JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Comparison of Volatiles of Cultured and Wild Sea Bream (*Sparus aurata*) during Storage in Ice by Dynamic Headspace Analysis/Gas Chromatography–Mass Spectrometry

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Cultured and wild sea bream were compared for differences in their volatile components over a 23 day storage period in ice. A total of 60 compounds in cultured and 78 compounds in wild sea bream were tentatively identified (in addition to this, there were 23 unknowns in cultured and 29 unknowns in wild sea bream volatiles). These included aldehydes, ketones, alcohols, aromatics, terpenes, furans, sulfur-containing compounds, an acid, and miscellaneous compounds. Although selection of best fish is a subjective matter, more aldehydes, ketones, aromatics, and terpenes were found in wild sea bream as compared to that of its cultured counterpart. Both sea bream samples exhibited complex volatile profiles over the entire storage period. The combination of several classes of volatile compounds, dependent upon their concentrations and odor thresholds, is responsible for the distinctive and unique flavor of fresh cultured and wild sea bream. Relative concentrations of several compounds (trimethylamine, piperidine, methanethiol, dimethyl disulfide, dimethyl trisulfide, 1-penten-3-ol, 3-methyl-1-butanol, and acetic acid) increased continually throughout the storage period, and these may have the potential to be used as indicators of sea bream quality.

KEYWORDS: Cultured and wild sea bream; volatile compounds; DHA/GC-MS; quality indicators; storage period; ice

INTRODUCTION

Gilthead sea bream (*Sparus aurata*) is one of the main marine fish species aquacultured around the Mediterranean coast, and its production has increased ~9-fold during the past decade (9657 metric tons in 1992 and 81 965 metric tons in 2001). In contrast, the wild capture has remained nearly constant (8529 metric tons in 1992 and 9552 metric tons in 2001) (*1*). The popularity of sea bream is mainly due to its distinctive aroma, desirable taste, and high nutritional quality. However, increasing production has raised concerns over the quality, aroma, and taste of cultured sea bream, especially in comparison with its wild counterpart. In addition, there is a common belief among consumers that wild fish possess a more desirable aroma and taste than cultured species. Nonetheless, there are no research results currently available on the volatile compounds of cultured and wild sea bream following storage in ice. Therefore, it is of considerable interest to find out the existing differences in volatile compounds between the two types of fish.

Volatile compounds contributing to the characteristic aroma of fish can be measured to evaluate the freshness of fish (*2*). The study of fresh fish flavor has attracted much attention due to its importance to consumer acceptability of fresh products. Volatile aroma compounds are generated by enzymatic reaction, lipid autoxidation, microbial action, and environmentally and thermally derived reaction products (*3*). Fresh fish aromas are characterized as being mild, green, delicate, and having melon-like and plant-like notes that are easily recognized and highly valued by consumers (*4*−*7*). Volatile compounds contributing to these aromas include carbonyl-containing compounds and alcohols (for example, those with 5, 6, 8, 9, and 11 carbons), which are primarily derived from the polyunsaturated fatty acids of fish lipids via lipoxygenase activity (*4*, *5*, *7*). It is evident that volatile carbonyls and alcohols are important contributors to freshly harvested fish aroma.

The aromas of fresh fish are very delicate and perishable; hence, the study of their deterioration and off odor development is important. By contrast to fresh fish, spoiled fish aromas are described as being rancid, fishhouse-like, sulfurous, stale, putrid, and ammonia-like (*5*, *7*). Upon cooking, processing, and storage, the volatile compounds of fish change dramatically. During the storage of fish, microbial and autolytic activities generate and/or accumulate a number of undesirable volatiles, which may mask the fresh aroma of fish. Accumulation of these volatiles together with other non-volatiles (nitrogenous and non-nitrogenous) cause changes in the flavor and taste of the stored

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10.1021/jf0483826 CCC: $30.25
Published on Web 03/01/2005

fish (*5*−*8*). Volatile compounds of fish at different storage periods have been studied by many researchers (*9*−*13*). These studies showed that different classes of volatile compounds may be used for indexing freshness/spoilage status of fish.

Measurement of volatile compounds can be accomplished by various headspace techniques (*13*−*15*), but dynamic headspace analysis (DHA) and purge-and-trap coupled with gas chromatography−mass spectrometry (GC-MS) have gained popularity as effective, sensitive, and rapid techniques for the isolation and study of fish volatiles (*9*, *16*). Associated advantages are ease of sample preparation, reduced sample size, and occurrence of low levels of artifacts (*10*, *14*−*16*).

The objectives of this study were to evaluate the effect of storage in ice on the volatile compounds of cultured and wild sea bream, to assess and compare changes in the volatile compounds over storage periods, and to identify marker compounds that could possibly be used as quality indicators.

MATERIALS AND METHODS

Fish Samples. Cultured gilthead sea bream, *S. aurata* (average weight and length: 418 ± 71 g and 250 ± 12 mm, respectively), used in this study were cultivated in net cages (located in Messolongi lagoon, Greece) and harvested (∼1 year old) in November 1999. The commercial feed (LAKY, Nea Kerasounta, Prevezis, Greece) used contained 46% protein, 20% fat, 17.6% carbohydrate, 1.2% crude fiber, 8% moisture, and 7.2% ash. Wild sea bream (average weight and length: 407 ± 70 g and 265 ± 13 mm, respectively) were caught in the lagoon of the Aegean Sea. The times of harvest, shipping, handling, and storage were the same for both cultured and wild fish; all other factors (such as feed and environment) prior to capture for wild fish cannot be controlled. All chemicals were obtained from Sigma-Aldrich-Fluka Company Ltd. (Dorset, United Kingdom), unless otherwise specified.

Preparation of Fish Samples and Storage Conditions. Cultured gilthead sea bream were slaughtered by immersing in ice-cold water (hypothermia) and dispatched (packed into an insulated polystyrene box with ice) by TNT World Wide Express to the Food Research Center, University of Lincoln (United Kingdom), within 1 day of harvest. Wild sea bream were also dispatched at the same time in a similar manner. Six cultured or wild fish were immediately sampled (day 1), while the rest (whole fish) were repacked separately (cultured and wild) in flaked ice into polystyrene boxes provided with holes for drainage. Boxes were stored in a cold room (2−4 °C) for up to 23 days from the time of harvest at a fish-to-ice ratio of 2:1 (w/w), maintained throughout the storage period. Volatile analyses were performed on days 1, 5, 9, 12, 16, 19, and 23.

Preparation of Internal Standard (IS) and Deodorized Water. The IS (2,4,6−trimethylpyridine) was dissolved in high-performance liquid chromatography (HPLC) grade methanol at a concentration of 1000 ppm. The final concentration was adjusted to 5 ppm by dilution in deodorized water. HPLC grade water and filtered water gave many artifacts on the chromatogram; hence, deodorized water freshly prepared on a daily basis was used. To achieve this, HPLC grade water was boiled in an open flask until its volume was decreased to one-third of the original. The flask was covered with aluminum foil after boiling and during cooling.

DHA/GC-MS. Volatile compounds in fish were analyzed according to the DHA/GC-MS method of Alasalvar et al. (*9*) with slight modifications. Total ion chromatograms of volatiles were obtained using a Tekmar 3000 purge-and-trap concentrator (Tekmar Inc., Cincinnati, OH), a Star 3400 CX GC, and a Saturn GC/MS/MS 4D (Varian Associates Inc., Palo Alto, CA).

Minced fish sample (5 g) and 1 mL of deodorized water (containing 5 μg of IS, 2,4,6-trimethylpyridine) were placed into a 25 mL needle sparger tube (Tekmar Inc.). The sparger tube, which was mixed using a Vortex for ∼5 s, was immediately attached to the sampling port of a Tekmar 3000 purge-and-trap concentrator and then prepurged for 2.6 min to remove oxygen. It was then preheated at 50 °C for 1 min by a pocket heater (Tekmar Inc.) and purged with ultrahigh purity helium gas at a flow rate of 40 mL/min at 50 °C for 40 min to remove headspace volatiles, which were subsequently adsorbed on a Tenax trap No. 1 (Tekmar Inc.) maintained at room temperature (22 ± 2 °C) during purging. The trap was dry-purged for 10 min in order to remove water and then thermally desorbed at 200 °C for 4 min using helium gas at 1 mL/min. Desorbed compounds were automatically injected (in 0.75 min) into a WCOT fused silica GC column (CP-Wax 52 CB, 60 m × 0.25 mm i.d. × 0.25 μm film thickness; Chrompack, Middelburg, The Netherlands). The flow rate of the helium carrier gas was 1 mL/min. After each run, the Tenax trap was baked at 220 °C for 15 min to remove any possible residual volatile compounds.

Each sample was injected in the splitless (model 1078) mode (200 °C injection temperature; 75 s valve delay). The GC oven temperature was programmed from initial holding at 35 °C for 4 min and then from 35 to 203 °C at 3 °C/min.

MS conditions were as follows: ion source temperature, 180 °C; ionization energy, 70 eV; mass scan range, 33−300 amu; electron multiplier voltage, 1650 V; scan rate, 1000 ms; and ion mode, electron ionization. Furthermore, chemical ionization was also used to aid identification. Samples of white muscle from each of three fish from both cultured and wild sea bream were analyzed in each case, and the results were used for calculating mean values.

Compound Identification and Quantification. Tentative identifications were based on comparison of GC retention indices (RI), determined using *n*-alkanes (C_8−C_{15}) (*17*), and matching mass spectra of unknowns with those in the NIST 92 mass spectral database (Varian Associates Inc.). The relative concentration of a compound in the sample was calculated as follows:

$$\text{relative concentration (ng/g)} = \frac{(\text{peak area of unknown compound/peak area of IS}) \times 5000 \text{ ng of IS}}{\text{amount of fish (5 g)}}$$

Statistical Analysis. Results were expressed as mean values ± standard deviation (SD) ($n = 3$) on a fresh weight basis. Statistical significance (*t*-test: two-sample assuming equal variances) was determined using Microsoft Excel Data Analysis. Differences at $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

As part of a parallel study, comparative quality assessment of cultured and wild sea bream over 23 days of storage period in ice was investigated (*18*). Different postmortem patterns and rates were observed in the various adenosine 5′-triphosphate-breakdown compounds between the two sets of fish. The limit for acceptability of cultured and wild sea bream was ∼16−18 days. Sensory assessment showed that the fresh flavor characteristic of both fish was strong on days 1−5, slowly decreasing in intensity to a bland/relatively flavorless stage on 9−12 days. Off flavors were evident on days 16−18. As spoilage progressed, the off flavors increased in intensity and changed in character, until the fish became unacceptable on days ∼19−23 (*18*).

Volatile Compounds. A total of 60 compounds in cultured (10 aldehydes, 11 ketones, 12 alcohols, 12 aromatics, six terpenes, two furans, three sulfur-containing compounds, one acid, and three miscellaneous compounds) and 78 compounds in wild sea bream (12 aldehydes, 13 ketones, 12 alcohols, 24 aromatics, eight terpenes, two furans, three sulfur-containing compounds, one acid, and three miscellaneous compounds) were tentatively identified (**Table 1**). In addition to this, 23 compounds in cultured and 29 compounds in wild sea bream remained unidentified.

Cultured and wild sea bream exhibited complex volatile profiles. Results in **Table 1** show large differences between cultured and wild sea bream, and concentrations of several classes of compounds gradually increased (**Figure 1**) and several

Table 1. Comparison of Volatile Compounds from Cultured and Wild Sea Bream over a Storage Period in Ice (ng/g)[a]

compd name by class	MI[b]	RI[c]	cultured sea bream (day)				wild sea bream (day)			
			1	9	16	23	1	9	16	23
aldehydes										
butanal	MS[d], RI	875	tr[e]	tr	tr	23 ± 1	8 ± 3	13 ± 2	32 ± 3	19 ± 8
2-methylbutanal	RI	900	86 ± 17	179 ± 40	156 ± 19	130 ± 15	263 ± 13	240 ± 44	497 ± 180	464 ± 122
3-methylbutanal	MS, RI	914	31 ± 7	28 ± 10	48 ± 7	103 ± 33	119 ± 18	63 ± 35	107 ± 20	308 ± 104
hexanal	MS, RI	1076	36 ± 4	103 ± 32	33 ± 4	38 ± 6	86 ± 21	63 ± 27	53 ± 23	83 ± 24
heptanal	MS, RI	1178	21 ± 1	25 ± 19	20 ± 7	18 ± 7	9 ± 3	70 ± 28	69 ± 45	54 ± 30
octanal	MS, RI	1282	45 ± 9	43 ± 13	39 ± 9	30 ± 14	42 ± 8	94 ± 62	188 ± 68	88 ± 43
nonanal	RI	1387	99 ± 31	118 ± 11	65 ± 5	81 ± 6	172 ± 90	218 ± 138	373 ± 77	330 ± 237
(*E,E*)-2,4-hexadienal	RI	1394	181 ± 60	94 ± 9	44 ± 18	35 ± 3	nd	82 ± 22	54 ± 21	43 ± 23
(*E,E*)-2,4-heptadienal	RI	1492	55 ± 3	60 ± 12	90 ± 17	62 ± 10	147 ± 106	226 ± 145	237 ± 53	195 ± 85
benzaldehyde	MS, RI	1520	24 ± 13	15 ± 10	13 ± 7	91 ± 21	32 ± 1	11 ± 4	7 ± 1	35 ± 14
(*E*)-2-nonenal	MS, RI	1530	nd[f]	nd	nd	nd	20 ± 9	16 ± 9	17 ± 11	15 ± 9
(*E*)-2-decenal	MS, RI	1640	nd	nd	nd	nd	22 ± 10	19 ± 0	13 ± 1	11 ± 7
alcohols										
2-methyl-1-propanol	MS	1085	26 ± 9	45 ± 11	33 ± 3	138 ± 35	39 ± 0	143 ± 7	345 ± 37	275 ± 29
2-pentanol	MS, RI	1113	nd	13 ± 3	13 ± 5	12 ± 4	5 ± 1	11 ± 1	19 ± 1	15 ± 1
1-butanol	MS, RI	1137	113 ± 22	195 ± 28	146 ± 5	158 ± 53	180 ± 61	311 ± 88	299 ± 33	233 ± 30
1-penten-3-ol[i]	MS, RI	1153	120 ± 24	2222 ± 9	2932 ± 280	3265 ± 89	118 ± 11	386 ± 15	529 ± 80	1648 ± 180
3-methyl-1-butanol[i]	MS, RI	1199	nd	110 ± 29	123 ± 2	510 ± 94	35 ± 7	259 ± 8	672 ± 124	1083 ± 164
1-pentanol	MS, RI	1242	73 ± 12	194 ± 28	192 ± 15	128 ± 22	77 ± 17	251 ± 22	93 ± 13	95 ± 9
(*Z*)-2-penten-1-ol	MS, RI	1313	24 ± 1	407 ± 49	407 ± 25	218 ± 30	31 ± 2	217 ± 24	51 ± 2	31 ± 5
1-hexanol	MS, RI	1344	33 ± 2	135 ± 7	87 ± 1	84 ± 21	20 ± 4	290 ± 37	48 ± 5	43 ± 1
1-octen-3-ol	MS, RI	1442	33 ± 9	35 ± 2	34 ± 1	224 ± 10	175 ± 22	364 ± 22	102 ± 6	172 ± 20
1-heptanol	MS, RI	1446	tr	52 ± 12	33 ± 6	33 ± 8	24 ± 14	44 ± 4	22 ± 1	32 ± 16
2-ethyl-1-hexanol	MS, RI	1481	1304 ± 242	385 ± 161	356 ± 63	378 ± 128	2695 ± 460	1503 ± 356	897 ± 47	261 ± 60
(*E*)-2-octen-1-ol	MS, RI	1609	nd	22 ± 1	13 ± 4	16 ± 0	34 ± 5	40 ± 10	12 ± 1	22 ± 2
ketones										
2-propanone	RI	813	345 ± 210	148 ± 32	168 ± 16	171 ± 80	223 ± 9	170 ± 14	308 ± 33	222 ± 82
2-pentanone	MS, RI	971	28 ± 1	54 ± 12	48 ± 5	79 ± 32	35 ± 3	39 ± 9	17 ± 6	107 ± 41
2,3-butanedione	RI	976	20 ± 9	25 ± 3	12 ± 1	75 ± 11	20 ± 12	54 ± 15	60 ± 14	26 ± 2
3-methyl-2-pentanone	MS, RI	1011	nd	nd	nd	nd	nd	tr	tr	20 ± 4
2,3-pentanedione	MS, RI	1056	8 ± 2	70 ± 39	25 ± 2	40 ± 18	nd	14 ± 5	nd	6 ± 0
cyclopentanone	MS, RI	1176	tr	13 ± 5	10 ± 1	22 ± 5	42 ± 20	7 ± 1	9 ± 4	42 ± 14
2-octanone	RI	1281	nd	tr	28 ± 8	26 ± 12	91 ± 63	82 ± 3	58 ± 1	nd
6-methyl-5-hepten-2-one	MS, RI	1330	150 ± 30	104 ± 11	49 ± 7	82 ± 18	242 ± 168	348 ± 222	155 ± 47	422 ± 250
2-nonanone	MS, RI	1381	18 ± 0	8 ± 4	13 ± 1	20 ± 2	18 ± 8	27 ± 2	24 ± 17	15 ± 3
2-decanone	RI	1494	136 ± 49	139 ± 12	111 ± 24	177 ± 1	157 ± 20	297 ± 98	58 ± 23	73 ± 14
(*E,Z*)-3,5-octadien-2-one	MS, RI	1513	nd	nd	nd	nd	nd	22 ± 3	8 ± 3	7 ± 0
(*E,E*)-3,5-octadien-2-one	RI	1565	tr	13 ± 6	7 ± 1	23 ± 3	22 ± 10	24 ± 12	55 ± 24	18 ± 8
2-undecanone	MS, RI	1593	10 ± 1	19 ± 1	9 ± 1	23 ± 8	7 ± 1	13 ± 1	14 ± 8	7 ± 3
aromatics										
benzene	MS, RI	936	nd	nd	nd	nd	nd	27 ± 11	54 ± 17	21 ± 15
toluene	MS, RI	1034	50 ± 14	146 ± 19	101 ± 10	67 ± 40	42 ± 1	98 ± 10	75 ± 15	183 ± 8
ethyl benzene	MS, RI	1120	19 ± 3	64 ± 4	67 ± 6	43 ± 15	21 ± 2	89 ± 23	46 ± 5	30 ± 4
p-xylene	MS, RI	1134	37 ± 0	25 ± 0	61 ± 2	36 ± 11	20 ± 7	77 ± 13	48 ± 5	27 ± 4
propylbenzene	MS, RI	1200	nd	nd	nd	nd	50 ± 7	38 ± 5	nd	nd
1-ethyl-4-methyl-benzene	MS	1216	10 ± 0	tr	tr	nd	102 ± 26	69 ± 9	33 ± 5	5 ± 1
1-ethyl-3-methyl-benzene	MS	1218	30 ± 3	20 ± 12	17 ± 4	tr	167 ± 16	106 ± 21	45 ± 10	33 ± 8
1,3,5-trimethylbenzene	MS, RI	1237	26 ± 3	19 ± 0	10 ± 3	16 ± 8	113 ± 9	89 ± 14	37 ± 5	16 ± 4
styrene	MS, RI	1251	42 ± 1	29 ± 1	80 ± 3	56 ± 16	24 ± 15	37 ± 6	38 ± 12	29 ± 1
1-ethyl-2-methylbenzene	MS	1255	nd	nd	nd	nd	54 ± 4	32 ± 1	nd	nd
1,2,4-trimethylbenzene	MS, RI	1275	50 ± 7	21 ± 3	44 ± 12	43 ± 22	33 ± 4	216 ± 33	96 ± 21	39 ± 2
1,4-diethylbenzene	MS	1293	nd	nd	nd	nd	24 ± 1	nd	nd	nd
1-methyl-4-propylbenzene	MS	1295	218 ± 42	93 ± 14	85 ± 25	53 ± 14	41 ± 4	39 ± 3	nd	nd
1-methyl-3-propylbenzene	MS	1297	nd	nd	nd	nd	40 ± 1	34 ± 7	nd	nd
1-methyl-2-propylbenzene	MS	1299	nd	nd	nd	nd	24 ± 3	16 ± 4	nd	nd
1,3-diethylbenzene	MS	1301	nd	nd	nd	nd	7 ± 1	tr	nd	nd
1-ethyl-3,5-dimethylbenzene	MS	1319	nd	nd	nd	nd	65 ± 4	63 ± 8	tr	nd
α-methylstyrene	MS	1326	15 ± 5	22 ± 4	16 ± 2	6 ± 2	11 ± 6	tr	tr	nd
1-ethyl-2,4-dimethylbenzene	MS	1347	nd	nd	nd	nd	39 ± 1	22 ± 5	nd	nd
1-ethyl-2,3-dimethylbenzene	MS	1362	nd	nd	nd	nd	62 ± 4	46 ± 2	19 ± 6	nd
1,2,4,5-tetramethylbenzene	MS	1419	nd	nd	nd	nd	34 ± 2	24 ± 6	17 ± 2	nd
1,2,3,5-tetramethylbenzene	MS	1430	nd	nd	nd	nd	58 ± 17	50 ± 10	17 ± 7	nd
1,4-dichlorobenzene	MS, RI	1438	30 ± 6	216 ± 9	148 ± 26	43 ± 9	18 ± 2	tr	12 ± 2	20 ± 2
naphthalene	MS, RI	1743	27 ± 3	24 ± 4	23 ± 3	23 ± 5	18 ± 2	32 ± 1	24 ± 3	11 ± 5
terpenes										
α-pinene	MS, RI	1015	17 ± 1	28 ± 12	251 ± 20	175 ± 51	20 ± 1	tr	nd	50 ± 24
sabinene	RI	1101	nd	8 ± 2	18 ± 6	15 ± 2	4 ± 2	63 ± 10	34 ± 6	27 ± 5
3-carene	MS	1141	nd	nd	192 ± 27	161 ± 35	5 ± 0	nd	45 ± 1	115 ± 47
limonene	MS, RI	1191	183 ± 85	71 ± 21	146 ± 3	98 ± 30	23 ± 7	39 ± 14	32 ± 15	55 ± 29
p-cymene	RI	1264	51 ± 28	38 ± 12	37 ± 4	32 ± 8	45 ± 21	22 ± 15	14 ± 2	18 ± 6
camphor	MS, RI	1511	nd	nd	nd	nd	13 ± 5	12 ± 1	28 ± 16	13 ± 2
bornyl acetate	MS	1666	nd	nd	nd	nd	nd	tr	13 ± 7	nd
borneol	MS	1699	tr	tr	tr	tr	nd	19 ± 0	15 ± 3	nd

Table 1. (Continued)

compd name by class	MI[b]	RI[c]	cultured sea bream (day) 1	9	16	23	wild sea bream (day) 1	9	16	23
furans										
tetrahydrofuran	MS	854	51 ± 9	79 ± 13	127 ± 3	96 ± 32	79 ± 58	44 ± 4	117 ± 47	44 ± 31
2-ethylfuran	RI	949	150 ± 40	118 ± 23	101 ± 35	64 ± 19	122 ± 42	41 ± 6	75 ± 32	36 ± 2
S-containing compounds										
methanethiol[i]	MS	1043	nd	nd	7 ± 2	16 ± 1	nd	nd	26 ± 13	196 ± 79
dimethyl disulfide[i]	MS, RI	1068	20 ± 11	45 ± 2	225 ± 56	560 ± 63	nd	30 ± 8	69 ± 36	617 ± 49
dimethyl trisulfide[i]	MS, RI	1375	nd	nd	34 ± 9	198 ± 76	nd	nd	19 ± 14	465 ± 57
acid										
acetic acid[i]	MS, RI	1463	56 ± 19	72 ± 8	100 ± 1	104 ± 22	38 ± 15	78 ± 3	85 ± 2	142 ± 2
miscellaneous compounds										
trimethylamine[i]	MS		237 ± 47	504 ± 42	2402 ± 703	3716 ± 83	468 ± 139	1141 ± 76	3523 ± 202	5322 ± 650
piperidine[i]	MS	795	23 ± 7	26 ± 1	35 ± 5	38 ± 1	37 ± 5	41 ± 10	64 ± 7	88 ± 9
chloroform	RI	1020	123 ± 32	86 ± 7	122 ± 49	210 ± 137	155 ± 59	73 ± 9	159 ± 22	75 ± 40
2,4,6-trimethyl-pyridine (IS)[g]	MS, RI	1355								
total unknowns			677 (13.1%)[h]	1551 (18.5%)	1361 (12.2%)	1530 (11.0%)	810 (10.0%)	1229 (12.2%)	1107 (9.6%)	888 (5.9%)
total volatiles			5159 ± 244[j]	8378 ± 308[k]	11169 ± 1088[kl]	13911 ± 1260[l]	8098 ± 569[j]	10063 ± 610[j]	11487 ± 1062[jk]	15086 ± 837[k]

[a] Data are expressed as means ± SD ($n = 3$) on a fresh weight basis. The percentage relative standard deviation (RSD) ranges from 0 to 75%. Sampling days were reduced from 7 (days 1, 5, 9, 12, 16, 19, and 23) to 4 (days 1, 9, 16, and 23). [b] MI, methods of identification. [c] RI, retention indices, which were obtained from refs *21*, *27*, *30*, *32*, and *50–53*. The GC columns and methods used by these references were comparable. [d] MS, mass spectral data. [e] tr, trace. [f] nd, not detected. [g] IS, internal standard. [h] Numbers in parentheses indicate percent of unknown compounds in the total amount of volatiles. [i] Compounds that show increases are shown in **Figure 1**. [j–l] Means ± SD followed by the same letter, within a row, are not significantly different ($p > 0.05$).

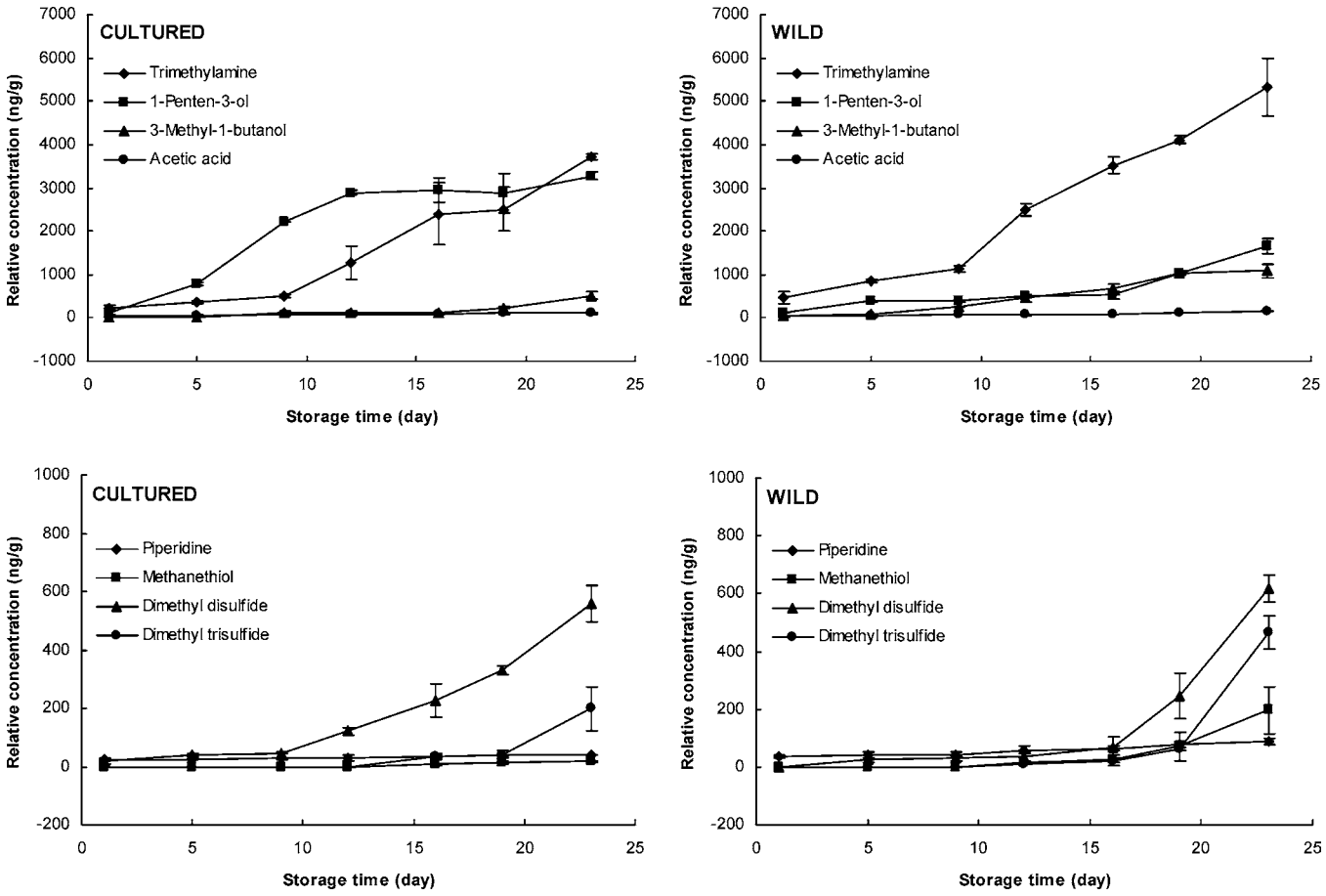

Figure 1. Volatile compounds of cultured and wild sea bream, which showed increases over a storage period in ice. Error bars show the variations of three determinations in terms of SD.

decreased during the 23 days of storage in ice. The relative concentration of total volatiles in cultured and wild sea bream was 5159 ± 244 and 8098 ± 569 ng/g on day 1, and these values increased significantly ($p < 0.05$) to 13911 ± 1260 and 15086 ± 837 ng/g on day 23, respectively. The total content of unknown compounds ranged from 5.9 to 18.5% of the total volatiles present in cultured and wild sea bream (**Table 1**). The combination of several classes of volatile compounds is responsible for the distinctive and unique flavor of fresh cultured and wild sea bream. The contribution of volatiles to flavor is dependent upon their recognition threshold values and concentrations. Although the selection of the "best" fish is a subjective

matter, more volatile compounds were found in wild sea bream than in cultured bream. The different classes of compounds found in both cultured and wild sea bream and their changes over the storage period along with their formation and general odor descriptions (where necessary) have been explained below.

Aldehydes. Ten aldehydes were found in cultured sea bream, and 12 were present in wild sea bream. The concentrations of (*E*)-2-nonenal and (*E*)-2-decenal were not detected in cultured sea bream. Aldehydes found in both fish showed fluctuation over the storage period. The odor thresholds of carbonyls compounds (aldehydes and ketones) are generally lower than those of alcohols (*4*, *19*). Therefore, aldehydes have an overriding effect on the flavor of many other substances, even when present in trace amounts (*20*). The majority of aldehydes, which have been reported as being green plant-like, grassy, dark chocolate, malty, fatty, sweet floral, apple-like, melon-like, nutty, and fruity, are present in various fresh fish and shellfish at various concentrations (*5*, *7*, *8*, *20*). The majority of aldehydes may be considered as lipid autoxidation products (*21*).

Ketones. Eleven ketones were found in cultured sea bream, and 13 were found in wild sea bream. Of these, 3-methyl-2-pentanone and (*E*,*Z*)-3,5-octadien-2-one were not detected in cultured sea bream. The concentration of most ketones showed fluctuation over the storage period. The most abundant compounds among identified ketones were 2-propanone, 6-methy-5-hepten-2-one, and 2-decanone. Generally, lower aroma threshold volatile ketones result in greater contributions to overall fresh fish-like odors (*4*). Ketones may be produced by thermal oxidation/degradation of polyunstaturated fatty acids (*4*), amino acid degradation (*22*), or microbial oxidation (*23*).

Alcohols. Twelve alcohols were detected in both cultured and wild sea bream. Among these, the concentrations of 1-penten-3-ol and 3-methyl-1-butanol (**Table 1** and **Figure 1**) increased during the storage of both fish, whereas that of 2-ethyl-1-hexanol decreased. The 2-ethyl-1-hexanol has been reported to decrease in sterile cold-smoked salmon (*24*). The increases in concentrations of 1-penten-3-ol and 3-methyl-1-butanol over the storage period of fish are in good agreement with those reported previously (*9*, *10*, *13*, *24*). Miller III et al. (*25*) observed that when sterile fish muscle blocks were inoculated with *Pseudomonas perolens*, 1-penten-3-ol and 3-methyl-1-butanol were produced and their concentrations increased during storage. These two compounds may be produced by microbial spoilage, and 1-penten-3-ol was the most noticeable compound detected in rancid sardine oil (*26*).

Among alcohols, 1-octen-3-ol (found in both cultured and wild sea bream), a degradation product of linoleic acid hydroperoxides, has been identified as one the major volatile alcohols in shellfish (*20*) and cooked alligator meat (*27*). Volatile alcohols are generally minor contributors to food flavor because of their high thresholds unless they are present at high concentrations or are unsaturated (*28*). They mostly possess fragrant, planty, rancid, and earthy odors (*29*) and contribute smoother qualities (*7*).

Aromatics. Among the aromatics identified, 12 compounds were detected in cultured sea bream and 24 compounds were detected in wild sea bream. The concentration of most aromatics decreased during the storage period (**Table 1**). Aromatics have been reported in various fish (*13*, *30*, *31*) and shellfish species (*20*, *21*, *32*). Although the origin of benzene derivatives is uncertain, Hsieh et al. (*19*) reported that these compounds might be transferred to crayfish from environmental pollutants.

Terpenes. Six terpenes in cultured and eight in wild sea bream were detected (**Table 1**), of which 3-carene, bornyl acetate, and berneol were identified for the first time in fish. Limonene was the only terpene reported in fish earlier (*13*, *31*). α-Pinene, sabinene, limonene, *p*-cymene, and camphor have been reported in various shellfish (*33*, *34*). These terpenes may have originated from alga or plants via the food chain (*34*). Unlike cultured sea bream, wild sea bream contained more terpenes. This may be due to the existing differences in the diets between the two fish. Terpenes present in cultured and wild sea bream have been reported as having pine, fruity, citrus, carrot top, carrot-like, and fresh green notes in carrots (*35*).

Furans. Two furans (tetrahydrofuran and 2-ethylfuran) were identified in both fish, of which the former has not been reported in seafoods. The compound 2-ethylfuran was in higher concentrations initially in both fish than tetrahydorfuran. Most furans, including 2-ethylfuran, have been reported to contribute burnt, sweet, bitter, cooked meat, and coconut-like flavors in some foods (*36*). Furan arises from the reaction of amino acids and sugars through Maillard and Strecker degradation (*37*).

Sulfur-Containing Compounds. Three straight chain sulfur-containing compounds (methanethiol, dimethyl disulfide, and dimethyl trisulfide) were found in cultured and wild sea bream and showed increases during the storage period (**Table 1** and **Figure 1**). These compounds were in trace amounts initially or were not detected but showed rapid increases after day 16 when both fish were considered at the limit of acceptability (*18*). Therefore, they might not contribute desirable volatile components for sea bream. Alasalvar et al. (*9*) studied the volatile aroma compounds in fresh and stored (5 days at 15 ± 2 °C) mackerel. Sulfur-containing compounds, which comprised 74% of the total peak area, were only detected in stored mackerel.

Sulfur-containing compounds are formed during processing and storage of foods and contribute both desirable and undesirable aromas, depending on their concentrations (*20*). The presence of high levels of methanethiol possessing putrid, rotten, and sulfurous notes generally has a negative impact on seafood quality (*20*, *38*, *39*). Both dimethyl disulfide and dimethyl trisulfide are often found in foodstuffs and usually affect overall food aroma because of their low threshold values (*40*). These two compounds, at higher concentrations, are associated with onion-like off flavors, cooked cabbage, spoiled odors, strong sulfurous, and putrid notes in marine products (*9*, *38*, *41*, *42*). Dimethyl disulfide was reported to arise from oxidation of methanethiol or bacterial degradation of methionine (*43*). Dimethyl trisulfide results from microbial action of *Pseudomonas* spp. (*25*) or bacterial contamination (*42*). Volatile sulfur compounds are usually associated with deteriorated seafoods (*7*).

Acid. Acetic acid, which was the only acid detected in both cultured and wild sea bream, showed an increasing trend during the storage period (**Table 1** and **Figure 1**). This increase was in good agreement with published results for stored mackerel (*9*) and Baltic herring (*12*). Volatile acids are formed from amino acids through bacterial fermentation, but lipid oxidation may also be responsible for the production of some of these acids (*44*). Aro et al. (*12*) stated that acetic acid along with other volatile acids, namely, propanoic, 2-methylpropanoic, and 3-methylybutanoic acids, are partially responsible for the increasing unpleasant odor during the storage of herring.

Miscellaneous Compounds. Three miscellaneous compounds, namely, trimethylamine (TMA), piperidine, and chloroform, were identified in both cultured and wild sea bream. Among these, the concentration of TMA and piperidine increased continually throughout the storage period in both fish (**Table 1** and **Figure 1**). TMA originates from the breakdown of TMA-

oxide by bacterial enzymes and is therefore related to bacterial spoilage of refrigerated marine fish (*45*). TMA values also increased for ice-stored sea bream (*46*). The formation of TMA in various fish and shellfish has been associated with off flavor and ammonia-like and fishhouse-like odors (*8*, *47*, *48*). High piperidine contents were reported in salmon just after spawning, and its level increased as spawning approached. Because of such off flavors, spawned salmon is rejected for consumption (*49*). Piperidine is a product formed by cyclization of cadaverine (*8*). Chloroform does not contribute to the aroma of sea bream, and this compound may be an artifact.

ACKNOWLEDGMENT

We thank Dr. Maria Alexis (NCMR-Greece) for acquiring and supplying the fresh cultured and wild sea bream.

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Received for review September 28, 2004. Revised manuscript received January 18, 2005. Accepted January 22, 2005. We acknowledge funding from the European Union (an INCO-COPERNICUS Project) for this study.

JF0483826