Critical Comparison of Three Olfactometric Methods for the Identification of the Most Potent Odorants in Cooked Mussels (*Mytilus edulis*)

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Three olfactometric methods (olfactometry global analysis, OSME, and AEDA) were compared to evaluate the main impact odorants of cooked mussels. The results obtained from these methods were very similar and well correlated. On the basis of the three techniques, 42 odor-active compounds were detected and 28 were identified. Among these compounds, 6 odorants seem to contribute actively to the aroma of mussels: 2,3-butanedione (**4**) (buttery, caramel-like odor), (*Z*)-4-heptenal (**14**) (boiled potato-like odor), (*E*)-2-penten-1-ol (**17**) (mushroom-like odor), 2-ethylpyrazine (**19**) (nutty odor), methional (**25**) (boiled potato-like odor), and (*E*,*E*)-2,4-octadienal (**32**) (cucumber-like odor).

Keywords: *Detection frequency; OSME; AEDA; aroma; olfactometry; mussel*

INTRODUCTION

In France, consumption of mussels is such that production does not meet the demands of French consumers, even though production was estimated to be ∼60000 tons in 1998. Yasuhara and Morita (1987) identified >100 volatile compounds in mussels (*Mytilus edulis*), among which were many esters, acids, phenylalkanes, and several alkylbenzenes. However, to our knowledge, no report on the potent odorants of mussels has been published.

Gas chromatography−olfactometry (GC-O) has been extensively used in aroma research and allows the direct determination of potent odorants in food. At the present time, olfactometric techniques can be classified into three categories: dilution methods; intensity method; and detection frequency method.

Dilution techniques, Charm analysis (Acree et al., 1984) and aroma extract dilution analysis (AEDA) (Ulrish and Grosch, 1987), are commonly applied and are suitable to screen the impact odorants of food. Both methods are based on GC-O of an aroma extract that is diluted until no odor is detected at the sniffing port. The principal difference between the two methods is that Charm analysis measures the dilution value over the entire time the compounds elute, whereas AEDA simply determines the maximum dilution value (Grosch, 1994).

The OSME method was developed by McDaniel et al. (1990) to measure the perceived odor intensity of a compound eluting from a GC. Four assessors sniffed the nondiluted extract on four replicates. Intensities were then averaged, which led to a consensus osmegram. This method is different from Charm analysis and AEDA in that OSME is not based on odor detection thresholds but on odor intensity.

Recently, Van Ruth et al. (1995) and Ott et al. (1997) developed a new technique, the olfactometry global analysis, which is based on detection frequency. Numerous panel members sniffed the nondiluted extract, and the individual aromagrams were summed. Peak heights are not linked to flavoring intensities but to their detection frequencies.

All of these methods have been used to determine potent odorants in food and to differentiate food products. Guichard et al. (1995) measured the intensity perceived by sniffing a model solution, using two different types of apparatus. They finally compared their results with those of Charm analysis and concluded that Charm histograms were very similar to those obtained with the intensity method. However, to our knowledge, no work that compares olfactometric methods in the case of a food product has been published.

The objective of this study is to compare the olfactometry global analysis, OSME, and AEDA and to identify the most potent odorants of cooked mussels.

MATERIALS AND METHODS

Materials. Mussels (*M. edulis*) were obtained from Bouchot culture of Mont Saint Michel bay (France). They remained 6 months at their breeding site. Once collected, mussels were washed and were immediately transported in refrigerated conditions to the laboratory and then stored at 4 °C.

Chemicals. Dichloromethane (GC quality) and all standard compounds were purchased from Sigma-Aldrich Chemical Co. except dimethyl sulfide, octanal, and xylene, which came from Merck,and 1-propanol, which was obtained from Prolabo.

Simultaneous Steam Distillation−Solvent Extraction (SDE). SDE was done in a Likens− Nickerson (Likens and Nickerson, 1964) apparatus as described by Tanchotikul and Hsieh (1991). After rinsing, 1.6 kg of mussels was cooked with a vapor cooker (Magimix M050) for 20 min; 350 g of decorticated mussels and 800 mL of purified water were transferred into a 2-L round-bottom flask. A 100-mL conical bottom flask containing 19 mL of redistilled water and 1 mL of a *p*-cymene solution at 2.5 μg/mL in dichloromethane (used as internal standard) was attached to the solvent arm of the SDE head. Sample and solvent were both heated to boiling point, and distillation/extraction was continued for 1 h. Extracts were stored at −20 °C until analysis to facilitate water removal. Before analysis, SDE extracts were dried over 3 g of anhydrous sodium sulfate and reduced to 4 mL in a Kuderna-Danish

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concentrator to exactly 0.5 mL under a gentle stream of nitrogen. Six SDE extracts were prepared and stored at −20 °C in glass vials. To improve identification by GC/MS and to have a higher sensitivity during olfactometric analyses, the six SDE extracts were pooled and concentrated to 1 mL under a gentle stream of nitrogen.

Gas Chromatography−Mass Spectrometry (GC-MS). A Hewlett-Packard (Palo Alto, CA) GC/mass selective detector (HP5890 II/HP5971) was used to analyze SDE extracts. Volatile compounds were separated using a fused silica gel capillary column (DB-Wax, 60 m length × 0.32 mm i.d. × 0.5 μm film thickness, J&W Scientific, Folsom, CA). GC conditions were as follows: 1-μL splitless injection (30-s valve delay); injector temperature, 250 °C; helium carrier gas at 1 mL/min; oven programmed from 40 to 250 °C at a rate of 3 °C/min, with initial and final hold times of 5 and 10 min. The quadrupole mass selective detector, with electronic impact ionization (ionization energy, 70 eV) and an electron multiplier voltage of 2000 V, operated in the scan mode, with a mass range of 30−300 amu, at 2.0 scans/s. The detector interface temperature was set at 280 °C, with the actual temperature in the MS source reaching 180 °C.

Compound identifications were based on comparison of retention indices (RI) (Van Den Dool and Kratz, 1963), mass spectra (comparison with standard MS spectra databases: NBS 75K and internal library of the laboratory), and odor properties.

Descriptive Analysis of Cooked Mussels and SDE Extract. The odor quality of the SDE extract of mussels was evaluated by a panel of nine judges previously trained to describe cooked mussel aroma. SDE extracts were presented to the panel on smelling strips. A list of descriptors previously determined by the judges as being necessary to describe the odor of the mussels samples was used. For the evaluation, panelists were asked to assess the odor of the extract and of cooked mussels used as a reference.

Gas Chromatography−Flame Ionization Detection−Olfactometry (GC-FID-O). The GC-FID-O system consisted of a 3400 Star GC (Varian, Palo Alto, CA) equipped with a capillary column (DB-Wax 30 m length × 0.32 mm i.d. × 0.5 μm film thickness, J&W Scientific, Folsom, CA), an FID at 280 °C, and a sniffing port supplied with humidified air at 40 °C. GC effluent was split 1/1 between the FID and sniffing port. Oven temperature was programmed from 40 °C for 2 min to 210 °C at 7.5 °C/min followed by a temperature increase of 4 °C/min up to 250 °C and a final time of 10 min. The temperature adjustment for the GC was not the same as for GC-MS, to minimize the sniffing time for panelists. Other GC conditions were the same as above.

Compounds identified by GC-MS were reinjected as chemical standards into the GC−sniffing system to check their retention indices and their odor qualities.

Olfactometry Global Analysis. A panel of nine judges [according to Pollien et al. (1997), who recommends that the ideal condition would be to have a panel of 8−10 assessors] trained in odor recognition and with experience in GC-O was selected. Sniffing was divided into two parts of 19 min. Each person participated in the sniffing of both parts but during two distinct sessions to avoid tiredness. The panelists were asked to assign odor properties to each compound detected. Detection of an odor at the sniffing port by fewer than four of nine assessors was considered as noise (Van Ruth et al., 1994). The nine individual aromagrams were summed, yelding the final aromagram (detection frequency versus RI).

OSME. The Osme method, as developed by McDaniel et al. (1990), was used to measure the perceived odor intensity of a compound. The panel of nine subjects was trained by olfactometry using standard compounds, over a period of three weeks, to evaluate aroma intensity using a nine-point intensity scale (1 = very week, 3 = week, 5 = moderate, 7 = large, 9 = extreme). Four judges selected for their repeatability during training and their high detection sensitivity during the olfactometric global analysis were chosen for OSME analyses. Sniffing conditions were the same as described for the global analysis, except that analysts were also asked to assess intensity (on a scale of nine points) for each odorant zone. The sample was evaluated four consecutive times by each of four assessors. Times and intensities of peaks detected at least twice for each subject were averaged (with an intensity of zero for odors not detected). Then, times and intensities of those peaks that were detected by at least three of the four panelists were averaged again and a consensus osmegram (averaged intensities versus RI) was created.

AEDA. In the AEDA method (Grosch, 1994), serial dilutions (1:3 in dichloromethane) of the extract were sniffed until odor-active regions were no longer detected. AEDA was performed by the two most sensitive and repeatable panelists selected during olfactometric global and OSME analyses. The result is expressed as a flavor dilution factor (FD factor = 3^{n-1}, with n = number of coincident responses), which is the ratio of the concentration of the odorant in the initial extract to its concentration in the most dilute extract in which odor was detected. Data from AEDA were also represented in graphs by reporting n (number of coincident responses) versus RI.

Statistical Treatment. Statistical analyses were carried out with Statgraphics Plus software (Manugistics, Inc., Rockville, MD). The three olfactometrics methods were compared by the Pearson correlation.

RESULTS AND DISCUSSION

The results of olfactometry global analysis, OSME, and AEDA are summarized in Table 1. Most of the volatile compounds were identified by GC-MS, retention index, and odor (both were compared with literature and chemical standards).

Olfactometry Global Analysis. Thirty-nine odorants were perceived by at least four of nine panelists (Figure 1), and 28 were identified. 2,3-Butanedione (peak 4), unknown 10, *o*-xylene (peak 13), (*Z*)-4-heptenal (peak 14), (*E*)-2-penten-1-ol (peak 17), 2-ethylpyrazine (peak 19), methional (peak 25), (*E*)-2-nonenal (peak 29), (*E*,*E*)-2,4-octadienal (peak 32), and unknown **38** were detected by eight or nine of nine judges. These 10 compounds may contribute actively to the global aroma of mussels.

OSME. Twenty-eight odor-active regions were perceived (Figure 2). Twenty-five of these odorants were already pointed out by the olfactometry global analysis. Three new odorants were detected (unknowns **2**, **40**, and **41**). They were not characterized earlier because they were perceived by only three analysts during the global analysis. Average intensities were between 1.38 (unknown **41**) and 7.19 (methional, **25**) on a scale of nine points. Ten compounds appeared with an average intensity ≥5: 2,3-butanedione (**4**), (*Z*)-4-heptenal (**14**), unknown **15**, (*E*)-2-penten-1-ol (**17**), 2-ethylpyrazine (**19**), unknown **21**, (*Z*)-3-hexen-1-ol plus dimethyl trisulfide (**22**), methional (**25**), (*E*)-2-nonenal (**29**), and (*E*,*E*)-2,4-octadienal (**32**).

Results of the Two Analysts Selected for AEDA. Thirteen compounds had FD factors ≥27 for judge 1 (Figure 3) and nine for judge 2 (Figure 4). AEDA revealed methional as the most potent volatile odorant for both judges (FD factor = 19683 for judge 1 and 6561 for judge 2). Most of the odorants, perceived at least until the third dilution step (i.e., FD factor ≥27) were common for both panelists. Judge 1 highlighted the importance of *m*-xylene (peak 11), 1-ethyl-2,3-dimethylbenzene (peak 20), (*E*)-2-nonenal (peak 29), and unknown **38**.

Critical Evaluation of Three Commonly Used Techniques for Olfactometry Analyses. The olfactometry global analysis, OSME, and AEDA were first compared using the Pearson correlation. For the sta-

Table 1. Odor-Active Compounds in Cooked Mussels

peak[a]	RI[b]	compound	method of identification	odor description[c]	global analysis[d]	OSME av intensity	AEDA FD factor judge 1	AEDA FD factor judge 2
1	729	dimethyl sulfide	MS, RI, odor,[h,i] standard	marine, sulfury	7	2.50	27	27
2	769	unknown		marine, sulfury, green	3	1.63	<3	<3
3	931	unknown		fruity, pyrogenous	6	2.19	3	<3
4	980	2,3-butanedione	MS, RI, odor,[h,i] standard	buttery, caramel	9	5.75	27	27
5	1052	1-propanol	MS, RI, odor,[h,i] standard	plastic	5	2.06	9	<3
6	1066	2,3-pentanedione	MS, RI, odor,[h,i] standard	buttery, grassy	5	nd[f]	<3	<3
7	1074	dimethyl disulfide	MS, RI, odor,[h,i] standard	sulfury	7	nd	<3	<3
8	1089	hexanal	MS, RI, odor,[h,i] standard	green, garlic	6	1.44	<3	<3
9	1099	unknown		grilled	7	1.88	<3	<3
10	1114	unknown		garlic	8	4.94	27	27
11	1148	*m*-xylene	MS, RI, standard	*e*	4	nd	27	<3
12	1177	3-penten-2-ol	MS, RI, odor,[i] standard	grilled	4	nd	<3	3
13	1189	*o*-xylene	MS, RI, odor,[i] standard	plastic-gas	8	4.19	9	3
14	1255	(*Z*)-4-heptenal	MS, RI, odor,[h,i] standard	boiled potato	8	6.88	2187	243
15	1280	unknown		garlic, sulfury	7	5.25	9	<3
16	1303	octanal	MS, RI, odor,[h,i] standard	citrus fruit, orange	6	3.88	9	<3
17	1318	(*E*)-2-penten-1-ol	MS, RI, odor,[i] standard	mushroom	9	6.31	81	81
18	1342	(*E*)-2-heptenal	MS, RI, odor,[h,i] standard	sulfury, grassy	6	2.88	<3	3
19	1358	2-ethylpyrazine	MS, RI, odor,[h,i] standard	nutty	8	6.00	243	81
20	1374	1-ethyl-2,3-dimethylbenzene	MS	plastic	6	nd	27	<3
21	1380	unknown		citrus fruit, green	7	5.25	<3	3
22	1391	(*Z*)-3-hexen-1-ol[j] + dimethyltrisulfide	MS, RI, odor,[h,i] standard	woody, green, marine	7	5.19	27	729
23	1413	2-butoxyethanol	MS, RI, odor,[i] standard	plastic	4	nd	<3	3
24	1451	(*E*)-2-octenal	MS, RI, odor,[h,i] standard	toasted, cucumber	5	3.06	9	<3
25	1477	methional	MS, RI, odor,[h,i] standard	boiled potato	8	7.19	19683	6561
26	1499	unknown	MS, RI	boiled potato, grassy	5	nd	<3	<3
27	1520	methylethenylpyrazine + (*E*,*E*)-2,4-heptadienal[j]	MS, RI, odor,[i] standard*	grassy, marine	6	nd	<3	<3
28	1533	2-nonanol	MS, RI, odor,[i] standard	plastic, fruity	4	nd	9	<3
29	1563	(*E*)-2-nonenal	MS, RI, odor,[i] standard	earthy	8	5.00	27	<3
30	1587	unknown		cucumber, earthy	4	1.44	9	3
31	1604	unknown		nutty	4	nd	<3	<3
32	1614	(*E*,*E*)-2,4-octadienal	MS, RI	cucumber, green	9	5.25	729	81
33	1656	unknown		nutty	7	1.56	<3	<3
34	1667	2-acetylthiazole	MS, RI, odor,[h,i] standard	grilled hazel nut	5	nd	3	<3
35	1735	ethylbenzaldehyde[g]	MS, odor[i]	fruity, anisic	6	nd	<3	<3
36	1764	naphthalene	MS, RI, odor,[h,i] standard	grilled, earthy	5	1.50	3	<3
37	1784	unknown		grilled, fruity	4	nd	<3	3
38	1800	unknown		nutty	8	4.50	27	3
39	1889	2-methylnaphthalene	MS, RI, odor,[i] standard	grilled, earthy	4	nd	<3	<3
40	1927	unknown		cucumber, grassy	3	2.13	<3	9
41	1948	unknown		grassy, boiled potato	3	1.38	3	<3
42	2038	2,6-dimethylnaphthalene	MS, RI, odor,[i] standard	grilled, boiled potato	5	2.25	9	9

[a] Numbers correspond to those in Figures 1−4. [b] Retention index on DB-Wax column. [c] Odor description as perceived by panelists during olfactometry global analysis, OSME, and AEDA. [d] Detection frequency (of nine panelists). [e] Odor detected without a common descriptor for most of the judges. [f] Odor not detected during OSME. [g] Position of the ethyl group not determined. [h] Furia (1980). [i] Odor of the standard. [j] In the case of coelution: compounds corresponding to the standard injected in GC.

tistical analyses, Table 1 was completed for intensities perceived by fewer than three judges or FD factors <3 for odorants revealed by the global analysis or OSME. Values of AEDA taken into account for the test were "*n*" values, which correspond to the number of coincident responses in the expression of the FD factor = 3^{n-1}. Results of the Pearson correlation are given (Table 2), through the correlation coefficient and the *p* value. The global analysis, OSME, and AEDA were compared two by two. Results of both judges of AEDA were also compared.

Results demonstrated that the three olfactometric methods were positively correlated, with *p* values ≅0.00001, which means that all of the methods were significantly linked.

The AEDA responses for judges 1 and 2 were well correlated. Nevertheless, correlations of AEDA with the two other methods showed that both judges had distinct sensitivities. Indeed, correlations of OSME or detection frequency with the responses of AEDA judge 1 were higher than correlations with AEDA judge 2, which translated to the highest sensitivity for judge 1. The main differences between the two both judges were due to the less odor-active compounds. Correlation between judges was better for the most potent odorants.

OSME was the method that best correlated with the other techniques with a correlation coefficient of 0.7984. Greater differences among the three techniques were due to compounds with weak average intensities (≤3) and FD factors ≤3, which were all the same perceived by six or seven assessors during the global analysis. These differences were due to the methods, which did not measure the same values and used different numbers of panelists and repeats.

These methods must be compared on the basis of three criteria: precision of the results, reproducibility, and easiness of use.

Van Ruth et al. (1995) pointed out that the number of assessors perceiving an odor at the sniffing port can be linked to correspond with odor intensity. Our results demonstrated that in most of the cases, detection frequency and intensity of one odor were well correlated.

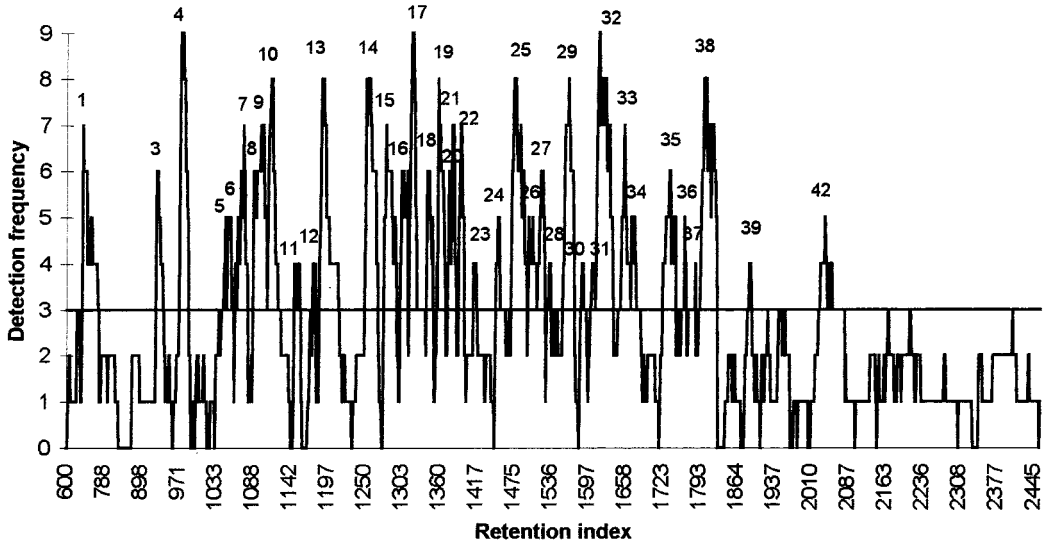

Figure 1. Aromagram of volatile compounds of cooked mussels obtained by the olfactometry global analysis. Peak numbers correspond to those listed in Table 1.

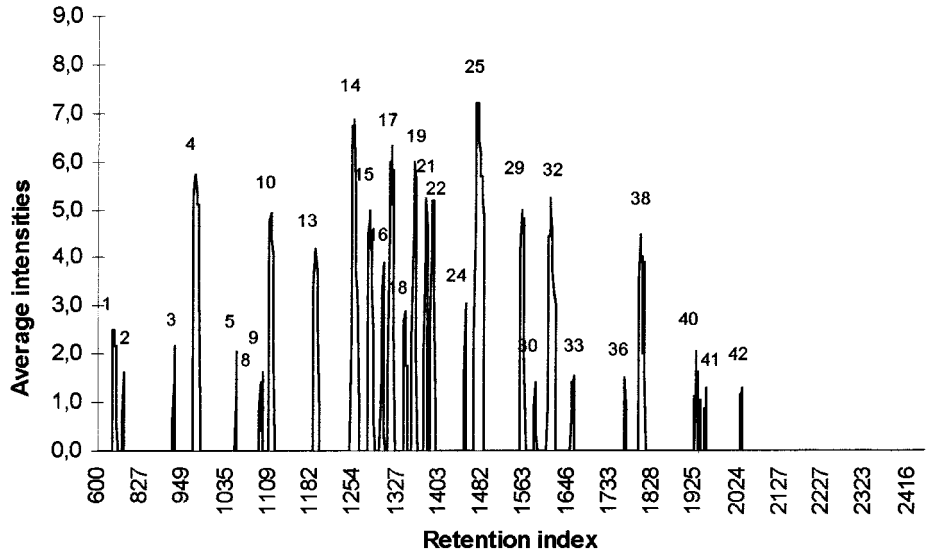

Figure 2. Osmegram of volatile compounds of cooked mussels, obtained by OSME. Peak numbers correspond to those listed in Table 1.

However, OSME is more precise than the two other methods. This can be illustrated by the odors **13** and **25**, both detected by eight analysts while using the olfactometry global analysis. OSME and AEDA showed that they did not contribute equally to the global aroma. Indeed, these odors had average intensities of 4.19 and 7.19, respectively, and FD factors of 9 and 19683 for judge 1. Likewise, AEDA is a bit less precise than OSME. An illustration of that are odors **24** and **30**. Both had FD factors of 9 (for judge 1) and intensities of 3.06 and 1.44, respectively. Meilgaard and Peppard (1986) showed that two components which are present at the same flavor unit level do not contribute equally to the flavor and that it is possible to underestimate the importance of some compounds in flavor contribution.

It is assumed that OSME is more precise than the other techniques, but the repeatability and reproducibility of the method must be taken into account. The panel used for the detection frequency method must have distinct sensitivities to be able to differentiate enough odors. This method offers the advantage of "smoothing" differences between or within individuals because each panelist participates in only 1/*n* of the final result, *n* being the number of panelists. Thus, two independent panels were able to generate similar aro-

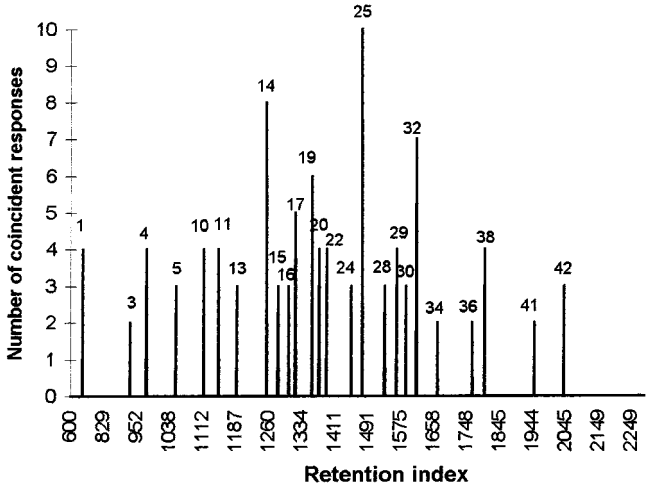

Figure 3. Flavor dilution chromatogram of volatiles isolated from cooked mussels, for judge 1 ($n \geq 2$). Peak numbers correspond to those listed in Table 1.

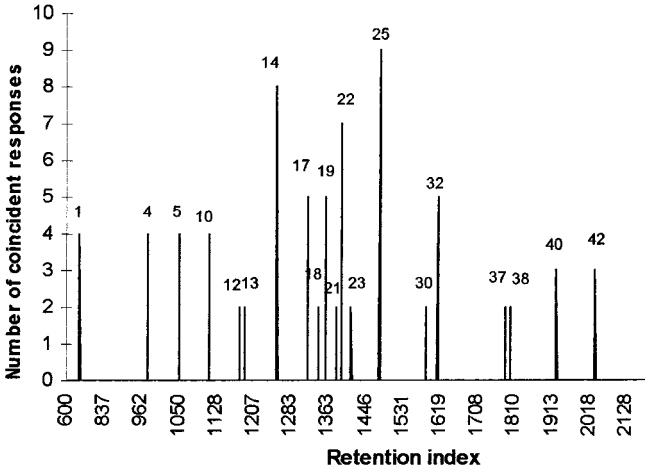

Figure 4. Flavor dilution chromatogram of volatiles isolated from cooked mussels, for judge 2 ($n \geq 2$). Peak numbers correspond to those listed in Table 1.

Table 2. Pearson Correlation between the Olfactometry Global Analysis (OGA), OSME, and AEDA[a]

	OGA	OSME	AEDA 1	AEDA 2
OGA	1			
OSME	0.7984 (0.00001)	1		
AEDA 1	0.6089 (0.00001)	0.7984 (0.00001)	1	
AEDA 2	0.5327 (0.0003)	0.7352 (0.00001)	0.7271 (0.00001)	1

[a] Correlation coefficient (p value).

magrams of a given product (Pollien et al., 1997). Research by da Silva et al. (1994) indicated that subjects using OSME were sensitive and reported odor intensity change with physical stimulus change. Our study verified this and can be seen in the good correlation of OSME with other methods. Our panelists gave sensitive and repeatable results, although there were some differences among panelists. For OSME and AEDA, responses were influenced by the quality of the panel. Results of AEDA showed that there were differences of sensitivity and odor detection between the two analysts, although they agreed in the rank order of the most odorant compounds. Indeed, threshold limits are known to vary greatly among individuals. Moreover, the panelist assessed an odor at the sniffing port against the background noise due to the chromatograph and therefore had to decide if he really perceived an odor or not; that is, the subject set a personal response criteria (Abbott et al., 1993). Grosch (1993) indicated that FD factors obtained by two assessors have been found to differ by two dilution steps at most. However, Abbott et al. (1993) claimed that the last dilution at which each individual detected an odor-active region was found to vary by up to four successive dilutions for the same retention index. In our case, FD factors differed by three dilution step maxima (odors **11**, **20**, **22**, and **29**). As shown by our results, judges participating in AEDA must have a very high sensitivity. Although AEDA has often been carried out by only one panelist, the presence of a second judge is necessary to confirm and complete the results.

These three methods must also be evaluated on their easiness of use. The olfactometry global analysis and AEDA need no training of the panel (except in odor recognition). On the contrary, members of the panel of OSME must first be trained in odor intensity. The global analysis needs nine injections on the CPG. OSME needs four replicates for each of the four analysts, which means 16 injections. For AEDA, 10 and 9 dilutions were necessary for judges 1 and 2, respectively, until no odors were detected, which represents 19 injections. The Global analysis is then almost twice as fast as OSME and AEDA.

Finally, the choice of an olfactometric method depends on the objective of the study, on the quality of the panel, and on the time scheduled for the analyses. The olfactometry global analysis allows one to obtain results in a short time with no specific panel. AEDA is more precise but more time-consuming than the global method. Its use requires at least one judge with a very high sensitivity. This technique is nevertheless suitable for obtaining detection threshold values. If results must be very precise, the use of OSME is necessary, but it requires four sensitive assessors who are repeatable and trained in odor intensity.

Identification of the Most Potent Odorants of Cooked Mussels. Here we discuss the precursors of odorant compounds and the positive or negative influence of these components on the flavor of cooked mussels. The presence of these odorants such as seafood volatiles found in previous studies is also discussed, especially if they were identified in other shellfish, cooked products, or extracts obtained by SDE. Focus was set on the most potent odorants.

Yasuhara and Morita (1987) identified >100 volatile compounds of mussel extracts obtained by vacuum distillation. Only five of the odorant compounds identified in the present study [2,3-pentanedione, (*E*)-2-penten-1-ol, hexanal, *o*- and *m*-xylene] were previously characterized by these authors.

In preliminary experiments, the odor quality of the SDE extract of cooked mussels was evaluated by the nine judges composing the panel of the olfactometric global analysis. They agreed that the odor of the extract was very similar to that of typical cooked mussels. This evaluation showed that mussels and SDE extract aroma were both described as boiled potato, white fish, crab, marine, and butter odors. In addition, the extract was characterized as having a grilled odor. This agreed with the results of the sniffing, compounds **14** and **25** being the most potent odorant with a boiled potato-like odor. Some sulfur-containing compounds (**1**, **7**) were described as having a sulfurous and marine-like odor. Odorant **4**, which was well perceived during olfactometry, and odorant **6**, both having a buttery odor, may contribute actively to the aroma of mussels. The grilled odor can be attributed to the extraction method. Compounds **9**, **12**, **36**, **37**, **39**, and **42** were described as having a grilled odor.

Five sulfur-containing compounds including dimethyl sulfide, disulfide, and trisulfide were identified in mussels extracts. These last three compounds, which were well perceived during the global analysis, may contribute to the overall aroma quality of flavor extracts because of their low threshold values (0.3−1.0, 0.16−12.0, 0.005−0.01 ppb; Leffingwell and Leffingwell, 1991), respectively. Dimethyl sulfide may have been produced from methionine, and dimethyl disulfide may have been thermally generated from methional (Ballance, 1961). Mussinan and Katz (1973) demonstrated the thermal generation of dimethyl trisulfide from cysteine. 2-Acetylthiazole and methional are commonly thermally generated. 2-Acetylthiazole, with a grilled, nutty odor, has already been identified as an impact odorant in several cooked marine products such as clam (Sekiwa et al., 1997) or spiny lobster tail meat (Cad-

wallader et al., 1995). Methional, with an odor threshold of 0.2 ppb (Guadagni et al., 1972), was the most potent odorant in cooked mussels. The formation of methional may have occurred during cooking via Strecker degradation of methionine (Forss, 1979). Our study confirms the odor evaluation of the SDE extract, which showed that the boiled potato-like odor of methional was considered to be an important component of the desirable aroma of cooked mussels.

There were two odor-active ketones (**4**, **6**) in mussel extract, both described as having a buttery odor. 2,3-Butanedione, which has a threshold value of 2.3−6.5 ppb (Leffingwell and Leffingwell, 1991), was an intense odorant. This Maillard reaction product (Hodge, 1967) was reported to contribute actively to the aroma of cooked turbot (Prost et al., 1998). This compound may contribute to the desirable flavor of cooked mussels.

Six alcohols were perceived in the SDE extract. Except for (*E*)-2-penten-1-ol, alcohols were not detected as potent odorants of mussels. This agreed with the findings of Heath and Reineccius (1986), who indicated that alcohols generally do not contribute to the overall aroma of food flavor because of their high threshold values unless they are present at high concentrations or are unsaturated. Alcohols may be formed by decomposition of secondary peroxides of fatty acids (Tanchotikul and Hsieh, 1989). (*E*)-2-Penten-1-ol, which is one of the main impact odorant in mussels, was described as having a mushroom-like odor. This was confirmed by the odor of the standard. This compound may be an important flavor component of cooked mussels and was previously identified in seafood SDE extracts as anchovy paste (Cha and Cadwallader, 1995) or turbot (Prost et al., 1998).

Nine odorant volatile aldehydes were identified. Aldehydes are generally responsible for a wide range of oxidized flavors. Most of these aldehydes (**8**, **16**, **18**, **24**, **27**, **29**, **32**) are the results of (*n*−3) PUFA oxidation. Alkanals and alkenals are known to contribute fatty−oily, slightly rancid odors (Vejaphan et al., 1988). Hexanal was described in this study as having a green, garlic-like odor. This compound could be coeluted with another compound having a garlic-like odor. Indeed, when the judges evaluated the odor of the standard, they described it only as green. (*E*)-2-Nonenal was an impact odorant of cooked mussels. This compound was usually found as having a tallowy, green odor (Milo and Grosch, 1993; Tanchotikul and Hsieh, 1989). In the present study, assessors characterized it with an earthy odor, which was confirmed by the odor of the standard. (*E*)-2-Nonenal was reported as a seafood volatile in SDE extracts of marine green algae (Sugisawa et al., 1990). The three olfactometric methods agreed in showing that (*Z*)-4-heptenal, with a boiled potato-like odor and a threshold value of 0.04 ppb (McGill et al., 1974), was one of the most potent odorants in mussel aroma. These authors have first suggested that the accumulation of (*Z*)-4-heptenal in cod was undesirable because it was associated with the cold storage cod flavor. However, Josephson and Linsay (1987) found that (*Z*)-4-heptenal exhibited a "cold boiled potato" aroma, and Chung and Cadwallader (1994) found that its presence in freshly cooked crab might be desirable. McGill (1974) pointed out that cooking was an important factor in the production of (*Z*)-4-heptenal in cod. Josephson and Lindsay (1987) demonstrated that (*Z*)-4-heptenal was formed by a water-mediated, retro-aldol condensation of (*E*,*Z*)-2,6-nonadienal, which was most likely enhanced during the distillation of volatiles through the combined effect of time and temperature. (*E*,*Z*)-2,6-Nonadienal was effectively found in mussel extract, but sniffing evaluation of it was not successful due to interference of another compound eluted in the same area of the chromatogram. Dienals are known to contribute pleasant fried−fatty aromas (Vejaphan et al., 1988). (*E*,*E*)-2,4-Octadienal was very well perceived during olfactometry, having a cucumber-like aroma. It has already been reported to contribute to the flavor of steamed rangia clam extracted by SDE (Tanchotikul and Hsieh, 1991).

There were two alkylpyrazines (compounds **19**, **27**) present in the SDE extract. Pyrazines, which are believed to contribute to the nutty, roasted, and toasted aromas of many foods (Maga and Sizer, 1973), could be formed by Maillard reaction and pyrolysis reactions through Strecker degradations in heat-processed foods from various sources such as amino acids (Wong and Bernhard, 1988). Pyrazines have already been reported as important aroma components in other thermally processed products such as crab (Hayashi et al., 1990) or crayfish (Baek and Cadwallader, 1996).

Three alkylbenzenes (**11**, **13**, **20**) and naphthalenes (**36**, **39**, **42**) were identified. Alkylbenzenes (**13**, **20**) had a plastic-like odor. Naphthalene and derivatives were perceived by only four or five assessors during the global analysis and did not have high average intensities. They do not contribute a lot to the overall aroma of mussels but may have a foreground undesirable odor. Yasuhara and Morita (1987) studied the volatile organic components in mussel for monitoring marine pollution. They identified many alkylbenzenes, among these xylene. Carotenoids are hypothesized to be the precursors of xylene (Josephson et al., 1991). Ogata and Miyake (1980) indicated that several C_3−C_9 alkylbenzenes and C_1−C_5 alkylnaphthalenes are the components in crude petroleum oil or petroleum-based products and can be used as chemical markers of oil pollution in fish and shellfish. Lee et al. (1972) reported a rapid uptake of naphthalene in marine fish. Xylene and naphthalene derivatives were previously identified in several crustaceans [crabmeat (Matiella and Hsieh, 1990); crayfish (Vejaphan et al., 1988)].

There were many unknown odor-active components. Some of them were not identified because they were low in quantity (**10**, **15**, **21**, **30**, **37**, **38**, **40**, **41**), coeluted (**2**, **31**) or were masked by the solvent (**3**). Unknown **9** was identified by MS as undecane, but injection of this compound in the same amount as in the SDE extract revealed that undecane was not an odorant. Likewise, the mass spectrum of unknown **26** was similar to that of 2-ethyl-1-hexanol, previously identified in clam (Tanchotikul and Hsieh, 1991; Sekiwa et al., 1997), but sniffing evaluation of the standard showed that it was not an odorant, probably due to its high threshold value (270000 ppb; Leffingwell and Leffingwell, 1991). Unknown **33** was tentatively identified by MS as being an alkylpyrazine, which could be confirmed by its nutty odor. Four of these unknowns may contribute to the global aroma of cooked mussel (**10**, **15**, **21**, **38**). Two of them had a garlic-like odor (**10**, **15**), another one had a citrus fruit, green-like odor (**21**), and the last one had a nutty odor (**38**).

Conclusion. Comparison of three commonly used techniques for olfactometry (olfactometry global analysis, OSME, and AEDA) showed that they were well

correlated, especially for the most potent odorants. Therefore, the choice of a method depends on the objective of the study, the quality of the panel, and the time scheduled for the analyses. It would be of great interest in a further study to have the same number of judges performing AEDA and OSME analyses to compare their mean values and to have this way a direct quantitative comparison between both methods.

The three methods were used to identify the most potent odorants of cooked mussels. On the basis of the three techniques, the aroma of mussels can be primarily attributed to six odorants: 2,3-butanedione (buttery, caramel-like odor), (*Z*)-4-heptenal (boiled potato-like odor), (*E*)-2-penten-1-ol (mushroom-like odor), 2-ethylpyrazine (nutty odor), methional (boiled-potato-like odor), and (*E*,*E*)-2,4-octadienal (green, cucumber-like odor). The most potent odorant was methional, which confirms the previous sensorial evaluation that showed a strong boiled potato-like odor for cooked mussels.

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