectrometry (GC-MS). *GC-MS Conditions.* Two microliters of the extracts was injected into an HP 5890 series II GC/HP 5971 mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA) (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 length \times 0.32 mm i.d. \times 0.5 μ m film thickness, J&W Scientific). The oven temperature was programmed from 40 to 250 °C at a rate of 4 °C/min, with initial and final hold times of 2 and 10 min, respectively. MSD (electronic impact ionization) conditions were as follows: ionization energy, 70 eV; mass range, 33–300 amu; 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.

Identification. Compound identifications were based on comparison of GC RI (*15*), mass spectra (comparison with standard MS spectra databases: NBS 75K and internal library of the laboratory), and odor properties. Chemical standards of identified compounds were reinjected in GC-MS and into the GC-sniffing.

RESULTS AND DISCUSSION

Representativeness of the Extract. Optimization. In the first experiment, a similarity test was performed on extracts obtained after each step of the distillationextraction-concentration process compared to cooked mussels. Extract A was directly obtained after hydrodistillation, in water. The assessment of this extract was essential to characterize the efficiency of the extraction technique. Extract B was obtained after liquid-liquid extraction by dichloromethane, prior to concentration. Further to this, the extract was concentrated and rediluted into dichloromethane (extract C) to test the effect of the concentration step. This step of redilution was necessary; otherwise, the extract had an excessively strong odor that prevented panelists from describing the odor correctly. Another concern was the choice of a suitable matrix for testing the olfactory character of the extracts. It is of great importance to assess the representativeness of the extracts in a matrix with characteristics similar to those of the original products. As mussels contain almost 80% water, the concentrated extract (in dichloromethane) was rediluted in purified water (extract D).

The results of similarity test and intensity evaluation on these four extracts are presented in Table 1. Similarities of extracts A–C were evaluated to be not significantly different at a level of 5%. Extract A had a very low intensity, near 0, because of its high dilution in water. It was assessed as being different from cooked mussels and had the lowest mark on the similarity scale, probably due to its low intensity. Extract B was also of low intensity, which led to a difficult characterization of the extract. By comparing extracts B and C, the effect of the concentration step was assessed. Similarity notes of these two extracts were not significantly different, which showed that the concentration

Table 1. Similarity of the Odors of Extracts A–D toCooked Mussel Reference and Odor Intensity Evaluation

extract similarity scaling (cm)		intensity scaling (cm)			
А	2.54^{a}	0.86^{a}			
В	3.18 ^a	2.74 ^a			
С	3.29 ^a	5.41 ^b			
D	6.10 ^b	6.04^{b}			

 a Scalings with the same superscript letter were not significantly different at a level of 5%.

step did not induce new thermally generated compounds. However, the similarity score obtained for extracts presented in dichloromethane were low, which gave grounds for thinking that the extract lost some of the characteristics of mussels during the volatile extraction process. Nevertheless, when the same concentrated extract was dissolved in water (extract D), it was evaluated differently from the three other extracts and was assessed as being representative of cooked mussels. The similarity note was not very high, maybe due to a psychological effect. Indeed, panelists differentiated the odor of mussels sniffed on smelling strips and that coming directly from cooked mussels. Le Quéré et al. (16) demonstrated that when panelists evaluated the odor similarity of a hidden cheese sample to the same cheese sample used as a reference, the odor of the hidden sample was not evaluated as similar to the odor of the reference sample.

To precisely characterize descriptors responsible for differences between extracts, we made sensory descriptive analyses on the four extracts plus mussels. Results of this sensory analysis are graphically represented by a factorial correspondence analysis (Figure 1). Axis 1, with a weight of 61.8%, could be defined as a "matrix" axis. Indeed, extracts A and D, presented in water to the panelists, were opposed to extracts C and B, both contained in dichloromethane. The second axis had a weight of 20.6% and could be defined as an intensity axis. This axis separated extracts A and B, which had the lowest intensities, and extract D, which was the more intense (Table 1). The paper note was probably due to the odor of the smelling strips soaked in the solvent. Cooked mussels were described by the panelists by the following descriptors: cooked mussel, cooked fish, boiled potato, cooked shellfish, and cooked crustaceouscrab. Extract A had a low intensity and was described by only a few descriptors. Although the similarity notes of extracts B and C were not significantly different, their odor descriptions were not the same for some descriptors. Extract C was more often defined by descriptors such as grilled/rubber/paper, cooked crustaceous-crab, or sulfury (= cooked cabbage). However, as extracts B and C were not presented to the assessors at the same intensity, this difference could be induced either from the intensity or from the concentration step. To investigate this, an evaluation was made of the odor of extract D. This extract was obtained after concentration by Kuderna-Danish, as extract C, but was rediluted in water after concentration. Extract D exhibited many of the same characteristics as mussels such as boiled potato, cooked mussels, cooked fish, or cooked crustaceous-crab.

We can conclude that the whole volatile extraction process was convenient for mussels and that the extract, reincorporated into a suitable matrix such as water, was representative of cooked mussels. In this case, we did not observe, contrary to Moio et al. (5), the lack of some

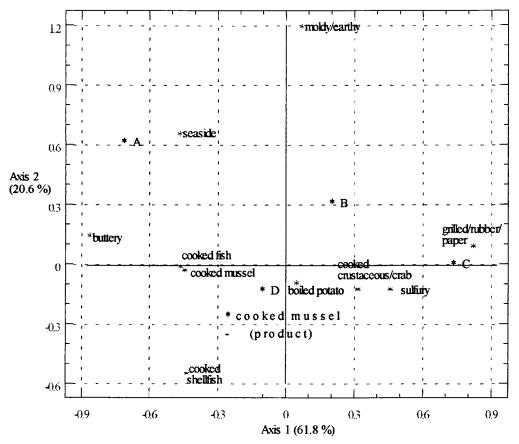


Figure 1. Factorial correspondence analysis of total weight of attributes to cooked mussels (product) and extracts A-D.

characteristics of the original product due to the concentration step. There still exists a difference between the odor of mussels and extract mainly due to a psychological effect. For the continuation of our studies, we kept the same distillation-extraction-concentration process, and the extracts were presented to the panelists in the same conditions as extract D.

Comparison of Wild Mussel and Bouchot Mussel Aroma. Ten descriptors were used to describe the aroma of mussels and corresponding extracts during descriptive analysis. Descriptors cited only once or twice were eliminated. The total weight of each of these descriptors is represented for both mussel batches in Figure 2. Bouchot mussels exhibited also a strong boiled potatolike odor, whereas wild mussels were more characterized by cooked crustaceous-crab-like odor. Seaside and sulfury descriptors were more used for wild mussels, whereas cooked fish and buttery odors were more characteristic of bouchot mussels. An analysis of the variance was performed to know which attribute made it possible to significantly discriminate wild mussels and bouchot mussels. Results are presented in Table 2 through the *p* value obtained first for the comparison of mussels and second for the comparison of both extracts. A p value of <0.05 meant that the corresponding descriptor significantly discriminated the two samples (mussel or extract). Two descriptors were judged to be significant when we compared mussels: cooked crustaceous-crab and boiled potato. The same analysis performed on both extracts showed the same results for these two descriptors. These results indicated that when a difference was detected between the products, it was also detected between the extracts on the same descriptors; these results also allow further comparative analyses to be performed on the extracts with the knowledge

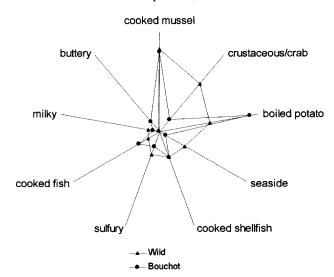


Figure 2. Total weight of sensory attributes of wild and bouchot mussels.

that the results obtained can be directly related to the aroma of mussels.

GC-O of Cooked Mussels. *Results of Olfactometry.* Odor-active compounds detected in wild and bouchot mussels are presented in Table 3. Thirty-three odors were detected; among them, 32 and 29 compounds were perceived by at least 4 of 10 panelists, respectively, in wild mussels and bouchot mussels. Twenty-three positive identifications were made by matching RI, mass spectrum, and odor quality to literature and chemical standards. Ten odor-active components were not identified because they were present at trace levels (peaks 12, 18, 20, 22, 24, and 32), because they coeluted (peaks

 Table 2. Descriptive Analysis of Wild Mussels and

 Bouchot Mussels and Their Corresponding Extracts

	<i>p</i> value			
descriptor	mussels	extracts		
cooked crustaceous-crab	0.0018	0.0799		
boiled potato	0.0179	0.0390		
seaside	0.1886	0.2878		
buttery	0.2409	0.5906		
sulfury	0.3105	0.1769		
cooked fish	0.4803	0.7517		
cooked mussel	0.9159	0.9225		
milky	0.6652	1.0000		
cooked shellfish	1.0000	0.1657		
paper/rubber/grilled	1.0000	0.9208		

1, 6, and 8), or because of a higher MS background at the higher temperature of GC separation (peak 31).

Of these 33 odorants, 7 were detected by at least 9 of 10 assessors in both extracts: unknown **6**, (*Z*)-4-heptenal (**11**), (*E*)-2-penten-1-ol (**15**), ethylpyrazine (**17**), dimethyl trisulfide (**19**), methional (**21**), and 2-acetyl-2-thiazoline (**30**). These 7 compounds may contribute actively to the aroma of cooked mussels due to their intense odor.

Difference between the Two Extracts. A difference of perception between two extracts by at least 3 of 10 assessors is significant (13). In our case, 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. One compound was

perceived by more judges in bouchot mussels: 3-methyl-2-butenal (**10**).

Relationship between Sensory Analysis and Olfactometry. We have tried to relate sensory attributes and odors detected during olfactometry. Boiled potato-like odor attributed to (Z)-4-heptenal (11), methional (21), and unknown 22 was a potent odor in both mussel extracts (Table 3). This odor was also a sensory characteristic of the aroma of cooked mussels, especially from bouchot mussels (Figure 2). During sensory analysis, boiled potato-like odor was found to be a significantly discriminating descriptor between wild and bouchot mussels (Table 2). (Z)-4-Heptenal and methional were detected by the majority of the panel in both extracts, which means that their concentrations were greater than the perception thresholds of the panel members but did not make it possible to determine if these compounds were more concentrated in either extract. (Z)-4-Heptenal was detected only at trace state in both extracts but was detected by the majority of the panel due to its low threshold value (0.04 ppb; 17). The odor of (Z)-4-heptenal was described by McGill et al. (17)as being similar to that of boiled potatoes. Likewise, Josephson and Lindsay (18) found that (Z)-4-heptenal exhibited a "cold boiled potato" aroma and that it was responsible for much of the aroma of boiling potatoes. McGill pointed out that cooking was an important factor in the production of (Z)-4-heptenal. Methional was formed by the Strecker degradation of methionine, during cooking (19). Petersen et al. (20) showed that

Table 3. Odor-Active Compounds in Wild Mussels (W) and Bouchot Mussels (B)

				Df		estimated concentration ^h			
peak	RI ^a	compound	methods of identification	odor description ^b	В	W	В	W	Ftest
1	930	unknown		fruity, pyrogenous	5	6	nd	nd	
2	980	2,3-butanedione	MS, RI, odor, ^{<i>e</i>,<i>f</i>} standard	buttery, caramel	7	9	3.0	9.5	***
3	1045	1-propanol	MS, RI, odor, ^{<i>e</i>,<i>f</i>} standard	fruity, plastic	5	5	1.8	0.4	***
4	1074	dimethyl disulfide	MS, RI, odor, ^{<i>e</i>,<i>f</i>} standard	sulfury	1	5	tr	tr	
5	1092	hexanal	MS, RI, odor, ^{<i>e</i>,<i>f</i>} standard	green	6	7	1.3	1.4	
6	1113	unknown		sulfury, garlic	10	9	nd	nd	
7	1150	<i>m</i> -xylene	MS, RI, odor, ^e standard	plastic	5	4	1.2	0.4	***
8	1171	unknown		plastic	5	6	nd	nd	
9	1197	heptanal	MS, RI, odor, ^{<i>e</i>,<i>f</i>} standard	citrus fruit, green	4	4	2.2	2.0	
10	1215	3-methyl-2-butenal	MS, RI	d	4	1	0.7	0.5	
11	1253	(Z)-4-heptenal	MS, RI, odor, ^{<i>e</i>,<i>f</i>} standard	boiled potato	9	10	tr	tr	
12	1274	unknown		sulfury, garlic	8	8	tr	tr	
13	1293	1,2,4-trimethylbenzene	MS, RI, odor, ^e standard	plastic	4	4	1.3	0.3	***
14	1303	octanal	MS, RI, odor, ^{e,f} standard	citrus fruit, orange	6	8	1.4	1.6	
15	1316	(E)-2-penten-1-ol	MS, RI, odor, ^e standard	mushroom	10	10	tr	tr	
16	1336	(E)-2-heptenal	MS, RI, odor, ^{<i>e</i>,<i>f</i>} standard	sulfury, grassy	6	6	tr	tr	
17	1354	ethylpyrazine	MS, RI, odor, ^{<i>e</i>,<i>f</i>} standard	nutty	10	10	0.2	0.4	
18	1373	unknown		green, fruity	6	9	tr	tr	
19	1390	dimethyl trisulfide	MS, RI, odor, ^f standard	sulfury, marine	9	10	nd	nd	
20	1451	unknown		nutty	6	8	tr	tr	
21	1477	methional	MS, RI, odor, ^{e,f} standard	boiled potato	9	10	4.8	6.0	
22	1496	unknown		boiled potato, grassy	7	8	tr	tr	
23	1532	2-nonanol	MS, RI, odor, ^e standard	fruity, solvent	3	4	tr	tr	
24	1576	unknown		moldy, earthy	8	6	tr	tr	***
25	1605	(E,E)-2,4-octadienal	MS, RI	cucumber	4	7	tr	1,7	
26	1650	1-acetylpyrazine	MS, RI, $odor^{f}$	nutty	8	10	1.1	1.5	
27	1660	2-acetylthiazole	MS, RI, odor, ^{<i>e</i>,<i>f</i>} standard	grilled hazelnut	6	4	1.7	2.8	
28	1681	4-methylthiazole	MS, RI	roasted, meaty	4	5	1.0	1.2	
29	1753	4-ethylbenzaldehyde	MS, RI, odor, ^e standard	fruity, anisic, minty	4	5	0.3	0.3	
30	1790	2-acetyl-2-thiazoline	MS, RI, odor ^g	grilled hazelnut	9	10	0.5	1.3	
31	1913	unknown		fruity, grassy	5	6	nd	nd	
32	1935	unknown		rubber, roasted	1	4	tr	tr	
33	2038	2,6-dimethylnaphthalene	MS, RI, odor, ^e standard	grilled, grassy	2	4	nd	nd	

^{*a*} Retention index on DB-Wax column. ^{*b*} Odor description as perceived by panelists during olfactometry. ^{*c*} Detection frequency (/10 panelists). ^{*d*} Odor detected without a common descriptor for most of the judges. ^{*e*} Odor of the standard. ^{*f*} Reference *33. ^g* Reference *23.* ^{*h*} Estimated concentration (in nanogram equivalents of collidine per gram of mussel); nd, not determined. ***, *F* test, significance level <1%.

methional was a key compound in the aroma of boiled potatoes, and it was even undetected in raw potatoes. The estimated concentrations of methional in both extracts were not significantly different. Through its low threshold value of 0.2 ppb (21), methional was already characterized as the most potent odorant in cooked mussel (3) and was previously reported as a necessary and significant component of the desirable aroma of cooked lobster (22) or cooked clam (23).

Five compounds (4, 6, 12, 16, and 19) were described during olfactometry by a sulfury odor, which had previously been characterized by panelists during sensory analysis. Shankaranarayana et al. (24) reported that sulfur-containing compounds gave strong, sulfurous, cooked cabbage odors in vegetables, meats, and marine products. Although it was not significant, the sulfury odor was more perceived in wild mussels than in bouchot mussels (Figure 2). This difference could be due to dimethyl disulfide (4), which was significantly more perceived in wild mussels during olfactometry. Dimethyl disulfide could be considered to be characteristic of wild mussels. This compound is often present in foodstuffs and usually affects overall food aroma because of its low threshold value of 12 ppb (25). It may have been thermally generated from methional (26)

As shown in Figure 2, mussels, especially bouchot mussels, were characterized by a buttery odor. The compound responsible for this odor was identified as 2,3butanedione (2), which exhibited a powerful buttery, caramel-like odor. To the contrary of results of sensory analysis, olfactometric analysis showed that the buttery odor was more perceived in wild mussels than in bouchot mussels. This discrepancy showed the difficulty of relating sensory and olfactometry results. Indeed, by olfactometry, odors are evaluated separately and out of the food matrix. For the sensory analysis, odors are blended and aroma compounds may interact with other constituents of the food matrix, which could modify their perception. Through its high detection frequency and its low detection threshold of 2.3-6.5 ppb (27), this compound may impart a key aroma for cooked mussels. 2,3-Butanedione is thermally generated through the Maillard reaction (28) and is a characteristic product in cooked seafood. It was also a contributor to the desirable flavor in cooked fish such as turbot (29) or tuna (30).

The rubber/roasted and grilled/grassy-like odors of unknown **32** and 2,6-dimethylnaphthalene (**33**) could be associated with the "rubber/grilled/paper"-like odor used for the description of mussel aroma during sensory analysis. This odor had a very little weight in the description of mussel aroma, which correlated with olfactometric results because these two odors were detected by only a few judges in wild mussels and were even almost undetected in bouchot mussels.

The cooked crustaceous—crab-like odor detected during sensory analysis (Figure 2) was not detected by olfactometry. However, it may be related to the marine note of dimethyl trisulfide (**19**), which was perceived by all of the panelists during olfactometric analyses. Dimethyl trisulfide, with a threshold value of 10 ppb (*25*), had been identified in many thermally processed molluscs such as prawn, crabmeat, and crayfish (*31*).

Although it was not detected by the panelists during sensory analysis, the green/cucumber/fruity notes were revealed to be important in the aroma of cooked mussels, especially in wild mussels. Indeed, 10 compounds (1, 3, 5, 9, 14, 18, 23, 25, 29, and 31) were described by a fruity and/or cucumber or green-like odor in both mussel batches. Two of them, unknown 18 with a green, fruity odor and (E, E)-2,4-octadienal (25) with a cucumber-like odor, had significantly higher detection frequencies in wild mussels than in bouchot mussels (Table 3).

Nutty odors seemed to play a major role in the aroma of mussels. Indeed, five nutty odors were detected in both extracts by olfactometry. These odors were due to two pyrazines (**17** and **26**), one unknown (**20**), 2-acetylthiazole (**27**), and 2-acetyl-2-thiazoline (**30**). It was not surprising to find pyrazines because they are a product of Maillard reactions and are extensively generated during cooking. They are usually found in cooked molluscs such as crab (*32*) or crayfish (*26*). Nevertheless, these nutty odors were not detected by sensory analysis, but they may contribute to the characteristic cooked flavor of mussels.

Although plastic-like odors of m-xylene (7) and 1,2,4trimethylbenzene (13) were not perceived during sensory analysis, they were detected by olfactometry. However, they were not detected by many judges due to their high detection thresholds and, thus, do not contribute much to the overall aroma of mussels. Aromatic hydrocarbons were suspected of coming from environmental pollutants.

The mushroom-like odor of (E)-2-penten-1-ol (**15**) and the moldy earthy-like odor of unknown **24** were perceived by the majority of the panel during olfactometry. These odors were detected by only one judge during sensory analysis and were thus eliminated from the results.

ACKNOWLEDGMENT

We gratefully acknowledge all colleagues for their contribution to sensory and olfactometric analyses and Mr. A. Chess for English revision.

LITERATURE CITED

- Yasuhara, A.; Morita, M. Identification of volatile organic components in mussel. *Chemosphere* 1987, *16* (10-12), 2559-2565.
- (2) Yasuhara, A. Comparison of volatile components between fresh and rotten mussels by gas chromatography-mass spectrometry. J. Chromatogr. 1987, 409, 251–258.
- (3) Le Guen, S.; Prost, C.; Demaimay, M. Critical comparison of three olfactometric methods for the identification of the most potent odorants in cooked mussels (*Mytilus edulis*). J. Agric. Food Chem. **2000**, 48, 1307–1314.
- (4) Abbott, N.; Etievant, P.; Langlois, D.; Lesschaeve I.; Issanchou, S. Evaluation of the representativeness of the odor of beer extracts prior to analysis by GC eluate sniffing. J. Agric. Food Chem. 1993, 41, 777–780.
- (5) Moio, L.; Chambellant, E.; Lesschaeve, I.; Issanchou, S.; Schlich, P.; Etievant, P. X. Production of representative wine extracts for chemical and olfactory analysis. *Ital. J. Food Sci.* **1995**, *3*, 265–278.
- (6) Etievant, P.; Moio, L.; Guichard, E.; Langlois, D.; Lesschaeve, I.; Schlich, P.; Chambellant, E. Aroma extract dilution analysis (AEDA) and the representativeness of the odour of food extracts. In *Trends in Flavour Research*; Maarse, H., van der Heij, D. G., Eds.; Elsevier Science: Amsterdam, The Netherlands, 1994; pp 179–190.

- (7) Guyot, C.; Bonnafont, C.; Lesschaeve, I.; Issanchou, S.; Spinnler, H.-E. Mise au point et validation d'une technique d'extraction des arômes du beurre permettant de conserver la typicité de l'odeur des produits (Optimization and validation of an extraction procedure preserving odor characteristics of butters). *Lait* **1998**, *78*, 351– 361.
- (8) Bernet, C.; Dirninger, N.; Etievant, P.; Schaeffer, A. Evaluation de la représentativité aromatique d'extraits de vins de gewurztraminer (Evaluation of the odor representativeness of gewurztraminer wines extracts). *Sci. Aliments* **1999**, *19* (6), 701–707.
- (9) Guillard, A.-S.; Le Quere, J.-L.; Vendeuvre, J.-L. Emerging research approaches benefit to the study of cooked cured ham flavour. *Food Chem.* **1997**, *59* (4), 567–572.
- (10) Charles, M.; Martin, B.; Ginies, C.; Etievant, P.; Coste, G.; Guichard, E. Potent aroma of two red wine vinegars. *J. Agric. Food Chem.* **2000**, *48*, 70–77.
- (11) Forss, D. A.; Holloway, G. L. Recovery of volatile compounds from butter oil. J. Am. Oil Chem. Soc. 1967, 44, 572–575.
- (12) Etievant, P. X.; Bayonove, C. L. Aroma components of pomaces and wine from the variety Muscat de Frontignan. J. Sci. Food Agric. **1983**, 34, 393–403.
- (13) 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 comparisons. J. Agric. Food Chem. **1997**, 45, 2630–2637.
- (14) van Ruth, S. M.; Roozen, J. P. Gas chromatography/ sniffing port analysis and sensory evaluation of commercially dried bell papers (*Capsicum annuum*) after rehydratation. *Food Chem.* **1994**, *51*, 165–170.
- (15) van den Dool, H.; Kratz, P. D. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. 1963, 11, 463.
- (16) Le Quéré, J.-L.; Septier, C.; Demaizières, D.; Salles, C. Identification and sensory evaluation of the characterimpact compounds of goat cheese. In *Flavour Science: Recent Developments*, Taylor, A. J., Mottram, D. S., Eds.; The Royal Society of Chemistry: London, U.K., 1996; pp 325–330.
- (17) 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.
- (18) Josephson, D. B.; Lindsay, R. C. Retro-aldol degradations of unsaturated aldehydes: Role in the formation of c4-heptenal from t2, c6-nonadienal in fish, oyster and other flavors. J. Am. Oil Chem. Soc. **1987**, 64 (1), 132– 138.
- (19) Forss, D. A. Review of the progress of diary science: mechanisms of formation of aroma compounds in milk and milk products. *J. Dairy Res.* **1979**, *46*, 691–706.

- (20) Petersen, M. A.; Poll, L.; Larsen, L. M. Comparison of volatiles in raw and boiled potatoes using a mild extraction technique combined with GC odor profiling and GC-MS. *Food Chem.* **1998**, *61* (4), 461–466.
- (21) Guadagni, D.; Buttery, R.; Turnbaugh, J. Odor thresholds and similarity ratings of some potato chip components. *J. Sci. Food Agric.* **1972**, *23*, 1435–1444.
- (22) 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.
- (23) Sekiwa, Y.; Kubota, K.; Kobayashi, A. Characteristic flavor components in the brew of cooked clam (*Meretrix lusoria*) and the effect of storage on flavor formation. J. *Agric. Food Chem.* **1997**, *45*, 826–830.
- (24) Shankaranarayana, M. L.; Raghavan, B.; Abraham, K. O.; Natarajan, C. P. Sulphur compounds in flavours. In *Developments in Food Science 3A. Food Flavours. Part A. Introduction*; Morton, I. D., MacLeod, A. J., Eds.; Elsevier Science Publishing: New York, 1982.
- (25) Buttery, R. G.; Guadagni, D. G.; Ling, L. C.; Seifert, R. M.; Lipton, W. Additional volatile components of cabbage, broccoli and cauliflower. J. Agric. Food Chem. 1976, 24, 829.
- (26) 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.
- (27) Leffingwell, J. C.; Leffingwell, D. GRAS Flavor Chemicals-Detection Threshold. *Perfum. Flavor.* **1991**, *16*, 1–19.
- (28) Hodge, J. E. Origin of flavour in foods. Nonenzymatic browning reactions. In *Chemistry and Physiology of Flavours*; Schultz, H. W., Day, E. A., Libbey, L. M., Eds.; AVI Publishing: Westport, CT, 1967; pp 465–491.
 (29) Prost, C.; Serot, T.; Demaimay, M. Identification of the
- (29) Prost, C.; Serot, T.; Demaimay, M. Identification of the most potent odorants in wild and farmed cooked turbot (*Scophtalamus maximus* L.). *J. Agric. Food Chem.* **1998**, 46, 3214–3219.
- (30) Cha, Y. J.; Cadwallader, K. R. Aroma-active compounds in Skijack tuna sauce. J. Agric. Food Chem. 1998, 46, 1123–1128.
- (31) Tanchotikul, U.; Hsieh, T. C.-Y. Volatile flavor components in crayfish waste. J. Food Sci. 1989, 54, 1515– 1520.
- (32) Hayashi, T.; Ishii, H.; Shinohara, A. Novel model experiment for cooking flavor research on crab leg meat. *Food Rev. Int.* **1990**, *6*, 521–536.
- (33) Furia, T. E. *CRC Handbook of Food Additives*, 2nd ed.; CRC Press: Boca Raton, FL, 1980; Vol. II, pp 259–316.

Received for review June 26, 2000. Revised manuscript received November 17, 2000. Accepted November 28, 2000.

JF000781N