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ARTICLE

Characterization of the Key Aroma Compounds in Cooked Grey Mullet (*Mugil cephalus*) by Application of Aroma Extract Dilution Analysis

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Aroma and aroma-active compounds of wild grey mullet (*Mugil cephalus*) were analyzed by gas chromatography−mass spectrometry−olfactometry (GC-MS-O). According to sensory analysis, the aromatic extract obtained by simultaneous distillation and extraction (SDE) was representative of grey mullet odor. A total of 50 aroma compounds were identified and quantified in grey mullet. Aldehydes were qualitatively and quantitatively the most dominant volatiles in grey mullet. Aroma extract dilution analysis (AEDA) was used for the determination of aroma-active compounds of fish sample. A total of 29 aroma-active compounds were detected in aromatic extract of grey mullet, of which 24 were identified. On the basis of the flavor dilution (FD) factor, the most powerful aroma active compounds identified in the extract were (*Z*)-4-heptenal and nonanal, which were described as the strong cooked fish and green-fruity odor, respectively.

KEYWORDS: Representativeness; olfactometry; aroma-active compounds; antioxidant activity; grey mullet

INTRODUCTION

Grey mullet (*Mugil cephalus* Linneaus, 1758) is a cosmopolitan in the coastal waters of the most tropical and subtropical zones. It is a diurnal feeder, consuming mainly zooplankton, dead plant matter, and detritus. Egypt is the largest producer of grey mullet in the world, and the other principal fisheries for mullet are the Republic of Korea, Italy, Tawian, and Israel (*1*). The total capture production in Turkey was 3345 tons in 2008, the Black Sea the leading coast of capture, with 1518 tons, followed by the Aegean Sea with 838 tons and the Mediterranean Sea with 606 tons (*2*).

The aroma is one of the important factors determining the quality of fish species. Fish has a characteristic and delicate aroma that is influenced by the species and the conditions used for its postharvest handling and storage. Important aroma compounds, characteristic of fresh fish species, are lipid-derived volatile compounds generated mainly by oxidative enzymatic reactions and autoxidation of lipids. The action of enzymes (lipoxygenases) on polyunsaturated fatty acids (PUFAs) produces carbonyls, aldehydes, and ketones, which are responsible for some of the fresh-like odors of fish (*3*, *4*).

Although several hundred chemically different aroma compounds have been identified in freshly harvested and processed fish, only a limited number are essential to overall fish aroma. The gas chromatography−olfactometry (GC-O) technique made it possible to divide identified volatile substances into odor-active and non-odor-active compounds regarding their existing concentrations in the studied sample (*5*). Many researchers have studied the odor-active compounds of fish species and other seafoods: Milo and Grosch (*6*) investigated the odor defects in boiled cod (*Gadus morhua*) and boiled trout (*Salmo fario*); Varlet et al. (*7*) compared the odor-active volatile compounds of fresh and smoked salmon; Senger-Emonnot et al. (*8*) determined the odor-active aroma compounds of sea fig (*Microcosmus sulcatus*); and Selli et al. (*9*) characterized the most odor-active compounds in cooked rainbow trout (*Oncorhynchus mykiss*) eliciting an off-odor. The achievement of aroma characterization of foodstuffs by olfactometric analysis depends largely on the extraction technique employed to isolate the aroma compounds from the food matrix. Therefore, the extraction method must be selected with the aim of producing extracts with odor as close as possible to that of the original product (*10*).

No work has yet been published in the literature to determine the aroma and aroma-active compounds of wild grey mullet (*M. cephalus*). Therefore, the aim of the present study was, first, to assess the representativeness of cooked grey mullet aromatic extract obtained by simultaneous distillation and extraction (SDE) using similarity and intensity tests and, second, to determine the aroma-active compounds by application of aroma extract dilution analysis (AEDA), a method that elucidates key odorants in complex volatile extracts (*11*, *12*) of this fish species. Furthermore, there is little information available about the antioxidant properties of fish samples in the literature. In the present study, the antioxidant activity of grey mullet sample was also studied.

MATERIALS AND METHODS

Reagents. The water used in the study was purified by a Millipore-Q system (Millipore Corp., Saint-Quentin, France). Diethyl ether, dichloromethane, sodium chloride, sodium sulfate, 2,3-pentanedione, hexanal, (*E*)-2-pentanal, (*E*)-2-hexanal, (*E*)-2-heptanal, nonanal, and (*E*)-2-octenal were obtained from Fluka (Buchs, Switzerland). 1-Penten-3-ol, β-myrcene,

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dl-limonene, (*Z*)-4-heptenal, 3-hydroxy-2-butanone, 2,5-dimethylpyrazine, 3-(methylthio)propanal, 2-furaldehyde, 1-octen-3-ol, 2-ethyl-3,5-dimethylpyrazine, (*E*,*E*)-2,4-heptadienal, (*E*)-2-nonenal, benzaldehyde, (*E*,*E*)-2,4-octadienal, γ-butyrolactone, (*E*,*E*)-2,4-nonadienal, (*E*)-undecenal, (*E*,*E*)-2,4-decadienal, hexadecanal, and 1,1-diphenyl-2-picrylhyrazyl (DPPH) were purchased from Sigma-Aldrich (Steinheim, Germany); methanol, octanal, 1-octanol, 4-nonanol, heptanal, decanal, and hexanoic acid came from Merck (Darmstadt, Germany).

Grey Mullet. Samples of wild grey mullet (*M. cephalus,* L., 1758) averaging 200−250 g were bought from Karatas province, on the eastern Mediterranean sea of Turkey. A total of 5 kg of fish was caught in March 2009 and manually slaughtered the same day by immersion in ice-cold water and transported under ice in insulated polystyrene boxes to the Biotechnology Laboratory at the Department of Food Engineering, University of Cukurova (Adana province), Turkey. For the aroma analysis, samples were manually eviscerated, filleted, and mechanically peeled the same day.

Extraction of the Volatile Compounds. The volatile compounds of cooked grey mullet were extracted by SDE in a Likens−Nickerson apparatus. This method has already shown its reliability for the extraction of volatile components of different fish species, such as mackerel, tuna, swordfish, eel, sablefish, and flounder (*4*) and fresh and smoked salmon (*7*). Grey mullet was finely minced and homogenized for 1 min in a household blender (Arcelik, Turkey). Then 30 g of grey mullet and 60 mL of 30% NaCl aqueous solution containing 40 μg of 4-nonanol as internal standard were placed in a 500 mL round-bottom flask attached to the appropriate arm of the SDE apparatus. A 50 mL round-bottom flask containing 25 mL of dichloromethane was linked to the other arm of the SDE apparatus. The steams were by the circulation of polyethylene glycol at −5 °C. Contents in the sample and solvent flasks were heated to boiling. The temperature of the dichloromethane flask was maintained by a water bath at 50 °C. The distillation−extraction was continued for 3 h. The volume of the extract was reduced to 5 mL by evaporating the solvent with a Vigreux apparatus and then to 200 μL under a gentle stream of nitrogen. Sample was extracted in triplicate, and the concentration of volatiles, as 4-nonanol equivalents, was obtained as a mean of three repetitions. 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 seven assessors (two females and five males between 22 and 43 years old) from the Biotechnology Laboratory at the Department of Food Engineering, University of Cukurova. The assessors were previously trained in odor recognition and sensory evaluation techniques and had experience in GC-O (*13*).

Sample Preparation and Presentation. Different methods can be used to evaluate the representativeness of the odor of aromatic extracts depending on the type of investigation. We used a cardboard smelling strip (reference 7140 BPSI, Granger-Veyron, Lyas, France) to check the representativeness of the extract obtained by SDE. The smelling strip has already given good results for the representativeness test of cooked mussel (*13*) and wild gilthead sea bream (*Sparus aurata*) extract (*14*). Two solvents (dichloromethane and diethyl ether) were evaluated for the representativeness using SDE. One and a half grams of small cubes of cooked grey mullet flesh was placed in a 15 mL brown-coded flask as a reference for representativeness tests. An aliquot of the fish aromatic extracts obtained from two solvents was adsorbed onto a cardboard smelling strip. After 1 min (the time necessary for solvent evaporation), the extremities of the strips were cut off and then placed in dark coded flasks (15 mL) and presented to the panel after 15 min. Dichloromethane and diethyl ether are very volatile solvents. After evaporation, no panelists detected the odor of the solvents. All of the samples were assessed at room temperature (20 °C) in neutral conditions.

Similarity Test. A similarity test was performed to evaluate the closeness between the odor of extract and the cooked grey mullet (reference sample). The panelists were instructed to sniff and memorize the aroma of the reference sample and, for the extract, to sniff the smelling strip 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.

GC-FID, GC-MS, and GC-O Analyses of Volatile Compounds. The 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 using deactivated capillary column (30 cm × 0.3 mm) heated at 240 °C and supplied with humidified air at 40 °C. 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 mode. Aroma compounds separated on a DB-Wax (30 m length × 0.25 mm i.d. × 0.5 μm thickness, J&W Scientific, Folsom, CA) column. A total of 3 μL of extract was injected in pulsed splitless (40 psi; 0.5 min) mode. This mode was chosen to minimize artifact formation by thermal degradation of analytes in the injection port. 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 DB-Wax 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.

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 aroma compounds were identified by comparing their retention indices and mass spectra on the DB-Wax column with those of a commercial spectra database (Wiley 6, NBS 75k) and of the instrument's internal library created from 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 (*15*).

AEDA. The original aroma extracts were analyzed by GC-MS-O using three experienced sniffers. For AEDA, the concentrated aromatic extract (200 μL) of grey mullet was stepwise diluted 1:1 using dichloromethane as the solvent to obtain dilutions of 1:1, 1:2, 1:4, 1:8, 1:16, and up to 1:2048 of the original extracts (*16*, *17*). Sniffing of dilutions was continued until no odorant could be detected by GC-MS-O. Each odorant was thus assigned a flavor dilution factor (FD factor) representing the last dilution in which the odorant was still detectable.

Antioxidant Activity Determination. *DPPH Radical Scavenging Method*. The antiradical capacity of the grey mullet fillet was estimated according to the procedure reported by Brand-Williams et al. (*18*), which was slightly modified by Sanchez-Moreno et al. (*19*). DPPH was dissolved in methanol at a final concentration of about 6×10^{-5} M. Five different concentrations were used for each assay. The decrease in absorbance of DPPH at 515 nm was measured at different time intervals by a Shimadzu UV-1700 spectrophotometer (Kyoto, Japan) until the reaction reached plateau (time at the steady state) at a temperature of 25 °C. The percentage of remaining DPPH at the steady state was plotted as a function of the concentration ratio of sample to DPPH to determine the effective concentration (EC_{50}). The time (minutes) needed to reach the steady state for EC_{50} (TEC_{50}) was used to calculate the antiradical efficiency defined as $AE = 1/(EC_{50} \times TEC_{50})$ (*19*). Three replicates were made for fish sample.

RESULTS AND DISCUSSION

Representativeness of the Extract. *Intensity and Similarity Evaluation*. The intensity scores of the aromatic extract obtained from dichloromethane and diethyl ether solvents on a smelling strip were found to be 70.8 and 61.4 mm on a 100 mm unstructured scale, respectively. The intensity scores of both extracts were quite high. The extract obtained from dichloromethane presented a higher intensity score than the diethyl ether extract. The differences of intensity score for two tests were found as statistically significant ($p < 0.05$). With regard to similarity evaluation, the

Table 1. Volatile Compounds of Grey Mullet (*Mugil cephalus*)

no.	LRI[a]	aroma compound	concentration[b] (mean ± SD)	identification[c]
1	1053	2,3-pentanedione	689 ± 2.88	LRI, MS, Std
2	1095	dimethyl disulfide	49 ± 1.57	LRI, MS tent.
3	1097	hexanal	747 ± 12.23	LRI, MS, Std
4	1110	(*E*)-2-pentenal	496 ± 1.66	LRI, MS, Std
5	1170	1-penten-3-ol	819 ± 11.39	LRI, MS, Std
6	1184	1-ethyl-1*H*-pyrrole	115 ± 4.12	LRI, MS tent.
7	1188	β-myrcene	65 ± 0.29	LRI, MS, Std
8	1199	heptanal	1295 ± 6.05	LRI, MS, Std
9	1234	*dl*-limonene	914 ± 11.99	LRI, MS, Std
10	1238	(*E*)-2-hexenal	267 ± 5.84	LRI, MS, Std
11	1244	4,4-dimethyl-2-pentene	246 ± 5.46	LRI, MS tent.
12	1251	(*Z*)-4-heptenal	101 ± 1.91	LRI, MS, Std
13	1277	3-hydroxy-2-butanone	309 ± 8.96	LRI, MS, Std
14	1287	octanal	324 ± 6.96	LRI, MS, Std
15	1294	2-octanol	34 ± 0.77	LRI, MS tent.
16	1310	methyl lactate	220 ± 6.89	LRI, MS tent.
17	1314	2,5-dimethylpyrazine	139 ± 4.92	LRI, MS, Std
18	1336	(*E*)-2-heptenal	90 ± 0.23	LRI, MS, Std
19	1336	2-acetyl-1-pyrroline	102 ± 2.79	LRI, MS tent.
20	1390	2,3,5-trimethylpyrazine	71 ± 0.71	LRI, MS tent.
21	1395	nonanal	320 ± 6.50	LRI, MS, Std
22	1408	(*E*)-2-octenal	227 ± 10.4	LRI, MS, Std
23	1448	3-(methylthio)propanal	150 ± 4.92	LRI, MS, Std
24	1452	2-furaldehyde	143 ± 5.04	LRI, MS, Std
25	1458	1-octen-3-ol	160 ± 1.44	LRI, MS, Std
26	1467	2-ethyl-3,5-dimethylpyrazine	94 ± 6.16	LRI, MS, Std
27	1483	(*E*,*E*)-2,4-heptadienal	463 ± 13.09	LRI, MS, Std
28	1497	decanal	150 ± 7.88	LRI, MS, Std
29	1532	(*E*)-2-nonenal	226 ± 5.26	LRI, MS, Std
30	1544	benzaldehyde	99 ± 3.61	LRI, MS, Std
31	1553	octanol	96 ± 0.30	LRI, MS, Std
32	1605	(*E*,*E*)-2,4-octadienal	229 ± 12.17	LRI, MS, Std
33	1612	γ-butyrolactone	580 ± 14.60	LRI, MS, Std
34	1666	2-cyclohexenol	287 ± 9.31	LRI, MS tent.
35	1681	(*E*,*E*)-2,4-nonadienal	35 ± 0.35	LRI, MS, Std
36	1740	2(5*H*)-furanone	231 ± 2.77	LRI, MS tent.
37	1754	(*E*)-2-undecenal	104 ± 2.31	LRI, MS, Std
38	1766	(*E*,*E*)-2,4-decadienal	87 ± 1.65	LRI, MS, Std
39	1823	isopropyl myristate	191 ± 9.30	LRI, MS tent.
40	1860	1-methylnaphthalene	28 ± 0.26	LRI, MS tent.
41	1868	hexanoic acid	16 ± 0.52	LRI, MS, Std
42	1914	2-methylnaphthalene	42 ± 2.90	LRI, MS tent.
43	2006	pantolactone	56 ± 0.45	LRI, MS tent.
44	2110	2-pentadecanone	169 ± 5.24	LRI, MS tent.
45	2130	2-phenoxyethanol	27 ± 0.54	LRI, MS tent.
46	2143	hexadecanal	44 ± 0.37	LRI, MS, Std
47	2152	1-hexadecanol	36 ± 0.27	LRI, MS tent.
48	2180	(*Z*,*Z*)-1,4-octadiene	311 ± 17.68	LRI, MS tent.
49	2198	2,5-dihydrothiophene	92 ± 2.22	LRI, MS tent.
50	2244	dihydro-4-hydroxy-2(3*H*)-furanone	75 ± 1.75	LRI, MS tent.
		total aroma compounds	11860	

[a] Linear retention index calculated on DB-WAX capillary column. [b] Concentration: results are the means of three repetitions as μg/kg. [c] Methods of identification: 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.

similarity score of dichloromethane extract (67.2 mm on a 100 mm unstructured scale) was found to be better than diethyl ether extract (40.3 mm on a 100 mm unstructured scale). Similarity scores of two solvents were found to be significantly different ($p < 0.05$). The similarity score of the dichloromethane extract was acceptable and quite high and also scored relatively more closely to the reference sample. On the basis of these results, we selected dichloromethane as extraction solvent of aroma compounds. As previously stated, it is of great importance to assess the representativeness of the aromatic extracts in a matrix with characteristics similar to those of the original product (*13*, *20*). From other studies we found similarity scores for rainbow trout (*O. mykiss*) of 51.1 mm (*9*), for wild gilthead sea bream (*S. aurata*) of 53.5 mm (*14*), for edible red algae (*Palmaria palmata*) between 25.9 and 42.3 mm by Le Pape et al. (*21*), and for cooked silurus (*Silurus glanis*) flesh between 22 and 55 mm by Hallier et al. (*22*). The results of our sensory analysis suggested that the extract from grey mullet was considered to be representative for the olfactometric analysis.

Volatile Compositions of Grey Mullet. The volatile compounds identified in grey mullet and linear retention index (LRI) values on DB-Wax column for these compounds are presented in **Table 1**.

Mean values (μg/kg) of the GC analyses of triplicate extractions and standard deviations are reported. A total of 50 compounds were identified and quantified in grey mullet. The fish had 11860 μg/kg volatile compounds, which included aldehydes (20), alcohols (7), nitrogenous compounds (5), lactones (4), ketones (3), terpenes (2), esters (2), alkenes (2), naphthalenes (2), sulfur compounds (2), and acid (1). Most of the volatile compounds identified in our study have already been identified in several freshwater and saltwater fish species. Aldehydes were the most dominant volatiles in grey mullet, as they accounted for the largest proportion of the total volatile compounds. Alcohols were the second largest volatile group in the grey mullet.

GC-O Results. The results of olfactometric analysis are summarized in **Table 2**. Application of the AEDA on the grey mullet extract revealed 29 aroma-active compounds in the FD factor range of 2−2048 (**Table 2**), including aldehydes (16), nitrogenous compounds (4), ketones (2), alcohol (1), lactone (1), and unknown compounds (5). The aroma-active compounds of grey mullet were predominantly aldehydes. Fish species are usually characterized by a sweet and delicate aroma. This aroma is given by the interaction of volatile aldehydes and alcohol compounds derived from the oxidative deterioration of n-3 and n-6 PUFA (*23*).

Aldehydes are the overwhelmingly largest aroma-active components of the grey mullet extract. Sixteen aldehydes were identified in this extract as aroma-active compounds (**Table 2**). These aldehydes are widespread, as they have already been found in many other fish species and sea products. Among the aldehydes, (*Z*)-4-heptenal (FD = 2048) and nonanal (FD = 2048) were the most powerful aroma-active compounds to contribute to the aroma profile of the grey mullet. The amounts of (*Z*)-4-heptenal and nonanal were 101 and 320 μg/kg, respectively. Both compounds were present at concentrations higher than their corresponding odor threshold values [0.8 μg/kg for (*Z*)-4-heptenal (*24*); 1 μg/kg for nonanal (*25*)]. The first has a cooked fish and the latter a green-fruity aroma. As previously stated, these compounds were detected in farmed and wild barramundi (*Lates calcarifer*) by Frank et al. (*26*), and unsmoked salmon (*Salmo salar*) by Varlet et al. (*7*) as aroma-active compounds. The odor threshold values of aldehydes are generally low; thus, they have important potential effects on the total flavor of fish species (*27*). In grey mullet extract, octanal (green-fatty) and (*E*)-2-octenal (oily fishy) constituted the second important group of volatile aldehydes. The FD factor of these aldehydes was also quite high (FD = 1024). The next most important aroma-active aldehyde in terms of FD factor was (*E*)-2-nonenal, with a fishy and earthy note. The FD factor value of this compound was 512. (*E*)-2-Nonenal is a lipid peroxidation product derived from oxidized ω-6 PUFAs such as linoleic acid in fish species. This aldehyde has been described in the literature as earthy and cucumber in cooked fillets of European catfish (*S. glanis*) by Hallier et al. (*28*); earthy and wet earth in rainbow trout (*O. mykiss*) by Selli et al. (*9*); and fatty and green in stewed beef/vegetable gravy by Christlbauer and Schieberle (*29*). Interestingly, Maarse and Grosch (*30*) reported that the cardboard off-flavor in butter oil is caused by an increase of (*E*)- and (*Z*)-2-nonenal. Among the aldehydes, hexadecanal (fatty, chemical) and (*E*)-2-pentenal (green plant) had the lowest FD factors detected by GC-O. FD factors of these compounds were 2 and 8, respectively. Similarly, (*E*)-2-pentenal has been previously identified in sardine (*Sardinops melanostica*) as a green note (*31*).

1-Ethyl-1*H*-pyrrole, 2,5-dimethylpyrazine, 2-acetyl-1-pyrroline, and 2-ethyl-3,5-dimethylpyrazine were identified as aroma-active nitrogenous compounds in the studied sample. Similar compounds have previously been reported in Baltic herring (*Clupea harengus membras*) by Aro et al. (*33*) and salted−dried white herring (*Ilisha elongate*) by Chung et al. (*34*). On the basis of the FD factor value, the most powerful nitrogenous compound in grey mullet was 2-acetyl-1-pyrroline, described as having a popcorn and oily odor. The FD factor of this compound was 1024. The lowest FD factor (FD = 8) for nitrogenous compounds was determined for the plastic, burnt--smelling 2,5-dimethylpyrazine. 2-Acetyl-1-pyrroline has been cited as a major aroma-active compound in a boiled carp fillet (*Cyprinus carpio* L.) by Schlüter et al. (*35*) and in skipjack tuna sauce by Cha and Chawallader (*36*), providing a popcorn-like odor. This compound has also been identified in bread crust by Schieberle and Grosch (*37*) and as an aroma-active compound in popcorn by Schieberle (*38*, *39*), who assumed that ornithine and proline were key precursors of 2-acetyl-1-pyrroline.

2,3-Pentanedione (FD = 4) and 2-pentadecanone (FD = 32) were detected as aroma-active ketone compounds in grey mullet extract. The first has a caramel-fruity and the latter a burnt-plastic odor. As previously stated (*28*), 2,3-pentanedione was detected with a buttery and caramel odor in cooked fillet of European catfish (*S. glanis*) and a fruity odor in salted−dried white herring (*34*). Chung et al. (*34*) reported that thermal degradation, oxidation of fat, degradation of amino acids, and Maillard reaction were possible mechanisms of formation of ketone compounds.

The other two aroma-active compound identified in grey mullet were 1-penten-3-ol (green, fishy odor) and pantolactone (burnt, chemical odor). FD factors of these compounds were 128 and 8, respectively. 1-Penten-3-ol was also found in refrigerated sardine (*S. melanostica*) and identified as the compound that provides major contribution to the paint and chemical-like odor. This compound was generated from PUFA by the influence of an enzyme such as lipoxygenase or hydroperoxidase (*31*).

Table 2. Aroma-Active Compounds of Grey Mullet by Application of AEDA

no.	LRI[a]	aroma compound	odor description[b]	FD factor[c]
1	1053	2,3-pentanedione	caramel, fruity	4
2	1078	unknown	chemical, varnish	128
3	1110	(*E*)-2-pentenal	green plant	8
4	1170	1-penten-3-ol	green, fishy	128
5	1184	1-ethyl-1*H*-pyrrole	roasty, chemical	32
6	1199	heptanal	green, fatty	128
7	1238	(*E*)-2-hexenal	green, fruity	64
8	1251	(*Z*)-4-heptenal	cooked fish	2048
9	1270	unknown	chemical, plastic	8
10	1287	octanal	green, fatty	1024
11	1314	2,5-dimethylpyrazine	plastic, burnt	8
12	1336	2-acetyl-1-pyrroline	pop corn, oily	1024
13	1395	nonanal	green, fruity	2048
14	1408	(*E*)-2-octenal	oily, fishy	1024
15	1448	3-(methylthio)propanal	potato, chemical	256
16	1467	2-ethyl-3, 5-dimethylpyrazine	roasty	64
17	1483	(*E*,*E*)-2,4-heptadienal	fatty	256
18	1490	unknown	plastic	2
19	1497	decanal	green	256
20	1532	(*E*)-2-nonenal	fishy, earthy	512
21	1549	benzaldehyde	almond	128
22	1605	(*E*,*E*)-2,4-octadienal	fatty, green	128
23	1714	unknown	fatty	2
24	1754	(*E*)-2-undecenal	plastic, oily	256
25	1759	unknown	spicy	4
26	1766	(*E*,*E*)-2,4-decadienal	cooked vegetable, fatty	32
27	2006	pantolactone	burnt, chemical	8
28	2110	2-pentadecanone	burnt, plastic	32
29	2143	hexadecanal	fatty, chemical	2

[a] Linear retention index calculated on DB-WAX capillary column. [b] Odor description as perceived by panelists during olfactometry. [c] FD factor is the highest dilution of the extract at which an odorant is determined by aroma extract dilution analysis.

Five unknown compounds may contribute to the global aroma of grey mullet. Unknown 2 (LRI = 1078), with a chemical−varnish note, was detected in grey mullet with the highest FD factor value (FD = 128). Other unknown compounds are not likely to have an important impact on the aroma of studied fish sample due to their low FD factors.

Antioxidant Activity. In our study, the antioxidant activities of grey mullet fillets were evaluated using DPPH free radical scavenging assays. This method is recommended by many authors (*18*, *19*) as an easy and accurate assay for measuring the antioxidant activity of food samples. EC_{50} is inversely related to the antioxidant capacity of a compound, as it expresses the amount of antioxidant needed to decrease the radical concentration by 50%. The lower the EC_{50} value, the higher the antioxidant activity of a compound (*19*). The EC_{50} value of grey mullet was found to be quite high (85 mg/mL). In the literature, limited study has been done on fish and seafood antioxidant activity determination. The EC_{50} value of lamb meat obtained from Segureña ewe was found as 227.96 mg/mL by Monino et al. (*32*). TEC_{50} is the time need to reach the steady state to EC_{50} concentration. Time at steady state depends on the reactivity of antioxidants and the concentrations used. The antiradical efficiency (AE) is a new parameter for the measurement the free radical scavenging of samples, and it combines the EC_{50} and TEC_{50} (*19*). The AE value of grey mullet was very low (7×10^{-5}). As previously reported, AE values for rosemary, black currant seeds (*40*), and Moro orange juice (*41*) were 0.45×10^{-3}, 0.35×10^{-3}, and 50.5×10^{-3}, respectively. On the basis of our results, wild grey mullet has a very low antioxidant activity value.

In conclusion, this first study revealed key aroma components that are responsible for the overall aroma of the wild grey mullet (*M. cephalus*). A total of 29 aroma-active compounds in the FD factor range of 2−2048 were determined by application of the AEDA method. Aldehydes were found as the majority of aroma-active compounds. The most powerful aroma-active compounds detected in the extract were (*Z*)-4-heptenal and nonanal, which were described as strong cooked fish and green fruity-like, respectively. Additionally, this fish species has a very low antioxidant activity value based on DPPH free radical scavenging assays.

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