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Journal of Chromatography A, 1056 (2004) 201–208
JOURNAL OF CHROMATOGRAPHY A
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New gas chromatography–olfactometric investigative method, and its application to cooked *Silurus glanis* (European catfish) odor characterization

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Available online 28 July 2004

Abstract

A new gas chromatography–olfactometric method, gas chromatography–global olfactometry omission detection (GC–GOOD), was applied to dynamic headspace odor extracts of *Silurus glanis* (European catfish). The GC–GOOD method is based on the omission test theory and uses a gas chromatograph coupled with a three-way valve and an a flame ionization detector. The GC–GOOD method enabled the identification of key families of volatile compounds in the *S. glanis* global odor and the elucidation of the interactions occurring between these families. Significant main effects were observed for the families of volatile compounds exhibiting cooked odor, grassy odor and alcohol, solvent and plastic odors. Omission of these families involved a loss of odor similarity.
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Keywords: *Silurus glanis*; Fish; Food analysis; Olfactometry; Detection, GC; Volatile organic compounds

1. Introduction

Among sensory attributes, odor perception is one of the foremost criteria used by the consumer to assess the quality of a food product. Odors enable the evaluation not only of acceptance but also of preference of food [1]. In this context, one of the most important purposes in food research is to identify the volatile compounds that are responsible for these odors.

Food odors are composed of a large number of volatile compounds and only a small fraction contributes to their global odor [2,3]. An interesting technique that enables odor active volatile compounds to be distinguished from the whole range of volatiles present in their relative concentrations in food product extracts is gas chromatography–olfactometry (GC–O) [3,4]. GC–O, proposed by Fuller et al. as early as 1964 [5], consists of sniffing the gas chromatographic effluent of an odor extract of food. Therefore, the division of identified volatile compounds into odor active and non-odor active volatile compounds is guaranteed by the use of the human nose as a detector [6]. GC–O has been widely used and many methods have been developed to enhance the quality and expressiveness of the results [3]. They may be classified into four categories [7]; dilution analysis methods, such as combined hedonic response measurement (CHARM) [8] and aroma extraction dilution analysis (AEDA) [9]; detection frequency methods [10]; posterior intensity methods [11] and time-intensity methods, such as OSME [12]. GC–O is often combined with flame ionization detection (FID) and mass spectrometry (MS), which allow, respectively, the quantification and the identification of the volatile compounds. In our study, the GC–O method selected was a detection frequency method described by Le Guen et al. [1].

The major problem of GC–O is that volatile compounds are assessed separately. Indeed, this approach does not allow information to be obtained about the behavior of these compounds in a mixture [6] and the role played by the different components in the global odor is not elucidated [13]. Many studies [14–18] have shown that the analysis of the mixture is essential because of the phenomena of hypo-additivity (also named the masking effect), additivity or hyper-additivity (also named the synergistic effect) which can occur between volatile compounds.

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doi:10.1016/j.chroma.2004.06.044

To date, the only complementary methodology that existed to clarify the relative impact of the components of a complex mixture was omission tests [14]. This methodology consists of measuring the sensory effect of the mixture components by sensory comparison of the complete mixture with a mixture in which some components were omitted [19]. Many authors have used omission tests on different food products to study the relative importance of some components on the global food flavor, which represents the combination of taste (retronasal way), odor (nasal way) and mouthfell [20], on the global food taste [13,14,19,21–23], and on the global food odor [24–29].

In methodologies used by these authors, GC–O is often combined with GC–FID and GC–MS, respectively to quantify and identify the odor active volatile compounds perceived. After GC–O, there are three critical steps that are very time-consuming [14,30]. The first step is the construction of an odor model by mixing pure volatile compounds in the proportions found in food product extracts [13]. In most cases, this may be problematic because some volatile compounds are often present at trace levels and, consequently, are difficult to identify and quantify. Moreover, some volatile compounds are tedious to synthesize or expensive [14,30]. The second step is to produce a matrix model with compositional and sensory properties as close as possible to the crude product [27,30]. Indeed, as every food component may have a role in the perception of the global odor, it is necessary to perform omission tests on a representative matrix model [18,19]. Some matrix components may also be tedious to synthesize or expensive and the construction of the matrix itself may be problematic [14]. The third step is to perform sensory studies on the matrix model containing the odor model to validate that they are representative of the crude food product [6,30].

A new gas chromatographic method developed in our laboratory, using the omission test theory, has enabled the influence of the volatile compounds in a mixture on the global odor perception to be elucidated. This new method, which we have named gas chromatography–global olfactometry omission detection (GC–GOOD), allows the evaluation of an odor mixture of selected natural volatile compounds in their own relative concentrations in a food product extract and an assessment of the effect of their absence on the global odor of the product. In addition, the GC–GOOD method saves a lot of time and avoids the problem of volatile compound supply. The purpose of this study is to present the GC–GOOD method and the results obtained for its application to the *S. glanis* odor.

2. Experimental

2.1. Chemicals

Water was purified by a Milli-Q system (Milliport). PTFE bags came from Interchim (Montlucon, France).

2.1.1. S. glanis samples

S. glanis samples (European catfish) were supplied by Technologies Aquacoles Géothermiques (TAG, France) which reared them for one year in indoor concrete ponds with renewed geothermal water.

Fish were caught and manually slaughtered the same day, then filleted using the same protocol. The average weight of the fillets was 450 g (σ_{n-1} = 100), which represents the commercial size of this product. Fillets were transported under ice in polystyrene boxes. They were wrapped in aluminum foil, vacuum-packed and stored at −80 °C before analysis.

2.2. GC–GOOD method

2.2.1. Silurus sample preparation

Fillets were thawed just before the analyses. The bags containing fillets were immersed in water at 25 °C for 20 min. A transversal section was finely cut out of the middle of the fillet. Twenty grams of this raw fillet and 20 ml of ultrapure water were introduced into a 100-ml glass flask. The glass flask was placed in a heating ring at 60 °C to cook the fillet sample during the 60 min of dynamic headspace extraction. The sample was agitated by a magnetic stirrer to ensure homogeneous cooking.

2.2.2. Dynamic headspace extraction

A purge and trap concentrator (model LSC 2000, Tekmar, Cincinnati, OH, USA) was used. The glass flask containing the fillet sample was fixed to the purge and trap concentrator. The headspace of the fish sample was purged with helium at 60 ml min^{-1} for 60 min and swept into a porous adsorbent polymer (Tenax) trap. Volatile compounds were thermally desorbed by heating the trap at 200 °C. They were cryofocused at −40 °C using carbon dioxide on a capillary interface before being simultaneously injected into a gas chromatograph by heating the interface at 250 °C [31].

2.2.3. Gas chromatography analysis

A gas chromatograph (Star 3400, Varian, Palto Alto, CA, USA) was used. The volatile compounds were separated on a capillary column (DB-wax, 30 m × 0.32 mm i.d., 0.5 μm thick, J&W Scientific, Folsom, CA, USA) with the following oven temperature program: from 40 °C for 5 min to 160 °C at 10 °C min^{-1} followed by a temperature increase of 15 °C min^{-1} to 230 °C [32].

2.2.4. Volatile compound omission system

Each volatile compound could be removed from the global odor thanks to the GC–GOOD system. The GC effluent was split 1:2 between a FID system and a three-way valve. This valve enabled the volatile compounds to be directed to a PTFE bag or to be omitted. The control of the omission was assumed by an FID bound to a computer, which allowed the simultaneous visualization of the elution of the volatile compounds. A heating sheath prevented the condensation of the volatile compounds in the capillary column directing them

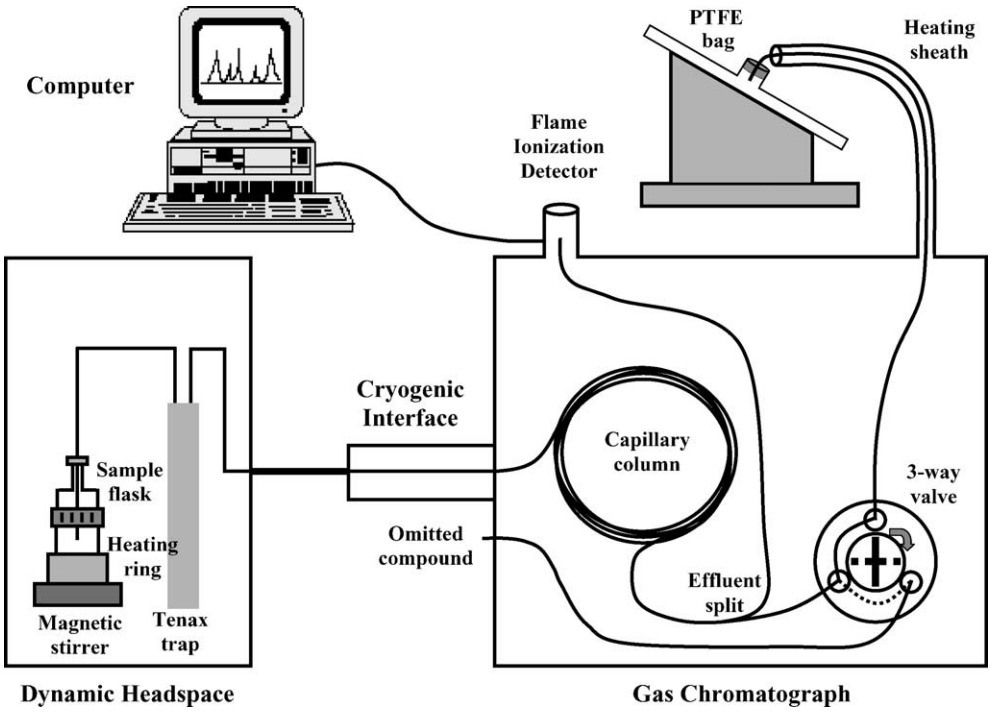

Fig. 1. Diagram of the GC–GOOD system.

to the PTFE bag. This column was air-tightly bound to the PTFE bag (Fig. 1).

2.2.5. GC–GOOD sample collection

The GC–GOOD samples were collected in PTFE bags, which have already been used by Atanasova et al. [33] to study odor samples. At the beginning of the desorption, the PTFE bag was empty thus it filled progressively with the desorption. Each odor sample was evaluated by two judges. Blanks were performed to verify the absence of interfering odors in the PTFE bags.

2.3. Sensory analyses of GC–GOOD extracts

2.3.1. Judging panel

The panel was composed of six judges from our laboratory (five women and one man, between 25 and 45 years old). They were all involved in fish odor evaluation. As part of this study, they were trained more specifically in the recognition of cooked silurus fillet odor. Training was divided into five sessions. The first session consisted of generating odor descriptors for dynamic headspace extracts of cooked *S. glanis* fillets. A list of 11 consensual odor descriptors (boiled potato, undergrowth, hay, cut grass, hot milk, buttery, moldy, hard-boiled egg, cooked cabbage, rancid and amine-like) was established. The three following sessions consisted of training the judges to use the 11 odor descriptors [34]. These took place in a sensory room [AFNOR V-09-105, 1987], in isolated booths, under natural light at room temperature. A last session was performed to accustom the judges to the use of the PTFE bags.

2.3.2. Similarity test

A similarity test was performed to evaluate the closeness between the global odor extract of *S. glanis* and the global odor extract from which some volatile compounds were omitted (omitted sample) thanks to the GC–GOOD system. To compare them, both odor samples were presented to the judges at each session, the sample containing all the volatile compounds extracted from *S. glanis* being used as an odor reference. The judges were instructed to smell the global odor of the reference and of the omitted sample. They were asked to assess the similarity of the omitted sample on an unstructured scale of 100 mm with a number at each end, 0 at the left corresponding to an omitted sample very different from the reference, 100 at the right end corresponding to an omitted sample identical to the reference. Each response was quantified by a score from 0 to 100, corresponding to the distance in millimeters from the left end. The closer the score was to 100, the more similar was the omitted sample [25,35].

2.3.3. Evaluation of the intensity of the GC–GOOD extracts

The six judges were instructed to assess the odor intensity of the omitted sample and of the reference. Judges used an unstructured scale consisting of a 100 mm horizontal line with a number at each end, 0 at the left end corresponding to “no odor”, 100 at the right end corresponding to “very strong odor” [35].

2.3.4. Quantitative descriptive analysis of the GC–GOOD extracts

A quantitative descriptive analysis was performed to describe the differences between the omitted samples [20]. The list of 11 consensual odor descriptors, generated to describe the odor of dynamic headspace extracts of cooked *S. glanis* fillets, was used. For each omitted sample, the judges assessed the intensity of each odor descriptor on an unstructured scale. The scale consisted of a 100 mm horizontal line with a number at each end, 0 at the left end corresponding to a weak intensity and 100 at the right end corresponding to a strong intensity [34].

2.4. Statistical treatment

Data acquisition and statistical treatment were performed with Statgraph 5.0 software. For the similarity scores, main effects and first order interactions between the five families were estimated by multiple linear regression analysis and an analysis of variance (ANOVA) was used to test the significance of these estimates. For the odor descriptors, principal component analysis (PCA) was performed on the average scores using the covariance matrix. Active variables were the odor descriptors and illustrative variables were the addition/omission variables for each family and the similarity rating.

3. Results and discussion

3.1. Gas chromatography–global olfactometry omission detection system

In the GC–GOOD system, a three-way valve enabled volatile compounds contained in GC effluent to be directed to a PTFE bag or to be omitted. Compared to other methods used until now, GC–GOOD system allowed to elucidate participation of volatile compounds naturally present in food products to global odor. This new system avoided the volatile compounds synthesis step which is not always possible and often imperfect (production of impurities or enantiomers particularly). Moreover, GC–GOOD system allowed for the own relative concentrations of volatile compounds in food product odor extracts.

The three-way valve was manually handled from a position to the other by an operator. This handling was synchronized with the simultaneous visualization of the FID signal corresponding to the volatile compounds elution. Synchronization of the visualization with crossing of volatile compounds in the three-way valve was ensured by adequate deactivated capillary column lengths and flows between the effluent split and the FID system and between the effluent split and the three-way valve (Fig. 1). Optimization of deactivated capillary column lengths was performed with a solution composed of the following standard odorous volatile compounds: dimethyl sulfide, 2-butanone, 2,3-butanedione, hexanal, (Z)-3-hexen-1-ol and 1-nonanal (Aldrich, Deisenhofen, Germany).

3.2. Preliminary studies

In a preliminary study (submitted for publication in J. Sci. Food Agr.), the odor representativeness of the *S. glanis* dynamic headspace extracts was checked. Indeed, as the volatile compounds are analyzed outside their food matrix, this verification is an indispensable step [6]. Then, the 65 volatile compounds present in *S. glanis* dynamic headspace extracts were identified by GC–FID (retention index, *I*) and GC–MS. They were quantified by GC–FID using an internal standard (5 μl of p-cymene at 300 μg/ml in methanol). The GC–O method described by Le Guen et al. [1] enabled 19 odor active volatile compounds to be distinguished from these 65 compounds.

3.3. Omission strategy

The omission procedure was performed on selected volatile compounds chosen from the 65 identified in the *S. glanis* dynamic headspace extracts. Selection was achieved by using two criteria. First, the 19 volatile compounds perceived significantly [36] during the GC–O analysis were kept whatever their concentrations. Secondly, as Patterson et al. [15] have indicated that two or more individual volatile compounds, each at levels too weak to be perceived on their own, could be able to do so in concert, the 23 volatile compounds present at a high relative concentration (i.e. more than 5% of the internal standard) were also kept, even if they were not perceived during the GC–O analysis. Only 33 out of the 65 volatile compounds fulfilled at least one of these two criteria (Table 1). As in this case it was the omission effect that was being studied, the 32 remaining unselected volatile compounds were always put in the PTFE bag.

To study the effect of the 33 selected volatile compounds, multiple omissions were performed. This strategy was used to determine whether combinations of several volatile compounds could have an effect on the global odor extract characteristics [14]. Single omissions, which would have been more time-consuming, would not allow the observation of such an effect [13]. Moreover, a volatile compound omitted alone has often a slight and non-significant effect [14,37]. Multiple omissions were probably the best method to obtain meaningful results with a minimum of repetitive testing [14].

Therefore, the 33 selected natural volatile compounds present in their relative concentrations were distributed into five families (Table 1); volatile compounds with sulfury/moldy odors (dimethyl sulfide, geosmin, dimethyl disulfide, camphene, 2-methyl isoborneol, unknown (I = 1427) and (E)-2-nonenal); cooked odor [unknown (I = 1150), (Z)-4-heptenal, heptanol + methional and 4-methyl thiazole]; grassy odor [2,3-butanedione, unknown (I = 1010), 2,3-pentadione and 1-nonanal]; green/floral/fruity odors (alpha pinene, hexanal, heptanal, limonene, 1-pentanol, octanal, 2-

Table 1
Volatile compound families selected for omission tests

A	B	C	D	E
Sulfury/moldy	Cooked	Grassy	Green/floral/fruity	Alcohol/solvent/plastic
Dimethyl sulfide[b]	Unknown (I = 1150)[a]	2,3-Butanedione[a,b]	Alpha pinene[b]	Ethyl acetate[a,b]
Geosmin[b]	(Z)-4-heptenal[a,b]	Unknown (I = 1010)[a,b]	Hexanal[a,b]	Unknown (I = 809)[a,b]
Dimethyl disulfide[b]	Heptanol[b] + methional[b]	2,3-Pentadione[a,b]	Heptanal[a,b]	2-Butanone[b]
Camphene[b]	4-Methylthiazole[a]	1-Nonanal[b]	Limonene[a]	(E)-2-octene[b]
2-Methylisoborneol[b]			1-Pentanol[b]	Unknown (I = 1012)[a]
Unknown (I = 1427)[a]			Octanal[a]	(E,E,Z)-1,3,5-Octatriene[a,b]
(E)-2-nonenal[a]			2-Nonanol[a]	Styrene[b]
			1-Nonanol[a]	1-Hexanol[b]
				Unknown (I = 1565)[a]

[a] Volatile compound significantly perceived during the GC–O analysis.
[b] Volatile compound present at a concentration greater than 5% of the internal standard.

nonanol and 1-nonanol) and alcohol/solvent/plastic odors [ethyl acetate, unknown (I = 809), 2-butanone, (E)-2-octene, unknown (I = 1012), (E,E,Z)-1,3,5-octatriene, styrene, 1-hexanol and unknown (I = 1565)]. Thanks to the GC–GOOD system, the five families could be selectively omitted. Omissions were directed by an experimental design. With these five families, a half-fraction of a complete factorial design (2^{5-1}) led to 16 different mixtures. To evaluate the repeatability of the method and the judge assessments, a 17th mixture, containing all the volatile compounds present in *S. glanis* dynamic headspace extracts and so identical to the odor reference sample, was added (Table 2).

3.4. Similarity results

The average similarity scores obtained for the seven mixtures compared to the reference sample containing all the volatile compounds are reported in Table 2. The analysis of the experimental design allowed the estimation of the main effects and first order interactions between the five families. Very significant main effects were observed for the families B (P-value <0.001), C (P-value <0.001) and E (P-value <0.001). Family A also had a significant main effect but it was less important (P-value = 0.053) than for families B, C and E. That was explained by the high average similarity score of 72/100 obtained for the mixture 15, in which only family A was omitted, because this result seemed to indicate that the similarity score was not modified by omission of family A. Omission of these four families of volatile compounds involved a loss of odor similarity. Omission of family D did not modify significantly this similarity. The volatile compounds associated with sulfury, moldy, cooked, grassy, alcohol, solvent and plastic odors played a key role in the global *S. glanis* odor, while those associated with green, floral and fruity odors were of only secondary importance. Significant first order interactions were noted between the families B and C (P-value <0.03), B and D (P-value <0.01) and A and C (P-value <0.01). There was a synergistic effect between the families B and C, and the families B and D. Omission of a family in these duos increased the omission effect of the other family. Therefore, whereas family D had no significant effect on its own, it affected the global *S. glanis* odor by enhancing the effect of family B. For families A and C, there was a masking effect. Omission of family A (or C) decreased the omission effect of family C (or A, respectively).

Table 2
Experimental design and similarity results of the omitted samples compared to the reference sample containing all the volatile compounds (average score out of 100)

Mixture	Family A	Family B	Family C	Family D	Family E	Similarity
1	0	0	0	0	0	24
2	1	0	0	0	1	56
3	0	1	0	0	1	31
4	1	1	0	0	0	27
5	0	0	1	0	1	61
6	1	0	1	0	0	26
7	0	1	1	0	0	43
8	1	1	1	0	1	61
9	0	0	0	1	1	17
10	1	0	0	1	0	25
11	0	1	0	1	0	20
12	1	1	0	1	1	58
13	0	0	1	1	0	35
14	1	0	1	1	1	42
15	0	1	1	1	1	72
16	1	1	1	1	0	53
17	1	1	1	1	1	88

Two average similarity scores were particularly remarkable. First, mixture 1, which contained only the 32 unselected volatile compounds, obtained an average similarity score of 24/100. This average score was relatively low and showed that these compounds, which were not significantly perceived during the GC–O analysis and were present at a concentration lower than 5% of the internal standard, were of only little importance in the global *S. glanis* odor. Nevertheless, this average score also showed that they did play a role and confirmed the Patterson et al. [15] assertion about the possible perception in concert of two or more individual volatile compounds not perceived on their own. Secondly, mixture 17, which contained all the volatile compounds present in *S. glanis* dynamic headspace extracts, obtained an average similarity score of 88/100. As this average score was not

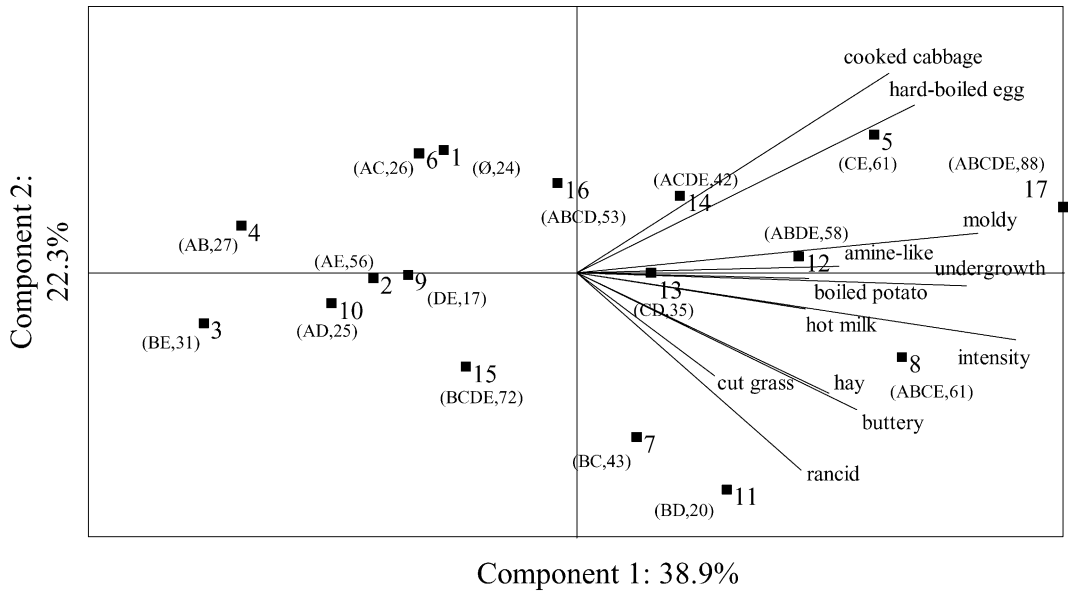

Fig. 2. Biplot of the principal component analysis: scores of the mixtures and loadings of the odor descriptors. The family composition and the similarity score of the mixtures are given in brackets.

100/100 while the judges compared the odor similarity of a sample similar to the odor reference, this result confirmed the Le Quéré et al [38] study. Indeed, they demonstrated that when judges assessed the odor similarity of a hidden cheese sample to the same cheese sample used as a reference, the hidden sample odor was not assessed as similar to the odor of the reference sample. However, as this average score was close to 100/100, it could also be said that the method was repeatable and that the judges were well trained.

3.5. Quantitative descriptive analysis results

3.5.1. Principal component analysis presentation

To precisely characterize the family addition/omission effect on the global odor of the different mixtures, a PCA was performed. To show the relative position of the mixtures, the biplot representing the scores of the mixtures and the loadings of the odor descriptors is presented in Fig. 2. To illustrate the correlations occurring between addition/omission variables for each family, similarity rating and odor descriptor loadings, the plot of the correlations of the illustrative variables (addition/omission variables for each family and similarity rating) with the first two axes of the PCA is presented in Fig. 3. In these plots, component 1, with a weight of 38.9%, could be defined as a "similarity" axis and component 2, with a weight of 22.3%, could be defined as an "odor" axis.

3.5.2. Family addition/omission variables and similarity rating correlation

These plots confirmed that omission of an odor family decreased the mixture similarity. Indeed, the family addition/omission variables and the similarity rating were positively correlated (Fig. 3) and the mixtures containing four families tended to be more represented by high component 1 values, equivalent to high similarity scores, than the mixtures containing two families (Fig. 2).

3.5.3. Similarity rating and 11 odor descriptor loadings correlation

A positive correlation was observed between similarity rating and the 11 odor descriptor loadings (Fig. 3). This was confirmed by the fact that the average similarity scores tended to increase with component 1 increasing values

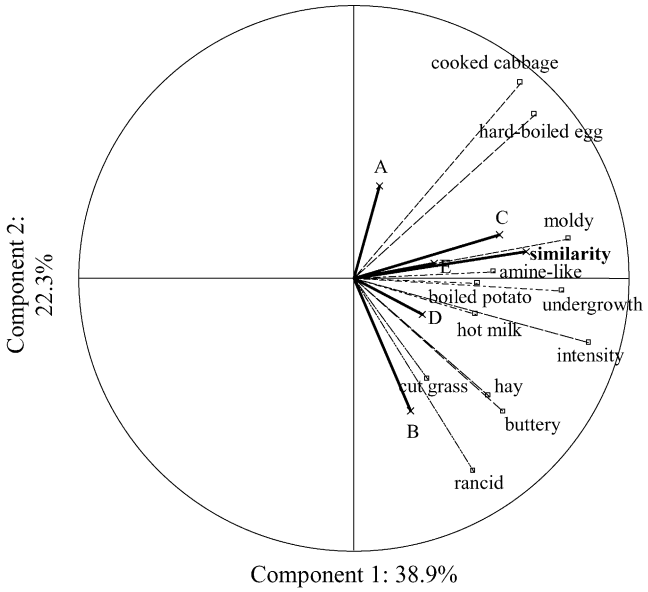

Fig. 3. Plot of the correlations of the variables (illustrative variables: addition/omission variables for each family and similarity rating; active variables: loadings of the odor descriptors) with the first two axes of the principal component analysis.

(Fig. 2). Therefore, the more intense the trained judges perceived the odor of the descriptors, the more similar they assessed the mixture. This result showed that the trained judges had assessed the odor of the mixtures well in comparison with the odor of the reference, which they evaluated as being the most odorous. The positive correlation between intensity and similarity ratings confirmed this result (Fig. 2).

3.5.4. Eleven odor descriptor loadings and family addition/omission variables correlation

The 11 odor descriptor loadings were positively correlated with family addition/omission variables (Fig. 3). Therefore, the omission from the mixture of a family decreased the perceived intensity of the odor descriptors. More particularly, some interesting phenomena could be observed. There was a great proximity between family A, which represented sulfury and moldy odors, and the odor descriptors cooked cabbage, hard-boiled egg and moldy. Omission of family A from the mixture decreased the perceived intensity of these odor descriptors. This is confirmed by the fact that the mixtures containing family A were more represented by high component 2 values, equivalent to an intense odor of cooked cabbage, hard-boiled egg and moldy (Fig. 2) than by low ones. Such a phenomenon was observed between family D, which groups volatile compounds presenting green, floral and fruity odors, and the odor descriptor cut grass. The importance of the mixture effects of the volatile compounds could also be observed. Although family B, which represented cooked odor, and family C, which represented grassy odor, were positively correlated to the odor descriptors boiled potato and buttery, respectively, there was not a great proximity between these families and these respective odor descriptors. Boiled potato and buttery odors seemed to be due to a combination of several families. Unfortunately, elucidation of the precise combinations occurring in the formation of the odor of each descriptor has proved difficult to date and will have to be specifically investigated in further studies. This was mainly due to the fact that, although the judges had received good training, their perception of the odor descriptors in the mixtures was not always consensual. Lawless [37] has indicated that such a phenomenon is inevitable, particularly because of individual differences in sensitivity to specific volatile compounds.

4. Conclusions

For the first time to our knowledge, the GC–GOOD system has enabled the selection of natural volatile compounds present in an odor extract (other extraction methods could be used) and the assessment of their global odor when they were mixed in their own extracted relative concentrations. The use of GC–GOOD will answer many queries occurring in the field of odorous perception of volatile compound mixtures.

The GC–GOOD system enabled the identification of key families of volatile compounds in global *S. glanis* odor and the interactions between these families were elucidated. Families A (sulfury and moldy odors), B (cooked odor), C (grassy odor) and E (alcohol, solvent and plastic odors) played a key role in the global *S. glanis* odor while family D (green, floral and fruity odors) was only of secondary importance. Synergistic and masking effects were also observed between these five families.

In further studies, the effect of the volatile compounds present in each family will be examined and other combinations will be studied. Thus, the role of each volatile compound in the global odor of *S. glanis* will be elucidated.

Acknowledgements

We thank B. Grevet (Technologies Aquacoles Géothermiques, France) for the supply of silurus, J. Marcel (Institut Technique de l'Aviculture, France) and J.M. Poli (Experimental farm of the Association pour le Développement de l'Aquaculture en Région Centre, France) for the project coordination, OFIMER (Office National Interprofessionnel des Produits de la Mer et de l'Aquaculture, France) for financial support and all the members of the odor evaluation panel of ENITIAA (Ecole Nationale d'Ingénieurs des Techniques des Industries Agricoles et Alimentaires, France).

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