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Influence of Rearing Conditions on the Volatile Compounds of Cooked Fillets of *Silurus glanis* (European Catfish)

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Volatile compounds of cooked fillets of *Silurus glanis* reared under two conditions occurring in France were studied. They were extracted by dynamic headspace, identified by gas chromatography/mass spectrometry, and quantified by gas chromatography−flame ionization detection. Odor active volatile compounds were characterized by gas chromatography−olfactometry. Sixty volatile compounds were detected in dynamic headspace extracts, among which 33 were odor active. Rearing conditions affected their estimated concentrations and their odor intensities, but very few qualitative differences were exhibited (only seven volatile compounds were concerned). A good correlation between quantitative and olfactometric results is shown. 2-Methylisoborneol and (*E*)-2-hexenal were less represented in OUTDOOR extracts, while 2-butanone was less represented in INDOOR extracts. In addition, olfactometric results can be closely related to those previously obtained by sensory analysis. Boiled potato sensory odor of the silurus cooked fillets can be related to (*Z*)-4-heptenal and methional, and buttery odor can be related to 2,3-butanedione, an unknown compound (RI = 1010), and 2,3-pentadione.

KEYWORDS: ***Silurus glanis*; freshwater fish; volatile compounds; dynamic headspace; gas chromatography−olfactometry**

INTRODUCTION

Silurus is a freshwater fish similar to the United States catfish. Its flesh is white without herringbones and possesses a high food value (*1*). As in recent years, consumers have been turning more and more toward freshwater fish, and their production has been increasing by more than 140% between 1990 and 2000 (*2*). In this context, silurus is of real economic interest.

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 of acceptance and preference of food (*3*). Silurus odor is often characterized by great heterogeneity (*4*). Such variability has already been observed in catfish and is mainly due to off-flavor problems (*5*). Thus, the study of the volatile compounds that are responsible for the silurus odor properties appeared necessary.

In France, silurus can be reared under two different conditions. The first enables silurus of commercial size to be obtained in 1 year and consists of rearing in indoor concrete ponds with renewed geothermal water. With the second method, which involves rearing in outdoor ponds with no renewal of water, 2 years are necessary to obtain silurus of commercial size. The comparison of volatile compounds contained in cooked fillets coming from these two types of silurus represents an original piece of work. To date, while odor differences between wild and reared fish are widely reported in the literature, little information is available on the effect of different rearing conditions on the flesh odor of fish (*6*).

Gas chromatography/olfactometry (GC-O) is often used to study food product odor (*7*). GC-O, proposed by Fuller et al. as early as 1964 (*8*), enables odor active volatile compounds to be distinguished from the whole range of volatiles in their own relative concentrations in food products. It consists of using the human nose as the detector by making a judge smell the gas chromatographic effluent of an odor extract of the food product (*9*). The extraction of the volatile compounds contained in the food product is required before this instrumental analysis. This extraction step necessitates checking the similarity between the odor of the extracts and that of the initial products (*10*). Indeed, as the study of the volatile compounds is carried out on the extract, it is essential to ensure that this extract has odor characteristics close to those of the initial food product. In our study, the extraction method selected was dynamic headspace. This technique has already been used for the extraction of volatile compounds of fish (*11*) and more specifically of catfish (*12*). A previous study of the odor similarity of dynamic headspace extracts demonstrated the reliability of this extraction method to characterize the odor active volatile compounds of cooked fillets of silurus (*13*). GC-O is often performed in association with gas chromatography/mass spectrometry (GC/MS) and gas chromatography−flame ionization detection (GC-FID), which allow, respectively, the identification and quantification of volatile compounds. Moreover, olfactometric analysis is often preceded by sensory analysis, which enables the global

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Table 1. "SARB Spécial HP" Composition (Quantities for 100 g of Food)

components	SARB spécial HP
crude protein	50 g
crude fat	12 g
crude ashes	9 g
cellulose	1 g
vitamin A	23100 U.I.
vitamin D3	2750 U.I.
vitamin E	149 U.I.

odor of the product to be described. A correlation between instrumental and sensory data is often essential to have a better knowledge of human nose perception. Such correlation has been widely used by authors studying fish odor (*11*, *14*). To complete these investigations, the study of the origin of the volatile compounds responsible for the odor of cooked fillets of silurus was of great interest. Indeed, nonvolatile compounds, mainly represented by the three large biochemical families, lipids, proteins, and carbohydrates, may lead to volatile compounds through enzymatic and chemical actions (*15*). Thus, the odor quality of fish fillets is dependent on these nonvolatile compounds designated more commonly as odor precursors. Many studies have proved that the main odor precursors in fish fillets are fatty acids, because of their high sensitivity to oxidative deterioration (enzymatic and chemical) (*16*).

The aim of this study is to characterize and compare volatile compounds of cooked fillets of silurus reared under two conditions occurring in France. First of all, volatile compounds contained in odor representative extracts obtained by dynamic headspace are identified and quantified. To explain the differences between both silurus sets, the possible origin of the volatile compounds responsible for the odor of cooked fillets of silurus is identified and correlations between the fatty acid and the volatile compound compositions of silurus fillets are established. Then, odor active volatile compounds are characterized by GC-O and these results are related to the volatile compound concentrations. Finally, olfactometry results are compared with those from sensory analysis, to relate odor sensory attributes to odor active volatile compounds.

MATERIALS AND METHODS

Reagents. All water was purified using a Millipore-Q system (Millipore Corp, Billerica, MA). Butylated hydroxytoluene and trifluoroboride/methanol (14%) were purchased from Aldrich (St. Louis, MO). Chloroform, methanol, hexane, and toluene were purchased from Merck (Germany). Sodium chloride and anhydrous sodium sulfate were purchased from Cluzeau (France). All of the standard compounds were purchased from Aldrich, except dimethyl sulfide, *p*-xylene, heptanal, octanal, and 1-octanol, which came from Merck.

Silurus. Silurus were reared under two different conditions. The ADARC experimental farm (Association pour le Développement de l'Aquaculture en Région Centre, France) reared silurus for 2 years in outdoor ponds with no renewal of water (the water temperature could fall below 10 °C in winter and rise above 25 °C in summer). The TAG company (Technologies Aquacoles Géothermiques, France) reared fish for 1 year in indoor concrete ponds with renewed geothermal water (the water temperature ranged between 27 and 31 °C). In both rearing conditions, the same food was used, "SARB spécial HP", which is employed by almost all silurus breeders in France. Its composition is reported in **Table 1**. For simplicity and clarity, fillets supplied by the ADARC experimental farm are called "OUTDOOR" and fillets supplied by the TAG company are called "INDOOR" in the rest of this paper.

Fish were caught and manually slaughtered the same day and then filleted using the same protocol for the different rearing conditions. Fish were manually eviscerated and filleted and mechanically peeled, and finally, fillets were manually trimmed. The average weight of the fillets was 400 g ($\sigma_{n-1} = 70$), which represents the commercial form of this product. Fillets were transported under ice in polystyrene boxes. They were wrapped in aluminum foil, vacuum-packed, and stored at −80 °C until analysis.

Fatty Acid Analyses. Lipids contained in silurus fillets were extracted by a mixture of chloroform/methanol (2/1, v/v) using the method of Folch et al. (*17*). Lipid extractions were performed nine times on nine different fillets coming from each silurus set on a crushed sample of 10 g. After the transmethylation of the fatty acids by a mixture of trifluoroboride/methanol (14%) according to Morrisson and Smith's method (*18*), the fatty acids were identified and their proportion in silurus fillets was determined as described by Pennarun et al. (*19*). The quantity of each fatty acid was expressed as a percentage of total identified fatty acid.

Odor Sensory Analyses. The panel was composed of eight judges from our laboratory, all involved in fish odor evaluation and more specifically trained in the recognition of cooked silurus fillet odor. Samples of 40 g taken from the central parts of fillets were cut into 1 cm cubes and steamed for 15 min. Cooked samples were put into 100 mL coded brown glass flasks, which were then closed and presented to the judges. Silurus odor was characterized by quantitative descriptive analysis as described by Hallier et al. (*13*).

Volatile Compound Analyses. *Sample Preparation.* Fillets were thawed just before analysis. 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. Five microliters of a methanolic solution of 300 μg mL^{-1} of *p*-cymene was added as an internal standard (IS). 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.

Dynamic Headspace Extraction. A purge and trap concentrator (model LSC 2000, Tekmar Inc., Cincinnati, OH) 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 for 2 min (*11*). This extraction method enabled us to obtain an average similarity mark of 52/100 ($\sigma_{n-1} = 22$), 100/100 corresponding to an odor extract identical to the cooked fillet reference, which is comparable with those obtained by other authors with other extraction methods (*13*).

Identification. Extraction and desorption of volatile compounds were performed as described above. A gas chromatograph (GC, HP 5890 II, Hewlett-Packard Co., Palo Alto, CA) coupled with a mass spectrometer (MS) equipped with an electronic impact source [HP 5971 II, mass selective detector (MSD), Hewlett-Packard Co.] was used. Volatile compounds were separated on a capillary column (DB-wax, 30 m in length, 0.32 mm i.d., 0.5 μm thick, J & W Scientific, Folsom, CA) with the following oven temperature programming: from 40 °C for 5 min to 160 °C at 10 °C/min followed by a temperature increase of 15 °C/min to 230 °C. The helium carrier gas flow was 1 mL min^{-1} (26.2 cm s^{-1}). The parameters of the MSD were as follows: electron impact mode, 70 eV; temperature of interface, 250 °C; ion source temperature, 180°C; mass range, *m*/*z* 33−300 amu; and scan rate, 1.9 s^{-1} (*11*).

Volatile compounds were identified by comparing their mass spectra with those of two libraries, a commercial one (NBS 75k) and an internal one of our laboratory, or with those of chemical standards injected in the same conditions if they were available. The identification was confirmed by comparison of their retention indices, calculated according to Van Den Dool and Kratz (*20*), with those found in the literature.

Quantification. Chromatographic separation of volatile compounds was performed as described above. A gas chromatograph (Star 3400, Varian, Palo Alto, CA) equipped with a flame ionization detector (FID) was used. The temperature of the detector was set at 250 °C. An IS (*p*-cymene, 1.5 μg) was used, and the quantity of each volatile compound was expressed as a percentage of this IS (*11*). Six extractions

were performed for each silurus fillet set analyzed to check the reproducibility of the method.

Olfactometry. The same apparatus and the same analysis conditions as described in the quantification part were used. The GC effluent was split 1:1 (v/v) between the FID and a sniffing port supplied with humidified air at 40 °C whose flow was 600 mL min^{-1}. Olfactometry consisted of making judges smell the effluent. According to the guidelines of Pollien et al. (*21*), a panel of nine trained judges (in our case, eight females and one male aged 25−55 years) was used. The judges were trained for fish odor detection and description using numerous standard compounds with various odors and thresholds. Each judge sniffed the 18 min of the chromatogram. Because of this short period of sniffing, the judges stayed alert (*11*). Each judge was asked to indicate when an odor was detected, to give an odor descriptor, and to assess odor intensity on a scale of 1−9 (1 = very weak odor intensity, and 9 = very strong odor intensity) (*22*). Two olfactometry methods were used as follows: frequency of detection (FD) and time intensity (TI). For the FD method, results were expressed as % NIF (nasal impact frequency) (*21*). A NIF of 100% means that the volatile compound considered was detected by all of the judges. According to van Ruth et al. (*23*), a response lower than 33.3% NIF is considered as noise. The FD method enabled odor active volatile compounds to be easily distinguished from the whole range of volatiles within a minimum period of time and with no specific training of the panel (*10*). For the TI method, results were expressed as average intensity computed for all of the nine judges. The odor intensity of a volatile compound not detected by a judge was considered as equal to zero. The TI method, thanks to the use of variance analysis, enabled significant differences between products to be highlighted.

To identify volatile compounds responsible for the odor perceived by the judges among volatile compounds identified in the odor extracts, three criteria were used (*11*) as follows: (i) The retention index of the volatile compounds had to be close to that of the odor reported by the judges; (ii) the odor descriptors used by the judges to describe the identified volatile compounds had to be similar to those reported in the literature; and (iii) the odor reported by the judges for the corresponding standard volatile compound, when it was available, had to be similar to that reported in the odor extracts.

Statistical Treatment. Data acquisition and statistical treatment were performed with Statgraph 5.0 software (Manugistics, Rockville, MD). For silurus odor sensory profiles, a multiway analysis of variance (multiway ANOVA) was performed on marks given by the judges for each odor descriptor. Three factors were studied as follows: session, judge, and rearing environment. The confidence level for the statistical treatments was 90%. Proportions of fatty acids (% total identified fatty acid), estimated concentrations (% IS), and odor intensities (TI method) of volatile compounds were averaged, respectively, with nine, six, and nine analyses for each silurus fillet set. A one-way ANOVA (rearing condition factor) was performed on proportions, estimated concentrations, and odor intensities. Proportions, estimated concentrations, and odor intensities evaluated for each silurus fillet set were then compared by least significance difference tests (LSD test). The confidence level for the statistical treatments was 95%. For estimated concentrations, traces were considered as equal to zero.

RESULTS AND DISCUSSION

Identification and Quantification of Volatile Compounds by GC/MS and GC-FID and Possible Origins of These Compounds. Sixty volatile compounds were detected in extracts of cooked fillets of silurus obtained by dynamic headspace (**Table 2**). Aldehydes and alcohol were the major chemical classes detected with, respectively, 12 and 11 volatile compounds. The extracts also contained five ketones, five aromatic hydrocarbons, five sulfur compounds, and five hydrocarbon compounds. Thirteen volatile compounds were not identified, and four compounds belonged to diverse chemical classes. Two main reasons could explain why some compounds were not identified. Volatile compounds could be present at low estimated concentrations so that the MS background was too high to obtain interpretable mass spectra (unknowns **22**, **26**, **38**, and **54**). They could also be coeluted with another compound that makes their identification difficult to perform (unknowns **3**, **4**, **11**, and **45**). To date, no study has been published concerning volatile compounds of silurus but many authors have analyzed volatile compounds in fish. Most of the volatile compounds identified

Table 2. Influence of the Rearing Conditions on the Volatile Compounds of Cooked Fillets of Silurus

peak[c]	compound	RI[d]	methods of identification	estimated concn (% IS)	
				OUTDOOR	INDOOR
1	dimethyl sulfide	737	MS, RI, O, st	75.7	79.6
2	ethyl acetate	762	MS tent, RI, O	59.5	53.8
3	unknown	785		11.9	9.0
4	unknown	809		35.2	32.9
5	2-butanone	841	MS tent, RI, O	20.6[a]	7.6[b]
6	(*E*)-2-octene	861	MS tent, RI	5.9	3.3
7	2,4-octadiene	886	MS tent, RI	5.3	5.8
8	geosmin	912	MS, RI, st	7.4	4.8
9	2,3-butanedione	947	MS, RI, O, st	20.9[a]	31.9[b]
10	unknown	976		6.4[a]	3.4[b]
11	unknown	1010		10.4[a]	35.6[b]
12	unknown	1012		1.1	4.5
13	dimethyl disulfide	1015	MS, RI, st	11.7[a]	17.3[b]
14	2,3-pentadione	1050	MS, RI, O, st	13.0[a]	18.5[b]
15	hexanal	1092	MS, RI, O, st	103.5	81.5
16	1,3,5-octatriene	1100	MS tent, RI, O	3.0[a]	7.4[b]
17	2-methylisoborneol	1112	MS, RI, st	17.9[a]	60.6[b]
18	*p*-xylene	1120	MS, RI, st	6.3[a]	2.0[b]
19	3-heptanone	1131	MS, RI, st	1.4	0.7
20	1-penten-3-ol	1146	MS, RI, st	2.6	1.4
21	heptanal	1148	MS, RI, O, st	41.4[a]	51.4[b]
22	unknown	1150		0.0	tr
23	α-terpinene	1152	MS tent, RI, O	0.0	tr
24	limonene	1154	MS, RI, O, st	2.2	2.3
25	eucalyptol	1200	MS tent, RI, O	2.1[a]	0.0[b]
26	unknown	1212		0.0[a]	0.8[b]
27	2-pentylfuran	1224	MS tent, RI	0.0	0.3
28	(*E*)-2-hexenal	1228	MS, RI, O, st	0.8[a]	3.3[b]
29	1-pentanol	1232	MS, RI, st	3.0[a]	9.5[b]
30	(*Z*)-4-heptenal	1233	MS, RI, O, st	5.6	5.1
31	styrene	1236	MS tent, RI	2.5	2.7
IS	*p*-cymene	1242	MS, RI, st	100.0	100.0
32	unknown	1263		5.6[a]	8.0[b]
33	octanal	1267	MS, RI, O, st	6.4	7.6
34	(*E*)-2-penten-1-ol	1303	MS, RI, O, st	0.8	1.5
35	(*E*)-2-heptenal	1323	MS, RI, st	0.0	0.4
36	1-ethyl-2,3-dimethylbenzene	1352	MS tent, RI, O	2.1	2.8
37	6-methyl-5-hepten-2-one	1355	MS, RI, O, st	2.6[a]	4.1[b]
38	unknown	1358		0.6	1.0
39	1-hexanol	1361	MS, RI, st	61.6	67.6
40	dimethyl trisulfide	1363	MS, RI, O, st	tr	0.3
41	1-nonanal	1367	MS, RI, st	38.9	24.8
42	unknown	1375		4.7	3.2
43	1,2,4,5-tetramethylbenzene	1400	MS tent, RI	1.3	0.8
44	(*E*)-2-octenal	1425	MS, RI, st	0.0	1.5
45	unknown	1427		2.2	2.6
46	1-octen-3-ol	1444	MS, RI, st	3.4	4.6
47[e]	heptanol + methional	1462	MS, RI, O, st	9.9[a]	15.2[b]
48	(*E*,*E*)-2,4-heptadienal	1470	MS, RI, st	4.7	5.9
49	2-ethyl-1-hexanol	1518	MS, RI, st	2.2	4.1
50	decanal	1521	MS, RI, st	8.3	10.9
51	benzaldehyde	1549	MS, RI, st	2.1	4.7
52	(*E*)-2-nonenal	1556	MS, RI, O, st	2.8[a]	4.4[b]
53	1-octanol	1563	MS, RI, st	6.3	9.5
54	unknown	1565		tr	1.0
55	2-acetyl pyridine	1602	MS tent, RI	0.6	1.5
56	1-nonanol	1639	MS, RI, O, st	3.8[a]	5.7[b]
57	unknown	1701		5.0[a]	11.7[b]
58	4-methylthiazole	1709	MS tent, RI, O	2.0[a]	3.4[b]
59	naphthalene	1792	MS, RI, st	2.4	2.7

[a,b] Values in the same row with different superscripts are significantly different ($p < 0.05$). [c] Numbers are the same in **Tables 1** and **2**. [d] Retention index (Kovats index) on DB-WAX column. MS tent, tentatively identified by MS; RI, retention index; O, olfactometry; st, standard; tr, traces. [e] Heptanol was identified by MS, and methional was perceived during olfactometry.

Table 3. Influence of the Rearing Environment on the Fatty Acid Composition of Fillets

	proportion as % of the total fatty acid	
fatty acid	OUTDOOR	INDOOR
14:0	4.0[a]	4.8[b]
16:0	18.7	19.0
17:0	0.2	0.2
18:0	5.5[a]	4.6[b]
20:0	0.1	0.0
∑SFA	**28.5**	**28.7**
14:1n-5	0.3	0.3
16:1n-7	7.7[a]	6.6[b]
18:1n-9 trans	23.1	23.0
18:1n-9 cis	5.4[a]	4.5[b]
20:1n-9	4.7	5.4
∑MUFA	**41.1**	**39.7**
18:2n-6	6.5[a]	6.0[b]
20:2n-6	0.5	0.5
20:4n-6	1.1	1.0
∑n-6 PUFA	**8.2**	**7.4**
18:3n-3	1.0	1.1
20:3n-3	0.1	0.0
20:5n-3	7.6[a]	7.9[b]
22:6n-3	13.5[a]	15.1[b]
∑n-3 PUFA	**22.2**	**24.2**

[a,b] Values in the same row with different superscripts are significantly different ($p < 0.05$).

in our study have already been identified in several freshwater and saltwater fish species.

There were very few qualitative differences between both silurus fillet sets. Indeed, nearly the same volatile compounds were identified in OUTDOOR and INDOOR extracts. Both extracts were rich (average estimated concentrations higher than 10% of IS) in dimethyl sulfide (**1**), ethyl acetate (**2**), unknown (**4**), 2-butanone (**5**), 2,3-butanedione (**9**), unknown (**11**), dimethyl disulfide (**13**), 2,3-pentadione (**14**), hexanal (**15**), 2-methylisoborneol (**17**), heptanal (**21**), 1-hexanol (**39**), 1-nonanal (**41**), and heptanol + methional (**47**).

One-way ANOVA demonstrated that 22 volatile compounds were characterized by quantitative differences according to the rearing conditions (**Table 2**). Eighteen of them were present in lower estimated concentrations in OUTDOOR extracts [2,3-butanedione (**9**), unknown (**11**), dimethyl disulfide (**13**), 2,3-pentadione (**14**), 1,3,5-octatriene (**16**), 2-methylisoborneol (**17**), heptanal (**21**), unknown (**26**), (*E*)-2-hexenal (**28**), 1-pentanol (**29**), unknown (**32**), 6-methyl-5-hepten-2-one (**37**), heptanol + methional (**47**), (*E*)-2-nonenal (**52**), 1-nonanol (**56**), unknown (**57**), and 4-methylthiazole (**58**)], while four were present in lower estimated concentrations in INDOOR extracts [2-butanone (**5**), unknown (**10**), *p*-xylene (**18**), and eucalyptol (**25**)].

All of these volatile compounds could be responsible for odor differences perceived between both silurus cooked fillet sets. Thus, it was very interesting to study their possible origin to attempt to justify these differences. Especially of interest was the correlation between volatile compound and fatty acid compositions of silurus fillets (results in submission, the main ones of which are presented in **Table 3**).

Six volatile compounds could arise from fatty acid degradation. 1,3,5-Octatriene (**16**) and (*E*)-2-hexenal (**28**) are known to be formed by the oxidation of n-3 polyunsaturated fatty acids (PUFAs) (*24*). These two compounds were present in higher estimated concentrations in INDOOR extracts than in OUTDOOR ones. These results could be explained by the higher proportion of 20:5n-3 and 22:6n-3 measured in INDOOR fillets than in OUTDOOR ones (respectively, 7.9 and 15.1 in INDOOR fillets against 7.6 and 13.5 in OUTDOOR ones). Heptanal (**21**) could be formed by n-6 PUFA oxidation but could also come from n-9 monounsaturated fatty acid (MUFA) oxidation. 1-Pentanol (**29**) and (*E*)-2-nonenal (**52**) could be produced during n-6 PUFA oxidation (*25*). These three volatile compounds were present in higher estimated concentrations in INDOOR extracts than in OUTDOOR ones. These results could not be directly related to n-6 PUFA and n-9 MUFA proportions because they were lower in INDOOR fillets than in OUTDOOR ones. This lack of relationship between estimated concentrations of volatile compounds produced by fatty acid oxidation and fatty acid proportions may be explained by the fatty acid autoxidation mechanism as described by Elmore et al. (*26*).

Among the other volatile compounds that could be responsible for the potential odor differences perceived between both silurus cooked fillet sets, some are known to arise from proteins and carbohydrates, in particular through the cooking process. 2,3-Butanedione (**9**) and 2,3-pentadione (**14**) may be thermally produced through the Maillard reaction (*27*). Dimethyl disulfide (**13**) and methional (**47**) may be formed by the Strecker degradation of methionine during cooking (*28*). Others could come from the environment. *p*-Xylene (**18**) is a pollutant that may come from gas exhausts or be produced naturally by some plants through polysaccharide degradation (*29*). 2-Methylisoborneol (**17**) may be produced not only by some actinomycetes (*Streptomyces*) but also by some cyanobacteria (*Oscillatoria*), which may be present in large amounts in rearing water (*30*).

Consequently, further investigations would be required to elucidate precisely the mechanisms generating the odor of cooked fillets of *S. glanis*. The objectives of these further studies would be the identification of the origin of the volatile compounds contributing to this odor by studying the fatty acid metabolism of silurus, by quantifying precisely the amino acids contained in silurus flesh, or by quantifying the volatile compounds contained in rearing water.

Characterization of Odor Active Volatile Compounds by GC-O and Correlation with Quantitative Results. The 33 volatile compounds detected by at least 33.3% of the judges (FD method) are given in **Table 4** with their odor description and their odor intensity (TI method). Fifteen odor active volatile compounds were perceived with appreciable odor intensities (average odor intensity higher than 4 on a scale of 9) by judges in the extracts [2,3-butanedione (**9**), unknown (**11**), 2,3-pentadione (**14**), hexanal (**15**), 1,3,5-octatriene (**16**), heptanal (**21**), (*Z*)-4-heptenal (**30**), octanal (**33**), (*E*)-2-penten-1-ol (**34**), 1-ethyl-2,3-dimethylbenzene (**36**), dimethyl trisulfide (**40**), unknown (**45**), heptanol + methional (**47**), unknown (**54**), and 1-nonanol (**56**)].

One-way ANOVA revealed that 11 volatile compounds were characterized by different odor intensities according to the rearing conditions (**Table 4**). The odor of eight of them was perceived as being less intense in OUTDOOR extracts [unknown (**22**) (boiled potato and green odors), α-terpinene (**23**) (citrus fruit odor), unknown (**26**) (no common odor descriptor), (*E*)-2-hexenal (**28**) (green odor), unknown (**32**) (fish and sulfury odors), 6-methyl-5-hepten-2-one (**37**) (citrus fruit and green odors), 1-nonanol (**56**) (green and floral odors), and 4-methylthiazole (**58**) (cooked meat odor)], while the odor of three compounds was perceived as being less intense in INDOOR extracts [2-butanone (**5**) (solvent and plastic odors), eucalyptol (**25**) (mint odor), and unknown (**42**) (moss odor)].

Among the 15 volatile compounds perceived with appreciable odor intensities, six were present in high estimated concentra-

Table 4. Influence of the Rearing Conditions on the Odor Active Volatile Compounds of Cooked Fillets of Silurus

peak[f]	compound	odor description	odor intensity	
			OUTDOOR	INDOOR
1	dimethyl sulfide	sulfury,[c−e] marine[c−e]	0.9	0.4
2	ethyl acetate	alcohol[c,d]	3.6	4.1
4	unknown	no common descriptor[c]	1.3	2.4
5	2-butanone	solvent,[c,d] plastic[c,d]	2.2[a]	0.0[b]
9	2,3-butanedione	buttery,[c−e] caramel[c−e]	3.9	5.4
11	unknown	buttery,[c] caramel[c]	4.0	4.8
12	unknown	plastic[c]	0.0	1.9
14	2,3-pentadione	buttery,[c−e] caramel[c−e]	4.8	5.6
15	hexanal	green,[c−e] garlic[d]	5.9	6.5
16	1,3,5-octatriene	plastic,[c,d] green[c,d]	4.8	5.4
21	heptanal	green,[c−e] floral[c−e]	4.2	3.4
22	unknown	boiled potato,[c] green[c]	0.0[a]	3.9[b]
23	α-terpinene	citrus fruit[c,d]	0.0[a]	3.6[b]
24	limonene	lemon[c−e]	3.3	1.5
25	eucalyptol	mint[c,d]	2.6[a]	0.0[b]
26	unknown	no common descriptor[c]	0.0[a]	1.6[b]
28	(*E*)-2-hexenal	green[c−e]	0.2[a]	1.0[b]
30	(*Z*)-4-heptenal	boiled potato,[c−e] cooked fish[c,d]	6.8	6.2
32	unknown	fish,[c] sulfury[c]	0.0[a]	7.6[b]
33	octanal	citrus fruit[c−e]	4.7	4.6
34	(*E*)-2-penten-1-ol	mushroom[c−e]	6.0	7.2
36	1-ethyl-2,3-dimethylbenzene	grilled[c,d]	4.9	5.7
37	6-methyl-5-hepten-2-one	citrus fruit,[c−e] green[c−e]	0.0[a]	2.6[b]
40	dimethyl trisulfide	sulfury[c−e]	7.2	8.2
42	unknown	moss[c]	0.3[a]	0.0[b]
45	unknown	grilled,[c] floral[c]	4.6	5.1
47	heptanol + methional	green,[c−e] boiled potato[c−e]	4.3	5.6
52	(*E*)-2-nonenal	earthy,[c−e] cucumber[c−e]	3.8	3.7
54	unknown	synthetic cloth[c]	3.0	5.2
56	1-nonanol	green,[c−e] floral[c−e]	4.9[a]	6.3[b]
57	unknown	no common descriptor[c]	1.7	1.4
58	4-methylthiazole	cooked meat[c,d]	3.0[a]	4.6[b]

[a,b] Values in the same row with different superscripts are significantly different ($p < 0.05$). [c] Odor description assigned during olfactometry. [d] Odor description given by the literature. [e] Odor description (quality and intensity) checked by a standard compound. [f] Numbers are the same in **Tables 1** and **2**.

tions in the extracts [(2,3-butanedione (**9**), unknown (**11**), 2,3-pentadione (**14**), hexanal (**15**), heptanal (**21**), and heptanol + methional (**47**)]. All of them, except the volatile compound (**11**) (buttery and caramel odors), which was not identified, are known to affect the overall odor of food because of their low perception thresholds (p.t.). 2,3-Butanedione (**9**) [p.t. = 2.3−6.5 ppb (*31*)] and 2,3-pentadione (**14**) [p.t. = 1.3−6.9 ppb (*31*)], with buttery and caramel odors, are known to be characteristic contributors to the desirable odor of cooked fish such as turbot (*32*). Hexanal (**15**), with green and garlic odors [p.t. = 4.5 ppb (*33*)] and heptanal (**21**), with green and floral odors [p.t. = 3 ppb (*33*)], are generally considered to be off-flavors of fish products (*3*). Hexanal (**15**) is more particularly known to be involved in oxidized fish odor (*24*). The two coeluted volatile compounds heptanol + methional (**47**), with green and boiled potato odors [p.t. = 3 and 0.2 ppb, respectively (*31*)], seem to be less known to contribute actively to fish odors but have already been identified in many food products (*7*, *10*).

The nine remaining volatile compounds [1,3,5-octatriene (**16**), (*Z*)-4-heptenal (**30**), octanal (**33**), (*E*)-2-penten-1-ol (**34**), 1-ethyl-2,3-dimethybenzene (**36**), dimethyl trisulfide (**40**), unknown (**45**), unknown (**54**), and 1-nonanol (**56**)] were present in lower estimated concentrations in the extracts than the first six. Most of these volatile compounds are characterized by low p.t. values, which could explain why they were perceived with strong odor intensities in the extracts. (*Z*)-4-Heptenal (**30**), with boiled potato and cooked fish odors [p.t. = 0.07 ppb (*31*)] and (*E*)-2-penten-1-ol (**34**), with mushroom odor (p.t. not yet evaluated to our knowledge), have already been identified as contributors to the odor of cooked turbot (*32*). Octanal (**33**), with citrus fruit odor [p.t. = 0.07 ppb (*31*)], is generally considered to be an off-flavor of fish products (*3*). Dimethyl trisulfide (**40**), with sulfury odor [p.t. = 1.5 ppb (*34*)] has already been identified in many thermally processed fish and mollusks (*28*). 1-Nonanol (**56**), with green and floral odors (p.t. not yet evaluated to our knowledge), has been identified as contributing to the odor of gilthead sea bream (*14*) and crayfish (*28*). 1,3,5-Octatriene (**16**), with plastic and green odor, and 1-ethyl-2,3-dimethylbenzene (**36**), with grilled odor (p.t. values not yet evaluated to our knowledge), seem to be less known to contribute actively to fish odors.

Among the 11 volatile compounds perceived as having significantly different odor intensities in the extracts, eight also presented significantly different estimated concentrations [2-butanone (**5**), eucalyptol (**25**), unknown (**26**), (*E*)-2-hexenal (**28**), unknown (**32**), 6-methyl-5-hepten-2-one (**37**), 1-nonanol (**56**), and 4-methylthiazole (**58**)]. There was agreement between quantitative and olfactometric results for all of these volatile compounds. That is to say, an estimated concentration increase corresponded to an odor intensity increase and the converse.

For the three other volatile compounds [(unknown (**22**), α-terpinene (**23**), and unknown (**42**)], although significant differences in odor intensity tended to be found again at the level of estimated concentrations, the differences observed on these estimated concentrations were not significant at the confidence level of 95%. This seems to indicate that for these volatile compounds, trained judges were able to perceive minute estimated concentration differences.

Eight volatile compounds [(2,3-butanedione (**9**), unknown (**11**), 2,3-pentadione (**14**), 1,3,5-octatriene (**16**), heptanal (**21**), heptanol + methional (**47**), (*E*)-2-nonenal (**52**), and unknown (**57**)] presenting estimated concentrations significantly different in the extracts, were not perceived as having significantly different odor intensities. A hypothesis able to explain these results is based on the interpretation of graphs: odorant intensity perception according to estimated concentration. These graphs, proposed by Acree (*35*), show that there are two zones where a significant increase in estimated concentrations may not correspond to a significant increase in odor intensities perceived, because of the weakness of the graph slope. These are zones close to the p.t. and the saturation threshold, respectively.

Relationships between Olfactometry and Sensory Analysis. Eleven odor descriptors were used by the judges to describe the odor of cooked fillets of silurus during sensory analysis (cut grass, hot milk, hard-boiled egg, undergrowth, moldy, cooked cabbage, rancid, boiled potato, hay, buttery, and amine-like). A one-way ANOVA (rearing condition factor) performed on the marks given by the judges to describe OUTDOOR and INDOOR silurus cooked fillet odors indicated that, at a confidence level of 90%, no significant effect was observed for the five odor descriptors boiled potato, undergrowth, buttery, rancid, and amine-like. Both silurus cooked fillet sets were characterized by a medium odor intensity for boiled potato and buttery and a low odor intensity for undergrowth, rancid, and amine-like. On the contrary, a significant rearing condition effect was identified for the six other odor descriptors [hay (P value = 0.09), cut grass (P value < 0.01), hot milk (P value = 0.09), moldy (P value < 0.01), hard-boiled egg (P value = 0.04), and cooked cabbage (P value = 0.09)]. OUTDOOR cooked fillets presented a significantly stronger odor of cut grass, hay, and hot milk, while INDOOR cooked fillets presented a significantly stronger odor of moldy, hard-boiled egg, and cooked cabbage. Consequently, it was interesting to note that there were many relationships between sensory odor descriptors and volatile compound odors.

Boiled potato sensory odor could be attributed to three odor active volatile compounds [unknown (**22**), (*Z*)-4-heptenal (**30**), and methional (**47**)]. (*Z*)-4-Heptenal (**30**) and methional (**47**) were perceived with appreciable odor intensities, which were not significantly different in OUTDOOR extracts and in INDOOR ones. Unknown (**22**) was perceived only in INDOOR extracts. Sensory analysis results showed that boiled potato odor was perceived with medium odor intensity and that there was no significant difference between OUTDOOR and INDOOR cooked fillet odors. Thus, (*Z*)-4-heptenal (**30**) and methional (**47**) were mainly responsible for the boiled potato sensory odor of the silurus cooked fillets.

Silurus cooked fillets were characterized by medium buttery sensory odor without significant differences between OUTDOOR and INDOOR fillets. Three volatile compounds [2,3-butanedione (**9**), unknown (**11**), and 2,3-pentadione (**14**)], exhibiting an appreciable buttery odor without significant difference between both extracts, were thus most likely responsible for this odor.

Moss and earthy odors of unknown (**42**) and (*E*)-2-nonenal (**52**) could be associated with the undergrowth sensory odor of silurus cooked fillets. This odor had a little weight in the sensory description of these fillets, which correlated with olfactometric results. Indeed, unknown (**42**) and (*E*)-2-nonenal (**52**) were perceived with relatively low odor intensities. No significant difference was detected between OUTDOOR and INDOOR fillets. Olfactometry showed that the odor of unknown (**42**) was perceived more intensely in OUTDOOR extracts than in INDOOR ones, but this difference was very weak and no significant difference was perceived for odor intensities of (*E*)-2-nonenal (**52**).

The large number of volatile compounds described by a green odor very probably explains why the cut grass odor was perceived as being important in the odor of silurus cooked fillets during sensory analysis. Indeed, the green odor of volatile compounds could be associated with the cut grass sensory odor. Eight volatile compounds [hexanal (**15**), 1,3,5-octatriene (**16**), heptanal (**21**), unknown (**22**), (*E*)-2-hexenal (**28**), 6-methyl-5-hepten-2-one (**37**), heptanol (**47**), and 1-nonanol (**56**)] were described by a green odor. Four of them (**22**, **28**, **37**, and **56**) were perceived with a significantly lower odor in OUTDOOR extracts than in INDOOR ones, while no significant difference was perceived for the four others. These results seem to be in contradiction with those of the sensory analysis, which indicated that OUTDOOR fillets exhibited a more intense odor of cut grass than INDOOR ones. In olfactometry, volatile compound odors are evaluated separately and outside the food matrix. In sensory analysis, they are blended so that volatile compounds may interact with other constituents of the food matrix. Both phenomena could greatly modify odor perception and thus explain this slight discrepancy (*10*).

Three volatile compounds [dimethyl sulfide (**1**), unknown (**32**), and dimethyl trisulfide (**40**)] were described by a sulfury odor. According to Shankaranarayana et al. (*36*), such sulfur compounds give strong sulfurous, cooked cabbage, hard-boiled egg odors in vegetable, meat, and marine products. Cooked cabbage and hard-boiled egg sensory odors were detected in silurus cooked fillets and were perceived as being more intense in INDOOR fillets than in OUTDOOR ones. No significant difference was perceived for dimethyl sulfide (**1**) and dimethyl trisulfide (**40**) between both extracts. Thus, this difference could be due to unknown (**32**), which was perceived with a significantly more intense odor in INDOOR extracts than in OUTDOOR ones.

No volatile compounds were perceived during olfactometry as exhibiting odors of rancid, amine-like, hay, hot milk, or moldy, whereas these odors were used to describe silurus cooked fillet odor during sensory analysis. Although these odors could not be related to one volatile compound in particular, they most likely resulted from an odor combination of several volatile compounds. Many studies have shown that the mixing of volatile compounds could modify their odor properties and that their perception alone or in a mixture could be very different. Moreover, Patterson et al. (*37*) 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. For instance, for moldy sensory odor, there were two nonodor active volatile compounds [geosmin (**8**) and 2-methylisoborneol (**17**)] that are well-known as being able to exhibit a moldy odor. The combination of these two volatile compounds, each detected by GC-FID and not detected during olfactometry, could be responsible for the moldy sensory odor of silurus cooked fillets. This seemed to be confirmed by the fact that, while estimated concentrations of geosmin (**8**) were not significantly different in both extracts, 2-methylisoborneol (**17**) was present in significantly higher estimated concentrations in INDOOR extracts than in OUTDOOR ones. Indeed, INDOOR silurus cooked fillets presented a significantly stronger odor of moldy than OUTDOOR ones.

In conclusion, the volatile compounds analysis showed that there were very few qualitative differences between both silurus

fillet sets but that there were many quantitative ones. This analysis also enabled us to identify the key volatile compounds, which characterized the odor of cooked fillets of silurus reared under both conditions. The quantitative differences highlighted between both silurus fillet sets were perceived by the judges during sensory analysis, and they would very likely be by consumers. Consequently, breeders could use the rearing conditions to improve the odor quality of silurus cooked fillets.

ABBREVIATIONS USED

ANOVA, analysis of variance; FD, frequency of detection; GC-FID, gas chromatography−flame ionization detection; GC/MS, gas chromatography/mass spectrometry; GC-O, gas chromatography/olfactometry; INDOOR, fillets supplied by the TAG company; IS, internal standard; LSD, least significance difference; MSD, mass selective detector; MUFAs, monounsaturated fatty acids; NIF, nasal impact frequency; OUTDOOR, fillets supplied by the ADARC experimental farm; p.t., perception threshold; PUFAs, polyunsaturated fatty acids; TI, time intensity; transferred, fillets transferred from the ADARC experimental farm to the TAG company.

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