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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of Aroma-Active Compounds in Rainbow Trout (*Oncorhynchus mykiss*) Eliciting an Off-Odor

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The aroma-active and off-flavor compounds of cooked rainbow trout (*Oncorhynchus mykiss*) were analyzed by sensory and instrumental analyses. Sensory analysis shows that the aromatic extract obtained by vacuum steam distillation was representative of rainbow trout odor. To obtain more information on odorants of volatile compounds, analyses were conducted on two gas chromatography columns of different polarities (DB-5 and DB-Wax). The results of the gas chromatography−olfactometry analysis showed that 38 odorous compounds were perceived when the DB-5 column was used and 36 with the DB-Wax column. Of these, 31 with the DB-5 and 28 with the DB-Wax were identified. (*E*)-2-Nonenal, 2-ethyl-1-hexanol, 2-methylisoborneol, geosmin, 2-methylnaphthalene, and 8-heptadecene were described as off-flavor compounds by the sniffing assessors. The most powerful off-flavor compounds identified in the extract were 2-methylisoborneol and geosmin, which were described as strong musty and earthy odors, respectively.

KEYWORDS: Representativeness; olfactometry; aroma-active compounds; off-flavor; rainbow trout

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) belongs to the taxon Teleostei and the family Salmonidae. The natural habitat of this species is the cold waters of North America, but it has been successfully introduced throughout the world. It is easily identified by its broad reddish band or "rainbow", which runs along its side from head to tail. The brightness of color varies according to environment and feed (*1*, *2*). The total European production in 2000 was estimated to be over 323 700 tons, with Norway as the leading producer with 60 000 tons followed by France and Italy with 47 500 and 43 400 tons, respectively (*3*).

The aroma is one of the most important factors to determine the character and quality of fish species; hence, the study of aroma-active and off-flavor compounds of fish is important because of the effects on consumer acceptance and preference. Many factors can affect the off-flavor compounds of fish including (i) the microbial and autoxidative spoilage of fresh material due to inappropriate handling and storage, (ii) the adsorption of volatile organic compounds from external sources, and (iii) the bioaccumulation of substances present either in the fresh diet or in its environment (*4*, *5*). The most common off-flavors in freshwater fish are caused by the production of odorous metabolites that are assimilated by the fish from water (*6*). Off-flavors in freshwater and aquacultured fish were reviewed in the literature (*7*−*9*). The majority of the problems are caused by geosmin and 2-methylisoborneol compounds, which are rapidly adsorbed by fish and stored predominantly in fat tissue (*6*). These two compounds, resulting in unpleasant earthy and musty off-flavors, have been identified in catfish (*7*, *9*, *10*) and *Nile tilapia* (*11*).

Fish aroma contains hundred of components belonging to very heterogeneous groups. These components are generated by enzymatic reaction, lipid autoxidation, microbial action, and environmentally and thermally derived reactions (*12*). Only a small fraction of this large number of volatiles in fish actually contributes to the overall aroma. With the aid of an "olfactometric technique", aroma-active volatiles and off-flavor compounds can be detected in the complex mixture of hundreds of aroma compounds. The technology of gas chromatography−olfactometry (GC-O) made it possible to divide identified volatiles into odor-active and non-odor-active compounds with regard to their existing concentration in the studied sample (*13*). Several studies were conducted to investigate the aroma of fish and other seafoods by GC-O: Milo and Grosch (*14*) investigated the odor defects in boiled cod (*Gadus morhua*) and boiled trout (*Salmo fario*). Le Guen et al. (*15*) compared three olfactometric methods for the identification of the most potent odorants in cooked mussel (*Mytilus edulis*). Sérot et al. (*16*) studied the effect of dietary lipid sources on odor-active compounds in

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muscle turbot (*Psetta maxima*). Pennarun et al. (*17*) identified the origin of the character-impact compounds of raw oyster *Crassostrea gigas*. Triqui (*18*) examined the sensory and flavor profiles of hake (*Merluccius merluccius*) during ice storage. The most important step in GC-O analysis is the preparation of an aromatic extract representing the sample. The odor representativeness can be defined as the similarity between the extract odor and the product odor. The test of the representativeness is based on the sensory evaluation of the extract and the product by a trained panel (*19*). The selection of the isolation method is the foremost criterion to obtain the best representation for the studied sample. Vacuum steam distillation followed by solvent extraction is the most widely applied technique to isolate the volatile compounds. This technique has been shown to be a reliable method for the isolation of the volatile compounds of mussel (*15*), turbot (*16*), and oyster (*17*). Additionally, Conte et al. (*20*) reported that the vacuum distillation method was a popular sample preparation technique for the instrumental analysis of the geosmin and 2-methylisoborneol in catfish tissue because of its cryogenic cold-trap collection.

To date, no work has been published in the literature on the aroma-active compounds of cooked rainbow trout (*O. mykiss*) with a representativeness evaluation of its extract. Therefore, the aim of this study was first to assess the representativeness of cooked rainbow trout extract obtained by vacuum steam distillation and second to characterize the most odor-active compounds in cooked rainbow trout eliciting an off-odor.

MATERIALS AND METHODS

Reagents. The water used in the study was purified by a Millipore-Q system (Millipore Corp., Saint-Quentin, France). Diethyl ether, sodium chloride, and sodium sulfate were obtained from Fluka (Buchs, Switzerland). All standard compounds were purchased from Aldrich (Steinheim, Germany) except octanal, 1-octanol, and heptanal, which came from Merck (Darmstadt, Germany), and geosmin and 2-methylisoborneol, which were obtained from Supelco (Bellefonte, PA).

Rainbow Trout. Off-flavor trout (*O. mykiss*) of commercial size, averaging ~1.5 kg, were obtained from processing facilities in Brittany (France). Off-flavor fish was checked by sensorial analysis performed by professional flavor checkers in the farm. Fish were caught and manually slaughtered the same day by immersion in ice-cold water and transported under ice in insulated polystyrene boxes. Fish were manually eviscerated and filleted and mechanically peeled. In our laboratory portions of 200−250 g of fish were prepared and frozen at −80 °C prior to instrumental analysis.

Extraction of the Volatile Compounds. Vacuum steam distillation was performed in a low-pressure distillation apparatus modified from the one designed by Forss and Holloway (*21*) as previously described by Etiévant and Bayonove (*22*). Rainbow trout was finely minced and homogenized for 1 min in a household blender (Moulinex, France). After mincing, 250 g of trout was cooked using a glass flask in a water bath (Memmert, Germany) at 80 °C for 20 min. Then 250 g of cooked trout, 600 mL of ultrapure water, and 50 g of NaCl were transferred to a 6 L round-bottom flask maintained at 30 °C. Distillation was continued for 4 h under a pressure of 5 mbar until no water was left in the flask. Most of the volatiles were collected in a 4 L round-bottom flask by means of condensers maintained at 0 °C. The more volatile compounds were accumulated in three traps immersed in liquid nitrogen at −196 °C. After distillation, the contents of the 4 L flask and traps were pooled and extracted with 3 × 40 mL of diethyl ether at 0 °C with magnetic stirring for 15 min. After dehydration by anhydrous sodium sulfate, the pooled organic extract was reduced to 5 mL in a Kuderna−Danish concentrator fitted with a Snyder column (Supelco, Saint Quentin, France) and then to 0.5 mL under a gentle stream of nitrogen. The whole process was repeated three times. The extracts were then stored at −20 °C in a glass vial equipped with a Teflon-lined cap before the analysis.

Sensory Analysis/Representativeness of the Extract. *Panel.* The panel was composed of 10 assessors (7 females and 3 males between 22 and 50 years of age) from LBAI ENITIAA, Laboratoire de Biochimie Industrielle et Alimentaire-Ecole Nationale d'Ingénieurs des Techniques des Industries Agricoles et Alimentaire. The assessors were previously trained in odor recognition and sensory evaluation techniques and had experience in GC-O. As for the training sessions on cooked trout descriptors, the first session took place in an ordinary room to generate descriptors for the cooked trout. The panel generated the descriptors of cooked trout. A list of nine consensus odor descriptors (fishy, fatty, green, earthy, musty, cooked potato, marine, and cooked) was established. The other sessions took place in a sensory room (*23*) in isolated booths under natural light color at room temperature.

Sample Preparation and Presentation. Two grams of cooked trout (80 °C at 20 min) was placed in a 15 mL brown coded flask as a reference for the panelists. For presenting the extract, we developed a simple and rapid technique for evaluating the representativeness of the trout extract using a 100 mL glass syringe (Hamilton, NV). The most commonly used solvents do not enable sensory analysis because of their toxicity and/or odors. The presence of solvents such as ether or dichloromethane makes the sensory evaluation of the extract impossible by masking odor. To solve this negative effect of the solvent, we used a method developed in our laboratory and described by Hallier et al. (*10*). However, for easier use, the Teflon bag that was previously used to collect the volatile compounds was replaced with a glass gas syringe having an airtight seal. This technique permits assessment of the global aroma quality of the extract for which the solvent odor was eliminated. The GC effluent was split 1:1 between the three-way valve and the FID system bound to computer. One of the three ways was related to a deactivated capillary column connected to the gas syringe and another to the atmosphere. During the elution of the solvent, after the peaks of the solvent were checked on the computer, the eluted gas was sent into the atmosphere, then the valve was turned and volatile compounds were collected in the syringe. Before the injection of the extracts, the syringe was completely empty; therefore, it was filled progressively by desorption. The content of each syringe was sniffed by pushing progressively its piston by two panelists.

Similarity Test. A similarity test was performed to evaluate the closeness between the odor of the extract and the cooked trout (reference sample). The panelists were instructed to sniff and memorize the aroma of the reference sample and for the extract, to sniff the syringe odor and determine the similarity of their odors. A 100 mm unstructured scale was used anchored with "very different from the reference" on the left and "identical to the reference" on the right. The position of the sample on the unstructured scale was read as the distance in millimeters from the left anchor. Results were analyzed with an analysis of variance with Statgraphics Plus software (Manugistic, Inc. Rockville, MD).

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

Descriptive Analysis of the Cooked Trout and Its Extract. A list of nine descriptors (fishy, fatty, green, earthy, musty, cooked potato, marine, and cooked) that describe the aroma of cooked trout was previously determined by the panelists and subsequently used to describe the extract. The extract and reference sample were presented to the panel, and assessors were asked to describe the odorous characteristics of each sample by evaluating the intensity of each given descriptor on an unstructured scale of 100 mm anchored at the left end with "no odor" and at the right end with "very strong odor". The intensity notes were given by averaging the distance in millimeters from the left anchor to the marks of the judges. Results were analyzed with an analysis of variance with Statgraphics Plus software.

GC-FID, GC-MS, and GC-O Analyses of Volatile Compounds. The gas chromatography (GC) system consisted of an Agilent 6890 chromatograph equipped with a flame ionization detector (FID) (Wilmington, DE), an Agilent 5973 network-mass selective detector (MSD) (Wilmington, DE), and a Gerstel ODP-2 (Baltimore, MD)

sniffing port supplied with humidified air at 40 °C using a deactivated fused silica capillary (30 cm × 0.3 mm). This system allowed us to simultaneously obtain a FID signal for the quantification, an MS signal for the identification, and the odor characteristics of each compound detected by sniffing port. GC effluent was split 1:1:1 among the FID, MSD, and sniffing modes. Volatile compounds were separated on two different capillary columns: (1) DB5-MS (30 m length × 0.32 mm i.d. × 0.5 μm thickness, J&W Scientific, Folsom, CA) and (2) DB-Wax (30 m length × 0.25 mm i.d. × 0.5 μm thickness, J&W Scientific Folsom). Each extract (3 μL) was injected in splitless mode into both capillary columns. Injector and FID detectors were set at 270 and 280 °C, respectively. The flow rate of carrier gas (helium) was 1.5 mL min^{-1}. The oven temperature of the DB5-MS column was first increased from 50 to 200 °C at a rate of 5 °C min^{-1} and then to 260 °C at 8 °C min^{-1} with a final hold at 260 °C for 5 min. For the DB-Wax column, the oven temperature was increased from 50 to 260 °C at 4 °C min^{-1} with a final hold at 260 °C for 5 min.

The same oven temperature programs were used for the mass-selective detector. The MS (electronic impact ionization) conditions were as follows: ionization energy of 70 eV, mass range *m*/*z* of 30−300 amu, scan rate of 2.0 scan s^{-1}, interface temperature of 250 °C, and source temperature of 180 °C.

The volatile compounds were identified by comparing their retention index and their mass spectra on the two columns (DB5 and DB-Wax) with those of a commercial spectra database (Wiley 6, NBS 75k) and the instrument's internal library created from the previous laboratory studies. Some of the identifications were confirmed by the injection of the chemical standards into the GC-MS system. Retention indices of the compounds were calculated by using an *n*-alkane series (*24*).

With regard to determining 2-methylisoborneol (2-MIB) and geosmin, the mass spectrometers were run in selected ion monitoring (SIM) mode because of their ultratrace level (*25*). Ions at *m*/*z* 95, 135, and 168 are monitored for 2-MIB, and those at *m*/*z* 112, 126, and 182 are monitored for geosmin. In addition, standard compounds of 2-MIB and geosmin were injected into the GC-MS to compare retention times of both compounds.

Frequency of Detection Method. A panel of 8 judges trained in odor detection and recognition and having experience in GC-O was selected from the 10 previous panelists. Sniffing of the chromatogram was divided into two parts of 20 min. Each panelist participated in the sniffing of both parts, but during two distinct sessions in order to remain alert. The panelists were asked to assign odor properties of each odorant detected. Detection of an odor at the sniffing port by fewer than three of the eight assessors was considered to be noise (*26*). The eight individual aromagrams were summed, yielding the final aromagram (detection frequency versus RI).

Time Intensity Method. The time intensity method (*16*) was used to measure the odor intensity of the compounds detected. The same panelists used before were trained to evaluate aroma intensity using a nine-point intensity scale (1 = very weak intensity, 3 = weak intensity, 5 = moderate intensity, 7 = strong intensity, and 9 = very strong intensity) (*15*). Sniffing conditions were the same as for frequency of detection, except that the panelists were also asked to assess intensity (according to the nine-point scale) for each odorous area. Times and intensities of areas detected by at least three panelists were averaged, and a consensus aromagram (averages versus RI) was created.

RESULTS AND DISCUSSION

Sensory Analysis. *Odor Sensory Profiles.* Eight odor descriptors were used by the panelists to describe the odor of cooked trout and its extract (fishy, fatty, green, earthy, musty, boiled potato, marine, cooked fish) **(Figure 1)**. Seven of these descriptors were also used by Sérot et al. (*27*) to describe the odor of brown trout (*Salmo trutta*). These authors also used alga, grass-hay, phenol, and salmon-like descriptors. Our panelists did not perceive these odors in rainbow trout samples. Extract was compared to cooked trout by 10 panelists.

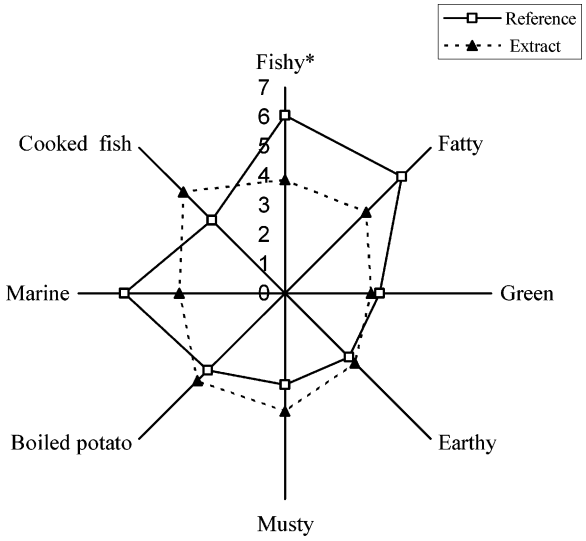

Figure 1. Odor sensory profiles of cooked rainbow trout and extract (units: centered average marks given by the judges on the 100 mm unstructured scales). The asterisk indicates significant difference for reference extract at a confidence level of 95%.

As can be seen in **Figure 1**, the sensory profile of extract is very similar to that of the reference sample. The LSD multiple-comparison tests, which compared any two means at the confidence level of 95%, showed that there were no statistically significant differences for green, earthy, musty, boiled potato, marine, and cooked fish descriptors between the reference and extract. Statistically significant difference was found for only the fishy descriptor.

Intensity and Similarity Evaluation. The intensity mark of the extract obtained by vacuum steam distillation was found to be acceptable (58.7 mm on a 100 mm unstructured scale). The extract presented good odor representativeness in comparison with the odor reference with a similarity score of 51.1 mm on a 100 mm unstructured scale. Similar results were found in apple extract (between 49.1 and 53.4 mm) by Mehinagic et al. (*28*), in orange juice extract (between 51.0 and 63.00 mm) by Rega et al. (*29*), and in oyster extract (mean score of 58.9 mm) by Pennarun et al. (*17*). These results show a close relationship between the odor properties of trout sample and those of the corresponding extracts. The use of an olfactometric method is therefore applicable in the determination of the odor-active compounds of trout.

GC-O Results. The isolation of volatile compounds of rainbow trout (*O. mykiss*) was performed using vacuum steam distillation. A representative extract of the original product was obtained by this extraction method. To characterize the volatile compounds, analyses were conducted on two GC columns of different polarities (DB-5 and DB-Wax). The results of the olfactometry analysis on DB-5 and DB-Wax columns showed that 38 and 36 odorous compounds were perceived, and 31 and 28 of these compounds were identified, respectively. Among these compounds found on the two different column types, 19 were common to both columns.

Aroma descriptors and linear retention index values on both columns for all compounds are presented in **Tables 1** and **2**. Aldehydes and alcohols were the most abundant aroma-active compounds in the volatile fraction of rainbow trout. Six compounds on the DB-5 column [heptanal, nonanal, 2-methylisoborneol, (*E*,*E*)-2,4-decadienal, geosmin, and an unknown (LRI = 1620) compound] and five compounds on the DB-Wax column [1-octen-3-ol, 2-methylisoborneol, 8-heptadecene, (*E*,*E*)-

Table 1. Aroma-Active Compounds of Cooked Rainbow Trout Tissue (DB-5 Column)

LRI	compound	odor description[a]	intensity[b]	detection freq[c]	methods of identification[d]
894	3-heptanone	roasty	4	6	LRI, MS, Std
902	heptanal	green	6	8	LRI, MS, Std
910	(*Z*)-4-heptenal	boiled potato, cooked	6	5	LRI, MS, Std
926	diethyl disulfide	sulfury, roasty	8	7	LRI, MS tent.
935	α-pinene	green, smoky	5	7	LRI, MS tent.
966	benzaldehyde	hazelnut, roasty	7	5	LRI, MS, Std
976	(*Z*,*Z*)-1,5-octadien-3-ol	cooked, mushroom	6	6	LRI, MS tent.
982	1-octen-3-ol	mushroom	7	7	LRI, MS, Std
992	(*E*,*E*)-2,4-heptadienal	boiled potato, grassy	5	7	LRI, MS, Std
1001	octanal	green, fruity	6	7	LRI, MS, Std
1049	2-ethyl-1-hexanol	roasty, earthy	5	6	LRI, MS, Std
1063	unknown	roasty, amine	3	4	
1073	unknown	no common descriptor	4	6	
1090	(*E*)-3-hepten-2-ol	green, cucumber	5	7	LRI, MS tent.
1105	nonanal	green, floral	6	8	LRI, MS, Std
1126	unknown	earthy, roasty, leather	6	5	
1146	2-methyl decahydronaphthalene	pungent, animal	4	6	LRI, MS tent.
1160	unknown	sulfury, garlic	4	4	
1172	benzyl acetate	violet, green	5	6	LRI, MS tent.
1189	2-(2-butoxyethoxy)ethanol	floral, minty	5	5	LRI, MS tent.
1197	2-methylisoborneol	musty, earthy	7	8	LRI, MS, Std
1206	decanal	plastic, green, floral	4	4	LRI, MS, Std
1228	1,2-dimethyldecahydronaphthalene	cucumber, garlic	6	5	LRI, MS tent.
1307	undecanal	minthy, fruity	5	7	LRI, MS, Std
1318	2-methylnaphthalene	plastic, earthy	5	5	LRI, MS, Std
1322	(*E*,*E*)-2,4-decadienal	cucumber, green, plastic	5	8	LRI, MS, Std
1340	2,6,10,14-tetramethylpentadecane	green, cooked	6	5	LRI, MS, Std
1367	2-(dodecyloxy)ethanol	fresh, aromatic plant	4	6	LRI, MS tent.
1416	2,6-dimethylnaphthalene	sweat, plastic	4	5	LRI, MS tent.
1430	2,7-dimethylnaphthalene	green, roasty, caramel	5	6	LRI, MS tent.
1427	*trans*-caryophyllene	rotten, moss	4	7	LRI, MS tent.
1439	geosmin	earthy	6	8	LRI, MS, Std
1492	unknown	paper, rubber	3	6	
1620	unknown	plastic, roasty	4	8	
1678	8-heptadecene	earthy, moss	3	6	LRI, MS tent.
1865	unknown	green, flowery, fresh	4	6	
1971	hexadecanoic acid	fresh, fruity	3	5	LRI, MS tent.
2013	hexadecanoic acid ethyl ester	green, fruity, fatty	3	7	LRI, MS tent.

[a] Odor description as perceived by panelists during olfactometry. [b] Average intensity. [c] Detection frequency (eight panelists). [d] LRI, linear retention index; MS tent., tentatively identified by MS; Std, chemical standard. When only MS or LRI is available for the identification of a compound, it must be considered as an attempt of identification. The odor given above corresponds to the odor detected by the judges for its retention time but not with certainty to the compound that we try to identify.

2,4-decadienal, and geosmin] can be considered as the most potent odorants of rainbow trout as they were detected by all eight assessors.

As indicated in **Tables 1** and **2**, the most-odor active compounds were the aldehydes. Nine and 11 aldehydes were identified on the DB-5 and DB-Wax columns, respectively. Heptanal, (*Z*)-4-heptanal, benzaldehyde, (*E*,*E*)-2,4-heptadienal, octanal, nonanal, decanal, and (*E*,*E*)-2,4-decadienal were common to both columns. On the basis of detection frequency results, heptanal (green odor), nonanal (green, floral odor), and (*E*,*E*)-2,4-decadienal (cucumber, green odor) were detected by all eight assessors when the DB-5 column was used; only (*E*,*E*)-2,4-decadienal (cucumber, green odor) was detected when the DB-Wax column was used. Therefore, these aldehydes might be of great importance for rainbow trout aroma. The aldehydes are common compounds for many fish and other sea products such as oyster (*17*), turbot (*26*), scallop (*30*), sardine (*31*), and salmon and cod (*32*). Within the aldehydes, (*E*)-2-nonenal could be considered as the off-flavor compound, the sniffing assessors assigning this compound earthy and wet earth odors. This compound was separated only on the DB-Wax column and detected by six assessors. The intensity value of this compound was 6. In the literature, Sérot et al. (*16*) and Le Guen et al. (*15*) indicated that (*E*)-2-nonenal was characterized by earthy odor for turbot and mussel, respectively. Similarly, Hartvigsen et al. (*33*) reported that (*E*)-2-nonenal is an off-flavor compound in fish oil enriched mayonnaise.

A total of six alcohol compounds were identified on the basis of GC-O carried out on both columns, and two of them (1-octen-3-ol and 2-ethyl-1-hexanol) were common to both separation conditions. Generally, these compounds are formed by a lipoxygenase-initiated peroxidation of the n-3 and n-6 polyunsaturated fatty acids, which are present in fish tissue (*34*). 1-Octen-3-ol (mushroom odor) could be an important contributor in this fraction on the basis of its very strong intensity value (*7*). In addition to its intensity value, this alcohol was perceived by seven and eight assessors when the DB-5 and DB-Wax columns, respectively, were used. Among the alcohols, 2-ethyl-1-hexanol was detected by only six sniffing assessors as an off-flavor compound and described as having a roasty and earthy odor. This alcohol was previously identified in raw oyster (*17*), cooked catfish (*25*), sardine (*31*), and fresh and smoked salmon (*35*). Interestingly, 2-ethyl-1-hexanol was also identified in fried bacon and fried pork loin, providing a wet and earthy odor (*36*).

Respectively, four and three terpene compounds were detected in trout extract analyzed on DB-5 and DB-Wax columns including α-pinene, 2-methylisoborneol, *trans*-caryophyllene, and geosmin. α-Pinene was separated on only the DB-5 column. Among the terpene compounds, 2-methylisoborneol and geosmin are important off-flavor compounds in trout tissue, providing

Table 2. Aroma-Active Compounds of Cooked Rainbow Trout Tissue (DB-Wax Column)

RI	compound	odor description[a]	intensity[b]	detection freq[c]	methods of identification[d]
1091	hexanal	green, floral	3	5	LRI, MS, Std
1144	1-penten-3-ol	green, floral	3	5	LRI, MS, Std
1182	heptanal	green	3	7	LRI, MS, Std
1217	diethyl disulfide	sulfury, roasty	4	6	LRI, MS tent.
1247	(*Z*)-4-heptenal	boiled potato, cooked	5	5	LRI, MS, Std
1291	octanal	green, fruity	3	6	LRI, MS, Std
1305	(*Z*)-2-pentenol	mushroom, cooked	5	7	LRI, MS, Std
1339	1-hexanol	green	6	4	LRI, MS, Std
1353	unknown	potato, roasty	4	5	
1389	nonanal	green, floral	7	7	LRI, MS, Std
1430	1-octen-3-ol	mushroom	7	8	LRI, MS, Std
1438	unknown	no common descriptor	3	5	
1461	(*E*,*E*)-2,4-heptadienal	boiled potato, grassy	6	6	LRI, MS, Std
1470	2-ethyl-1-hexanol	roasty, earthy	5	6	LRI, MS, Std
1515	decanal	plastic, green, floral	5	7	LRI, MS, Std
1525	benzaldehyde	hazelnut, roasty	6	5	LRI, MS, Std
1532	(*E*)-2-nonenal	earthy, wet earth	5	6	LRI, MS, Std
1540	1-octanol	green, roasty	4	5	LRI, MS, Std
1552	unknown	green, minthy	4	4	
1587	(*E*,*Z*)-2,6-nonadienal	cucumber, green	6	5	LRI, MS, Std
1602	2-methylisoborneol	musty, earthy	6	8	LRI, MS, Std
1622	*trans*-caryophyllene	rotten fruit, moss	4	7	LRI, MS tent.
1664	2,6,10,14-tetramethylpentadecane	green, cooked	3	5	LRI, MS, Std
1670	unknown	sulfury, roasty	5	7	
1685	tetrahydro-2-methylnaphthalene	green, moss, floral	3	7	LRI, MS tent.
1718	8-heptadecene	earthy, moss	3	8	LRI, MS tent.
1751	1-heptadecene	flowery, anise	5	4	LRI, MS tent.
1763	(*E*,*E*)-2,4-decadienal	cucumber, green	5	8	LRI, MS, Std
1821	geosmin	earthy	7	8	LRI, MS, Std
1877	2-methylnaphthalene	plastic,earthy	8	6	LRI, MS, Std
1972	unknown	musty, sweat	4	4	
2003	unknown	animal, marine	3	6	
2012	2,6-dimethylnaphthalene	sweat, plastic	3	5	LRI, MS tent.
2131	unknown	musty, rotten	5	5	
2214	unknown	plastic, roasty	4	7	
2223	hexadecanoic acid methyl ester	green, fruity, fatty	3	4	LRI, MS tent.
2131	unknown	musty, rotten	5	5	

[a] Odor description as perceived by panelists during olfactometry. [b] Average intensity. [c] Detection frequency (eight panelists). [d] LRI, linear retention index; MS tent., tentatively identified by MS; Std, chemical standard. When only MS or LRI is available for the identification of a compound, it must be considered as an attempt of identification. The odor given above corresponds to the odor detected by the judges for its retention time but not with certainty to the compound that we try to identify.

musty and earthy odors, respectively. These odorants were detected by all eight assessors with an intensity value of >6. On the basis of detection frequency and intensity value, both compounds may contribute actively to the aroma of rainbow trout, giving strong earthy and musty odors. 2-Methylisoborneol and geosmin are known to be produced by some species of actinomycetes and cyanobacteria (*10*). Sensory thresholds of these compounds were estimated to be 0.6 μg kg^{-1} for 2-methylisoborneol (*37*) and 0.9 μg kg^{-1} for geosmin (*38*) in rainbow trout. As the concentrations of these odorous volatiles were too low for identification, we used SIM mode mass spectra to detect them. Similarly, these volatiles have been reported to be important off-flavor compounds by many researchers in catfish tissue (*10*, *25*), *Nile tilapia* (*11*), apple juice (*39*), and farmhouse Cheddar cheese (*40*). Other aroma-active terpenes, α-pinene (green, smoky odor) and *trans*-caryophyllene (rotten, moss odor), might originate from plant materials that the trout consumed.

With regard to naphthalene compounds, 2-methylnaphthalene and 2,6-dimethylnaphthalene were separated on both columns. These compounds could be produced from the degradation of plant materials by microorganisms and accumulated in the fish tissues as environmental contaminants (*30*). Within naphthalene compounds, 2-methylnaphthalene was determined as an off-flavor compound by the assessors, providing a plastic and earthy note. The intensity value of this compound was 5 when analysis was performed on the DB-5 column and 8 when analysis was performed on the DB-Wax column. As previously stated, this compound was detected with an earthy and grilled odor in cooked mussel (*15*) and a moss and plastic odor in smoked salmon (*35*).

2,6,10,14-Tetramethylpentadecane, 8-heptadecene, and 1-heptadecene were detected as alkene and alkane compounds. 1-Heptadecene was separated on only the DB-Wax column. Some of these compounds were previously determined in Baltic herring (*41*) and black bream and rainbow trout (*42*). 8-Heptadecene was identified as the compound that provides undesirable contribution to the moss and earthy odors in rainbow trout extract. It does not have a strong impact on the overall odor because it is perceived by a low-intensity value (*3*). This odorant has been reported earlier as a leather note in smoked salmon and as plastic and moss notes in unsmoked salmon by Varlet et al. (*35*).

Esters, benzyl acetate, and hexadecanoic acid ester have been detected in cooked trout extracts. Benzyl acetate, detected only when the DB-5 column was used, is especially important for a pleasant violet and green note. These compounds might be products of esterification of the alcohols with carboxylic acids that are formed by microbial and degradation of lipids (*43*).

Seven and eight unknown compounds were separated on the DB-5 and DB-Wax columns, respectively. Among the unknown compounds, LRI = 1620 in the DB-5 column was determined

by all of the assessors as a plastic and roasty note. Two unknown compounds on the DB-Wax column were perceived by seven assessors and were described as sulfury-roasty (LRI = 1670) and plastic-roasty (LRI = 2214). Unknown LRI = 1126 on the DB-5 column, with an earthy, leather, roasty odor, and unknown LRI = 1972 and LRI = 2131 on the DB-Wax column, with musty-sweat and musty-rotten odors, respectively, may contribute to the global aroma of cooked trout as providing unpleasant notes.

In conclusion, the sensory evaluation results correlated well with the instrumental analysis. Results showed that the complex combination of several aroma-active compounds contributes to the aroma profile of the cooked rainbow trout. Totals of 38 and 36 odorous compounds were perceived on the DB-5 and DB-Wax columns, respectively. Among these, (*E*)-2-nonenal, 2-ethyl-1-hexanol, 2-methylisoborneol, geosmin, 2-methylnaphthalene, and 8-heptadecene were described as off-flavor compounds by the sniffing assessors. The most powerful off-flavor compounds identified in the extract were 2-methylisoborneol and geosmin, which were described as strong musty and earthy odors, respectively.

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