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Aroma Extracts from Oyster *Crassostrea gigas*: Comparison of Two Extraction Methods

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The study of the aroma of oysters is of great economic interest in France because it enables their organoleptic quality to be verified. The aim of this study is to optimize the extraction methods of the volatile compounds of oysters *Crassostrea gigas* in order to obtain an extract with an odor as close as possible to that of the original oysters'. Oyster aroma is rarely studied, and its sensory profile has not been investigated to date. Two extraction methods were studied: vacuum hydrodistillation carried out at 20 °C with noncrushed oyster using ultrapure water and dynamic headspace carried out using noncrushed oyster during a 30 min purge. They were compared with regard to their sensory characteristics by a panel of seven judges, all trained in seafood aroma recognition. This study has shown that vacuum hydrodistillation is the better method to obtain an extract closest in aroma to the oyster reference.

KEYWORDS: Oyster; aroma; vacuum hydrodistillation; dynamic headspace; authenticity

INTRODUCTION

In France, the consumption of oysters is traditional during the holiday season. France is the largest producer of oysters *Crassostrea gigas* in Europe (*1*). The study of their quality through their organoleptic characteristics is of real economic interest, particularly during the current food crisis. The analysis of the aroma is a convenient method for checking the quality of these mollusks. Nevertheless, few studies (*2*−*4*) are found in the literature concerning oyster aroma. Cha (*2*) has compared the effect of hydrolysis by a protease on the aromatic composition of the oyster *Crassostrea virginica*. Piveteau et al. (*3*) have studied the aroma of oyster *C. gigas* by gas chromatography coupled with olfactometry in order to identify the most potent odorants. Josephson et al. (*4*) have studied the aroma of oysters *C. gigas* to show the biosynthetic pathways of the polyunsaturated fatty acids as aromatic precursors.

In France, oysters are consumed raw. The study of raw oysters is difficult because they are greatly susceptible to autoxidation, easily identifiable by a strong green odor. Our work lies in the search for a convenient extraction method for the volatile compounds of oysters *C. gigas*, which yields an extract as close as possible to the original odor of the oyster. Aroma extraction methods using low temperatures should be favored. However, many of them are based on the steam distillation of the volatile compounds, which involves heating the product. Extraction methods such as simultaneous distillation−extraction involve boiling the sample to recover the volatile compounds in an organic solvent, leading to artifacts responsible for roasted odors (*5*, *6*).

Two extraction methods have been studied: vacuum hydrodistillation and dynamic headspace. Vacuum hydrodistillation is interesting because of the use of low-temperature extraction at ∼20 °C. Indeed, this technique is usually used for the extraction of raw products, such as tomatoes (*7*) and wine (*8*), but it has never been used for seafoods such as oysters. This technique enables both the low and high boiling point components to be extracted. Dynamic headspace allows the extraction of volatile compounds at a temperature close to that of vacuum hydrodistillation. However, it enables only the low boiling point components to be extracted. This technique has already been used for the extraction of the volatile compounds of oysters (*3*, *4*).

These two optimized methods are compared to identify the best method for the extraction of the volatile compounds of oysters *C. gigas*. The originality of our work is based on the optimization of these two extraction methods in relation to the authenticity of the aroma extracts. In the field of aroma, the study of odor authenticity is an essential first step. Indeed, the assessment of the authenticity of the extract is necessary before all quantitative, qualitative, and olfactometric analyses. Some researchers do attach importance to checking the characteristics of the odor extract with the original product. Very few have studied aroma authenticity in order to characterize the most potent odorants by gas chromatography coupled with olfactometry [Sarrazin et al. (*9*) on coffee, Bernet et al. (*10*) on Gewurztraminer wine, Charles et al. (*11*) on red wine vinegar, and Escudero and Etievant (*12*) on Champagne]. To date, however, no work has been published on oyster aroma authenticity.

Moreover, it is very difficult to reconstitute an aroma with volatile compounds outside its matrix. Thus, different authors

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have tried to put the extract in a matrix similar to that of the original product. For example, Charles et al. (*11*) diluted red wine vinegar extracts in water, Etievant et al. (*13*) incorporated an emulsion in cheese extract, and Abbott et al. (*14*) diluted beer extracts in water to obtain an adequate final ethanol concentration. During the assessment of odor extract characteristics, judges were misled by different means to test their performance and to better evaluate the extracts. In addition, owing to an original technique performed in our laboratory, the study of the odor characteristics of headspace extract was made possible. This has never been found in the literature.

This study aims to obtain a reliable method for the extraction of the volatile compounds of oysters *C. gigas* that gives an extract with an odor very similar to that of the original product to characterize the most potent odorant in a further study.

MATERIALS AND METHODS

Reagents. All water used was purified by a Milli-Q system (Millipore Corp.). Dichloromethane was purchased from Aldrich and was of 99.9% HPLC grade purity. Sodium sulfate anhydrous was purchased from Panreac Quimica. *p*-Cymene was purchased from Aldrich. Odor evaluation blotter strips came from the SARL H.Granger-Veyron.

Oysters. Adult oysters *C. gigas* were obtained from the Vendée. Vendée is a French area on the Atlantic coast known for its oyster production. Oysters were collected from a marine farm from September to December 2000. Following collection, they were transported under refrigerated conditions and stored at 4 °C in our laboratory before analysis. Previous analyses had shown that no changes in the organoleptic quality of oysters occurred during 10 days. However, for practical purposes, we chose to keep oysters under refrigerated conditions for 6 days. Analyses were performed on live oysters, identified by their closed shells. Oysters were opened up just before extractions.

Methods. *Vacuum Hydrodistillation (VD).* Vacuum hydrodistillation was performed using the method of Forss and Holloway (*15*). Two hundred grams of raw oyster flesh was placed in a 6-L flask with 300 mL of ultrapure water. The temperature of the 6-L flask containing the oysters was 20 °C, whereas the collector flask was at 2 °C. Condenser columns were at −1 °C. Three traps were cooled by liquid nitrogen at −196 °C. The residual pressure was maintained at 600 Pa for 4 h and 30 min. This time was necessary to evaporate all of the aqueous phase (300 mL of added water and ∼70% of oyster intrinsic water). The contents of the collector flask and of the three traps were pooled and extracted with 3 × 30 mL of freshly distilled dichloromethane. The organic extract was dried using 20 g of sodium sulfate anhydrous, and then it was concentrated, using a Kuderna-Danish apparatus, to 10 mL and under a nitrogen stream to 1 mL. The extract thus obtained was sealed with a Teflon cap and stored at −20 °C prior to use. Extractions were carried out in triplicate.

Dynamic Headspace (DH). A purge and trap concentrator (model LSC 2000, Tekmar Inc., Cincinnati, OH) was equipped with a capillary interface for cryofocusing. Fifteen grams of raw oyster flesh was introduced into a flask. The headspace of the sample was purged with helium at 60 mL/min for 30 min at 25 °C (*3*) and swept into a porous adsorbent polymer (Tenax) trap. Volatile compounds were cryofocused at −40 °C using carbon dioxide and thermally desorbed at 195 °C. The extract was collected at the end of the interface.

Quantification. A gas chromatograph (Star 3400, Varian, Palo Alto, CA) equipped with a flame ionization detector was used. The volatile compounds were separated on a capillary column (DB-Wax, 30 m in length × 0.32 mm i.d. × 0.5 μm thickness, J&W Scientific, Folsom, CA). The helium carrier gas flow was 1 mL/min. The injector and the detector were set at 250 °C. The oven temperature was programmed from 50 °C for 5 min, to 80 °C at 1 °C/min, followed by a temperature increase of 10 °C/min to 250 °C. An internal standard (*p*-cymene, 16 μg) was used for the quantitative analysis of the dynamic headspace extract.

Sensory Analyses. *Panel.* The panel consisted of seven judges, all trained in seafood aroma recognition. They were recruited in our biochemistry laboratory. A preliminary session took place in an ordinary room to generate descriptors of fresh oysters. The other sessions took place in a sensory room [AFNOR V-09-105 (1987)], in isolated booths, under red light at ambient temperature.

Preliminary Session. The panel generated descriptors of fresh oysters. The list of descriptors was refined after discussion with the panel to eliminate repetitive descriptors and to agree on the definition of each descriptor (*16*). A list of seven consensual descriptors (seaside, oyster, seaweed, grass, mud, floral, and cucumber) was established. To evaluate the authenticity of the extracts, the descriptor "cardboard" was added to this list but was not identified in fresh oysters by judges. This descriptor, associated with the odor of a piece of cardboard presented to the judges, was useful for them to evaluate the characteristics of extracts. A list of eight descriptors was then established for the evaluation of the aroma authenticity of the extracts.

VD Preparation of the Sample. Organic samples of the three extracts were pooled together to obtain 3 mL of extract in dichloromethane. Two hundred microliters of this extract was diluted in 5 mL of ultrapure water. This sample was shaken vigorously to create an emulsion. Odor blotter strips were dipped into the emulsion and then placed in brown flasks. The period between this soaking and the closing of the flask was 30 s. During this time, the last traces of the solvent on the odor blotter strips evaporated. Each flask was surrounded by a piece of aluminum foil and covered with white tulle to mask the contents of the flask. Each flask was coded with a three-digit number.

VD Profile Quantitative Descriptive Analysis and Similarity Test. Eight different extracts were assessed by the seven judges. Each extract was assessed twice by a judge to verify the results. At each session, each subject smelled four extracts and one oyster, all placed in a brown flask as described above. A list of eight descriptors previously established by our panel was used to assess the odor of extracts and oysters. Panelists assessed the intensity of each given descriptor on an unstructured scale. The scale consisted of a 10 cm horizontal line with a verbal anchor at each end (left end, weak intensity; right end, strong intensity). The subjects were asked to rate how close the odor in the flask was to the internal reference of a typical fresh oyster on an unstructured scale of 10 cm (0 on the left, no typical similarity with the fresh oyster; 10 on the right end, odor typical of the fresh oyster) (*9, 10, 17*). Each response was quantified by a mark from 0 to 10 of 10. The closer the mark is to 10, the more authentic is the extract.

DH Preparation of the Sample. Volatile extract was collected at the end of the dynamic headspace interface, which consisted of a piece of deactivated silica column. This piece of column passed through a needle set in a rubber cap sealed on a brown flask. The end of the column dipped into 3 mL of ultrapure water placed in the flask. As a result, the volatile compounds were desorbed in the water. Each flask was surrounded by a piece of aluminum foil and was coded with a three-digit number. Only one extract was evaluated by each judge. Thus, the extraction was carried out seven times (once for each of the seven judges).

DH Profile Quantitative Descriptive Analysis and Similarity Test. At each session, each subject was asked to smell one extract and one oyster. The panelist evaluated the intensity of the eight given descriptors (the same as for vacuum hydrodistillation). In the same way, they assessed the similarity of the extract odor compared to a fresh oyster.

Comparison of the Two Methods VD and DH: Preparation of the Samples and Sensory Tests (Profile Quantitative Descriptive Analysis and Similarity Test). Three flasks were presented. Each flask was brown, surrounded by a piece of aluminum foil and coded with a three-digit number. The first flask contained an odor blotter strip impregnated with the vacuum hydrodistillation extract. The second one contained the dynamic headspace extract in 3 mL of ultrapure water. The third one contained an oyster in order to set the oyster reference in the same evaluation conditions as the extracts. The panelists could not distinguish the differences between the three flasks because of the darkness of the flasks and because we had taken care to equilibrate the flasks to the same weight using small pieces of glass. We had previously confirmed that this did not affect the odor in the flask. The panelists smelled each of the three flasks and evaluated the intensity of each of the eight given descriptors (as before) on an unstructured scale of 10 cm. They assessed the similarity of the contents of the three flasks (which contained VD

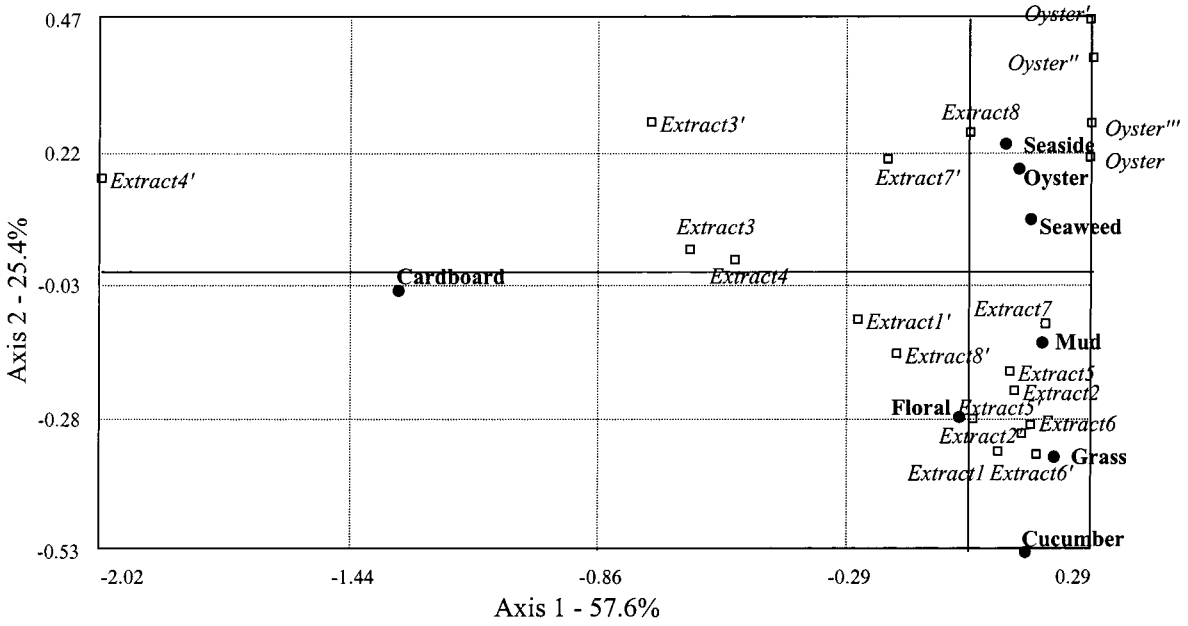

Figure 1. Factorial correspondence analysis: relative position of eight extracts and four oyster references and eight odors in the plane formed by the first and second axes. Extracts were assessed in duplicate; numbers correspond to trials as shown in Table 1. Oyster references were assessed four times.

Table 1. Completed Factorial Design and Similarity Marks for the Optimization of the Vacuum Hydrodistillation

trial	temp (°C)	oysters	aqueous phase	similarity marks[a]
1	20	crushed	juice	3.31 ± 1.90
2	30	crushed	juice	3.83 ± 1.32
3	20	noncrushed	juice	1.78 ± 2.37
4	30	noncrushed	juice	1.16 ± 1.22
5	20	crushed	ultrapure water	3.98 ± 1.82
6	30	crushed	ultrapure water	4.57 ± 1.82
7	20	noncrushed	ultrapure water	5.35 ± 1.63
8	30	noncrushed	ultrapure water	3.50 ± 2.31

[a] Mean ± standard deviation/10, $n = 14$.

extract, HD extract, and one oyster reference) with a visual fresh oyster that is the reference product. Each flask was assessed twice by each judge.

Statistical Treatment. Data acquisition and statistical treatment (variance analysis ANOVA) was performed with Statgraph 4.0 software. The factorial correspondence analysis (FCA) was carried out on Sgwin software. To evaluate the reproducibility of the assessment of aroma authenticity, the data of the FCA were carried out on nonaveraged data. The eight products evaluated in duplicate for the hydrodistillation were considered as 16 individual samples (*18*).

RESULTS AND DISCUSSION

Optimization of the Vacuum Hydrodistillation. The vacuum hydrodistillation was carried out using the method of Forss and Holloway (*15*). Three parameters were optimized: the extraction temperature, the use of crushed or noncrushed oyster, and the addition of oyster juice or ultrapure water. The last parameter was chosen to study the impact of oyster juice, which contains aromatics, on extracts. A completed factorial design was then performed ($2^3 = 8$ trials). The eight trials were carried out as shown in Table 1. Each trial was done in triplicate to limit the batch effect. The authenticity of the eight extracts was evaluated twice by the seven judges. Similarity marks are given in Table 1. A multiple variance analysis was performed on the similarity mark, studying two parameters: extract and assessor. These both have a statistically significant effect on the similarity mark at 95% confidence level (ANOVA). The "assessor" effect usually exists due to different utilizations of the scale by the assessors or because of a different perception of the odor by the judges (*9*). The eight extracts are statistically different at a confidence level of 95%. Moreover, two further parameters have a statistical effect on the similarity marks at $p < 0.05$. These parameters are the use of crushed or noncrushed oyster and the addition of oyster juice or ultrapure water. The similarity marks (Table 1) show that trial 7, which was carried out at 20 °C with noncrushed oyster and using ultrapure water, is the nearest to typical fresh oyster.

A factorial correspondence analysis was performed using the quantitative descriptive analysis (Figure 1): intensities given for each descriptor were pooled for the seven judges. The sum of the intensities of each descriptor was used to perform the FCA. We noted a good inertia of the FCA because it represents 83% of the whole information. The FCA could distinguish three groups. The first one is composed of trials 3 and 4, corresponding to extracts using noncrushed oyster with oyster juice. This group is characterized by a cardboard note. The second group is composed of extracts 1, 2, 5, and 6, using crushed oysters. These extracts are described by aromatic notes such as mud, grass, floral, and cucumber. The last one included extracts 7 and 8, which used noncrushed oysters and ultrapure water. These last two extracts are near fresh oyster and were characterized by seaweed, oyster, and seaside notes. All eight extracts were assessed in duplicate. There is low variation between the relative position of the duplicate extracts, except for extracts 4 and 4′. During the evaluation of extract 4, two of the seven judges were hindered by the low and similar intensities of both fresh oyster and extract 4. These judges had assimilated extract 4 to oyster, but not extract 4′, which explains the difference between the extracts.

It is interesting to observe a correlation between the similarity marks and the quantitative descriptive analysis. In fact, axis 1 distinguishes extracts made with ultrapure water (trials 7 and 8) or oyster juice (trials 3 and 4), whereas axis 2 distinguishes extracts made with crushed oysters (trials 1, 2, 5, and 6) or

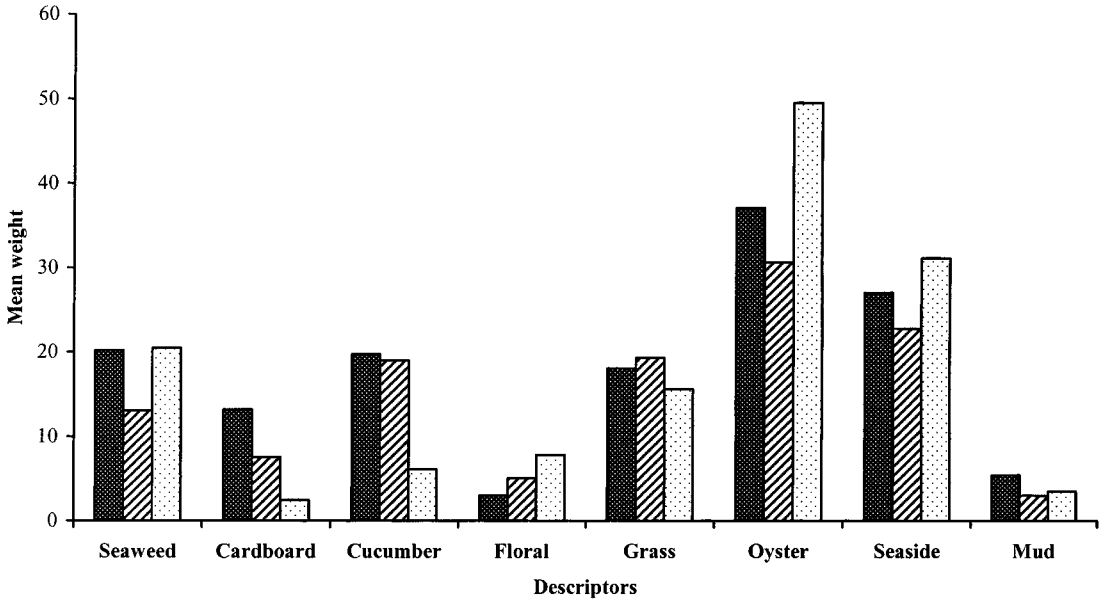

Figure 2. Sensory profile of extracts obtained by vacuum hydrodistillation (black bars with white dots) and dynamic headspace (slashed bars) and of oyster reference (white bars with black dots).

noncrushed oysters (trials 3, 4, 7, and 8). These same parameters distinguish statistically the similarity marks. The distinction of axis 1 could be explained by the presence of proteins in the oyster juice. They are responsible for foaming at the beginning of the extraction, and these proteins could interact with the volatile components. Therefore, the volatile compounds could be retained, and the weak odor intensity of extracts made with oyster juice (trials 3 and 4) could then be explained. They were characterized by a cardboard note that could be provided by the odor blotter strips. Another theory could explain the cardboard odor. The cardboard note is greater for extracts obtained with noncrushed oysters using oyster juice (trials 3 and 4) than for extracts obtained with noncrushed oysters using ultrapure water (trials 7 and 8). This note could be a taint due to the oxidation of the oyster juice. This phenomenon could generate off-flavor characteristics of cardboard or paper odor (*19*, *20*). This taint was higher for extracts using oyster juice (300 mL of oyster juice and 80% of intrinsic water of oysters that is assimilated to oyster juice) rather than ultrapure water (80% of oyster juice).

The distinction between extracts from crushed or noncrushed oyster (axis 2) can be explained by an enzymatic hydrolysis phenomenon. The crushing of the oyster provokes some enzymatic hydrolysis reactions. Polyunsaturated fatty acids (PUFA) contained in oysters are known to be the most important precursors of volatile carbonyl compounds in seafood (*4*). These PUFA can be degraded by several processes: autoxidation and enzymatic hydrolysis (*21*). Many volatile carbonyl compounds are responsible for odors such as grass, cucumber, and floral.

In conclusion, an extraction at 20 °C with noncrushed oyster using ultrapure water provides the optimal parameters for vacuum hydrodistillation with regard to aroma authenticity.

Optimization of the Dynamic Headspace. A preliminary study was carried out to determine the support in which the extract was collected. The seven judges smelled an extract made using noncrushed oyster with a purge time of 60 min. The extract obtained was collected in an empty flask or in a flask containing 3 mL of ultrapure water. The odor of the extract collected in 3 mL of ultrapure water was less irritating in global odor intensity. Moreover, oysters are constituted of ~80% water, so the collection of volatile compounds in water comes closer to the natural matrix of oyster. Therefore, all extracts were collected in a flask containing 3 mL of ultrapure water.

Table 2. Completed Factorial Design and Similarity Marks for Optimization of the Dynamic Headspace

trial	purge time (min)	oysters	similarity marks[a]
1	60	noncrushed	4.90 ± 1.62
2	60	crushed	4.44 ± 2.08
3	45	noncrushed	3.56 ± 2.05
4	45	crushed	3.57 ± 2.55
5	30	noncrushed	4.89 ± 1.62
6	30	crushed	3.56 ± 2.50

[a] Mean ± standard deviation/10, $n = 14$.

Two parameters were optimized: the purge time and the use of crushed or noncrushed oysters. Extraction temperature (25 °C) and purge flow (60 mL/min) were fixed as described by Piveteau et al. (*3*). The trials were performed as a completed factorial design as shown in Table 2. Each trial was performed seven times, once for each of the seven judges. The similarity marks for the six trials were very close (Table 2). There is no statistically significant difference between the six extracts at a confidence level of 95%. As for vacuum hydrodistillation, a factorial correspondence analysis was done using the results of the quantitative descriptive analysis. The extracts are very different from the oyster reference. Indeed, oyster references were characterized by fresh notes such as seaside, seaweed, and oyster, whereas these six extracts were described by cucumber, grass, and floral descriptors. Enzymatic reactions responsible for the green notes such as cucumber and grass (*21*) were greater than for the vacuum hydrodistillation method. These results did not allow the best parameters to be identified, so the study was completed by a quantitative analysis. To be homogeneous with vacuum hydrodistillation, the extractions for the quantitative analysis were done with noncrushed oysters. An increase in the purge time increases the quantity of volatiles collected (*22*). The purge time was then studied. Extractions were carried out during 30 and 60 min, respectively. The extractions were performed in triplicate for each purge time using an internal standard (16 μg of *p*-cymene). Quantification is based on the

size of the area of all volatile compounds. No statistical difference was observed at $p < 0.05$ for the quantitative analysis between the two experiments. This method has been said to have a lack of reproducibility (*22*), which could explain this observation.

In conclusion, dynamic headspace extractions were carried out with noncrushed oysters during 30 min to limit the duration of the experiment.

Validation of the Most Authentic Extract. The two extraction methods (vacuum hydrodistillation and dynamic headspace) were compared using their respectively optimized parameters. The extracts thus obtained were compared in relation to both their similarity to the oyster reference and their sensory profile. Their similarity marks to the oyster reference were $5.89 \pm 1.88/10$, $n = 14$, and $4.61 \pm 2.24/10$, $n = 14$, for vacuum hydrodistillation and dynamic headspace, respectively. The statistical difference between the extracts was studied using ANOVA. This analysis shows that there is a statistical difference between the dynamic headspace extract and the oyster reference but none between the vacuum hydrodistillation extract and the oyster reference. Moreover, the sensory profile (Figure 2) illustrates that the mean weight for seaside, oyster, and seaweed descriptors for oysters is nearer the vacuum hydrodistillation extract than the dynamic headspace extract.

To conclude, with regard to the similarity of the extracts and the sensory profile, vacuum hydrodistillation is the most suitable method for the extraction of the volatile compounds of oysters *C. gigas* to achieve a characteristic aroma. The similarity mark of this extract is acceptable, although it is rather far from the reference, which shows that it is difficult to obtain an extract very close to the original product. Similar results were obtained by Escudero and Etievant (*12*), who obtained a mean score of 47.4 mm on an unstructured scale of 100 mm for Champagne extracts, and by Le Quere et al. (*23*), who obtained a mean score of 44 mm on an unstructured scale of 100 mm for goat cheese extracts. These authors used an unstructured scale of 100 mm where the left end anchor corresponded to an odor very similar to the reference. All of these results show that it is difficult to reconstitute an aroma outside its matrix, using volatile compounds.

Sensory Difference. Owing to an original technique, the headspace extract could be collected at the end of the interface of the Tekmar apparatus, thus enabling its odor characteristics to be assessed. This has not previously been reported in the literature to our knowledge. During the assessment of authenticity of the different extracts (VD extract, DH extract, and oyster reference), we took care to mask the contents of the flasks, using aluminum foil and white tulle, and to equilibrate the weight of each flask so as not to influence the judges. Moreover, each extract was evaluated twice. This original technique shows the credibility of our panel because they all distinguished the extracts from the oyster reference. Our judges are reproducible in their assessment; their marks were similar from one evaluation to another. The advantage of the quantitative descriptive analysis is that the panel gives an intensity for each descriptor. The results given by this analysis enable a sensory profile (Figure 2) of extracts and of oyster to be established. An ANOVA study was performed to discriminate all of the descriptors from the different extraction methods at $p < 0.05$. Descriptors such as seaweed, cardboard, floral, grass, seaside, and mud showed no significant statistical difference between the oyster and the two extraction methods. However, it is interesting to observe the greater intensity of the cardboard note for vacuum hydrodistillation. This aromatic note could be generated by the oxidation of oyster juice (e.g., by the intrinsic water of oyster) or could be emphasized by the use of the odor blotter strip. Indeed, this note could be provided by oxidation. Some molecules generated by oxidation are responsible for off-flavors such as cardboard or paper (*19*, *20*). There is a statistically significant difference at $p < 0.05$ between the oyster reference and the extract obtained by vacuum hydrodistillation for the cucumber note. The cucumber odor is very intense in the extracts obtained by both methods compared to the oyster reference. Some other authors (*3*, *4*) have shown that cucumber odor is a key potent odorant of oysters *C. gigas*. These authors have identified carbonyl compounds responsible for cucumber odor. The presence of the carbonyl compounds in extracts is due to the high level of PUFA in oyster. By way of autoxidation and enzymatic reactions, these PUFA synthesize these aromas (*21*). In the same way, a significant statistical difference at a confidence level of 95% is observed between the oyster reference and the extract obtained by dynamic headspace for the oyster descriptor. This latest observation strengthens the choice of the most authentic method: vacuum hydrodistillation. This technique has already been used with success for several products but only for *Rangia* clams in seafood (*24*). Thus, this method could be used for a large range of products, and it is particularly well-adapted for the extraction of the volatile compounds of oysters *C. gigas*.

Vacuum hydrodistillation is a more suitable method for oysters than dynamic headspace with regard to the similarity marks and the oyster descriptor in the sensory profile. The optimization of this technique has allowed us to obtain a reliable extraction method that produces an extract with good authenticity. This study will subsequently enable us to characterize the key odorant compounds of fresh oysters *C. gigas*.

ABBREVIATIONS USED

ANOVA, analysis of variance; DH, dynamic headspace; FCA, factorial correspondence analysis; PUFA, polyunsaturated fatty acid; VD, vacuum hydrodistillation.

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